Maroun Bou Khalil, Weimin Hou, Hu Zhou, Fred Elisma, Leigh Anne Swayne, Alexandre P. Blanchard, Zemin Yao, Steffany A.L. Bennett, and Daniel Figeys*

Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, 451 Smyth Road, Ottawa, ON, Canada K1H 8M5

Received 16 April 2009; accepted 5 August 2009

Published online 7 October 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mas.20294

Lipid mediators participate in signal transduction pathways, proliferation, apoptosis, and membrane trafficking in the cell. Lipids are highly complex and diverse owing to the various combinations of polar headgroups, fatty acyl chains, and backbone structures. This structural diversity continues to pose a challenge for lipid analysis. Here we review the current state of the art in lipidomics research and discuss the challenges facing this field. The latest technological developments in mass spectrometry, the role of bioinformatics, and the applications of lipidomics in lipid metabolism and cellular physiology and pathology are also discussed. © 2010 Wiley Periodicals, Inc., Mass Spec Rev 29:877–929, 2010

Keywords: *lipidomics; tandem mass spectrometry; systems biology; bioinformatics; identification; quantification*

I. A LIPIDOMICS APPROACH

Lipids exhibit immense combinatorial and structural diversity. This vast range of unique chemical entities encodes for distinct functions within biological systems. Lipids play essential roles in cell structure and organization, signaling events, and trafficking, and sorting of macromolecules. However, the study of the role of lipids has been complicated by their structural diversity and by considerable technical challenges associated with distinguishing pathogenic from non-pathogenic lipid species within samples that contain several thousand lipid isoforms. ''The large-scale analysis of lipid profiles in cells and tissues'' (Piomelli, Astarita, & Rapaka, 2007) was made possible by the dawn of lipidomics (Han & Gross, 2003; Spener et al., 2003). However, lipid biology and homeostasis are impacted by the physicochemical properties of lipids, the physical basis of lipid behavior (i.e., lipid–lipid and lipid–protein interactions), local concentrations and compartmentalization of lipids, lipid transporters and enzymes/proteins involved in lipid metabolism, as well as by cellular response to intrinsic and extrinsic stimuli. Therefore, ''the full characterization of lipid molecular species and their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation'' (Spener et al., 2003) represents a balanced and objective view of the lipidomics field as a whole.

Empowered by technological advances in mass spectrometry-based applications, the lipidomics field has gained momentum over the years. Mass spectrometry has been successfully coupled to front-end separation or used directly (shotgun lipidomics) to allow the identification of specific molecular lipid species and their quantification. The integration and statistical analyses of the sheer volume of new lipidomic data are facilitated by advances in bioinformatics. Lipidomics is now considered an integral part of systems biology, an interdisciplinary field that focuses on the systematic study of complex interactions in biological systems. The ultimate goal of scientists around the world is to bridge ''omics'' sciences and cross-link available fingerprints of genes, transcripts, proteins, and metabolites to get a closer look at the cell interactome and function. The integration of lipidomics with genomics, proteomics and metabolomics will thus provide a powerful tool to decode molecular mechanisms of lipid-associated disorders, and to identify biomarkers and novel therapeutic targets.

This review on lipidomics will be of interest to the analytical biosciences, biochemical, medical, and pharmaceutical communities. It will commence with an overview of lipid biology (structure, classification, physicochemical properties, isolation and processing, cellular functions), and then move onto the new horizons made possible by mass spectrometry and bioinformatics in lipidomics research that have begun to unravel how lipids contribute to cell and body function, and disease pathogenesis. This review will end with a discussion of challenges facing modern lipidomics research. It is hoped that this review will act as a bridge for researchers and clinicians across multiple disciplines to generate novel approaches to disease diagnostics.

II. CLASSIFICATION OF LIPIDS

To support the emerging field of lipidomics, a comprehensive classification system for lipids with a universal platform compatible with bioinformatics requirements was proposed in 2005 by Fahy and co-workers (Fahy et al., 2005). This classification system has been under the leadership of the International Lipid Classification and Nomenclature Committee (ILCNC) and focused primarily on mammalian lipids. Recently the classification system has been updated to encompass lipid structures from non-mammalian sources such as plants, bacteria, and fungi (Fahy et al., 2009). This classification system distinguishes fatty acyls from other polyketides, glycerophospholipids from other glycerolipids, and sterol lipids from other prenols. As a result, lipids were regrouped under the following eight categories that cover eukaryotic and prokaryotic sources: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides (Fig. 1). Each category contains distinct classes, subclasses, subgroups, and subsets of lipid molecules (LipidMapsConsortium, 2009). The characteristics of each lipid class are outlined below.

**Correspondence to:* Daniel Figeys, Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, 451 Smyth Road, Ottawa, ON, Canada K1H 8M5.
E-mail: dfigeys@uottawa.ca

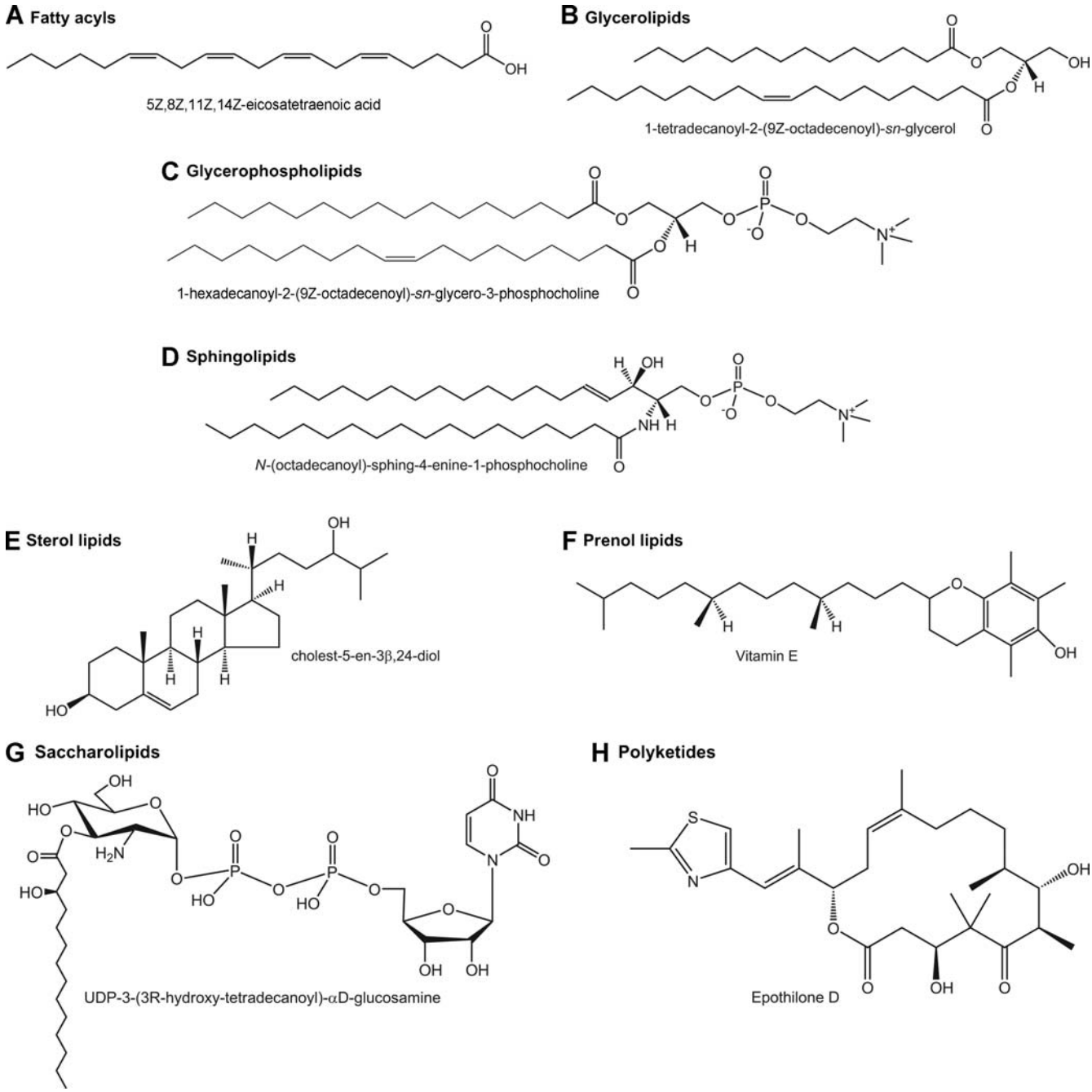


FIGURE 1. Representative structures of lipid classes.

