Advances in Mass Spectrometry for Lipidomics

Stephen J. Blanksby[1] and Todd W. Mitchell[2]

[1]School of Chemistry and [2]School of Health Sciences, University of Wollongong, Wollongong, New South Wales 2522, Australia; email: stephen_blanksby@uow.edu.au, todd_mitchell@uow.edu.au

Annu. Rev. Anal. Chem. 2010. 3:433–65

First published online as a Review in Advance on April 1, 2010

The *Annual Review of Analytical Chemistry* is online at anchem.annualreviews.org

This article's doi: 10.1146/annurev.anchem.111808.073705

1936-1327/10/0719-0433$20.00

Key Words

lipids, electrospray ionization, liquid chromatography, collision-induced dissociation, imaging mass spectrometry, ion mobility

Abstract

Recent expansion in research in the field of lipidomics has been driven by the development of new mass spectrometric tools and protocols for the identification and quantification of molecular lipids in complex matrices. Although there are similarities between the field of lipidomics and the allied field of mass spectrometry (e.g., proteomics), lipids present some unique advantages and challenges for mass spectrometric analysis. The application of electrospray ionization to crude lipid extracts without prior fractionation—the so-called shotgun approach—is one such example, as it has perhaps been more successfully applied in lipidomics than in any other discipline. Conversely, the diverse molecular structure of lipids means that collision-induced dissociation alone may be limited in providing unique descriptions of complex lipid structures, and the development of additional, complementary tools for ion activation and analysis is required to overcome these challenges. In this article, we discuss the state of the art in lipid mass spectrometry and highlight several areas in which current approaches are deficient and further innovation is required.

1. INTRODUCTION

Lipidomics is concerned with the identification and quantification of lipids within a given biological system; it also seeks to elucidate how individual molecular species affect lipid metabolism and the function (or dysfunction) of the system as a whole. The field has recently emerged as a crucial component in the broader push to arrive at an integrated picture of the role of genes, proteins, and metabolites that fully describes cellular function. The evolution of contemporary lipidomics and its increased prominence in systems biology over the past ten years have been the subject of several reviews and perspective articles (1–3). From the literature, two themes emerge as drivers of the current renaissance in lipid biochemistry. First, new insights into functional interactions in biochemical systems have revealed the specific nature of lipid-lipid and lipid-protein interactions. Therefore, lipids can no longer be considered as simple baskets for proteins; rather, they have emerged as key players with distinctive biochemical roles and biophysical properties. Second, the availability of new analytical tools and, in particular, recent developments in lipid mass spectrometry (MS) are providing the ability to track changes in individual lipids at very low levels and to take quantitative snapshots of significant portions of the lipidome.

Ultimately, lipidomics must quantitatively describe all lipids and their functions at the cellular level (2). This requirement presents the formidable challenge to the analyst of identifying and quantifying lipids at vanishingly low concentrations in systems containing many hundreds and perhaps even thousands of structurally diverse lipids. In a recent study employing state-of-the-art MS technologies, Ejsing et al. (4) identified 342 distinct molecular lipids (and quantified 250 of these) in extracts from yeast. Although this number is impressive, it may ultimately reflect only a fraction of the full lipid diversity in these organisms.

Historically, lipids were defined by their physical properties—specifically their solubility in nonpolar solvents—rather than by the common motifs among their molecular structures. Recently, investigators have attempted to refine this definition: Fahy et al. (5, 6) have suggested a classification based on lipid biosynthesis, namely "hydrophobic or amphipathic small molecules that may originate entirely or in part by carbanion-based condensations of thioesters (e.g., fatty acyls) and/or by carbocation-based condensation of isoprene units (e.g., sterols)" (5, p. 840). Even within this more specific definition, lipids encompass a broad range of molecular structures (**Figure 1**). Unlike other classes of biomolecules that can be considered as permutations on a common and finite set of monomers (e.g., proteins and oligonucleotides), complex lipids such as glycerophospholipids and sphingolipids are composites of a wide range of building blocks that can give rise to a bewildering array of combinations. Permutations that may arise only from common eukaryotic lipid motifs give rise to more than 180,000 theoretical phospholipid structures that could be present in a given cell or tissue extract (7). Note that this number does not include complexity that may arise from consideration of isomeric lipids that differ only in double-bond position, backbone substitution (e.g., *sn* position, where *sn* stands for stereospecific numbering), or stereochemistry (e.g., *cis* and *trans* double bonds) (8). The need to develop analytical tools that can readily tackle such a diverse range of molecular structures is a key reason that lipidomics has lagged behind her sister disciplines of genomics and proteomics.

Although both chromatography and nuclear magnetic resonance have played a role in advancing lipidomics, it is inarguably the recent advances in MS that have propelled the field forward (1–3). In contemporary lipidomics, MS has been deployed in two ways, namely (*a*) targeted lipidomics, which focuses on the identification and quantification of a single lipid or subset of lipids in a tissue or cellular extract, and (*b*) so-called global lipidomics, which aims to identify and quantify all the

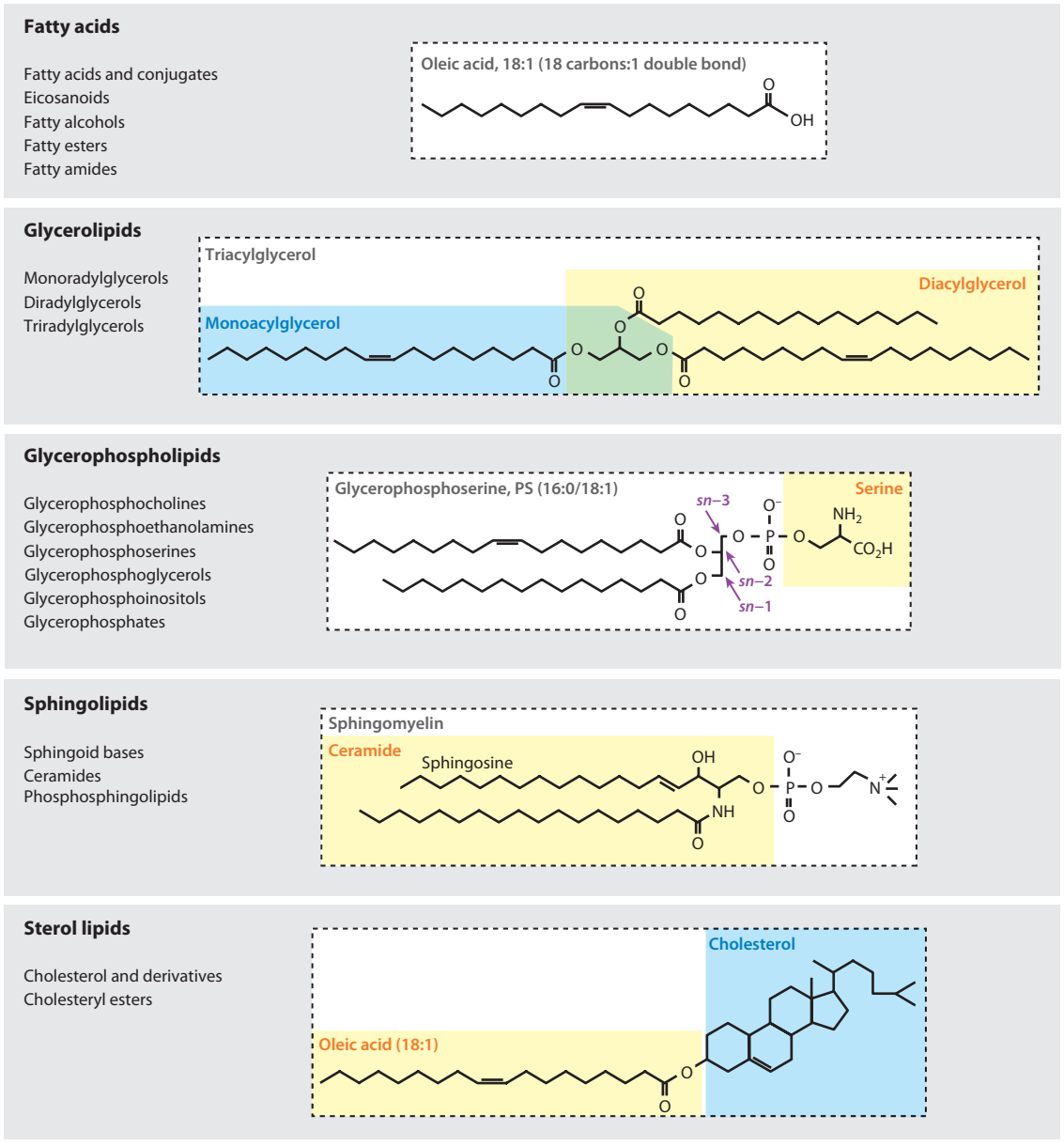

Figure 1

Common lipid classes and representative structures. Adapted with permission from Reference 3.

lipids in a system (9). Both approaches have been advanced by innovations in MS and the parallel evolution of associated tools for data analysis. In this article, we discuss the state of the art in MS for lipidomics and highlight where, in our view, further efforts and innovations are required to overcome some of the challenges unique to this field.

2. IONIZATION OF LIPIDS FOR MASS SPECTROMETRIC ANALYSIS

2.1. Gas Chromatography–Mass Spectrometry

Electron ionization (EI): process in which vapor-phase molecules are ionized by energetic electrons (70 eV) that strip a valence electron from the analyte, resulting in the formation of an $M^{\cdot+}$ molecular ion with concomitant fragmentation

Field ionization (FI): when molecules passing through a very high electric field (~12 keV) are ionized by removal of an electron via quantum tunneling to produce an $M^{\cdot+}$ molecular ion with very little associated fragmentation

For low-molecular-weight lipids such as fatty acids and sterols, the use of gas chromatography–mass spectrometry (GC-MS) with electron ionization (EI) is a mainstay for lipid identification and quantification (10). This technique has not changed significantly over the past 40 years, and although it is widely used, it has some limitations. For example, the extensive fragmentation that takes place during EI requires the complete chromatographic separation of components prior to MS analysis; further, the high energies (70 eV) involved in EI can facilitate structural rearrangement that results in indistinguishable mass spectra for isomeric lipids. Hejazi et al. (11) investigated the use of lower EI energies for the analysis of fatty acid methyl esters (FAMEs) and found that isomeric lipids, which produced indistinguishable mass spectra at 70 eV, can be differentiated at 30 eV with only a fourfold loss in sensitivity. The low-energy EI spectra were used to uniquely assign the double-bond geometries (i.e., *cis* or *trans*) of all three double bonds in isomeric α-linoleic acid methyl esters. This relatively simple approach may have the potential for use in other isomeric lipids that differ in terms of their geometry and/or position of double bonds—a common motif in lipid biochemistry.

Although field ionization (FI) was used for lipid analysis in the mid-1970s (12), it has fallen out of favor due to significantly lower and less stable ion currents. The recent combination of FI with orthogonal acceleration time-of-flight (TOF) MS, however, has overcome many of these difficulties (13). When applied to the analysis of FAMEs, FI produces molecular ions with little associated fragmentation. It has been suggested that under these soft-ionization conditions the mass-to-charge (m/z) scale can be presented as a second dimension of fractionation (**Figure 2*a***). Hibbert and coworkers (14) have undertaken such two-dimensional analyses of FAMEs using GC with a highly polar column to achieve polarity separation and FI-MS in place of a conventional boiling-point fractionation step. The advantages of such an approach are that (*a*) complex FAMEs can be separated in two dimensions to reveal coeluting species and (*b*) the FAMEs become grouped—vertically by carbon number and horizontally according to degree of unsaturation and double-bond stereochemistry (i.e., *cis* and *trans*)—allowing the possibility of de novo identification of fatty acids.

Advances in the speed of TOF analyzers (generally at the expense of resolution) have facilitated the rapid adoption of EI mass spectrometers as fast, selective detectors for comprehensive two-dimensional GC (GC × GC) (15). Such techniques couple two GC separations via a modulator and can thus provide two complementary fractionation steps in addition to the structural information from the EI mass spectrum. For example, Jover et al. (16) identified 30 simple lipids, including fatty acids and fatty alcohols, from the GC × GC analysis of a hydrolyzed and derivatized extract from lanolin (**Figure 2*b***). The extra level of separation provided by GC × GC also permits, at least in some cases, de novo identification of lipids from EI mass spectra, even within a complex matrix.

2.2. Electrospray Ionization

Early analysis of complex, high-molecular-weight lipids [e.g., glycerolipids, glycerophospholipids, and sphingomyelins (SMs)] was conducted by fast atom bombardment (FAB) MS. Indeed, much of what we now know about the fragmentation of ionized lipids is based on seminal work that used FAB coupled with tandem mass spectrometers (17). Although FAB made a major contribution to lipid MS, it was fundamentally limited by (*a*) low overall sensitivity, (*b*) the presence of matrix ions, and (*c*) significant source fragmentation that precluded

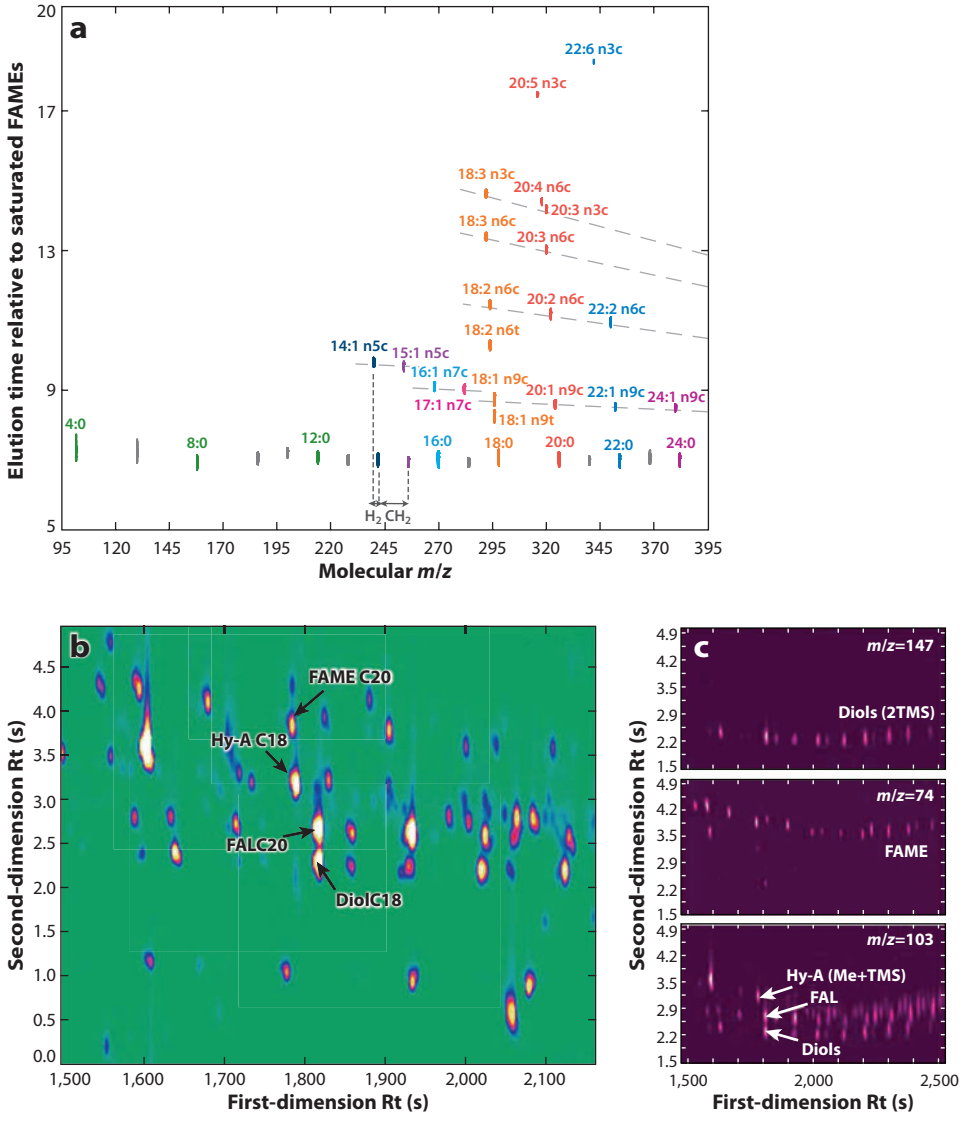

Figure 2

(*a*) A two-dimensional representation of data acquired by gas chromatography–field ionization–mass spectrometry (GC-FI-MS) for a mixture of fatty acid methyl esters (FAMEs). The chromatographic elution time (determined relative to saturated FAMEs) from a polar GC column is plotted against the *m/z* of the molecular ion generated by FI-MS. FAMEs with the same number of carbons are shown with like colors and are vertically aligned; the dashed lines indicate horizontal trends associated with the degree of unsaturation. Reproduced with permission from Reference 14. (*b*) A two-dimensional plot from GC × GC analysis of a hydrolyzed and derivatized (by methylation of fatty acids and silylation of fatty alcohols) wool lanolin extract obtained via a fast electron ionization (EI) mass spectrometer as a detection system. The nonpolar (*x* axis) and polar (*y* axis) chromatographic dimensions provide separation by chain length and degree and nature of unsaturation, respectively. (*c*) EI mass spectral data provide molecular identification and allow for the simplification of chromatographic data by class. Reproduced with permission from Reference 16.

quantitative analysis. The application of electrospray ionization (ESI) MS to the analysis of glycerophospholipids in the mid-1990s (18–20) overcame these problems. An early publication on the use of ESI for the examination of phospholipids reported an increase of two to three orders of magnitude in sensitivity over FAB, enabling the identification of more than 50 phospholipid species in human erythrocyte plasma membranes from less than 1 μl of blood (18). Recent developments in nanospray technologies have further increased the sensitivity of such analyses, and chip-based nanospray-array devices have significantly increased sample throughput (4). ESI has given rise to two main approaches for lipid analysis, namely online high-performance liquid chromatography–mass spectrometry (HPLC-MS) and direct-infusion ESI-MS; the latter is sometimes referred to as shotgun lipidomics.

Electrospray ionization (ESI): technique in which analytes in solution are volatilized and ionized under ambient conditions by infusion of the solution through a narrow-bore needle in the presence of a high electric field

Collision-induced dissociation (CID): when ionized molecules are mass selected and then subjected to an energetic collision with an inert gas (e.g., Ar, N_2, or He); conversion of ion-translational energy into internal energy results in dissociation of the molecule into charged and neutral fragments, and mass analysis of the former yields the CID spectrum

The shotgun lipidomic approach arises from the intrinsic soft-ionization behavior of ESI. It is particularly effective for polar lipids (e.g., phospholipids and sulfatides) because these species are readily ionized by either protonation $[M+H]^+$ or deprotonation $[M-H]^-$, depending on their molecular structure. As such, under typical ESI conditions each lipid gives rise to a single, intact anion or cation. The resulting spectrum can thus be considered as a profile of the lipids in the extract. The major concern with the shotgun approach is ion suppression, particularly when a crude lipid extract containing a broad matrix of lipids is diluted and infused directly into the mass spectrometer. Depending on the application, however, ion suppression may not be detrimental. An elegant example of the use of discriminatory ionization properties of lipids to simplify spectra is the intrasource separation of phospholipid classes (21, 22). However, if the target lipid is subject to unwanted ion suppression, several strategies may be implemented. These include (*a*) production of adduct ions in the electrospray source, (*b*) differential extraction, and (*c*) solid-phase extraction (SPE).

The first (and simplest) method for improving the ionization efficiency of nonpolar lipids involves the formation of adduct ions during ESI. This is achieved by doping the spray solvent with a small amount of an ionic salt and is often employed in the analysis of triacylglycerols, in which $[M+Li]^+$ (23), $[M+Na]^+$ (24), and $[M+NH_4]^+$ (25) ions are commonly formed following the addition of appropriate dopants. Adduct ions can also be employed to enhance desirable fragmentation pathways during collision-induced dissociation (CID; see below), and for this reason metal adducts of phospholipids (26, 27) and fatty acids (28) have also been analyzed. Although this approach can be advantageous in simple lipid extracts, it may increase spectral complexity if numerous lipids capable of forming adduct ions are present in the sample.

A recent example of the second (differential extraction) approach is the analysis of the yeast lipidome by Ejsing et al. (4). In this experiment, neutral lipids were first extracted via a 17:1 chloroform-to-methanol solvent mixture, whereas polar lipids were subsequently extracted in a 2:1 chloroform-to-methanol mixture. Analysis of these extracts through use of quadrupole/TOF and linear ion-trap/orbitrap mass spectrometers equipped with automated nanospray ion sources allowed the quantification of 21 different lipid classes and 250 molecular lipids from only 2 million cells. **Figure 3** shows all 21 quantified lipid classes and an example of the molecular data obtained (inositolphosphoceramides) in four strains of *Saccharomyces cerevisiae*. The level of information on the yeast lipidome obtained in this study is a compelling example of the power of the shotgun approach.

The third approach for reducing ion suppression is to perform a standard, total-lipid extract and then fractionate the extract via SPE. An example of this approach is the analysis of triacylglycerols by McAnoy et al. (25). In this experiment, lipids were extracted from RAW 264.7 macrophage-like cells and loaded onto an amino propyl column. The neutral lipids were then eluted with a 2:1 chloroform-to-isopropanol mixture. The removal of polar lipids allowed the acquisition

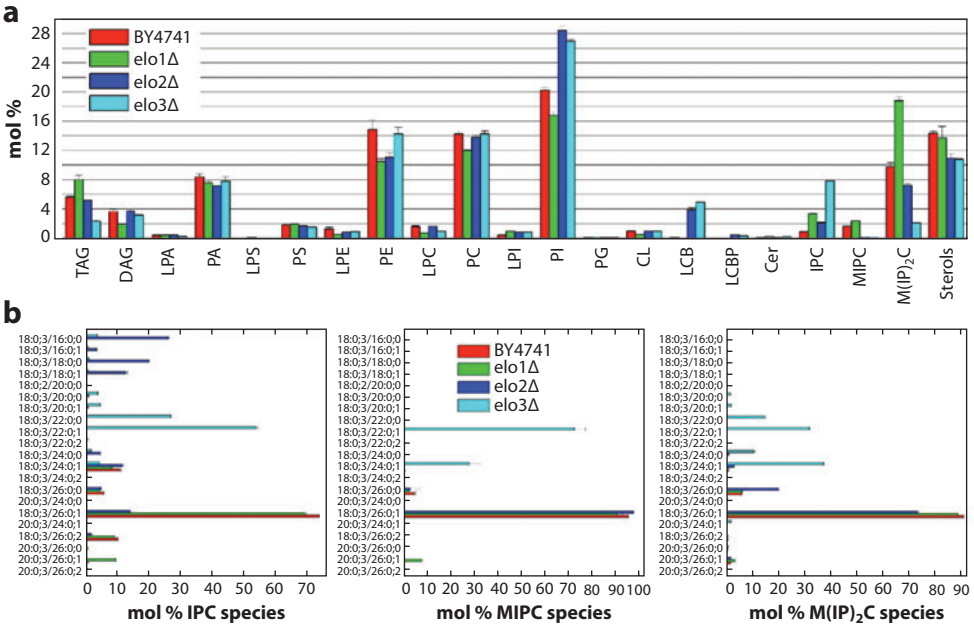

Figure 3

Comparative lipidomics of yeast strains: wild-type BY4741, elo1Δ, elo2Δ, and elo3Δ. (*a*) Lipid class composition. (*b*) Molecular composition of sphingolipid species. Error bars indicate ± standard deviation ($n = 4$). Abbreviations: Cer, ceramide; CL, cardiolipin; DAG, diacylglycerol; IPC, inositolphosphoceramide; LCB, long-chain sphingoid base; LCBP, LCB phosphate; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; LPS, lysophosphatidylserine; MIPC, mannosylinositol phosphorylceramide; PA, glycerophosphate; PC, glycerophosphocholine; PE, glycerophosphoethanolamine; PG, glycerophosphoglycerol; PI, glycerophosphoinositol; PS, glycerophosphoserine; TAG, triacylglycerol. Reproduced with permission from Reference 4.

of extremely rich triacylglycerol spectra (see **Figure 4**) and permitted the identification of over 50 molecular triacylglycerols.

Although both differential fractionation and SPE can greatly enhance the ability of ESI-MS to detect and even quantify neutral lipids, there are certain cases in which more rigorous purification of target lipids by chromatographic methods is required. Given the ease with which HPLC can be coupled to an ESI source, HPLC-MS is the chromatographic technique of choice and is often employed for the analysis of low-abundance signaling lipids such as endocanabinoids (cf. fatty amides) (29) and eicosanoids (30). Although it is beyond the scope of this review to comprehensively cover HPLC-MS analysis of lipids, an interesting development in the area is the use of nanoflow and ultrahigh-performance liquid chromatography MS (31). These techniques significantly increase the speed of the chromatographic analysis (with run times of ~10 min) and improve the sensitivity and resolution (32), which is clearly beneficial for large-scale lipidomic style analyses.

Matrix-assisted laser desorption/ionization (MALDI): technique in which analytes are desorbed and ionized from a solid or solvent matrix by irradiation with a laser beam

2.3. Matrix-Assisted Laser Desorption/Ionization

The initial reports on matrix-assisted laser desorption/ionization (MALDI) analysis of intact glycerophospholipid and sphingolipid species (33–35) appeared shortly after the early ESI papers, but

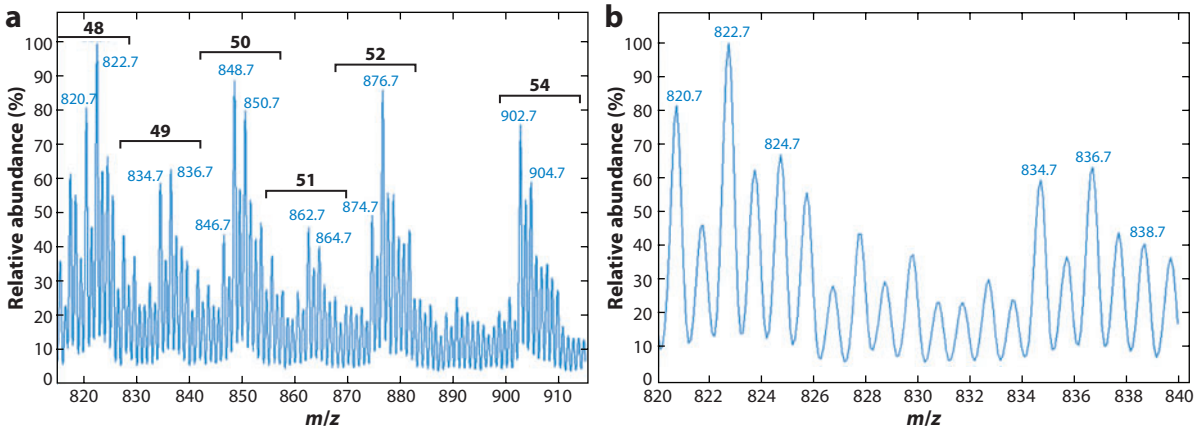

Figure 4

Electrospray ionization mass spectra of the neutral lipid fraction of RAW 264.7 cells obtained with a quadrupole linear ion-trap mass spectrometer showing (*a*) the triacylglycerol (TAG) region of the mass spectrum, and (*b*) the expanded *m/z* of the $[M + NH_4]^+$ corresponding to TAGs containing a total of 48 and 49 acyl carbon atoms. Reproduced with permission from Reference 25.

MALDI did not enjoy the same initial rise in popularity as ESI. Nevertheless, MALDI-MS of lipids has received increased attention in recent years (36–39), largely due to its ability to sample directly from tissue (40). As in the case of shotgun ESI, attention has mostly been focused on the application of MALDI to glycerophospholipid analysis, although this technique has also been applied to other lipid classes, including sphingolipids (44), triacylglycerols (45), diacylglycerols (46), cholesterol and cholesteryl esters (47), wax esters (48), and even free fatty acids (49). For a comprehensive review of this literature, see References 50 and 51.

MALDI's advantages include its tolerance of impurities, such as buffer salts, and its suitability to the analysis of large sample arrays that arise in biological analysis. Another point of difference with ESI is that MALDI analysis of phospholipids can give rise to positive ions for all subclasses, allowing a wider range of species to be observed in a single spectrum (41, 42). Unfortunately, under such conditions an individual lipid may appear as a complex array of ions (including $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, and even higher-order adducts), making even relative comparisons between samples extremely difficult. Furthermore, the dominance of desorption/ionization of glycerophosphocholines (PCs) in MALDI of lipid samples can suppress the formation and detection of other lipid ions (41), which is particularly problematic with respect to quantification. The signal-to-noise ratio of ionized lipids in a MALDI mass spectrum may be used in conjunction with appropriate internal standards as a reliable indicator of concentration (43). However, this approach is suitable only for simple lipid mixtures, and there is currently no simple and effective method of quantifying complex lipid extracts with MALDI. Moreover, purification of samples by HPLC must be conducted offline. These two limitations give ESI a distinct advantage over MALDI in both global and targeted lipidomics experiments. Despite these drawbacks, MALDI's ability to obtain lipid profiles directly from tissues ensures a valuable role for this technique in lipidomics (52). An example of the direct analysis of glycerophospholipids and SMs from different regions of the bovine lens is shown in **Figure 5** (53).

An important factor in obtaining reproducible MALDI spectra of lipids is the choice of matrix. Given the increasing popularity of MALDI for the analysis of lipids, the list of matrices used is rapidly expanding; an excellent overview of their respective strengths and weaknesses is provided in a recent review (50). The push to achieve higher spatial resolution in MALDI-imaging experiments

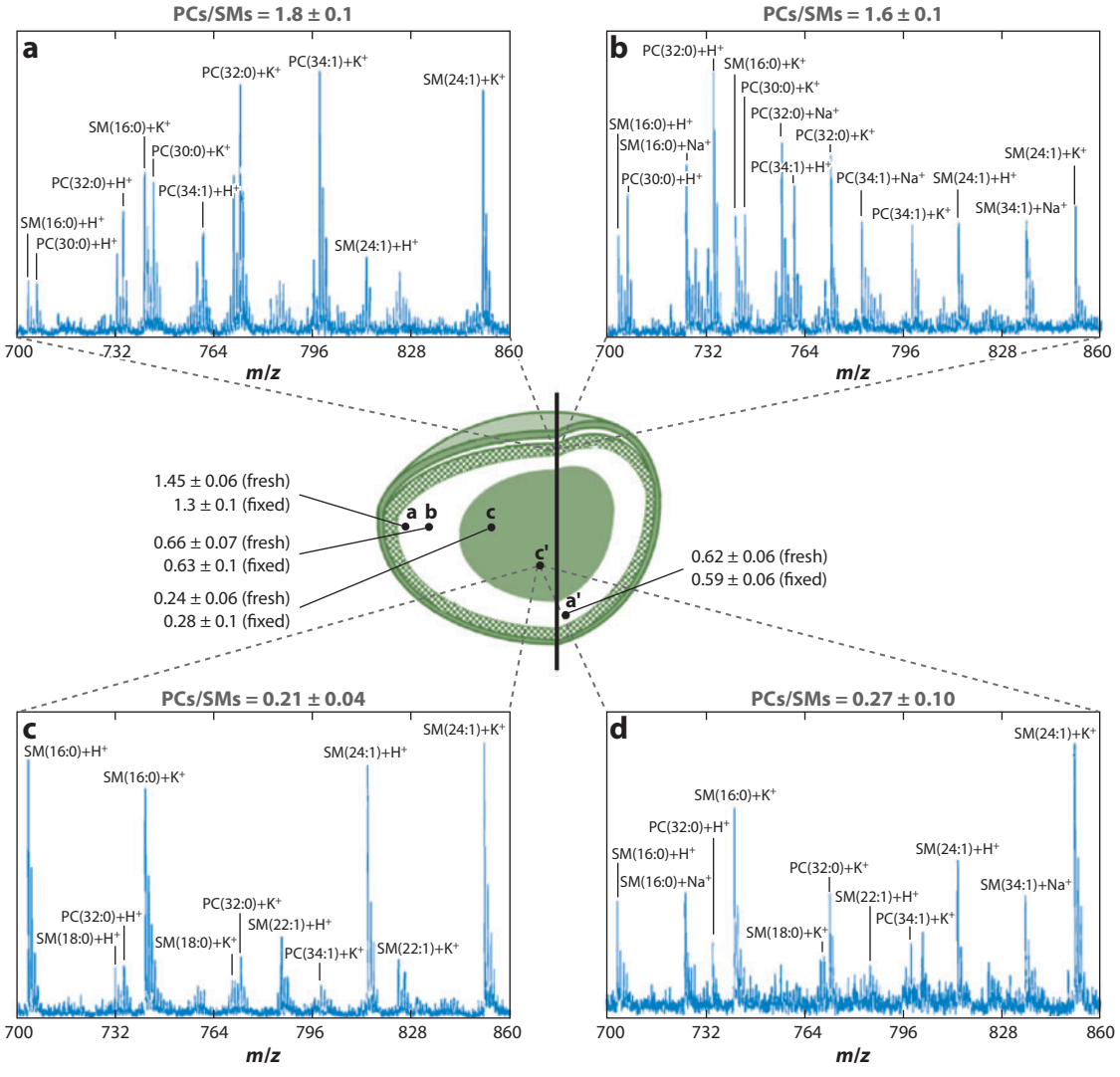

Figure 5

(*a–d*) Representative matrix-assisted laser desorption/ionization (MALDI) mass spectra obtained directly from (*center*) tissue slices showing the most abundant species of glycerophosphocholines (PCs) and sphingomyelins (SMs) in different regions of a bovine lens. One mass spectrum is the average of 50 to 100 traces. Reproduced with permission from Reference 53.