A. Fatty Acyls

The fatty acid group is the fundamental building block of complex lipids. It consists of a carboxylic acid with a long unbranched, hydrophobic aliphatic tail that is saturated or unsaturated. Fatty acids are structurally diverse and contain distinct classes (Fig. 2) that are defined at the molecular level based on the degree of branching, the number and position of double bonds, the chain length, the *cis–trans* isomer conformations, and the presence of functional groups (Small, 1986; Brennan & Nikaido, 1995; Ohlrogge & Jaworski, 1997; Vance & Vance, 2002). Most naturally occurring fatty acids are composed of an even number of carbons and occur predominantly in esterified form (e.g., waxes, oils). In plants, bacteria, and, more rarely, in some animals, fatty acids can be assembled with an odd number of carbon atoms. However, these fatty acids are much less abundant than those with even numbers of carbons. Cyclic fatty acids containing three to six carbon atoms, and heterocyclic rings containing oxygen or nitrogen, are also found in nature. Heteroatoms of oxygen, halogen, nitrogen, and sulfur can be also linked to the carbon chains.

There are 14 classes of fatty acyls defined by specific modifications to the core fatty acid (Fig. 2). The first class, fatty acids and conjugates, is composed of 17 subclasses (Fig. 3)

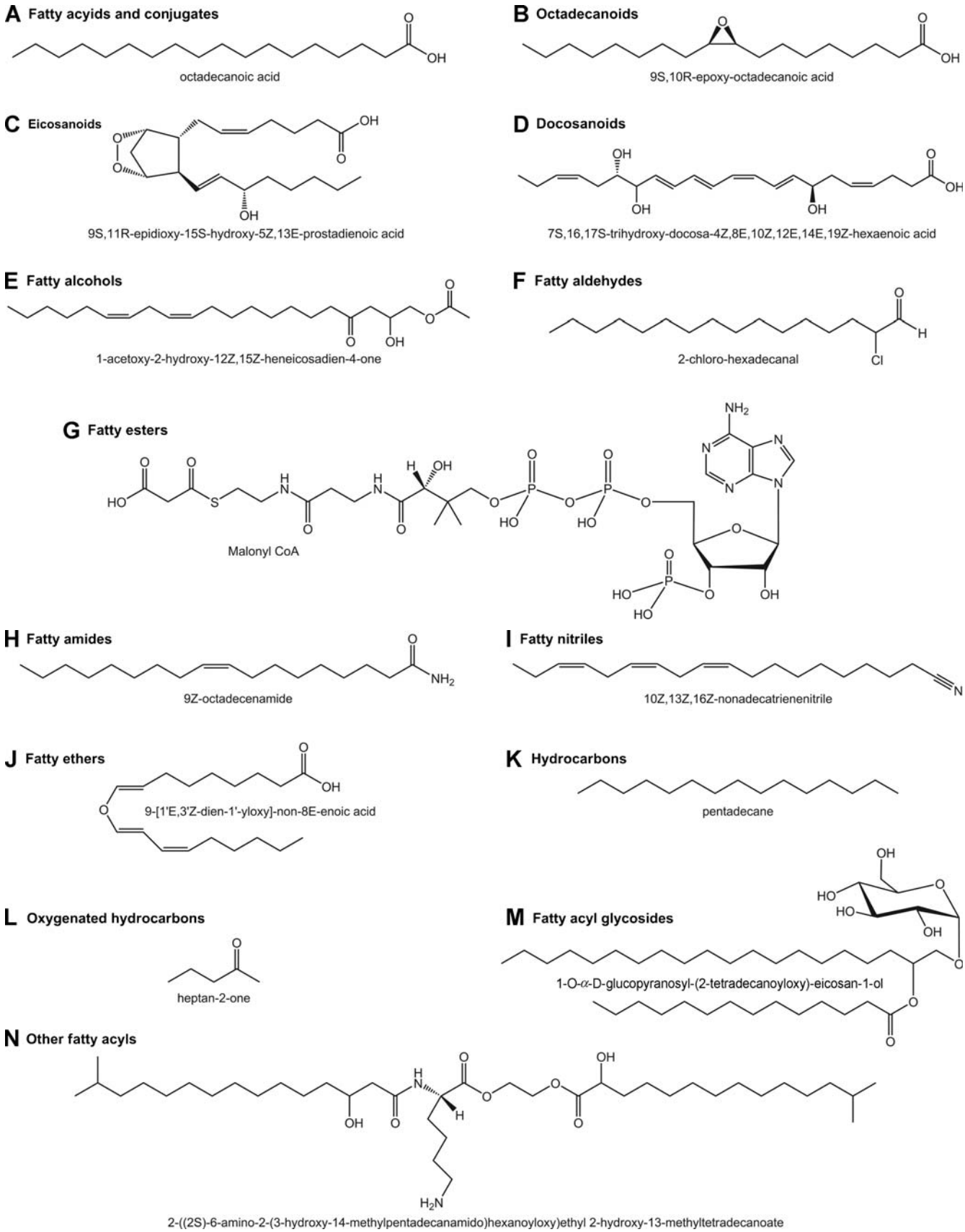


FIGURE 2. Representative structures of fatty acyls. Fatty acyls are composed of 14 classes: fatty acids and conjugates, octadecanoids, eicosanoids, docosanoids, fatty alcohols, fatty aldehydes, fatty esters, fatty amides, fatty nitriles, fatty ethers, hydrocarbons, oxygenated hydrocarbons, fatty acyl glycosides, and other fatty acyls.