(see below) has driven recent improvements in matrix-application procedures. Several techniques have been applied for the direct analysis of lipids from tissue, including (*a*) spray coating (54), (*b*) automated spotters (55), (*c*) dry coating (56), and (*d*) sublimation (57).

2.4. Ambient Ionization

In recent years, a new wave of ambient ionization techniques have been developed (58). The first was desorption electrospray ionization (DESI) (59), which has been applied particularly to lipid

analysis. Through use of this technique, lipid profiles can be obtained directly from biological tissue with little or no sample preparation (60). Moreover, ions are generated in ambient conditions, rather than inside the high-vacuum region of a mass spectrometer, which allows for further chemical and/or physical manipulation to aid in the desorption/ionization of lipids. In a recent example, DESI solvent was seeded with a small amount of betaine aldehyde, which forms a covalent adduct with cholesterol, thereby introducing a fixed-charge trimethylammonium moiety and significantly enhancing cholesterol detection via MS (**Figure 6**) (61).

Low-energy CID: typically refers to an energy regime <100 eV and is common to most mass spectrometers used in contemporary lipid analysis (e.g., triple quadrupole, quadrupole time-of-flight, quadrupole ion-trap, and Fourier transform mass spectrometers)

Another interesting development in the analysis of lipids directly from tissue is rapid evaporative ionization mass spectrometry (REIMS) (62). In this technique, ions are formed from the rapid thermal evaporation of biological tissue from a surgical electrode and extracted into the mass spectrometer (**Figure 7*a***). REIMS allows in situ analysis of tissue, and the resulting mass spectra reveal abundant positive and negative ions that are attributable to phospholipids (**Figure 7*b***). Statistical comparison of lipid profiles thereby obtained by DESI or REIMS can be used to distinguish healthy from diseased tissue without the need for laborious identification of individual molecular species (60, 62). The use of lipid profiles to "fingerprint" tissues may find significant future application in diagnostic pathology and may ultimately provide real-time feedback for surgeons.

3. STRUCTURAL CHARACTERIZATION OF LIPIDS BY MASS SPECTROMETRY

The combination of soft-ionization methods such as ESI and MALDI with tandem MS has undoubtedly been the most significant contribution to recent advances in lipidomics. Tandem MS with CID is a reliable diagnostic tool for the structural elucidation of lipids, and the use of related scanning approaches [e.g., precursor ion and neutral loss scans and multiple reaction monitoring (MRM)] has significantly enhanced analytical sensitivity in both global and targeted lipidomics. Although CID is likely to remain the mainstay of modern lipid analyses for the foreseeable future, several recent innovations can provide complementary information to that derived from CID and suggest exciting possibilities for future developments in lipid MS.

3.1. Collision-Induced Dissociation

Although we cannot provide a comprehensive review of lipid CID in this article (see References 63–65), consideration of some representative examples provides insight into how such data are used for structure elucidation. For acidic glycerophospholipids (**Figure 1**), which readily form negative ions, low-energy CID yields diagnostic fragmentation (65). As an example, the CID spectrum of a deprotonated glycerophosphoserine (PS) with an $[M\text{-}H]^-$ anion at *m/z* 760 is shown in **Figure 8** and reveals abundant product ions related to the fatty acyl substituents (66). These fragments appear as carboxylate anions, RCO_2^- (*m/z* 255 and 281), and product ions arising from neutral losses of these moieties (following head group elimination) as either fatty acids, RCO_2H (*m/z* 417 and 391), or ketene derivatives, R′CHCO (*m/z* 435 and 409). Given this fragmentation pattern, the CID spectrum clearly identifies the number of carbons and the degree of unsaturation of both fatty acyl substituents (i.e., 16:0 and 18:1), and thus by calculating the residual mass the glycerophospholipid subclass can also be deduced as PS. Additionally, the negative-ion CID of phospholipids often yields fragment ions that are characteristic of the head group itself; for example, the abundant ion at *m/z* 673 (**Figure 8**) arises from the neutral loss of the serine moiety (−87 Da) and confirms the lipid subclass.

PCs and SMs incorporate a fixed positive charge, and as such, the formation of negative ions cannot be achieved by deprotonation. As an alternative, $[M+Cl]^-$ chloride adducts of these compounds

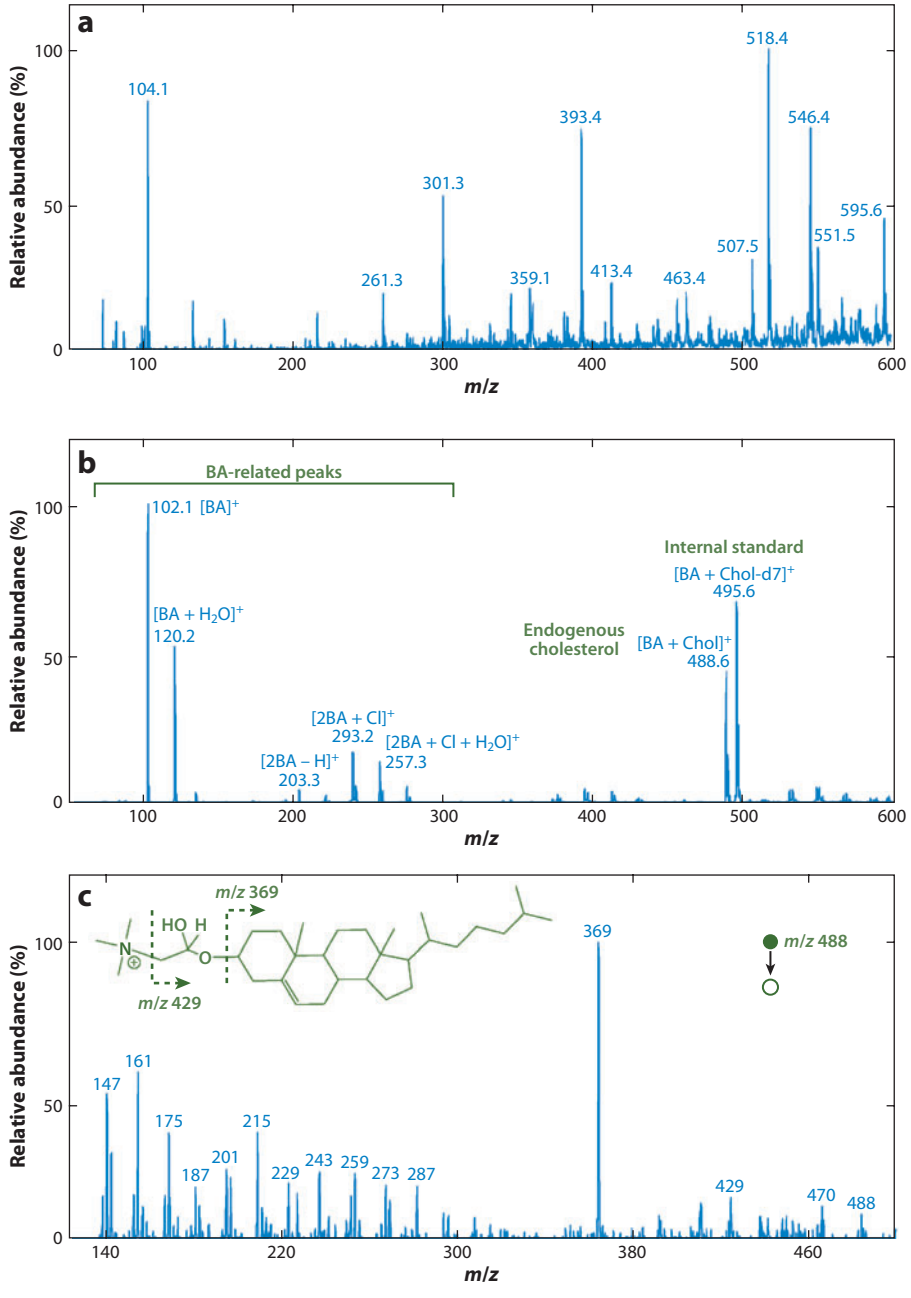

Figure 6

(*a*) Normal desorption electrospray ionization (DESI) spectrum obtained from a dried human serum spot, with $CH_3CN/CHCl_3$ (1:2) as the spray solvent. (*b*) Reactive DESI spectrum from a dried human serum spot doped with D_7-cholesterol as internal standard. A solvent of $CH_3CN/CHCl_3$ (1:2) with 50 ppm betaine aldehyde (BA) was used. (*c*) Product ion tandem mass spectrum of *m/z* 488. Reproduced with permission from Reference 61.

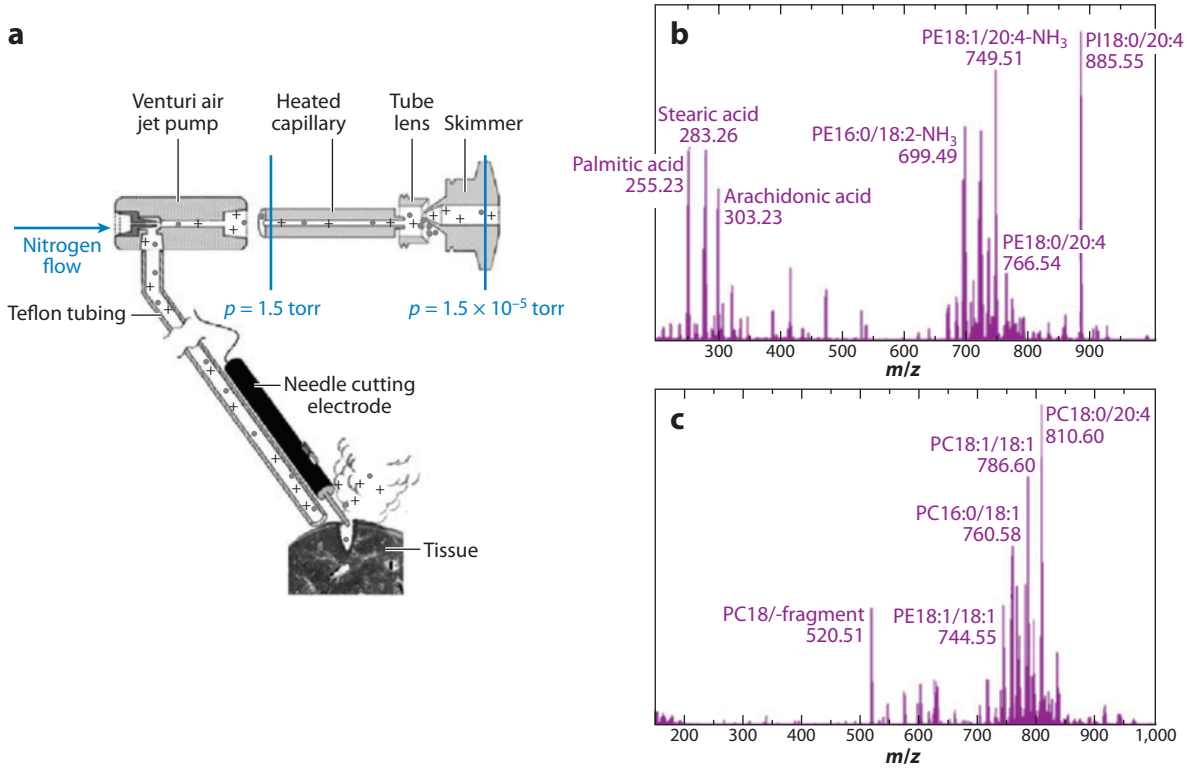

Figure 7

(*a*) Experimental setup for rapid evaporative ionization mass spectrometry (REIMS) tissue analysis. The ions are generated along the contact surface of the surgical electrode and transferred to the mass spectrometer via a Venturi pump. REIMS spectra of vital porcine hepatic tissue in (*b*) negative-ion and (*c*) positive-ion mode identify a range of phospholipids. Abbreviations: PC, glycerophosphocholine; PE, glycerophosphoethanolamine; PI, glycerophosphoinositol. Reproduced with permission from Reference 62.

have been generated by ESI along with other adduct anions, including formate $[M+HCO_2]^-$ and acetate $[M+CH_3CO_2]^-$, that are often present in the mobile phase during HPLC-MS analysis (67–69). Dissociation of $[M+A]^-$ anions (where A = Cl, HCO_2, or CH_3CO_2) results in the neutral loss of A-CH_3, yielding an $[M\text{-}CH_3]^-$ negative ion; subsequent dissociation provides an information-rich negative-ion CID spectrum that is particularly useful in the identification of the fatty acyl substituents.

It has long been accepted that the relative abundances of ions in phospholipid CID can also be used for structure elucidation (70). For example, the greater abundance of *m/z* 391 compared to *m/z* 417 in **Figure 8*a*** is consistent with the location of the 16:0 and 18:1 fatty acids substituted on the *sn*-1 and *sn*-2 positions (**Figure 1**), respectively. These assignments are based on studies of synthetic phospholipids, but such conclusions can be complicated by the difficulties in obtaining regiopure standards and the high likelihood of isomeric mixtures in naturally derived lipid extracts. Ekroos et al. (71) undertook the first systematic study on the application of CID for the relative quantification of *sn*-positional isomers in biological samples. By comparing the relative abundance of the product ions arising from ketene elimination of acyl substituents from the $[M\text{-}CH_3]^-$ ions of PCs and comparing them with enzymatic hydrolysis (using phospholipase A_2), the authors observed a straight-line correlation. When this calibration was applied to lipid extracts from human

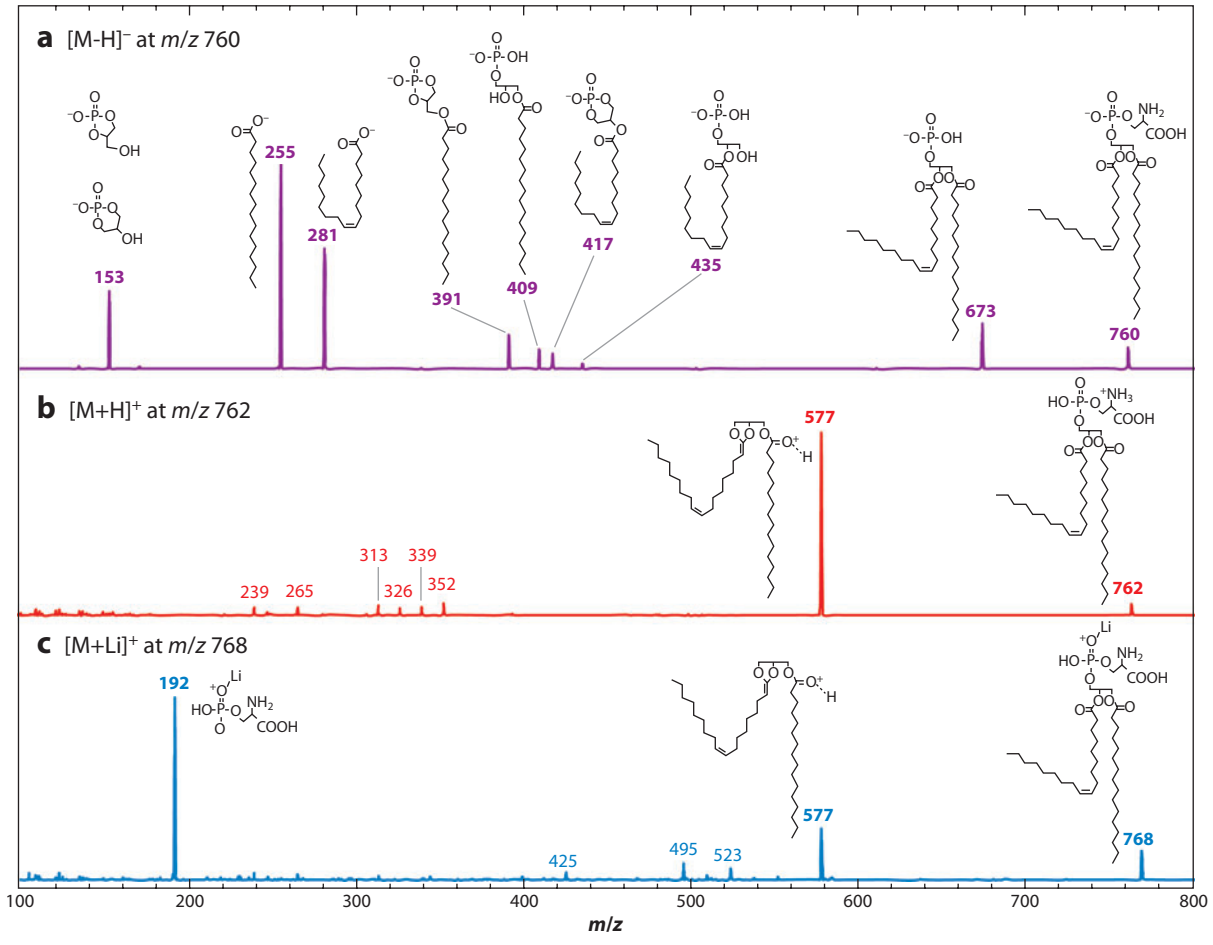

Figure 8

The low-energy collision-induced dissociation mass spectra of the glycerophosphoserine PS (16:0/18:1) as the following ions: (*a*) $[M-H]^-$ at *m/z* 760, (*b*) $[M+H]^+$ at *m/z* 762, and (*c*) $[M+Li]^+$ at *m/z* 768. The chemical structures of the major fragment ions are shown.

red blood cells, the presence of several regioisomeric PCs were identified. This study demonstrates that, without careful calibration, the abundances of fragment ions in CID spectra provide a guide as to the major *sn*-positional isomer present but do not exclude the presence of regioisomers in the extract. The study also points to the need to (*a*) develop new MS tools for the differentiation and relative quantification of isomers in complex mixtures and (*b*) better understand the molecular mechanisms by which lipids decompose under CID. Such mechanistic insight will provide greater power to the analyst in using the CID spectrum for structure elucidation. Significant work by Hsu & Turk (65) over the past ten years has provided valuable insight into the mechanisms underpinning the fragmentation pathways of deprotonated phospholipids; these mechanisms have recently been summarized (65). In general, isotopic labeling studies point to charge-driven fragmentation initiated by the negatively charged phosphate moiety. Further insight into the thermodynamics and kinetics of dissociation of ionized lipids and the structures of product ions and intermediates in the gas phase—from either experiment or theory—would resolve many mechanistic questions

and provide a solid foundation for the reliable interpretation of CID spectra in modern lipidomic applications.

Figure 8*b*,*c* shows the CID spectra from the $[M+H]^+$ (*m/z* 762) and $[M+Li]^+$ (*m/z* 768) positive ions formed from PS (16:0/18:1). These spectra are typical of the fragmentation of protonated and metallated phospholipids in that they are dominated by product ions arising from either the neutral loss of the head group (e.g., *m/z* 577 in **Figure 8*b*,*c***) or the formation of product ions from this moiety (e.g., *m/z* 192 in **Figure 8*c***) (66). Minor fragment ions arising from the fatty acyl substituents are also detectable, but they are usually too low in abundance to be assigned with confidence; CID analysis of anions is the method of choice to derive this structural information. Although the lack of structurally specific fragments in positive-ion mode may be considered as a disadvantage, the concentration of much of the ion abundance in one or two head group–specific product ions represents a specific advantage in sensitivity in precursor ion– and neutral loss–scanning protocols (see below).

In an effort to gain more structurally informative CID spectra in positive-ion mode, researchers have investigated the fragmentation of several different types of adduct ions. Ho et al. (26) examined the CID spectra of a wide range of mono- and divalent metal cation adducts (e.g., Li, Na, K, Sc, V, Cr, Sr, Mn, Co, Cu, and Zn) with phospholipids. The authors found that the $[M-H+Co]^+$ and $[M-H+Ni]^+$ metal adduct ions were particularly effective for some lipids, yielding fragment ions related to the fatty acyl chains with relative product ion abundances related to the *sn* position on the glycerol backbone (26). Although the use of divalent metals shows some promise for acidic glycerophospholipids, it did not prove effective for PCs because the divalent metal adduct ions could not be formed in the presence of the fixed-charge trimethylammonium moiety. O'Hair and coworkers (72, 73) found an ingenious solution to this problem by generating homodimeric cation complexes of PCs with an $[M_2+Cu(II)]^{2+}$ stoichiometry. CID of these complexes yields a rich fragmentation chemistry, clearly identifying the fatty acyl substituents and showing a clear preference for formation of fragment ions from the *sn*-2 position. The requirement of making and characterizing homodimer ions in a complex mixture is likely to limit applications of this approach. More recently, the $[M-H+Li_2]^+$ cations formed by ESI on acidic phospholipids in the presence of lithium salts gave a range of fragment ions upon CID. Supplemental activation of some of these product ions in MS^n experiments with an ion-trap mass spectrometer gave rise to detailed structural insight allowing (*a*) the near-complete elucidation of the molecular structure of the phospholipid class, (*b*) the stoichiometry of esterified fatty acids, (*c*) the identification of the most abundant *sn*-positional isomer, and (*d*) an indication of the position of unsaturation in the fatty acyl substituents (74).

Adduct ions formed by ESI or MALDI are also effective for the ionization and fragmentation of several classes of neutral lipids including triacylglycerols, wax, and cholesteryl esters. CID spectra of $[M+NH_4]^+$, $[M+Li]^+$, and especially $[M+Na]^+$ ions yield product ions that identify the ester-linked fatty acids; thus, the structure of the intact lipid can usually be deduced (25, 48, 75–77). In summary, the product ions observed in the CID of lipid adduct ions can be selected to produce either simple but selective fragmentation or information-rich fragmentation, depending on the nature of the adduct ion. This information can be obtained even at relatively low collision energies with conventional tandem mass spectrometers. These techniques are likely to play an increasingly important role in lipidomics; for this reason, additional investigation to explore other adduct ions and rigorously elucidate the mechanisms of these fragmentation processes is required to ensure that future structural conclusions are soundly based.

Although positive- and negative-ion CID spectra of complex lipids can provide near-complete structural characterization, they are somewhat less effective in determining the regiochemical assignment of unsaturation in these compounds. This drawback is illustrated in **Figure 9*a*,*b***, where

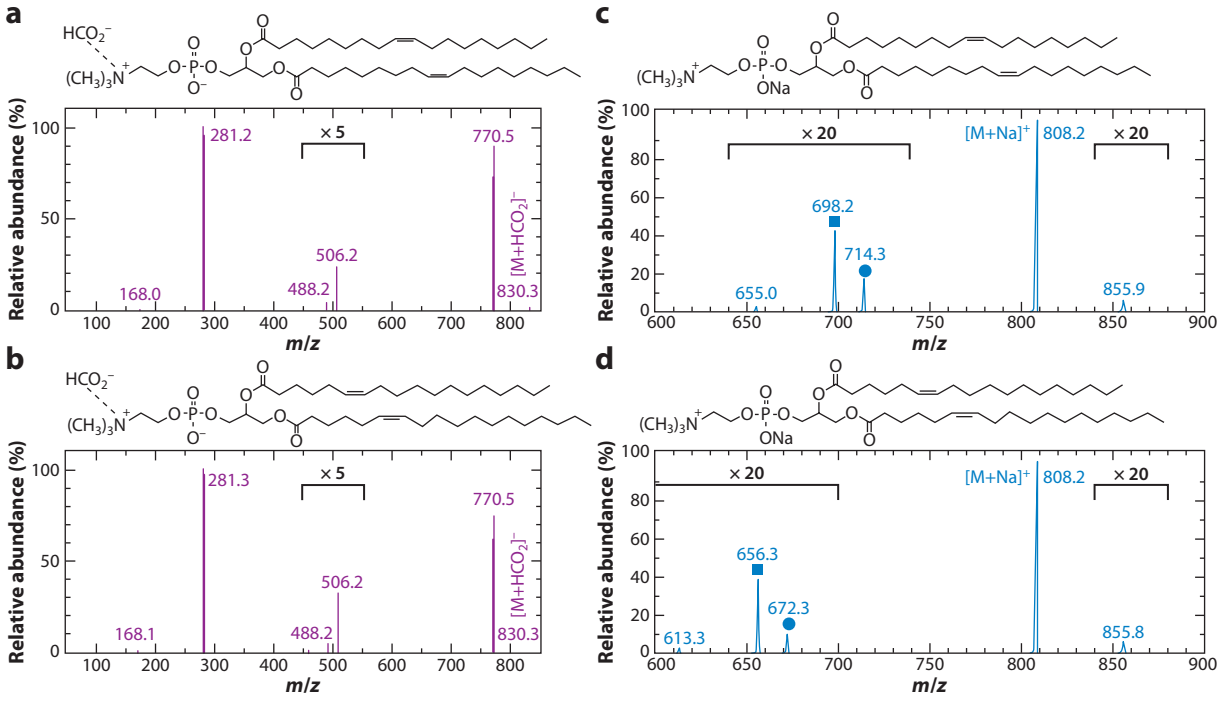

Figure 9

(*a*,*b*) The collision-induced dissociation (CID) spectra of the $[M+HCO_2]^-$ adduct anion arising from electrospray ionization of a 1-μM methanolic solution of (*a*) the glycerophosphocholine PC (9*Z*-18:1/9*Z*-18:1) and (*b*) its isomer PC (6*Z*-18:1/6*Z*-18:1) in the presence of formic acid and ammonia. Although these PCs differ in the position of their respective carbon-carbon double bonds, their CID spectra are indistinguishable. (*c*,*d*) The ozone-induced dissociation spectra of the $[M+Na]^+$ ions of the same species: (*c*) PC (9*Z*-18:1/9*Z*-18:1) and (*d*) PC (6*Z*-18:1/6*Z*-18:1). The two ions resulting from ozonolysis of the (*c*) *n*-9 and (*d*) *n*-12 double bonds (i.e., the double bonds are 9- and 12-carbons, respectively, from the methyl end of the acyl chains) are labeled with squares and circles. Reproduced with permission from References 24 and 144.

two PCs with distinct double-bond positions give rise to identical CID spectra. In some instances wherein the carbon-carbon double-bond position is more closely associated with heteroatoms, fragmentation associated with this functional group is observed, and the double-bond position can be determined (78–80). However, this situation is the exception rather than the rule.

CID of deprotonated or metal cationized fatty acids in the high collision-energy regime (i.e., >1 keV) on classical double-focusing mass spectrometers is known to lead to product ions that are characteristic of the position of double bonds or branching in the carbon chain (81, 82). Such approaches have also been applied to complex lipids such as glycerophospholipids and triacylglycerols; the resulting CID spectra provide a range of structural information that is not available in the lower-energy regime (17, 83). However, high-energy CID of ionized lipids has not been widely adopted because the requisite sector-based instruments are not readily available and are inefficiently coupled to ESI and MALDI sources. Interestingly, a new generation of commercial tandem TOF mass spectrometers (e.g., MALDI-TOF/TOF) operate in a collision-energy regime above 1 keV. Recent exploratory studies on CID of ionized lipids performed with MALDI-TOF/TOF mass spectrometers suggest that a rich fragmentation chemistry can be achieved at collision energies ranging from 2 to 20 keV. For the $[M-H+Li_2]^+$ cations of fatty acids, high-energy CID initiated charge-remote fragmentation that indicated the position of double bonds and branching

High-energy CID: typically refers to an energy regime above 1 keV and can be achieved on multisector mass spectrometers and some tandem time-of-flight instruments

in the hydrocarbon chain (84). For both triacylglycerols (77) and phospholipids (85), the CID of $[M+Na]^+$ ions gave products that indicated the position of substitution of the fatty acids on the glycerol backbone, but the carbon-carbon double-bond position could be determined only in favorable cases.

Although the large number of fragment ions benefits structure elucidation, it also increases the complexity of data analysis. This problem is exacerbated in complex extracts, wherein a single mass-selected ion population may include a range of isomeric lipids. In only one instance (77) has the method been applied to the structural identification of lipids in complex mixtures. In this case, the mass-gating ability of the TOF/TOF instrument used was limited to a 4-*m/z* window that presented problems in discriminating lipids differing by one or two double bonds. Improvement of instrumentation toward unit mass discrimination would greatly improve the utility of these mass spectrometers for lipidomics applications. Ideally, one could obtain both (*a*) the simple and informative low-energy CID spectrum to characterize the broad structural features of complex lipids (e.g., lipid subclass and fatty acid stoichiometry) and (*b*) the high-energy CID on some of the low-energy product ions (e.g., fatty acid carboxylate anions) to yield detailed insight into double-bond position. This MS^3 principle was elegantly demonstrated by Feneslau and coworkers (86) with a four-sector mass spectrometer; however, the implementation of such protocols on contemporary instrumentation is not currently possible.

3.2. Tandem Mass Spectrometry in Lipidomics

In global lipidomic approaches, the infusion of crude lipid extracts by ESI can result in exceedingly complex mass spectra with literally hundreds of different ionized lipids and many isobaric species. The specific fragmentation behavior of ionized lipids allows the use of precursor ion and neutral loss scans on triple quadrupole mass spectrometers to observe molecules with common structural motifs (e.g., lipid classes and subclasses) in a single spectrum with significantly reduced chemical noise (see **Figure 10**). Cole & Enke (87) and later Brugger et al. (88) demonstrated that the use of specific precursor ion and neutral loss scans on triple quadrupole mass spectrometers was a powerful tool for analysis of phospholipids in complex lipid extracts. Subsequent investigations of the fragmentation behavior of ionized lipids (see above) have led to a broad array of potential precursor ion and neutral loss transitions for phospholipids (see Reference 89 for a recent review). These scans can be used to target particular lipid classes, or they can be combined in a prescribed protocol to acquire an array of information on a lipid extract in a relatively short time period. In the latter case, the overlay of selected scans can allow the structural assignment of a large number of lipid molecules without requiring time-consuming product ion mass spectra to be obtained for each ionized lipid (**Figure 11**). Such protocols form the basis of many contemporary global lipidomic approaches, and the data analysis has been implemented in a number of software packages. Data-dependent analyses have also been employed in these protocols when the computer controlling the instrument triggers targeted scans on the basis of what is detected in preliminary acquisitions (90). This situation has the overall effect of decreasing the number of scans required for structural characterization and quantification and thus increases the sensitivity of the analysis.