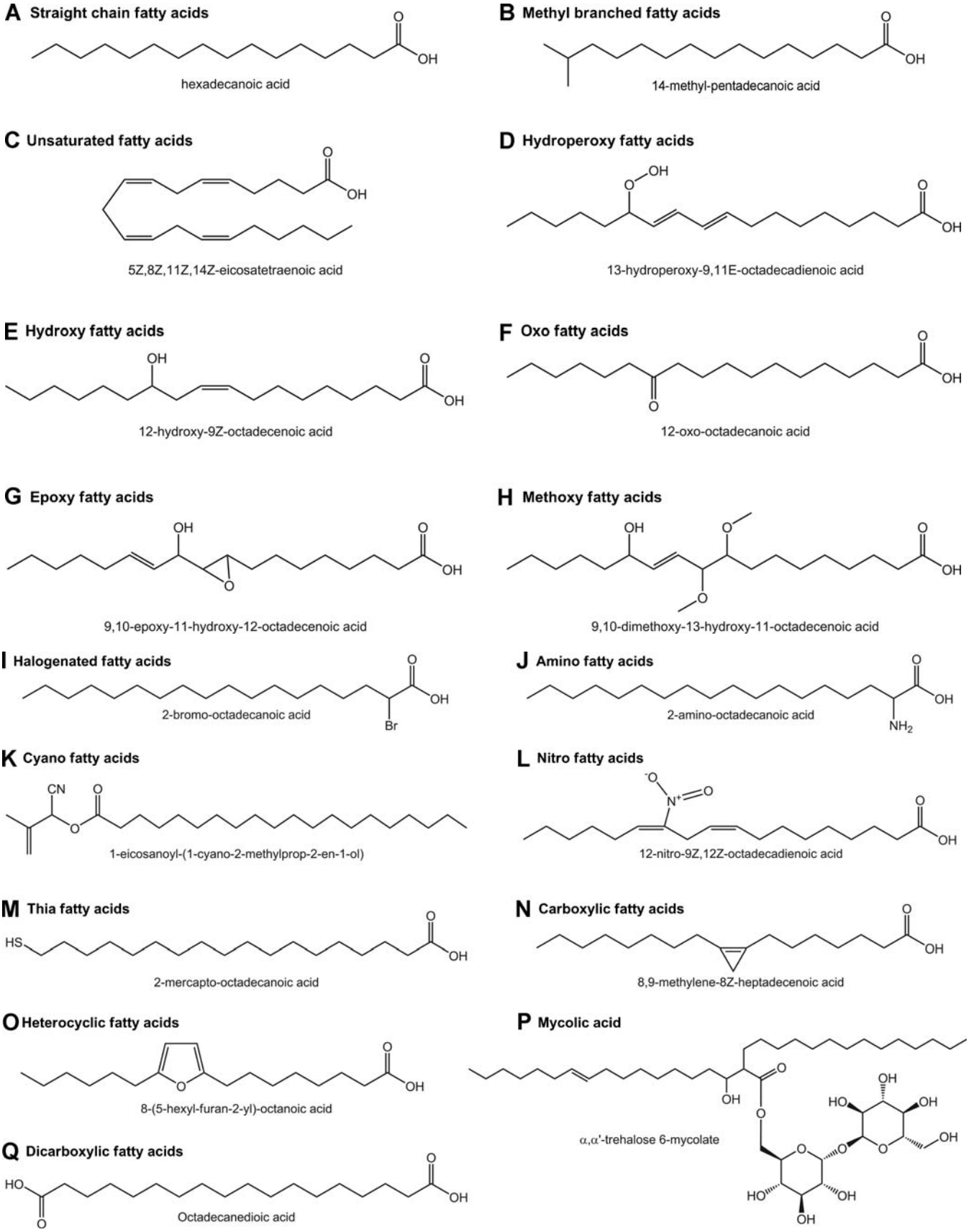


FIGURE 3. Representative structures of fatty acids and conjugates. This class of fatty acyls is subdivided into 17 subclasses: straight chain, methyl branched, unsaturated, hydroperoxy, hydroxy, oxo, epoxy, methoxy, halogenated, amino, cyano, nitro, thia, carbocyclic, heterocyclic, mycolic, and dicarboxylic fatty acids.

beginning with the straight-chain saturated fatty acids that contain a terminal carboxylic acid ($CH_3(CH_2)_nCOOH$, $n = 4–30$ or greater). Subsequent subclasses are further defined by one or more substituents (e.g., methyl substituents), conjugates, and branched-chain fatty acids such as the mycolic acids. More complex fatty acids with multiple functional groups, such as octadecanoids, can be divided into three subclasses that include 12-oxophytodienoic acid metabolites, lipids in the jasmonic acid pathway of plant hormone biosynthesis, as well as other octadecanoids (Agrawal et al., 2004). The eicosanoids class (Fig. 4) is derived from arachidonic acid and includes prostaglandins, leukotrienes, and other structural derivatives (Murphy & Smith, 2002). The docosanoids, which are derived from docosahexaenoic acid, contain 22 carbon atoms (Bazan, 1989). Other major lipid classes in the fatty acyl category include fatty acid esters subdivided into wax monoesters and diesters, cyano esters, and the lactones. The fatty ester class also has subclasses that include biochemical intermediates such as fatty acyl thioester-CoA derivatives, fatty acyl thioester-acyl carrier protein derivatives, fatty acyl carnitines (esters of carnitine), and fatty adenylates.

The fatty alcohols and fatty aldehydes are typified by terminal hydroxy and oxo groups, respectively. The fatty amides also include *N*-fatty acylated amines, unsubstituted amides, homoserine lactones, and *N*-acyl ethanolamines (commonly refered to as endocannabinoids). These subclasses confer distinct biological properties. For instance, fatty acyl homoserine lactones are fatty amides involved in bacterial quorum sensing (Roche et al., 2004). Finally, hydrocarbons, oxygenated hydrocarbons, fatty acyl glycosides, and long-chain ethers are also regrouped under the fatty acyls category.

B. Glycerolipids

This category of lipids encompasses all glycerol-containing lipids. Acylglycerols or the fatty acid esters of glycerol (e.g., mono, di, and triacylglycerols) are the best known glycerolipids (Coleman & Lee, 2004). Other subclasses are the glyceroglycans characterized by the presence of sugar residues attached to the glycerol backbone *via* a glycosidic linkage, including glycosylmonoradylglycerols and glycosyldiradylglycerols (Pahlsson

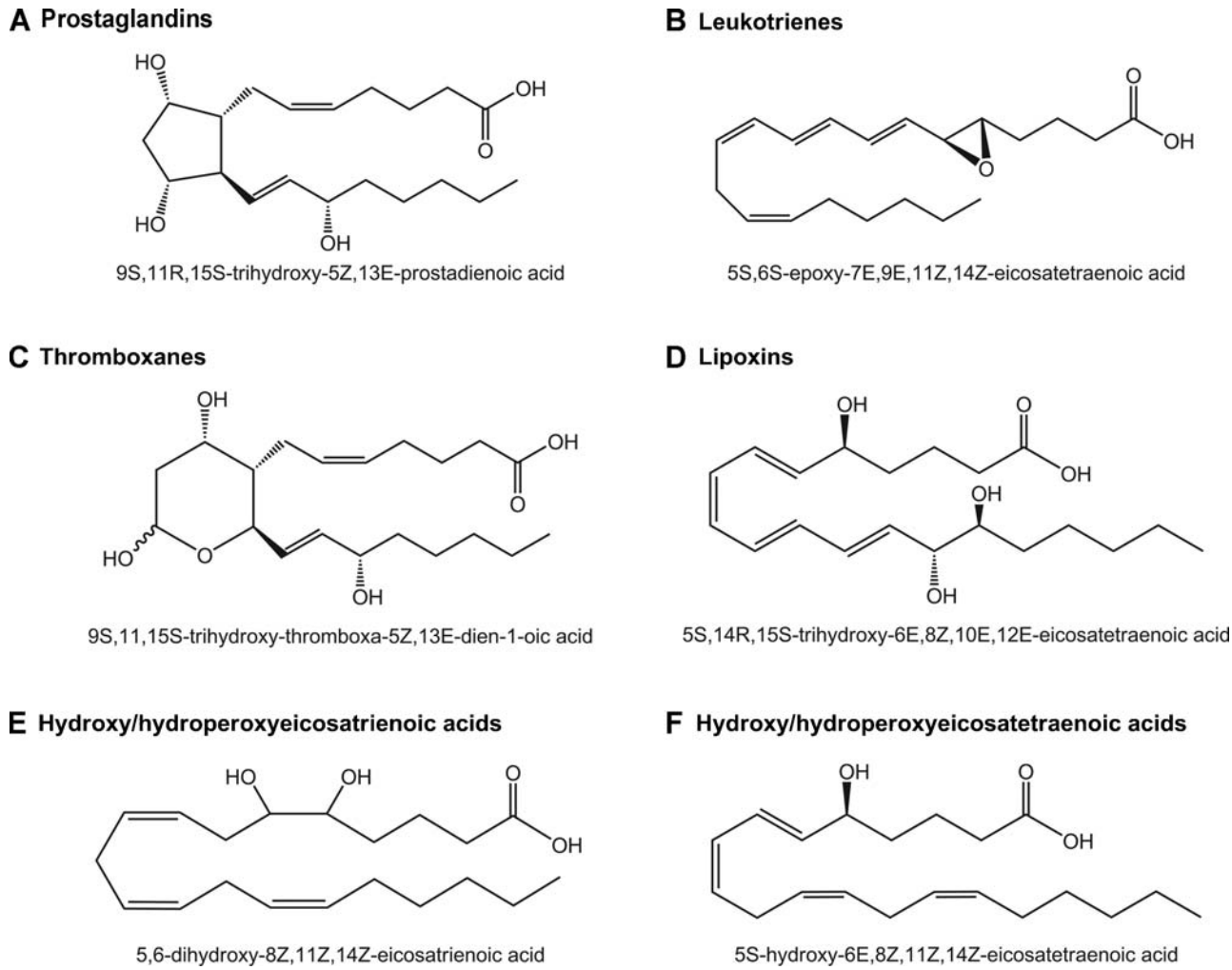


FIGURE 4. Representative structures of eicosanoids. There are 13 subclasses of eicosanoids: prostaglandins, leukotrienes, thromboxanes, lipoxins, hydroxy/hydroperoxyeicosatrienoic acids, hydroxy/hydroperoxyeicosatetraenoic acids, hydroxy/hydroperoxyeicosapentaenoic acids, epoxyeicosatrienoic acids, hepoxilin, levuglandins, isoprostanes, clavulones and derivatives, and other eicosanoids.