An alternative approach to identifying a lipid in the presence of other species of the same nominal mass is to increase the resolution of the mass analysis. For example, the selectivity and sensitivity of precursor ion–scan functions can be improved through the use of a quadrupole TOF mass spectrometer to achieve much greater mass accuracy on the selected product ion (91–93). A new generation of high-resolution mass spectrometers, particularly orbitrap and Fourier transform–ion cyclotron resonance (FT-ICR) instruments, are also proving to be powerful tools for lipidomics. Such instrumentation has been harnessed in a number of ways: (*a*) Utilizing

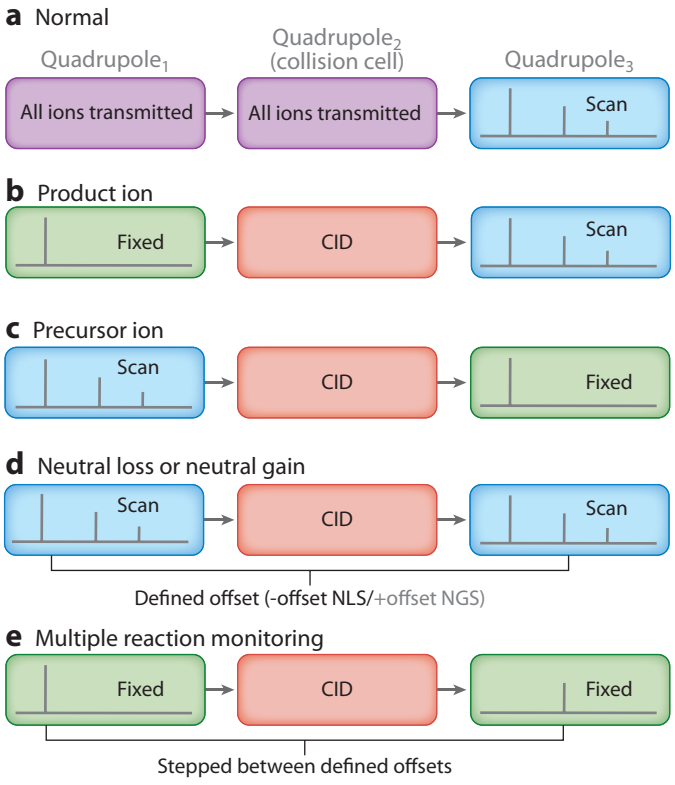

Figure 10

Schematic representation of the configuration of a triple quadrupole mass spectrometer for scan types commonly employed in lipidomic analyses, including (*a*) normal, (*b*) product ion, (*c*) precursor ion, (*d*) neutral loss or neutral gain, and (*e*) multiple reaction monitoring. Abbreviation: CID, collision-induced dissociation.

accurate mass measurements on a contemporary high-resolution mass spectrometer to determine elemental composition can often be performed in tandem with low-resolution CID analysis to confirm molecular connectivity (94–96), and (*b*) using the high-resolution mass spectrum as a detailed fingerprint of the lipid profile provides an information-rich data set to identify points of difference between samples by statistical analysis, which can then be targeted for further structural interrogation (97, 98).

In targeted lipidomics, the lipid or lipid class examined is often present at low levels relative to other species in the extract; thus, chromatography is essential to separate the target compound from a complex matrix. The specificity and sensitivity of online HPLC-MS applications are enhanced significantly by the use of MRM, in which a triple quadrupole mass spectrometer is programmed to a mass offset that is specific to the target lipid. In MRM experiments, a signal is detected only when an ionized lipid has the prescribed *m/z* ratio for both the precursor and product ions, thus minimizing chemical noise. Furthermore, in this configuration up to 100% of the target ions entering the instrument can be detected, increasing sensitivity and allowing detection and quantitation of lipids at very low levels in complex matrices. These approaches have been successfully employed in the quantification of low levels of endocannabinoids (99) and eicosanoids (30), among other lipid classes. Improvements in fast electronics in the new generation of triple quadrupole mass spectrometers mean that a significantly increased number of MRM transitions

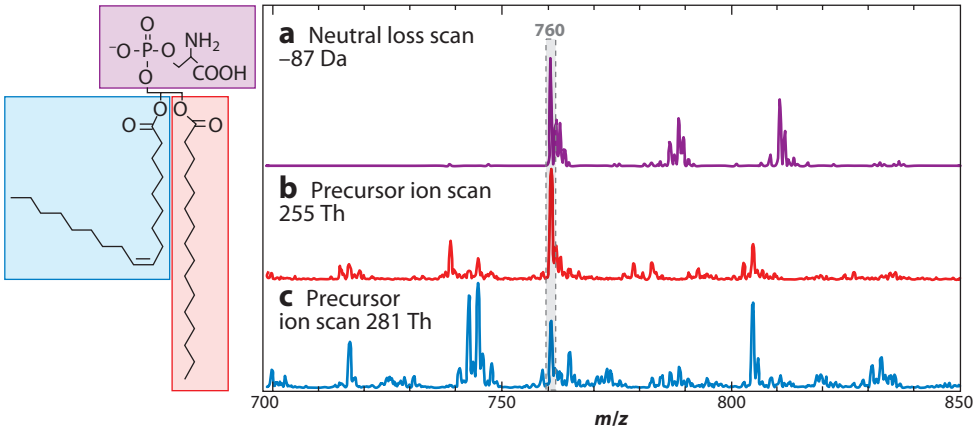

Figure 11

Negative-ion mass spectra obtained via scan functions commonly applied in the identification of phospholipids within complex extracts. (*a*) Neutral loss scan with an offset of -87 Da identifies phospholipids with the phosphoserine head group. (*b*) Precursor ion scan for *m/z* 255 identifies acidic phospholipids bearing a palmitate (16:0) acyl chain. (*c*) Precursor ion scan for *m/z* 281 identifies acidic phospholipids bearing an oleate (18:1) acyl chain. Observation of *m/z* 760 in all three scans identifies the presence of glycerophosphoserine (16:0/18:1) in the extract.

(and therefore lipids) can be monitored in a single liquid chromatogram. In a recent protocol for eicosanoid analysis, more than 100 MRM transitions were monitored in a 2-s interval (100), and indeed even larger sets of lipids can be quantified if the MRM transitions are preprogrammed to trigger at specific retention-time windows. All of these innovations are incrementally increasing the sensitivity of such methods.

3.3. Alternative Ion-Activation Methods

Although they are not widely used, ion-molecule reactions have been employed as an alternative to CID for molecular structure elucidation. In 1991, Cole & Enke (101) demonstrated that protonated phospholipids react with ethylvinyl ether in the collision cell of a triple quadrupole mass spectrometer to yield a series of adduct ions that uniquely identify the phospholipid head group. The authors also used this chemistry to undertake neutral gain scans on the triple quadrupole instrument to identify phospholipid classes in complex mixtures. More recently, gas-phase ion-molecule reactions of ionized lipids with ozone were investigated on a modified linear ion-trap mass spectrometer. Unsaturated lipid molecules were found to undergo ozone-induced dissociation (OzID) of carbon-carbon double bonds, and the resulting neutral loss provided the position of the double bond(s) in complex lipids such as phospholipids and triacylglycerols (24). The OzID mass spectra of two isomeric PCs that differ only in the position of their respective double bonds are shown in **Figure 9*c,d***. For each isomer, a pair of product ions that uniquely assign the position of the double bonds to *n*-9 and *n*-12 were observed. OzID carried out on CID product ions (i.e., CID/OzID) can be used to assign the double-bond position on individual fatty acyl chains within phospholipids (80). Indeed, the combined power of low-energy CID used both in parallel and in series with OzID holds some promise for complete top-down structure elucidation of complex lipids, including the assignment of double-bond position and *sn* position (24).

An alternative activation method for the elucidation of double-bond position in unsaturated lipids is known as covalent adduct chemical ionization (CACI) and is also based on ion-molecule

reactions (102). In this approach, the reagent is the reactive (1-methyleneimino)-1-ethylium cation $[CH_2NCCH_2]^+$ (formed in situ from chemical ionization of acetonitrile), which forms an $[M+54]^+$ adduct with a neutral unsaturated lipid via cycloaddition at the double bond(s). Mass selection and subsequent CID of the adduct result in fragment ions that are diagnostic of the position of the double bond. This approach has been demonstrated for FAMEs (102) and triacylglycerols (103), and it is a potent tool for lipid analysis. Given that the ion-molecule reaction precedes mass selection of the lipid, CACI is not directly compatible with shotgun lipidomics analyses of complex mixtures.

Ultraviolet photodissociation continues to be explored as an alternative activation method in biomolecular MS (104, 105). In a recent paper, Reilly and coworkers (106) described the photofragmentation of isomeric leukotriene lipids from irradiation of $[M+H]^+$, $[M+Na]^+$, and $[M-H]^-$ ions by an excimer laser (157 nm) in an ion-trap mass spectrometer. The product ions observed for these compounds upon photoexcitation were similar to those obtained in low- and high-energy CID experiments, and in all cases they allowed differentiation of the isomeric forms of the lipid. Compared to other classes of biomolecules, lipids have relatively poor absorption cross sections at visible and ultraviolet wavelengths, and thus wide application of photodissociation in lipidomics is unlikely. However, there may be niche applications for lipids with particular structural motifs, such as conjugated double bonds.

Electron-capture dissociation (ECD) and electron-transfer dissociation (ETD) methods have significantly influenced the field of proteomics by providing an alternative activation method that yields sequence-rich mass spectra from multiply charged peptides (107, 108). Both ECD and ETD are predicated on energetic charge reduction of multiply charged cations to drive fragmentation. In contrast to peptides, however, the relatively low number of basic sites in lipids typically results in only singly charged cations, and as such, ETD and ECD are not well suited to lipid-structure analysis. However, the fact that both ETD and ECD result in formation of radical cations presents intriguing possibilities for inducing fragmentation of the otherwise stable hydrocarbon chains inherent in lipids, which would provide greater structural information. McLuckey and coworkers (109) observed the ion-ion reaction of the $[M+Na_2]^{2+}$ ions of PCs with azobenzene radical anions in a linear ion-trap mass spectrometer. The resulting ETD spectrum provided structural information similar to that obtained by conventional CID. One prominent ion in the ETD spectrum was assigned as $[M+Na_2]^{\cdot+}$, and when subjected to subsequent CID analysis, this radical cation gave rise to complex spectral features. Some of the product ions could be tentatively assigned to dissociation of the polyunsaturated fatty acyl chain within the parent lipid. Experiments that use ECD on homodimeric cation complexes of PCs with divalent metals such as $[M_2+Mg(II)]^{2+}$ yielded a combination of charge-reduced $[M_2+Mg(II)]^{\cdot+}$ radical cations and neutral losses associated with free-radical fragmentation of the phospholipids (110). The latter product ions could be used to assign fatty acid stoichiometry to the parent phospholipid, but they were typically of low abundance. These preliminary studies suggest some possibilities for the application of ETD and ECD in lipid MS, but further research is needed to establish whether they can provide complementary insight into CID.

3.4. Ion-Mobility Mass Spectrometry

The combination of ion-mobility spectrometry with MS is showing increasing promise for biomolecular analysis with the possibility for discrimination of ionized biomolecules on the basis of collisional cross section—a measure of molecular shape and size—as well as *m/z* (111, 112). Although ion-mobility mass spectrometry (IM-MS) has been widely applied to large biomolecules, its application to lipids is currently limited. Two groups, using different IM-MS instrumentation,

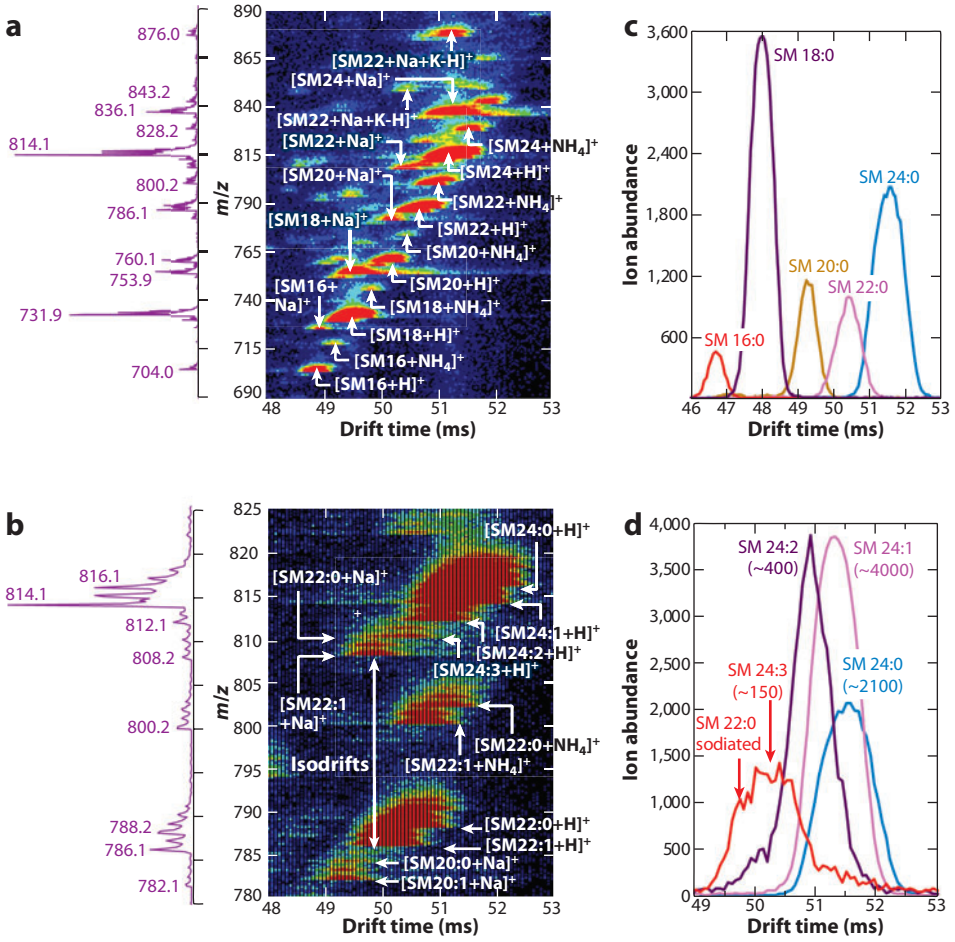

Figure 12

(*a*) A two-dimensional representation of ion-mobility mass spectrometry data obtained from electrospray ionization of a mixture of sphingomyelins, in which the ion-mobility time (in milliseconds) is presented on the *x* axis and the *m/z* scale is plotted on the *y* axis. The false color represents the increase in ion abundance from black to red; the latter indicates regions of high ion abundance. (*b*) A magnified representation of the mobility-versus-*m/z* plot in the region around *m/z* 814. (*c*,*d*) One-dimensional projections of the temporal data for the mobility time ranges of (*c*) 46 to 53 ms and (*d*) 49 to 53 ms. The ion abundances are indicated by the *y* axis, and the arrival distributions of different lipids identified from their *m/z* are shown in different colors. Reproduced with permission from Reference 113.

have demonstrated that cationized phospholipids can be grouped in the two-dimensional space bounded by mobility time and *m/z* (37, 113). This situation is illustrated in **Figure 12**, which shows a suite of cationized SMs generated by ESI that have been separated by both mobility time (*x* axis) and *m/z* (*y* axis). Projection of these data into the temporal axis only (**Figure 12*c***) shows near-baseline separation of SMs according to carbon-chain length; the longer chains have longer mobility times. Close examination of the data in a window around *m/z* 814 reveals that increasing unsaturation of these phospholipids subtly decreases their mobility times, thus

suggesting more compact gas-phase structures for polyunsaturated lipids (113). Examination of complex mixtures incorporating a range of different phospholipid classes reveals that the most significant trends in ion mobility arise from the increasing size of the head group following a general pattern: PA < PE < PG < PS < PC < SM < PI (where PA stands for glycerophosphate, PE stands for glycerophosphoethanolamine, PG stands for glycerophosphoglycerol, and PI stands for glycerophosphoinositol; PS, PC, and SM are defined above) (37, 113). Ion-mobility analysis is not dissimilar to rapid normal-phase chromatographic separation; however, the strong correlation between mobility and *m/z* (note that the peaks in **Figure 12** fall mostly on the diagonal) means that despite their speed, these technologies do not currently provide the same two-dimensional separation as conventional HPLC-MS analyses. Furthermore, current data do not yet indicate that IM-MS can provide structural information about ionized lipids that cannot already be gleaned from CID of mass-selected ions. Rather, the advantages of this technology lie in (*a*) temporal separation of isobaric lipids and thus potential deconvolution of overlapping CID spectra, (*b*) the ability to acquire time-aligned CID spectra, thereby allowing the simultaneous acquisition of these data for a wide range of ionized lipids (113), and (*c*) the simplification of complex spectra derived from direct analysis of tissue (e.g., MALDI imaging; see Section 5) (52). The recent commercialization of IM-MS instrumentation for both ESI and MALDI applications (114) suggests that these exciting possibilities are likely to be rapidly adopted in lipidomic analyses. Conceptually, IM-MS also has potential as a means to discriminate among the structures of isomeric lipid ions that cannot be readily differentiated by other MS means. For example, the principle of temporal separation of stereoisomers by IM-MS has been demonstrated, suggesting that in the future such methods could be used to provide detailed and hitherto unavailable information of lipid molecular structure (115, 116).

4. DATA ANALYSIS

MS-based lipidomics has developed rapidly over the past ten years and is now capable of producing enormous amounts of data in a relatively short time. These developments have highlighted the need for appropriate computational bioinformatics approaches to bypass the bottleneck of identifying and quantifying lipids from the raw chromatographic and mass spectral data. In this section, we describe the data-processing and statistical-analysis steps. A comprehensive discussion of bioinformatics approaches to lipidomics can be found in several excellent reviews (7, 117, 118).

4.1. Data Processing

A typical MS-based lipidomic workflow is illustrated in **Figure 13**, which shows that the initial step in processing raw data is lipid identification. Several specific lipid databases and software packages to achieve this end have been developed (**Table 1**). Software designed specifically for metabolomics, such as MZmine (119), may also be employed for lipid identification and quantification (120). Of particular note is the approach of the LIPID MAPS consortium, which has attempted to establish a standard system for lipid nomenclature (5, 6). If such a system were universally adopted, it would allow far simpler integration of current databases and data sets.

The second step in data processing is to normalize the data via a set of internal standards. To do so, the data must be corrected for the presence of isotopologues for two reasons: (*a*) to correct for contributions from the second isotope peak [M+2] of one lipid to the peak area of a lipid that is two mass units higher, that is, a lipid with one fewer double bond, and (*b*) because the natural abundance of isotopologues is higher for larger lipids relative to related lipids of lower mass. Note that analysis on a high-resolution mass spectrometer can resolve the contributions

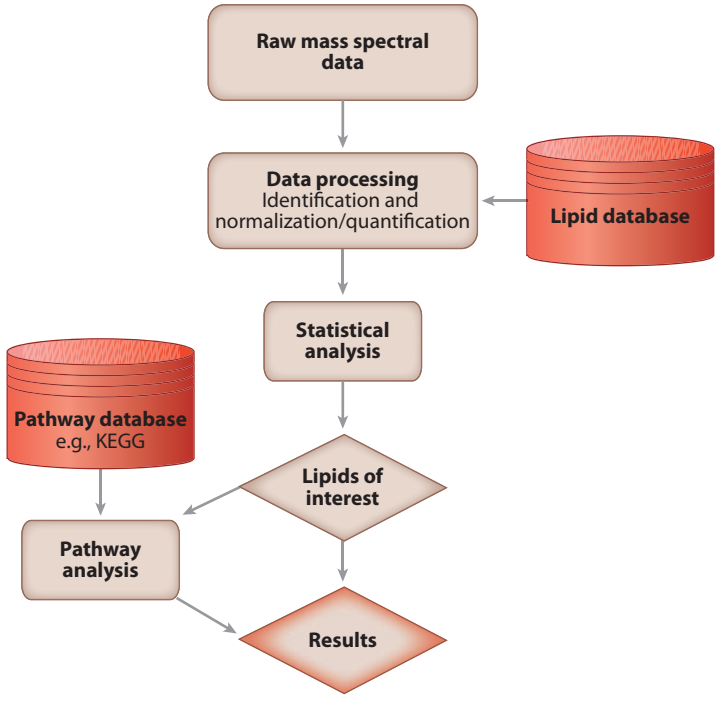

Figure 13

Lipidomics data-processing workflow. Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

of isotopologues and thus obviate the need for the first of these two corrections. Once these calculations have been performed, the identified lipids can then be quantified by comparison to appropriate internal standards (21, 121). **Table 1** lists several software packages that not only allow the identification of lipids from MS data but also incorporate built-in algorithms to perform isotope correction and quantification. Given the rapid expansion in the field of lipidomics, there is obvious commercial potential in producing a comprehensive lipid-analysis software package for mass spectral data, and several vendors appear to be moving toward releasing products that automate these functions.

4.2. Statistical Analysis

As shown in **Figure 13**, the third step in the lipidomic workflow is performing statistical analysis of the complex data sets produced. Univariate statistical tests are commonly applied in lipidomics for comparisons between experimental groups. However, such tests consider each lipid individually and therefore ignore the complex interactions that take place in the regulation of molecular lipids within and between lipid classes. To overcome such limitations, one may utilize multivariate analyses. One such method that is gaining popularity in lipidomics is principal-component analysis (PCA) (122). PCA is an unsupervised analysis that allows any structure in a data set to be observed without any prior knowledge or manipulation, so that trends in the data that would otherwise have been hidden by the restrictions imposed by supervised methods may be elucidated. The aim of many lipidomic analyses is to compare differences (in, for instance, diet, disease, or drug treatment) between groups, and in such cases it is important to employ a supervised multivariate analysis to identify these major differences. It is important to emphasize that raw mass spectral

Table 1 Databases and software packages for lipid analysis

Name	Comments	Web site	Reference
LMSD (LIPIDMAPS)	Open-access, Web-based search engine	**http://www.lipidmaps.org**	137
LipidBank	Open-access, Web-based search engine	**http://lipidbank.jp**	138
AMDMS-SL	A comprehensive freeware package designed for shotgun lipidomics; can be downloaded from the Web site	**http://www.shotgunlipidomics.com**	139
MZmine 2	A comprehensive freeware package designed for metabolomics studies; can be downloaded from the Web site	**http://mzmine.sourceforge.net**	119
LipidView	Formerly known as LipidProfiler; commercial software that performs complete data analysis recently released by MDS Sciex; is the most comprehensive lipid software currently available but is compatible only with analyst data	**http://www.mdssciex.com**	91
LIMSA	A comprehensive freeware package that can be downloaded from the Web; operates as a Microsoft Excel plug-in	**http://www.helsinki.fi/science/lipids/software.html**	140
Lipid Inspector	–	**http://www.mpi-cbg.de/research/research-groups/andrej-shevchenko.html**	90
LipidX	A subset of the systems biology freeware Systems X	**http://www.systemsx.ch**	141
LipidQA	Freeware package that can be downloaded from the Web	**http://msr.dom.wustl.edu/Personnel/Staff_Scientist_Song_Haowei.htm**	142
FAAT	Freeware for accurate mass fatty acid analysis	**http://www.genomecenter.ucdavis.edu/leary**	143

data may not reflect changes in concentration (see above); as such, it is essential to perform data normalization and quantification prior to statistical analysis (see **Figure 13**).

Although not widely employed in the lipidomics field, PLS/DA (partial least-squares discriminant analysis) has found favor in metabolomics studies for such comparisons (123). Other regression methods, such as LASSO (least absolute shrinkage and selection operator) and Elastic Net, are also ideally suited to analysis of lipidomic data sets in terms of ascertaining the relationship between variables (117). As analytical capabilities evolve (see Sections 3.3 and 3.4), new structural information will be obtained and data sets will become larger and more complex. Therefore, any bioinformatics strategies that are put into place will need to be adaptable so that new information can be added to databases and utilized for data interpretation.

5. MAPPING LIPID DISTRIBUTIONS

Imaging MS has become one of the most exciting areas in contemporary MS, generating a flurry of research activity driven by the advent of commercial instruments that can obtain position-correlated mass spectra from a two-dimensional substrate (124, 125). In particular, the ability of these technologies to visualize the distribution of molecules within tissue sections represents a powerful new tool for biomedical research that has the potential for clinical use as a diagnostic tool. MS imaging of lipids in tissue has been demonstrated by three separate desorption/ionization approaches, namely secondary ion mass spectrometry (SIMS), MALDI, and DESI. The

Secondary ion mass spectrometry (SIMS): technique in which atoms or molecules are desorbed and ionized from a sample surface as a result of bombardment by a primary beam of ions with kiloelectronvolt kinetic energies; the secondary ions thus formed are mass analyzed and detected by MS

application of each of these technologies to the MS imaging of lipids has recently been reviewed (126–128).

Through use of a focused beam of high-energy (>20-keV) primary ions, SIMS can desorb and ionize lipids directly from tissue samples without the need for pretreatment of the tissue section. Initially, it was thought that the high energy of the incident beam—usually an atomic metal ion—led to the formation of fragment ions rather than the ionization of intact lipids. The use of polyatomic clusters as primary ions (e.g., Au_3^+, Bi_3^+, and C_{60}^+) ameliorated this effect and allowed the detection of intact molecular species including cholesterol, fatty acids, phospholipids, and acylglycerols (129–131). **Figure 14*a–c*** shows MS images of coronal sections of mouse brain obtained via SIMS coupled with a TOF mass analyzer (130).

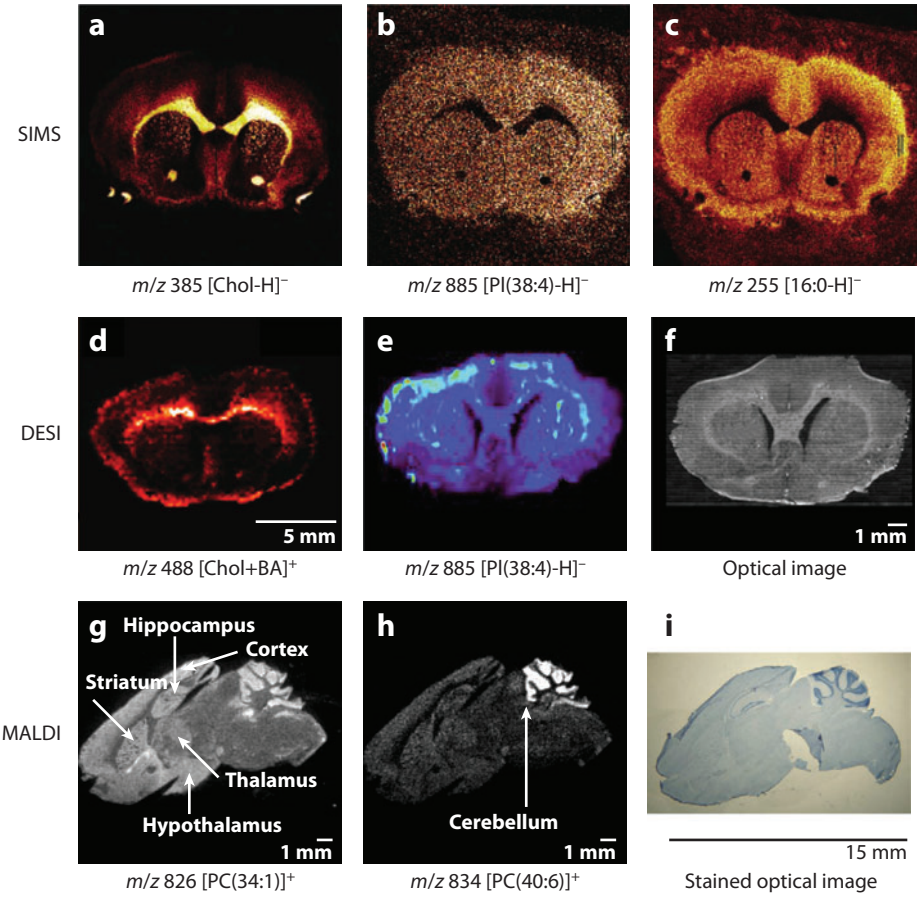

Figure 14

(*a–c*) Mass spectrometric images of a coronal section of mouse brain obtained via secondary ion mass spectrometry (SIMS). Abbreviation: PI, glycerophosphoinositol. Reproduced with permission from Reference 130. (*d–f*) Mass spectrometric and optical images of a coronal section of rat brain, where the former were obtained by desorption electrospray ionization (DESI) mass spectrometry. Cholesterol was imaged via reactive DESI with betaine aldehyde (BA) as a reagent. Reproduced with permission from References 61 and 128. (*g–i*) Mass spectrometric and optical images of a sagittal section of mouse brain, where the former were obtained via matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. Abbreviation: PC, glycerophosphocholine. Reproduced with permission from Reference 57.

Structural assignment of the ions is generally based on accurate mass measurements and existing knowledge of the lipid profile of the target tissue. Recently, CID spectra of protonated diacylglyceride secondary ions were obtained with a quadrupole TOF mass spectrometer, providing a direct connection between this powerful imaging technology and the structural insight available from CID (132). The spatial resolution of the images in **Figure 14*a–c*** is of the order of 1 μm and is limited by the relatively low abundance of the ionized lipids. Even with the use of polyatomic primary ions, low-mass fragment ions still dominate the SIMS spectra obtained from tissue sections; they are typically 100-fold more abundant than are intact lipid ions. This abundance can be exploited, as the greater ion abundances afforded by the fragment ions can be imaged with increased spatial resolution, albeit with a loss of molecular information. For example, in positive-ion SIMS analysis, *m/z* 184 is a major fragment arising from phosphocholine-containing lipids (131), whereas *m/z* 147 yields an image of cholesterol in macrophage cells (132). Of the currently available technologies, only SIMS has demonstrated the spatial resolution to image lipids (or at least their trademark fragment ions) at a cellular or even subcellular level (133).

Similarly, MALDI has been employed for MS imaging of lipids; the presence of the matrix—which absorbs the majority of the photon energy—allows for the gentle desorption/ionization of intact lipids directly from tissue sections. Conversely, the matrix also represents a source of potential pitfalls for imaging: (*a*) The solvent-based application of matrix may permit lipid migration prior to analysis, (*b*) the inhomogeneous crystallization of the matrix may lead to topographical artifacts in the resulting MS image, and (*c*) there may be ion-suppression effects from the choice of matrix itself. The factors affecting the direct measurement of lipids from tissues by use of MALDI are discussed above (see Section 2.4) and have recently been reviewed (52). If these issues are carefully addressed, then it is possible to obtain detailed images of intact ionized lipids from tissue sections via MALDI MS (e.g., **Figure 14*g*,*h***) (57). Phospholipids in particular can be effectively imaged by MALDI in both positive- and negative-ion mode (depending on the nature of the head group and the choice of matrix), although there may be fragmentation of cationized PE, PS, and PG (127). MALDI imaging has the advantage of already being well integrated in commercial mass spectrometers; thus, when MALDI is coupled with both accurate mass measurements and tandem MS, the molecular structure of ionized lipids can be confidently assigned. Furthermore, the coupling of MALDI with IM-MS (now also a commercial configuration) shows significant promise in separating different biomolecular classes (e.g., lipids and peptides) in the time domain, which simplifies the analysis of the complex mixture of ions formed from a single laser shot (134, 135).

DESI is the most recent of the lipid-imaging technologies and is also the most gentle of the three desorption/ionization protocols. DESI can sample lipids directly from a tissue section under ambient conditions, avoiding the use of matrix and its associated disadvantages. DESI imaging is particularly effective for phospholipids (e.g., **Figure 14*e***) that are readily ionized by the electrospray-like mechanism, and the ion currents are generally sufficient for tandem MS identification in either positive- or negative-ion mode. Also, one can image nonpolar lipid classes via DESI by seeding the incident spray with a derivatization agent in a so-called reactive DESI experiment. **Figure 14*d*** depicts cholesterol derivatized in situ with betaine aldehyde (as discussed in Section 2.5). In contrast to SIMS and MALDI, DESI uses an incident spray to sample the tissue surface and consequently has a lower spatial resolution than the former methods.

The state of the art in lipid imaging across the three technologies discussed above is a decrease in spatial information from SIMS (~1 μm) to MALDI (~10 μm) to DESI (~100 μm). Imaging MS in general approaches a fundamental limit at which the greater the spatial resolution achieved by the incident desorption/ionization source is (i.e., the spot size of the incident ion beam, laser beam, or spray) the lower is the number of molecules present and thus the greater sensitivity required

to mass analyze and detect them. These limits are currently being addressed through application of oversampling techniques (136) and careful statistical analysis of ion counts (133). New insight into the lipidome requires not only high spatial resolution but also certainty and specificity in the assignment of lipid structure. In these respects, MALDI and DESI imaging methods currently have distinct advantages over the high-energy SIMS process. Overall, despite the promise of these lipid-imaging technologies, further work is required to establish what physiological and biochemical conclusions should be drawn from MS imaging data. The question remains: Does a distribution map of ion abundance equate to localized lipid concentration within the tissue?