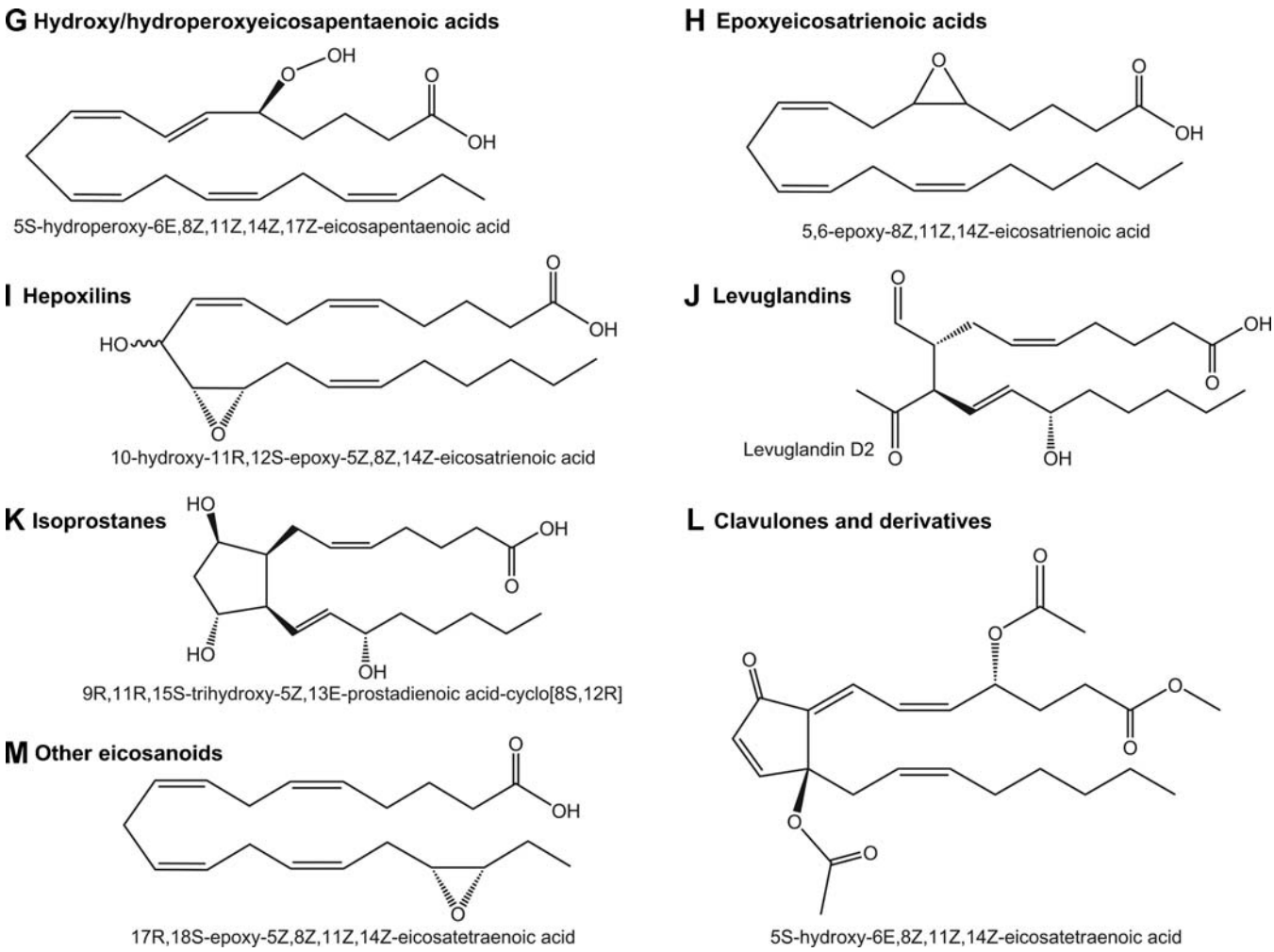


FIGURE 4. (*Continued*)

et al., 2001), and macrocyclic ether lipids present in the archaebacteria membranes (Koga et al., 1993).

C. Glycerophospholipids

Glycerophospholipids are ubiquitous in nature. They are key components of cellular membranes (Pereto, Lopez-Garcia, & Moreira, 2004) and serve as binding sites for cellular and extracellular proteins. Glycerophospholipid metabolites also function as second messengers involved in proliferation and apoptotic cell injury (Cronan, 2003). Glycerophospholipids are derivatives of *sn*-glycero-3-phosphoric acid that contains at least one *O*-acyl, or *O*-alkyl or *O*-alkyl-1′-enyl residue attached to the glycerol moiety, and a polar headgroup composed of a nitrogenous base, a glycerol, or an inositol unit. Naming of the glycerophospholipids involves stereospecific numbering (*sn*) (Hirschmann, 1960; IUPAC-IUB-CBN, 1967). The fatty acyl substituents at the *sn*-1 and/or *sn*-2 position are generally referred to as ''radyl'' groups. The double bond geometry of the radyl moieties is described with E/Z designation (instead of trans/cis), and species lacking one radyl group are denoted as ''lyso'' glycerophospholipids. In response to lipidomic researchers worldwide, the nomenclature of glycerophospholipid classes adopted by Fahy and co-workers (Fahy et al., 2005) has reverted to the universally accepted/used two letter ''phospholipid'' format (Table 1) in the updated comprehensive classification system for lipids (Fahy et al., 2009).

Glycerophospholipids are grouped into classes (Fig. 5) based on the composition of their polar headgroup at the *sn*-3 position of the glycerol backbone in eukaryotes and eubacteria or at the *sn*-1 position in archaea (Pereto, Lopez-Garcia, & Moreira, 2004). The major classes of glycerophospholipids found in mammalian cell membranes include glycerophosphatidic acids (PA), glycerophosphocholines (PC), glycerophosphoethanolamines (PE), glycerophosphoinositols (PI), glycerophosphoglycerols (PG), glycerophosphoserines (PS), and cardiolipins (CL) (Fig. 5). Each headgroup class is further differentiated into subclasses on the basis of the *sn*-1 and *sn*-2 substituents on the glycerol backbone. Fatty acids at the *sn*-1 position can be substituted by ether or vinyl ether moieties (plasmanyl and plasmenyl glycerophospholipids, respectively) in some of the classes. In the case of the glycerophosphoglycerols and glycerophosphoglycerophosphates (Fig. 5D,E) a second glycerol unit constitutes part of the headgroup, whereas for the glycerophosphoglycerophosphoglycerols (cardiolipins) a third

TABLE 1. Changes in the nomenclature of glycerophospholipids in the updated comprehensive classification system for lipids (reproduced from Fahy et al., 2009)

Class	Synonym	Nomenclature	
		Old	New
Glycerophosphocholines	Phosphatidylcholines	GPCho	PC
Glycerophosphoethanolamines	Phosphatidylethanolamines	GPEtn	PE
Glycerophosphoserines	Phosphatidylserines	GPSer	PS
Glycerophosphoglycerols	Phosphatidylglycerols	GPGro	PG
Glycerophosphoglycerophosphates	Phosphatidylglycerol phosphates	GPGroP	PGP
Glycerophosphoinositols	Phosphatidylinositols	GPIns	PI
Glycerophosphoinositol monophosphates	Phosphatidylinositol phosphates	GPInsP	PIP
Glycerophosphoinositol bis-phosphates	Phosphatidylinositol bis-phosphates	GPInsP2	PIP2
Glycerophosphoinositol tris-phosphates	Phosphatidylinositol tris-phosphates	GPInsP3	PIP3
Glycerophosphates	Phosphatidic acids	GPA	PA
Glyceropyrophosphates		GPP	PPA
Glycerophosphoglycerophosphoglycerols	Cardiolipins	CL	CL
CDP-glycerols		GCDP	CDP-DG
Glycosylglycerophospholipids		[glycan]GP	[glycan]GP
Glycerophosphoinositolglycans		[glycan]GPIns	[glycan]PI
Glycerophosphonocholine		GPnCho	PnC
Glycerophosphonoethanolamines		GPnEtn	PnE
Monoradylglycerophospholipids	Lysophospholipids		LPX[1]

[1] X denotes PC, PE, PA, etc.

[1]X denotes PC, PE, PA, etc.

glycerol unit is typically acylated at the *sn*-1′ and *sn*-2′ positions (Fig. 5L).

Inositol-containing glycerophospholipids (Fig. 5F–I) represent a unique and biologically diverse class. Phosphoinositides or phosphorylated derivatives of PIs are the best characterized inositol lipids. They play a wide variety of cellular roles in many eukaryotic cells. Less understood are ceramides containing inositol in fungi, and inositol glycolipids in pathogens. More detailed overviews are available for the classification, nomenclature, metabolism, and profiling of glycerophospholipids (Cronan, 2003; Coleman & Lee, 2004; Forrester et al., 2004; Ivanova et al., 2004).

D. Sphingolipids

Sphingolipids are often enriched in neural tissues (Piomelli, Astarita, & Rapaka, 2007). They play important roles in both signal transduction and cell recognition. This category of lipids is derived from the aliphatic amino-alcohol sphingosine or sphing-4-enine (Fig. 6A), which is synthesized *de novo* from serine and palmitoyl-CoA. Sphingolipids include ceramides, phosphosphingolipids (e.g., sphingomyelins), glycosphingolipids (e.g., cerebrosides and gangliosides), and other species that include protein adducts (Merill & Sandhoff, 2002; Taniguchi, Honke, & Fukuda, 2002). Ceramides (Fig. 6B) consist of a sphingosine moiety that is amide-linked to a fatty acyl group (Fahy et al., 2005). Ceramides are typically saturated or mono-unsaturated with chain lengths varying from 14 to 26 carbon atoms; the presence of a hydroxyl group on carbon 2 is fairly common. In mammals, ceramides are the common precursors of phosphosphingolipids (Fig. 6C) in which the ceramide backbone is *O*-linked to a charged headgroup (e.g., ethanolamine, serine, or choline). Insects contain mainly ceramide phosphoethanolamines, and fungi have phytoceramidephosphoinositols and mannose-containing headgroups. Glycosphingolipids (Chester, 1998; UPAC-IUB-JCBN, 1999) are further subclassified based on their carbohydrate moieties composition: (i) neutral glycosphingolipids (Fig. 6E) contain uncharged sugar residues such as glucose, galactose, *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), and fucose; (ii) acidic glycosphingolipids (Fig. 6F) contain phosphate or sulfate attached to neutral sugars or charged sugar residues such as sialic acid (*N*-acetyl or *N*-glycoloyl neuraminic acid). The latter are called gangliosides, and the number of sialic acid residues is usually denoted with a subscript letter (i.e., mono-, di-, or tri-) and a number reflecting the subspecies within that category; (iii) basic glycosphingolipids (Fig. 6G); and (iv) amphoteric glycosphingolipids (Fig. 6H). In addition, sphingoid base analogs are regrouped under sphingolipids because they are known to function as inhibitors or antagonists of sphingolipids. In some organisms, these analogs serve as surrogates for sphingolipids.

E. Sterol Lipids

The sterol category is subdivided into six classes (Fig. 7) according to biological functions. Cholesterol (Fig. 7A) and its derivatives are the most widely studied in mammalian systems. Along with glycerophospholipids and sphingomyelins, sterol lipids constitute important components of membrane lipids (Bach & Wachtel, 2003). Unique sterol lipids also exist in plant, fungal, and marine sources. Sterol lipids participate in different biological processes as hormones and as signaling molecules (Tsai & O'Malley, 1994). The C18 steroids include the estrogen family. The C19 steroids comprise the androgens such as testosterone and androsterone (Fig. 7B). The C21 subclass, containing a two carbon side chain at the C17 position, includes the progestogens, glucocorticoids and mineralocorticoids. The secosteroids comprise various forms of vitamin D

(Fig. 7C) (Jones, Strugnell, & DeLuca, 1998). Additional classes within the sterols category are the bile acids (Russell, 2003) and their conjugates (sulfuric acid, taurine, glycine, glucuronic acid, and others) (Fig. 7D).

F. Prenol Lipids

Prenol lipids (Fig. 8) are essential for cell survival in all organisms, and are synthesized from the five-carbon building units isopentenyl diphosphate and dimethylallyl diphosphate which are produced *via* the mevalonate pathway in animals and *via* the methylerythritol phosphate pathway in pathogenic bacteria, malaria parasites and plants (Rodriguez-Concepcion, 2004). Carotenoids are examples of prenol lipids that function as antioxidants and as precursors of vitamin A (Demmig-Adams & Adams, 2002). Another biologically important class of molecules is exemplified by the quinones and hydroquinones (Fig. 8B) which contain an isoprenoid tail attached to a quinonoid core of non-isoprenoid origin. Vitamin E, menaquinone (vitamin K2), and ubiquinone (coenzyme Q) are examples of this class (Ricciarelli, Zingg, & Azzi, 2001; Meganathan, 2001a,b). Polyprenols (Fig. 8C) and their phosphorylated derivatives play important roles in the transport of oligosaccharides across membranes. Polyprenol phosphate sugars and polyprenol diphosphate sugars function in extracytoplasmic glycosylation reactions (Raetz & Whitfield, 2002), in extracellular polysaccharide biosynthesis in bacteria (Raetz & Whitfield, 2002), and in protein *N*-glycosylation in eukaryotes (Helenius & Aebi, 2001; Schenk, Fernandez, & Waechter, 2001).

G. Saccharolipids

Saccharolipids are compounds in which fatty acids are linked directly to a sugar backbone that substitutes for the glycerol backbone present in glycerolipids and glycerophospholipids. Saccharolipids can occur as glycan or as phosphorylated derivatives in six classes (Fig. 9). The acylated glucosamine precursors of the lipid A (endotoxin) component of lipopolysaccharides in Gram-negative bacteria are the most familiar