FUTURE ISSUES

1. Modern MS analysis of lipids provides (*a*) for the direct observation of intact lipids with little or no fragmentation; (*b*) excellent sensitivity; (*c*) the ability to analyze crude mixtures without prior fractionation, often detecting hundreds of individual lipid molecules with very short analysis time; and (*d*) the detailed molecular structure of individual lipids, compared with traditional chromatographic techniques. Recent developments in MS imaging of lipids allow us to infer the spatial distribution of individual molecular lipids within tissues or even cells. Nevertheless, a concerted effort is still required in several areas if we wish to fully characterize the lipidome and its role in health and disease.
2. Our understanding of the dissociation mechanisms of ionized lipids upon CID is incomplete, which has led to assumptions in the assignment of molecular structure (e.g., the uncalibrated assignment of *sn* position based on CID ion abundances). Given the push toward software development for automated lipid identification and quantification, it is important that we address this issue posthaste so that such assumptions are not buried in the code and perpetuated ad nauseam.
3. We are still unable to determine the complete molecular structure of many naturally occurring lipids without large amounts of sample and exhaustive fractionation (e.g., double-bond stereoisomerism in complex lipids). Therefore, new and innovative MS methods for comprehensive structural analysis that are compatible with modern lipidomics techniques and workflows are still required.
4. Ongoing progress in the sensitivity, resolution, and mass accuracy of instrumentation—often driven by demands in other fields—will also have an incremental but important impact on the speed and precision of lipidomic analysis.
5. Continued work on data-processing technology is required to ensure that data acquired across different platforms can be integrated and compared and that any new structural information can be incorporated and utilized.
6. Imaging MS is showing significant promise as a tool for lipidomics, but further work is required to determine whether the spectacular images it produces can be confidently interpreted as lipid distributions.
7. As our knowledge of the lipidome increases, so will the requirement to understand why such diversity is required. Improving our understanding of the interaction of molecular lipids with other biomolecules may help resolve this issue and thereby have a significant impact on our understanding of cellular metabolism and its impairment by various pathologies.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors acknowledge the financial support of the Australian Research Council (grant numbers DP0986628 and LP098983) and thank Dr. Berwyck Poad for assistance in preparation of some of the figures for this manuscript.

LITERATURE CITED

1. Brown HA, Murphy RC. 2009. Working towards an exegesis for lipids in biology. *Nat. Chem. Biol.* 5:602–6
2. van Meer G. 2005. Cellular lipidomics. *EMBO J.* 24:3159–65
3. Wenk MR. 2005. The emerging field of lipidomics. *Nat. Rev. Drug Discov.* 4:594–610
4. Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, et al. 2009. Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. *Proc. Natl. Acad. Sci. USA* 106:2136–41
5. Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH Jr, et al. 2005. A comprehensive classification system for lipids. *J. Lipid Res.* 46:839–61
6. Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CRH, et al. 2009. Update of the LIPID MAPS comprehensive classification system for lipids. *J. Lipid Res.* 50:S9–14
7. Yetukuri L, Ekroos K, Vidal-Puig A, Oresic M. 2008. Informatics and computational strategies for the study of lipids. *Mol. Biosyst.* 4:121–27
8. Mitchell TW, Pham H, Thomas MC, Blanksby SJ. 2009. Identification of double bond position in lipids: from GC to OzID. *J. Chromatogr. B* 877:2722–35
9. Han X. 2009. Lipidomics: developments and applications. *J. Chromatogr. B* 877:2663
10. Christie WW. 2003. *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids*. Bridgewater, UK: Oily
11. Hejazi L, Ebrahimi D, Guilhaus M, Hibbert DB. 2009. Discrimination among geometrical isomers of α–linolenic acid methyl ester using low energy electron ionization mass spectrometry. *J. Am. Soc. Mass. Spectrom.* 20:1272–80
12. Wood GW, Lau PY. 1974. Analysis of intact phospholipids by field desorption mass spectrometry. *Biomed. Mass Spectrom.* 1:154–55
13. Hejazi L, Ebrahimi D, Hibbert DB, Guilhaus M. 2009. Compatibility of electron ionization and soft ionization methods in gas chromatography/orthogonal time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* 23:2181–89
14. Hejazi L, Ebrahimi D, Guilhaus M, Hibbert DB. 2009. Determination of the composition of fatty acid mixtures using GC × FI-MS: a comprehensive two-dimensional separation approach. *Anal. Chem.* 81:1450–58
15. Mondello L, Tranchida PQ, Dugo P, Dugo G. 2008. Comprehensive two-dimensional gas chromatography-mass spectrometry: a review. *Mass Spectrom. Rev.* 27:101–24
16. Jover E, Adahchour M, Bayona JM, Vreuls RJJ, Brinkman UAT. 2005. Characterization of lipids in complex samples using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. *J. Chromatogr. A* 1086:2–11
17. Jensen NJ, Tomer KB, Gross ML. 1987. FAB MS/MS for phosphatidylinositol, -glycerol, -ethanolamine and other complex phospholipids. *Lipids* 22:480–89
18. Han X, Gross RW. 1994. Electrospray ionization mass spectroscopic analysis of human erythrocyte plasma membrane phospholipids. *Proc. Natl. Acad. Sci. USA* 91:10635–39
19. Kim HY, Wang TC, Ma YC. 1994. Liquid chromatography/mass spectrometry of phospholipids using electrospray ionization. *Anal. Chem.* 66:3977–82

20. Kerwin JL, Tuininga AR, Ericsson LH. 1994. Identification of molecular species of glycerophospholipids and sphingomyelin using electrospray mass spectrometry. *J. Lipid Res.* 35:1102–14
21. Han X, Gross RW. 2005. Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. *Mass Spectrom. Rev.* 24:367–412
22. Han X, Gross RW. 2003. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. *J. Lipid Res.* 44:1071–79
23. Han X, Gross RW. 2001. Quantitative analysis and molecular species fingerprinting of triacylglyceride molecular species directly from lipid extracts of biological samples by electrospray ionization tandem mass spectrometry. *Anal. Biochem.* 295:88–100
24. Thomas MC, Mitchell TW, Harman DG, Deeley JM, Nealon JR, Blanksby SJ. 2008. Ozone-induced dissociation: elucidation of double bond position within mass-selected lipid ions. *Anal. Chem.* 80:303–11
25. McAnoy AM, Wu CC, Murphy RC. 2005. Direct qualitative analysis of triacylglycerols by electrospray mass spectrometry using a linear ion trap. *J. Am. Soc. Mass. Spectrom.* 16:1498–509
26. Ho YP, Huang PC, Deng KH. 2003. Metal ion complexes in the structural analysis of phospholipids by electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 17:114–21
27. Hsu FF, Bohrer A, Turk J. 1998. Formation of lithiated adducts of glycerophosphocholine lipids facilitates their identification by electrospray ionization tandem mass spectrometry. *J. Am. Soc. Mass. Spectrom.* 9:516–26
28. Afonso C, Riu A, Xu Y, Fournier F, Tabet J-C. 2005. Structural characterization of fatty acids cationized with copper by electrospray ionization mass spectrometry under low-energy collision-induced dissociation. *J. Mass Spectrom.* 40:342–49
29. Astarita G, Piomelli D. 2009. Lipidomic analysis of endocannabinoid metabolism in biological samples. *J. Chromatogr. B* 877:2755–67
30. Mesaros C, Lee SH, Blair IA. 2009. Targeted quantitative analysis of eicosanoid lipids in biological samples using liquid chromatography–tandem mass spectrometry. *J. Chromatogr. B* 877:2736–45
31. Rainville PD, Stumpf CL, Shockcor JP, Plumb RS, Nicholson JK. 2007. Novel application of reversed-phase UPLC-oaTOF-MS for lipid analysis in complex biological mixtures: a new tool for lipidomics. *J. Proteome Res.* 6:552–58
32. Wilson ID, Nicholson JK, Castro-Perez J, Granger JH, Johnson KA, et al. 2005. High resolution "ultra performance" liquid chromatography coupled to oa-TOF mass spectrometry as a tool for differential metabolic pathway profiling in functional genomic studies. *J. Proteome Res.* 4:591–98
33. Harvey DJ. 1995. Matrix-assisted laser desorption/ionization mass spectrometry of phospholipids. *J. Mass Spectrom.* 30:1333–46
34. Marto JA, White FM, Seldomridge S, Marshall AG. 1995. Structural characterization of phospholipids by matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* 67:3979–84
35. Harvey DJ. 1995. Matrix-assisted laser desorption/ionization mass spectrometry of sphingo- and glycosphingo-lipids. *J. Mass Spectrom.* 30:1311–24
36. Huang L, Estrada R, Yappert MC, Borchman D. 2006. Oxidation-induced changes in human lens epithelial cells: 1. Phospholipids. *Free Radic. Biol. Med.* 41:1425–32
37. Jackson SN, Ugarov M, Post JD, Egan T, Langlais D, et al. 2008. A study of phospholipids by ion mobility TOFMS. *J. Am. Soc. Mass. Spectrom.* 19:1655–62
38. Schiller J, Suss R, Fuchs B, Muller M, Zschornig O, Arnold K. 2006. Recent applications of MALDI-TOF mass spectrometry and ^{31}P-NMR spectroscopy in phospholipid research. *Future Lipidol.* 1:115–25
39. Ma X, Liu G, Wang S, Chen Z, Lai M, et al. 2007. Evaluation of sphingolipids changes in brain tissues of rats with pentylenetetrazol-induced kindled seizures using MALDI-TOF-MS. *J. Chromatogr. B* 859:170–77
40. Caprioli RM, Farmer TB, Gile J. 1997. Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS. *Anal. Chem.* 69:4751–60
41. Petkovic M, Schiller J, Muller M, Benard S, Reichl S, et al. 2001. Detection of individual phospholipids in lipid mixtures by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: Phosphatidylcholine prevents the detection of further species. *Anal. Biochem.* 289:202–16

42. Schiller J, Suss R, Petkovic M, Zschornig O, Arnold K. 2002. Negative-ion matrix-assisted laser desorption and ionization time-of-flight mass spectra of complex phospholipid mixtures in the presence of phosphatidylcholine: a cautionary note on peak assignment. *Anal. Biochem.* 309:311–14
43. Petkovic M, Schiller J, Muller J, Muller M, Arnold K, Arnhold J. 2001. The signal-to-noise ratio as the measure for the quantification of lysophospholipids by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Analyst* 126:1042–50
44. Guittard J, Hronowski Xiaoping L, Costello CE. 1999. Direct matrix-assisted laser desorption/ionization mass spectrometric analysis of glycosphingolipids on thin layer chromatographic plates and transfer membranes. *Rapid Commun. Mass Spectrom.* 13:1838–49
45. Asbury GR, Al-Saad K, Siems WF, Hannan RM, Hill HH Jr. 1999. Analysis of triacylglycerols and whole oils by matrix-assisted laser desorption/ionization time of flight mass spectrometry. *J. Am. Soc. Mass. Spectrom.* 10:983–91
46. Benard S, Arnhold J, Lehnert M, Schiller J, Arnold K. 1999. Experiments towards quantification of saturated and polyunsaturated diacylglycerols by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry. *Chem. Phys. Lipids* 100:115–25
47. Schiller J, Zschornig O, Petkovic M, Muller M, Arnhold J, Arnold K. 2001. Lipid analysis of human HDL and LDL by MALDI-TOF mass spectrometry and ^{31}P-NMR. *J. Lipid Res.* 42:1501–8
48. Vrkoslav V, Míková R, Cvacka J. 2009. Characterization of natural wax esters by MALDI-TOF mass spectrometry. *J. Mass Spectrom.* 44:101–10
49. Ayorinde FO, Garvin K, Saeed K. 2000. Determination of the fatty acid composition of saponified vegetable oils using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* 14:608–15
50. Fuchs B, Schiller J. 2009. Application of MALDI-TOF mass spectrometry in lipidomics. *Eur. J. Lipid Sci. Technol.* 111:83–98
51. Schiller J, Süß R, Arnhold J, Fuchs B, Leßig J, et al. 2004. Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry in lipid and phospholipid research. *Prog. Lipid Res.* 43:449–88
52. Jackson SN, Woods AS. 2009. Direct profiling of tissue lipids by MALDI-TOFMS. *J. Chromatogr. B* 877:2822–29
53. Rujoi M, Estrada R, Yappert MC. 2004. In situ MALDI-TOF MS regional analysis of neutral phospholipids in lens tissue. *Anal. Chem.* 76:1657–63
54. Chen Y, Allegood J, Liu Y, Wang E, Cachon-Gonzalez B, et al. 2008. Imaging MALDI mass spectrometry using an oscillating capillary nebulizer matrix coating system and its application to analysis of lipids in brain from a mouse model of Tay-Sachs/Sandhoff disease. *Anal. Chem.* 80:2780–88
55. Baluya DL, Garrett TJ, Yost RA. 2007. Automated MALDI matrix deposition method with inkjet printing for imaging mass spectrometry. *Anal. Chem.* 79:6862–67
56. Puolitaival SM, Burnum KE, Cornett DS, Caprioli RM. 2008. Solvent-free matrix dry-coating for MALDI imaging of phospholipids. *J. Am. Soc. Mass. Spectrom.* 19:882–86
57. Hankin JA, Barkley RM, Murphy RC. 2007. Sublimation as a method of matrix application for mass spectrometric imaging. *J. Am. Soc. Mass. Spectrom.* 18:1646–52
58. Cooks RG, Ouyang Z, Takats Z, Wiseman JM. 2006. Ambient mass spectrometry. *Science* 311:1566–70
59. Takats Z, Wiseman JM, Gologan B, Cooks RG. 2004. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* 306:471–73
60. Wiseman JM, Puolitaival SM, Takáts Z, Cooks RG, Caprioli RM. 2005. Mass spectrometric profiling of intact biological tissue by using desorption electrospray ionization. *Angew. Chem.* 44:7094–97
61. Wu C, Ifa DR, Manicke NE, Cooks RG. 2009. Rapid, direct analysis of cholesterol by charge labeling in reactive desorption electrospray ionization. *Anal. Chem.* 81:7618–24
62. Schäfer K-C, Dénes J, Albrecht K, Szaniszló T, Balog J, et al. 2009. In vivo, in situ tissue analysis using rapid evaporative ionization mass spectrometry. *Angew. Chem.* 48:8240–42
63. Murphy RC, Fiedler J, Hevko J. 2001. Analysis of nonvolatile lipids by mass spectrometry. *Chem. Rev.* 101:479–526
64. Pulfer M, Murphy RC. 2003. Electrospray mass spectrometry of phospholipids. *Mass Spectrom. Rev.* 22:332–64