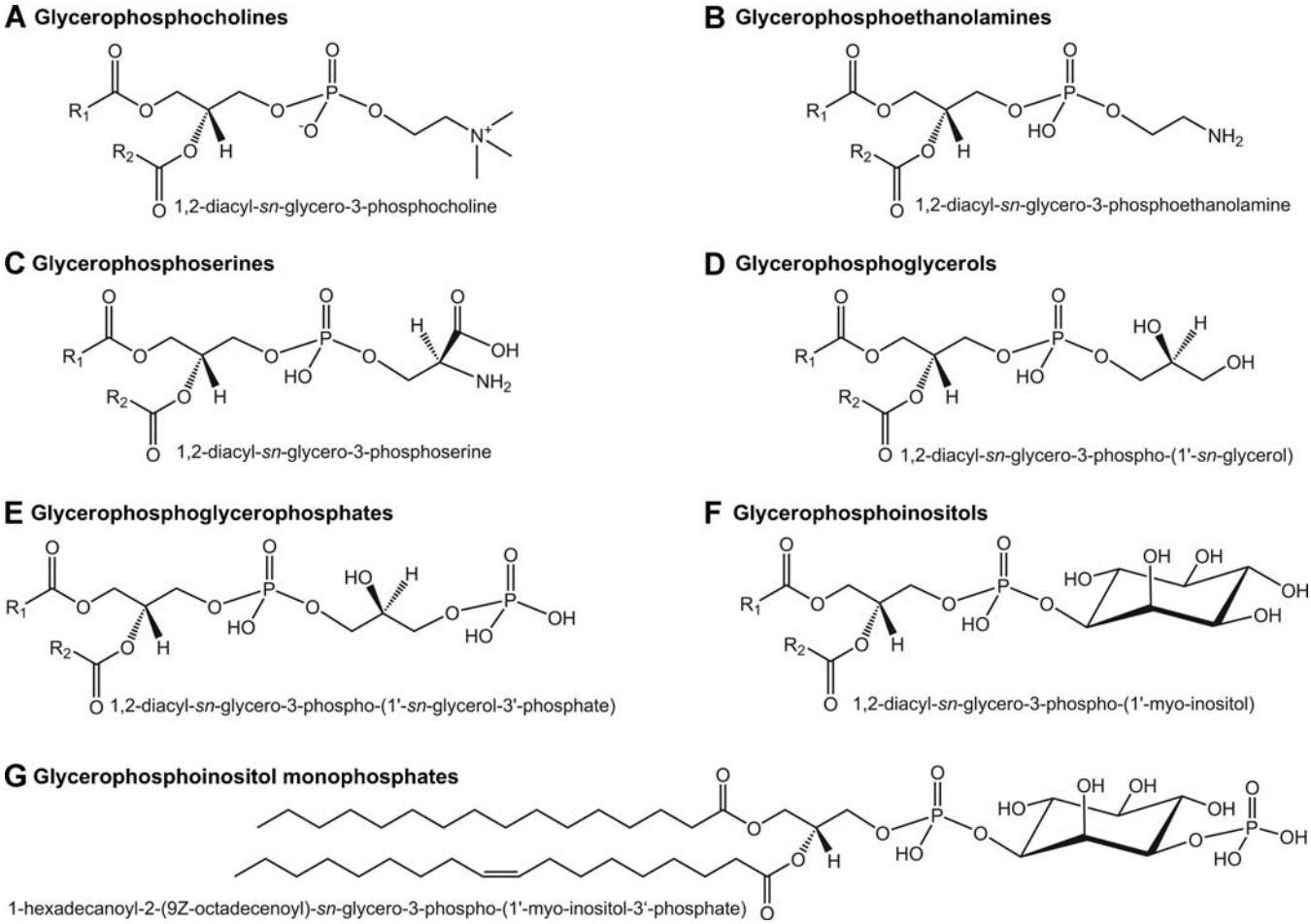


FIGURE 5. Representative structures of glycerophospholipids. The Lipid Map consortium has defined 21 classes of glycerophospholipids: PC, PE, PS, PG, glycerophosphoglycerophosphates, PI, PI monophosphates, PI bisphosphates, PI trisphosphates, glycerophosphates, glyceropyrophosphates, glycerophosphoglycerophosphoglycerols (cardiolipins), CDP-glycerols, glycosylglycerophospholipids, glycerophosphoinositolglycans, glycerophosphonocholines, glycerophosphonoethanolamines, caldarchaeols, glycerol-nonitol tetraether phospholipids, oxidized glycerophospholipids, and other glycerophospholipids. Subclasses within these groups are further defined by linkage to the glycerol backbone.

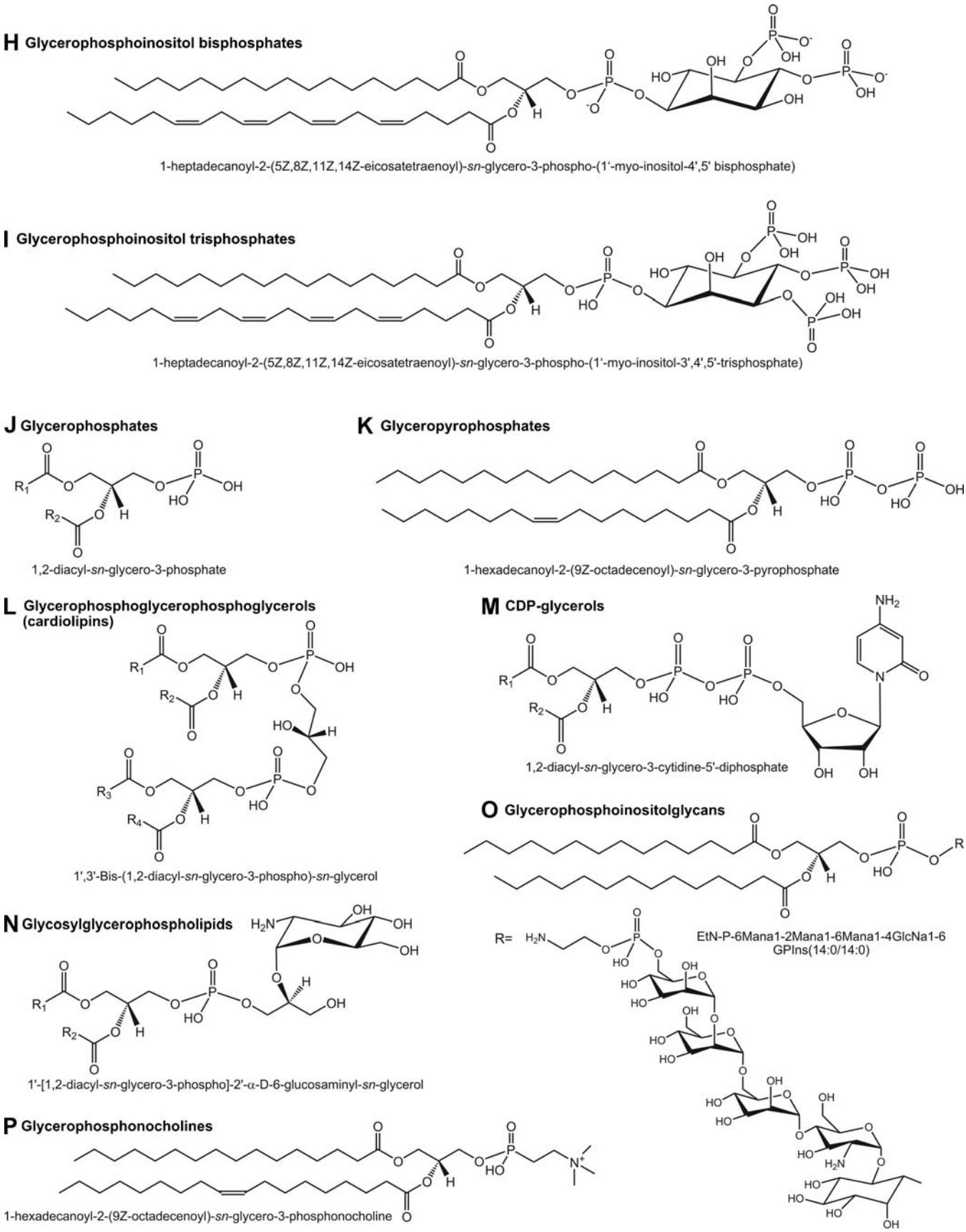


FIGURE 5. (*Continued*)

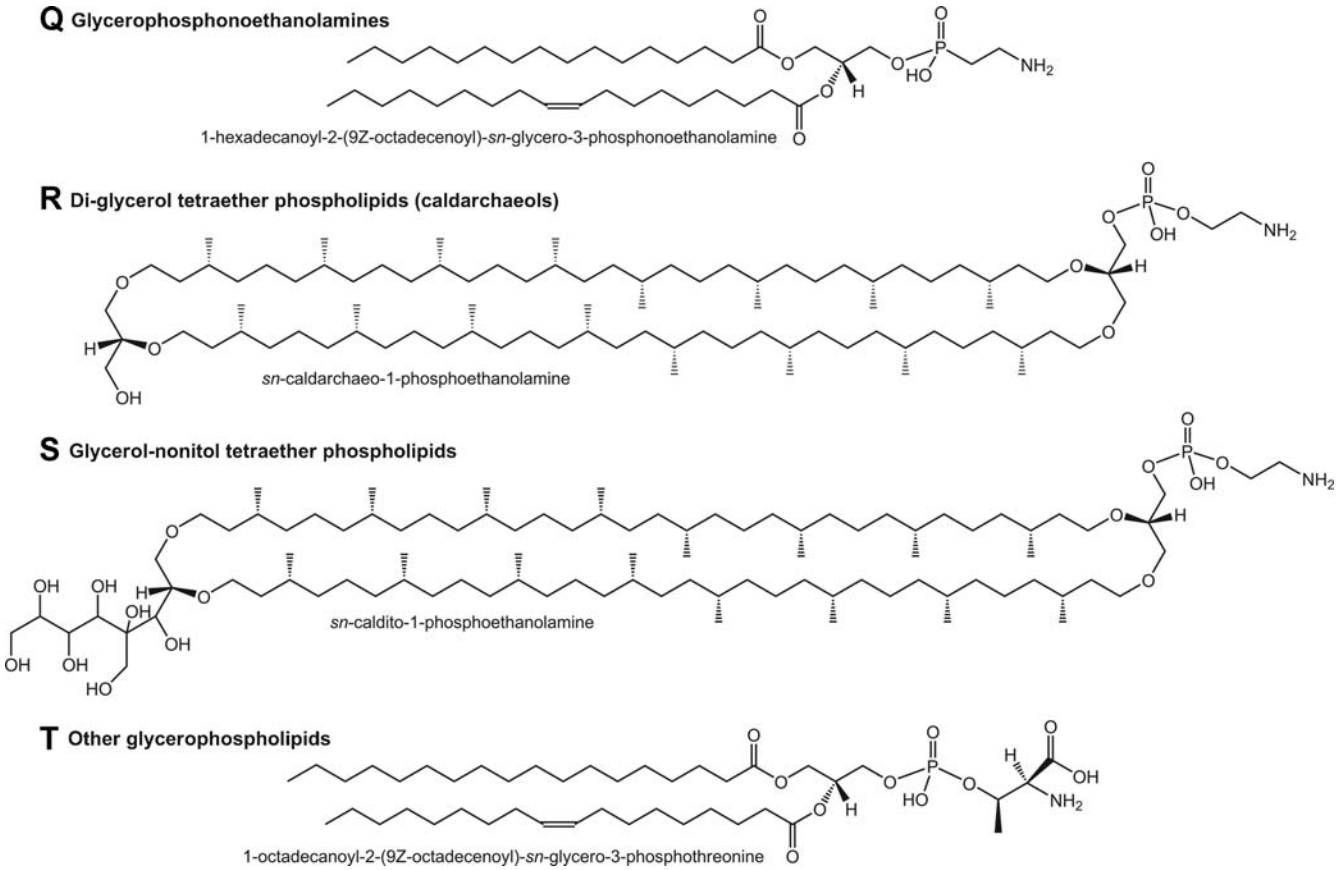


FIGURE 5. (*Continued*)