65. Hsu FF, Turk J. 2009. Electrospray ionization with low-energy collisionally activated dissociation tandem mass spectrometry of glycerophospholipids: mechanisms of fragmentation and structural characterization. *J. Chromatogr. B* 877:2673–95
66. Hsu F-F, Turk J. 2005. Studies on phosphatidylserine by tandem quadrupole and multiple stage quadrupole ion-trap mass spectrometry with electrospray ionization: structural characterization and the fragmentation processes. *J. Am. Soc. Mass. Spectrom.* 16:1510–22
67. Han XL, Gross RW. 1995. Structural determination of picomole amounts of phospholipids via electrospray ionization tandem mass spectrometry. *J. Am. Soc. Mass. Spectrom.* 6:1202–10
68. Harrison KA, Murphy RC. 1995. Negative electrospray ionization of glycerophosphocholine lipids: Formation of $[M\text{-}15]^-$ ions occurs via collisional decomposition of adduct anions. *J. Mass Spectrom.* 30:1772–73
69. Khaselev N, Murphy RC. 2000. Electrospray ionization mass spectrometry of lysoglycerophosphocholine lipid subclasses. *J. Am. Soc. Mass. Spectrom.* 11:283–91
70. Huang ZH, Gauge DA, Sweeley CC. 1992. Characterization of diacylglycerylphosphocholine molecular species by FAB-CAD-MS/MS—a general method not sensitive to the nature of the fatty acyl groups. *J. Am. Soc. Mass. Spectrom.* 3:71–78
71. Ekroos K, Ejsing CS, Bahr U, Karas M, Simons K, Shevchenko A. 2003. Charting molecular composition of phosphatidylcholines by fatty acid scanning and ion trap MS^3 fragmentation. *J. Lipid Res.* 44:2181–92
72. James PF, Perugini MA, O'Hair RAJ. 2007. Letter: Collision-induced dissociation of $[metal(L)_2]^{2+}$ complexes (metal = Cu, Ca and Mg) of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine allows distinction of the acyl groups at the *sn*1 and *sn*2 positions. *Eur. J. Mass Spectrom.* 13:433–36
73. James PF, Perugini MA, O'Hair RAJ. 2007. Size matters! Fragmentation chemistry of $[Cu(L)_n]^{2+}$ complexes of diacylglycerophosphocholines as a function of coordination number (n = 2–7). *Rapid Commun. Mass Spectrom.* 21:757–63
74. Hsu FF, Turk J. 2008. Structural characterization of unsaturated glycerophospholipids by multiple-stage linear ion-trap mass spectrometry with electrospray ionization. *J. Am. Soc. Mass. Spectrom.* 19:1681–91
75. Liebisch G, Binder M, Schifferer R, Langmann T, Schulz B, Schmitz G. 2006. High throughput quantification of cholesterol and cholesteryl ester by electrospray ionization tandem mass spectrometry (ESI-MS/MS). *Biochim. Biophys. Acta* 1761:121–28
76. Fitzgerald M, Murphy RC. 2007. Electrospray mass spectrometry of human hair wax esters. *J. Lipid Res.* 48:1231–46
77. Pittenauer E, Allmaier G. 2009. The renaissance of high-energy CID for structural elucidation of complex lipids: MALDI-TOF/RTOF-MS of alkali cationized triacylglycerols. *J. Am. Soc. Mass. Spectrom.* 20:1037–47
78. Zemski Berry KA, Murphy RC. 2004. Electrospray ionization tandem mass spectrometry of glycerophosphoethanolamine plasmalogen phospholipids. *J. Am. Soc. Mass. Spectrom.* 15:1499–508
79. Hsu F-F, Turk J. 2007. Differentiation of 1-O-alk-1′-enyl-2-acyl and 1-O-alkyl-2-acyl glycerophospholipids by multiple-stage linear ion-trap mass spectrometry with electrospray ionization. *J. Am. Soc. Mass. Spectrom.* 18:2065–73
80. Deeley JM, Thomas MC, Truscott RJW, Mitchell TW, Blanksby SJ. 2009. Identification of abundant alkyl ether glycerophospholipids in the human lens by tandem mass spectrometry techniques. *Anal. Chem.* 81:1920–30
81. Tomer KB, Crow FW, Gross ML. 1983. Location of double-bond position in unsaturated fatty-acids by negative-ion MS/MS. *J. Am. Chem. Soc.* 105:5487–88
82. Davoli E, Gross ML. 1990. Charge remote fragmentation of fatty-acids cationized with alkaline-earth metal-ions. *J. Am. Soc. Mass. Spectrom.* 1:320–24
83. Cheng CF, Gross ML. 1998. Complete structural elucidation of triacylglycerols by tandem sector mass spectrometry. *Anal. Chem.* 70:4417–26
84. Trimpin S, Clemmer DE, McEwen CN. 2007. Charge-remote fragmentation of lithiated fatty acids on a TOF-TOF instrument using matrix-ionization. *J. Am. Soc. Mass. Spectrom.* 18:1967–72
85. Simoes C, Simoes V, Reis A, Domingues P, Domingues MRM. 2008. Determination of the fatty acyl profiles of phosphatidylethanolamines by tandem mass spectrometry of sodium adducts. *Rapid Commun. Mass Spectrom.* 22:3238–44

86. Bryant DK, Orlando RC, Fenselau C, Sowder RC, Henderson LE. 1991. 4-sector tandem mass-spectrometric analysis of complex mixtures of phosphatidylcholines present in a human-immunodeficiency-virus preparation. *Anal. Chem.* 63:1110–14
87. Cole MJ, Enke CG. 1991. Direct determination of phospholipid structures in microorganisms by fast-atom-bombardment triple quadrupole mass spectrometry. *Anal. Chem.* 63:1032–38
88. Brugger B, Erben G, Sandhoff R, Wieland FT, Lehmann WD. 1997. Quantitative analysis of biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry. *Proc. Natl. Acad. Sci. USA* 94:2339–44
89. Cui Z, Thomas MJ. 2009. Phospholipid profiling by tandem mass spectrometry. *J. Chromatogr. B* 877:2709–15
90. Schwudke D, Oegema J, Burton L, Entchev E, Hannich JT, et al. 2006. Lipid profiling by multiple precursor and neutral loss scanning driven by the data-dependent acquisition. *Anal. Chem.* 78:585–95
91. Ejsing CS, Duchoslav E, Sampaio J, Simons K, Bonner R, et al. 2006. Automated identification and quantification of glycerophospholipid molecular species by multiple precursor ion scanning. *Anal. Chem.* 78:6202–14
92. Ekroos K, Chernushevich IV, Simons K, Shevchenko A. 2002. Quantitative profiling of phospholipids by multiple precursor ion scanning on a hybrid quadrupole time-of-flight mass spectrometer. *Anal. Chem.* 74:941–49
93. Stahlman M, Ejsing CS, Tarasov K, Perman J, Boren J, Ekroos K. 2009. High-throughput shotgun lipidomics by quadrupole time-of-flight mass spectrometry. *J. Chromatogr. B* 877:2664–72
94. Hein EM, Blank LM, Heyland J, Baumbach JI, Schmid A, Hayen H. 2009. Glycerophospholipid profiling by high-performance liquid chromatography/mass spectrometry using exact mass measurements and multi-stage mass spectrometric fragmentation experiments in parallel. *Rapid Commun. Mass Spectrom.* 23:1636–46
95. He H, Conrad CA, Nilsson CL, Ji Y, Schaub TM, et al. 2007. Method for lipidomic analysis: *p53* expression modulation of sulfatide, ganglioside, and phospholipid composition of U87 MG glioblastoma cells. *Anal. Chem.* 79:8423–30
96. He H, Nilsson C, Emmet MR, Ji Y, Marshall AG, et al. 2010. Polar lipid remodeling and increased sulfatide expression are associated with the glioma therapeutic candidates, wild type p53 elevation, and the topoisomerase-1 inhibitor, Irinotecan. *Glycoconj. J.* 27:27–38
97. Schwudke D, Hannich JT, Surendranath V, Grimard V, Moehring T, et al. 2007. Top-down lipidomic screens by multivariate analysis of high-resolution survey mass spectra. *Anal. Chem.* 79:4083–93
98. Koulman A, Woffendin G, Narayana VK, Welchman H, Crone C, Volmer DA. 2009. High-resolution extracted ion chromatography, a new tool for metabolomics and lipidomics using a second-generation orbitrap mass spectrometer. *Rapid Commun. Mass Spectrom.* 23:1411–18
99. Kingsley PJ, Marnett LJ. 2009. Analysis of endocannabinoids, their congeners and COX-2 metabolites. *J. Chromatogr. B* 877:2746–54
100. Deems R, Buczynski MW, Bowers-Gentry R, Harkewicz R, Dennis EA. 2007. Detection and quantitation of eicosanoids via high performance liquid chromatography–electrospray ionization-mass spectrometry. In *Methods in Enzymology*, Vol. 432: *Lipidomics and Bioactive Lipids: Mass-Spectrometry-Based Lipid Analysis*, ed. HA Brown, pp. 59–82. San Diego: Academic
101. Cole MJ, Enke CG. 1991. Fast-atom-bombardment tandem mass spectrometry employing ion-molecule reactions for the differentiation of phospholipid classes. *J. Am. Soc. Mass. Spectrom.* 2:470–75
102. Van Pelt CK, Brenna JT. 1999. Acetonitrile chemical ionization tandem mass spectrometry to locate double bonds in polyunsaturated fatty acid methyl esters. *Anal. Chem.* 71:1981–89
103. Xu Y, Brenna JT. 2007. Atmospheric pressure covalent adduct chemical ionization tandem mass spectrometry for double bond localization in monoene- and diene-containing triacylglycerols. *Anal. Chem.* 79:2525–36
104. Reilly JP. 2009. Ultraviolet photofragmentation of biomolecular ions. *Mass Spectrom. Rev.* 28:425–47
105. Ly T, Julian RR. 2009. Ultraviolet photodissociation: developments towards applications for mass-spectrometry-based proteomics. *Angew. Chem.* 48:7130–37
106. Devakumar A, O'Dell DK, Walker JM, Reilly JP. 2008. Structural analysis of leukotriene C-4 isomers using collisional activation and 157 nm photodissociation. *J. Am. Soc. Mass. Spectrom.* 19:14–26

107. Zubarev RA, Kelleher NL, McLafferty FW. 1998. Electron capture dissociation of multiply charged protein cations. A nonergodic process. *J. Am. Chem. Soc.* 120:3265–66
108. Syka JEP, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF. 2004. Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proc. Natl. Acad. Sci. USA* 101:9528–33
109. Liang XLR, Liu J, Leblanc Y, Covey T, Ptak AC, et al. 2007. Electron transfer dissociation of doubly sodiated glycerophosphocholine lipids. *J. Am. Soc. Mass. Spectrom.* 18:1783–88
110. James PF, Perugini MA, O'Hair RAJ. 2008. Electron capture dissociation of complexes of diacylglycerophosphocholine and divalent metal ions: competition between charge reduction and radical induced phospholipid fragmentation. *J. Am. Soc. Mass. Spectrom.* 19:978–86
111. Bohrer BC, Merenbloom SI, Koeniger SL, Hilderbrand AE, Clemmer DE. 2008. Biomolecule analysis by ion mobility spectrometry. *Annu. Rev. Anal. Chem.* 1:293–327
112. Kanu AB, Dwivedi P, Tam M, Matz L, Hill HH. 2008. Ion mobility–mass spectrometry. *J. Mass Spectrom.* 43:1–22
113. Trimpin S, Tan B, Bohrer BC, O'Dell DK, Merenbloom SI, et al. 2009. Profiling of phospholipids and related lipid structures using multidimensional ion mobility spectrometry-mass spectrometry. *Int. J. Mass Spectrom.* 287:58–69
114. Pringle SD, Giles K, Wildgoose JL, Williams JP, Slade SE, et al. 2007. An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/traveling wave IMS/OA-TOF instrument. *Int. J. Mass Spectrom.* 261:1–12
115. Dwivedi P, Wu C, Matz LM, Clowers BH, Siems WF, Hill HH. 2006. Gas-phase chiral separations by ion mobility spectrometry. *Anal. Chem.* 78:8200–6
116. Zhu ML, Bendiak B, Clowers B, Hill HH. 2009. Ion mobility–mass spectrometry analysis of isomeric carbohydrate precursor ions. *Anal. Bioanal. Chem.* 394:1853–67
117. Oresic M. 2009. Bioinformatics and computational approaches applicable to lipidomics. *Eur. J. Lipid Sci. Technol.* 111:99–106
118. Niemelä PS, Castillo S, Sysi-Aho M, Oresic M. 2009. Bioinformatics and computational methods for lipidomics. *J. Chromatogr. B* 877:2855–62
119. Katajamaa M, Miettinen J, Oresic M. 2006. MZmine: toolbox for processing and visualization of mass spectrometry based molecular profile data. *Bioinformatics* 22:634–36
120. Schwab U, Seppanen-Laakso T, Yetukuri L, Agren J, Kolehmainen M, et al. 2008. Triacylglycerol fatty acid composition in diet-induced weight loss in subjects with abnormal glucose metabolism—the GENOBIN study. *PLoS ONE* 3:e2630
121. Deeley JM, Mitchell TW, Wei X, Korth J, Nealon JR, et al. 2008. Human lens lipids differ markedly from those of commonly used experimental animals. *Biochim. Biophys. Acta* 1781:288–98
122. Jackson JE. 1991. *A User's Guide to Principal Components*. New York: Wiley
123. Laaksonen R, Katajamaa M, Paiva H, Sysi-Aho M, Saarinen L, et al. 2006. A systems biology strategy reveals biological pathways and plasma biomarker candidates for potentially toxic statin–induced changes in muscle. *PLoS ONE* 1:e97
124. Heeren RMA, Smith DF, Stauber J, Kukrer-Kaletas B, MacAleese L. 2009. Imaging mass spectrometry: hype or hope? *J. Am. Soc. Mass. Spectrom.* 20:1006–14
125. McDonnell LA, Heeren RMA. 2007. Imaging mass spectrometry. *Mass Spectrom. Rev.* 26:606–43
126. Brunelle A, Laprevote O. 2009. Lipid imaging with cluster time-of-flight secondary ion mass spectrometry. *Anal. Bioanal. Chem.* 393:31–35
127. Murphy RC, Hankin JA, Barkley RM. 2009. Imaging of lipid species by MALDI mass spectrometry. *J. Lipid Res.* 50:S317–22
128. Dill AL, Ifa DR, Manicke NE, Zheng OY, Cooks RG. 2009. Mass spectrometric imaging of lipids using desorption electrospray ionization. *J. Chromatogr. B* 877:2883–89
129. Touboul D, Halgand F, Brunelle A, Kersting R, Tallarek E, et al. 2004. Tissue molecular ion imaging by gold cluster ion bombardment. *Anal. Chem.* 76:1550–59
130. Sjovall P, Lausmaa J, Johansson B. 2004. Mass spectrometric imaging of lipids in brain tissue. *Anal. Chem.* 76:4271–78
131. Carado A, Kozole J, Passarelli M, Winograd N, Loboda A, et al. 2008. Biological tissue imaging with a hybrid cluster SIMS quadrupole time-of-flight mass spectrometer. *Appl. Surf. Sci.* 255:1572–75

132. Piehowski PD, Carado AJ, Kurczy ME, Ostrowski SG, Heien ML, et al. 2008. MS/MS methodology to improve subcellular mapping of cholesterol using TOF-SIMS. *Anal. Chem.* 80:8662–67
133. Piehowski PD, Davey AM, Kurczy ME, Sheets ED, Winograd N, et al. 2009. Time-of-flight secondary ion mass spectrometry imaging of subcellular lipid heterogeneity: Poisson counting and spatial resolution. *Anal. Chem.* 81:5593–602
134. McLean JA, Ridenour WB, Caprioli RM. 2007. Profiling and imaging of tissues by imaging ion mobility–mass spectrometry. *J. Mass Spectrom.* 42:1099–105
135. Jackson SN, Ugarov M, Egan T, Post JD, Langlais D, et al. 2007. MALDI–ion mobility–TOFMS imaging of lipids in rat brain tissue. *J. Mass Spectrom.* 42:1093–98
136. Jurchen JC, Rubakhin SS, Sweedler JV. 2005. MALDI-MS imaging of features smaller than the size of the laser beam. *J. Am. Soc. Mass. Spectrom.* 16:1654–59
137. Sud M, Fahy E, Cotter D, Brown A, Dennis EA, et al. 2007. LMSD: LIPID MAPS structure database. *Nucleic Acids Res.* 35:D527–32
138. Watanabe K, Yasugi E, Oshima M. 2000. How to search the glycolipid data in "LIPIDBANK for Web," the newly developed lipid database in Japan. *Trends Glycosci. Glycotechnol.* 12:175–84
139. Yang K, Cheng H, Gross RW, Han XL. 2009. Automated lipid identification and quantification by multidimensional mass spectrometry–based shotgun lipidomics. *Anal. Chem.* 81:4356–68
140. Haimi P, Uphoff A, Hermansson M, Somerharju P. 2006. Software tools for analysis of mass spectrometric lipidome data. *Anal. Chem.* 78:8324–31
141. Graessler J, Schwudke D, Schwarz PEH, Herzog R, Shevchenko A, Bornstein SR. 2009. Top-down lipidomics reveals ether lipid deficiency in blood plasma of hypertensive patients. *PLoS ONE* 4:e6261
142. Song HW, Ladenson J, Turk J. 2009. Algorithms for automatic processing of data from mass spectrometric analyses of lipids. *J. Chromatogr. B* 877:2847–54
143. Leavell MD, Leary JA. 2006. Fatty acid analysis tool (FAAT): an FT-ICR MS lipid analysis algorithm. *Anal. Chem.* 78:5497–503
144. Thomas MC, Mitchell TW, Deeley JM, Harman DG, Murphy RC, Blanksby SJ. 2007. Elucidation of double bond position in unsaturated lipids by ozone electrospray ionization mass spectrometry (OzESI-MS). *Anal. Chem.* 79:5013–22

Annual Review of
Analytical Chemistry

Volume 3, 2010

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Analytical Chemistry* articles may be found at http://arjournals.annualreviews.org/errata/anchem.