saccharolipids (Raetz & Whitfield, 2002). Also included in this category are the acyl aminosugars of rhizobia, which are soil bacteria that engage in a symbiosis with leguminous plants that produce nitrogen-fixing root nodules (Spaink, 2000). Additional saccharolipids include fatty acylated derivatives of glucose which are best exemplified by the acylated trehalose units of certain mycobacterial lipids (Brennan & Nikaido, 1995). Acylated forms of glucose and sucrose have been also reported in plants (Ghangas & Steffens, 1993).

H. Polyketides

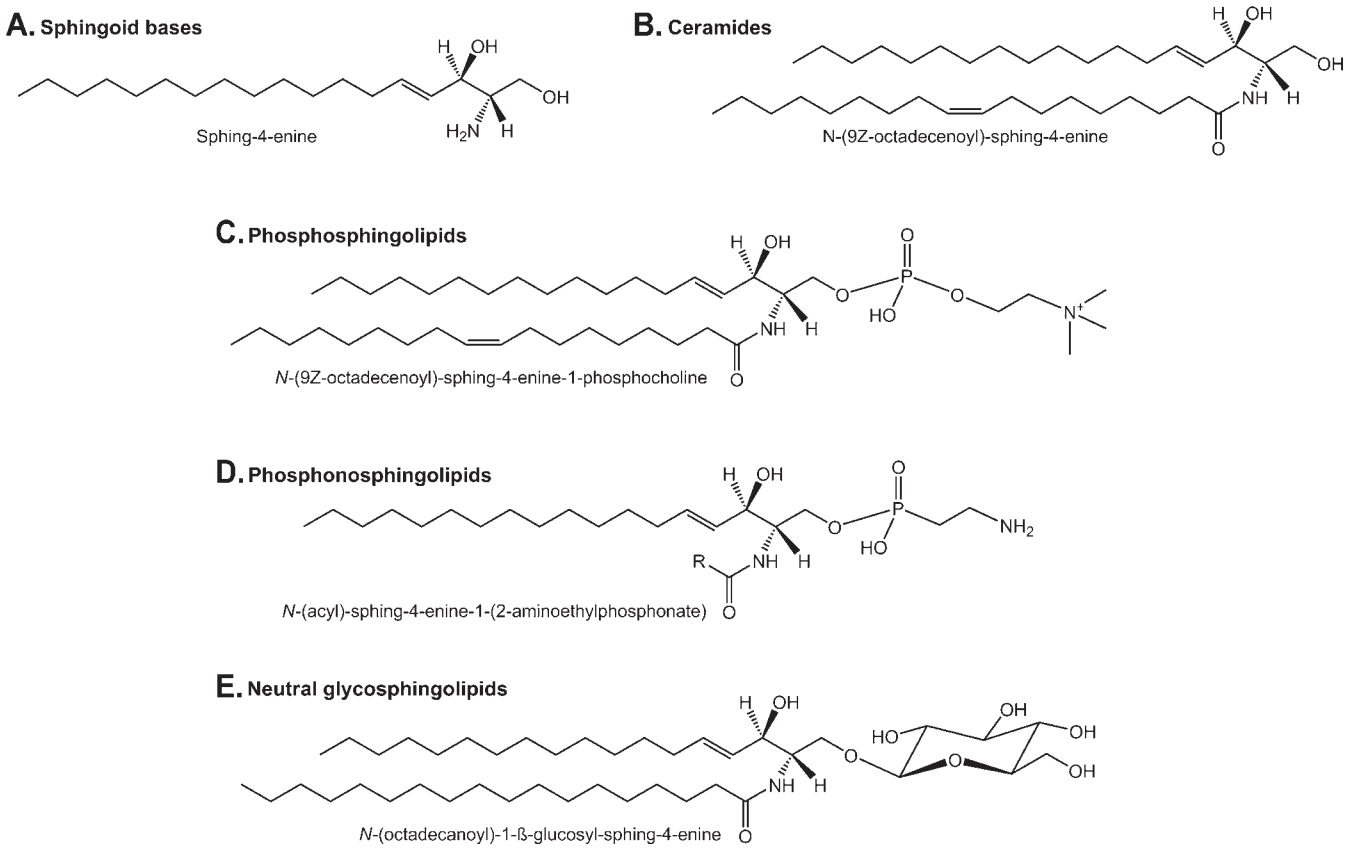
Polyketides (Fig. 10) are synthesized from propionyl-CoA and methylmalonyl-CoA by polyketide synthases in a process analogous to fatty acid biosynthesis (Khosla et al., 1999; Walsh, 2004). The different classes of polyketide synthases produce a great diversity of natural product structures. The class I polyketide synthases form constrained macrocyclic lactones ranging in size from 14 to 40 atoms, whereas class II and III polyketide synthases generate complex aromatic ring systems. Polyketide backbones are often modified by glycosylation, methylation, hydroxylation, oxidation, and/or other processes. Some polyketides are linked with non-ribosomally synthesized peptides to form hybrid scaffolds. Many commonly used antimicrobial, anti-parasitic, and anti-cancer agents are polyketides or polyketide derivatives (Reeves, 2003). Important examples of these drugs include erythromycins (Fig. 10A), tetracyclines (Fig. 10D), and anti-tumor epothilones. Other polyketides include potent toxins (Hopwood, 2004).

III. PHYSICAL PROPERTIES OF LIPIDS

Lipids are operationally classified as hydrophobic or amphipathic molecules that participate in different types of associations. Neutral or non-polar lipids (e.g., sterol esters, glycerolipids, hydrocarbons, and carotenoids) participate in non-covalent interactions through their hydrocarbon chains with other lipids and hydrophobic regions of proteins. Examples include fat in adipose tissue, chylomicrons, albumin-fatty acid complexes, etc. Polar lipids (e.g., glycerophospholipids, sphingolipids, and sterol lipids) interact with proteins *via* hydrogen bonding, electrostatic and/or hydrophobic forces as in cellular membranes, mitochondria, endoplasmic reticulum (ER), and serum lipoprotein complexes. Fatty acids, hydroxylic acids or complex branched acids are linked covalently as esters, amides, or glycosides to polysaccharide structures as in lipopolysaccharides of bacterial cell walls. At the lipid/water interface, hydrogen bonding and electrostatic interactions between polar headgroups are the predominant forces. At the lipid bilayer core, hydrophobic interactions govern the ordering of membranes (i.e., the compactness of acyl chains and sterol rings).

Polar lipids exhibit amphipathic character and have critical micelle concentrations in the nM range (Israelachvili, 1992). Thus, the amphipathic balance in polar lipids is weighted heavily in favor of the non-polar domain of the molecule (Heerklotz & Epand, 2001). In biological membranes, polar lipids form either bilayer or hexagonal-II structures under physiological conditions. The bilayer is stabilized by the exposure of the polar

Bou Khalil et al. (2009) Figure 6A-E



Bou Khalil et al. (2009) Figure 6F-I

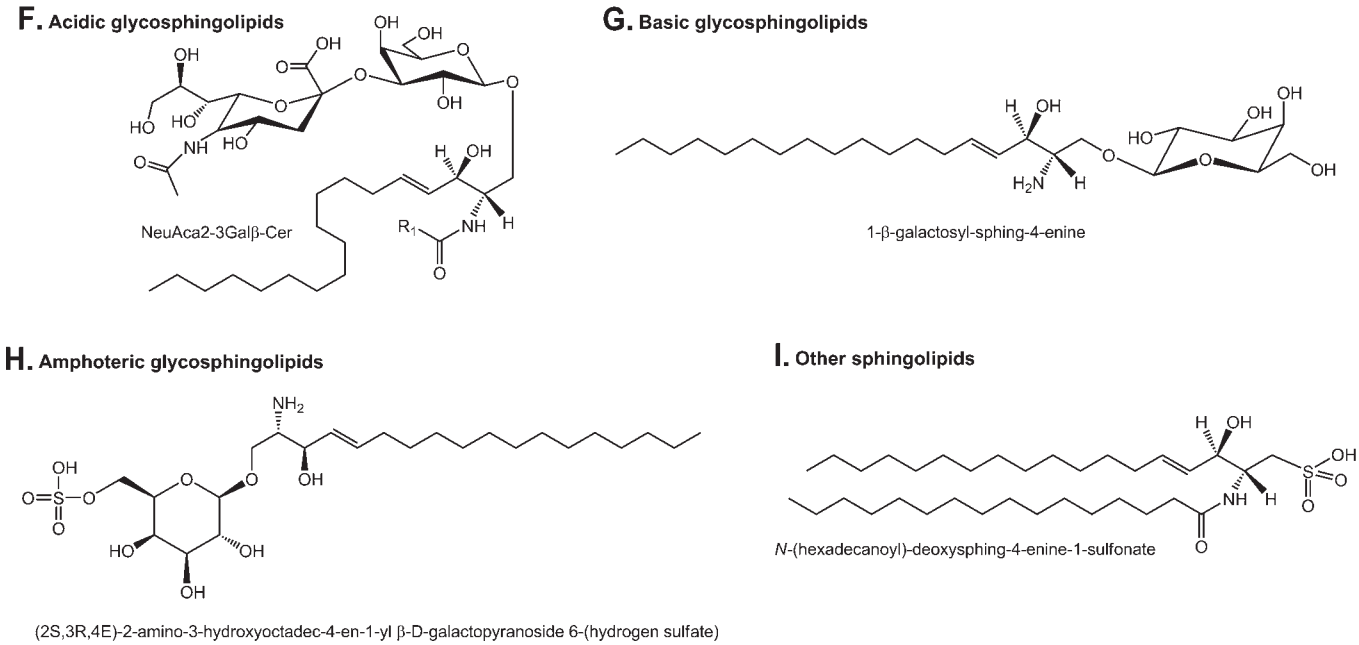


FIGURE 6. Representative structures of sphingolipids. There are 10 classes of sphingolipids: spingoid bases, ceramides, phosphosphingolipids, phosphonosphingolipids, neutral glycosphingolipids, acidic glycosphingolipids, basic glycosphingolipids, amphoteric glycosphingolipids, arsenosphingolipids, and other sphingolipids.

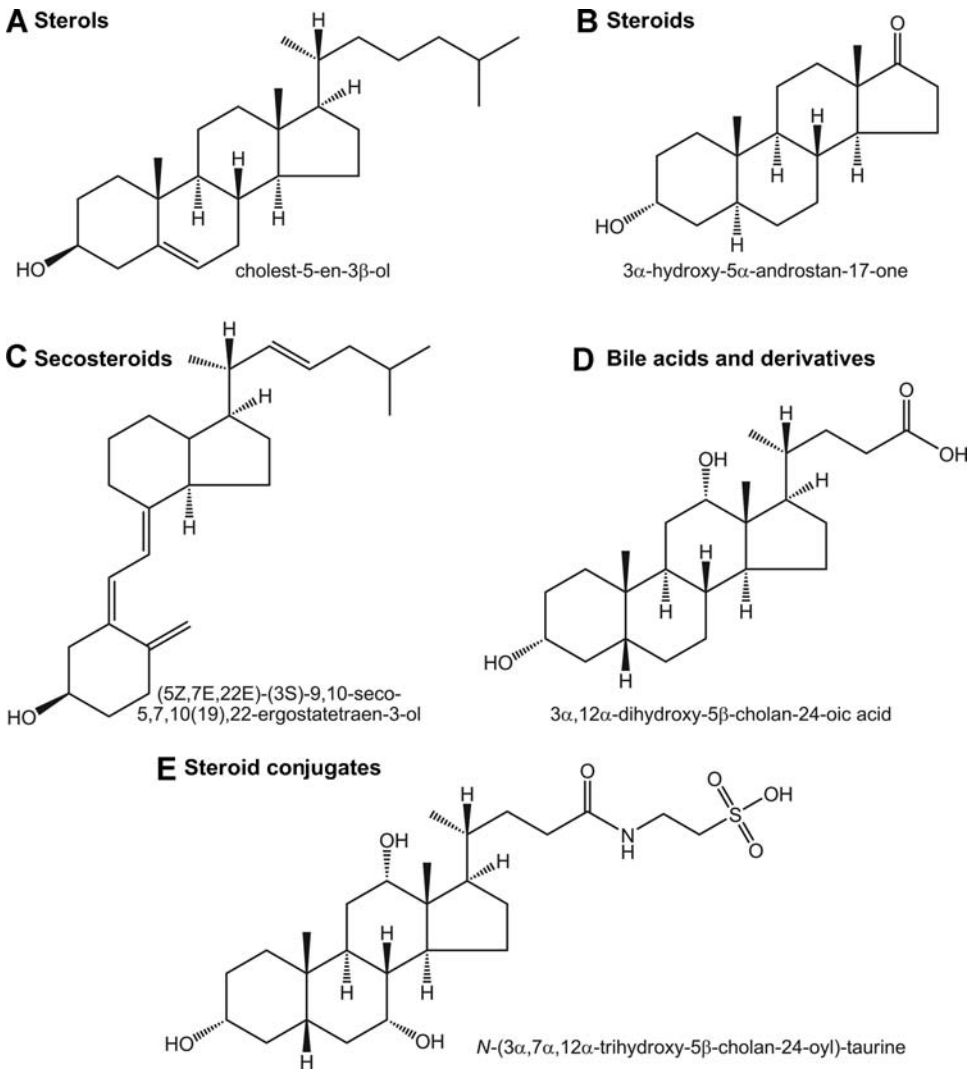


FIGURE 7. Representative structures of sterol lipids. This category is subdivided into six classes: sterols, steroids, secosteroids, bile acids and derivaties, steroid conjugates, and other sterols.

headgroup to the aqueous milieu, and by the sequestering of the hydrocarbon chains within the hydrophobic core (Tieleman & Marrink, 2006). The hexagonal-II structure consists of tubes of water formed by the lipid polar groups, and these tubes are packed into a two-dimensional hexagonal array. The amphipathic balance favors the hydrocarbon affinity, and typically the polar group is less hydrated than in bilayer-forming lipids. In living cells the membranes are comprised of polar and non-polar lipids within a lipid matrix that exhibits invariably a bilayer arrangement.

A. Polar Groups

Polar lipids are classified based upon their headgroup moieties. The simplest and most abundant structures of polar lipids are the galactosylglycerolipids. These are the dominant polar lipids of photosynthetic thylakoid membranes (e.g., mono- and di-galactosyldiacylglycerols). Other glycosylglycerides include sulphonated sugars such as sulphoquinovosyldiacylglycerol found abundantly in higher plant photosynthetic membranes. The sugar residues represent the polar groups of these lipids and provide hydrogen bonding potential. While the presence of one sugar residue in membrane lipids favors the formation of hexagonal-II structures, the presence of two sugar residues is conducive to the formation of bilayer structures under physiological conditions.

Glycerophospholipids, the most common membrane lipids, tend to form bilayer structures under physiological conditions. However, polyunsaturated PE form hexagonal-II structures. Some classes possess a net negative charge at pH 7 such as the inositol, serine, glycerol and ethanolamine phosphatides, while the choline phosphatides are zwitterionic.

Sphingolipids represent another large group of polar membrane lipids. Sphingomyelins are the most common sphingolipids and are characterized by a phosphocholine polar group. Most sphingolipids have sugar residues attached to the hydroxyl group of the ceramide. The attachment of phosphorylinositol to ceramide represents the sphingolipid analogue of phosphatidylinositol, and is a major component of sphingolipids in yeast (e.g., mannosylinositol- and mannosyldiinositol-

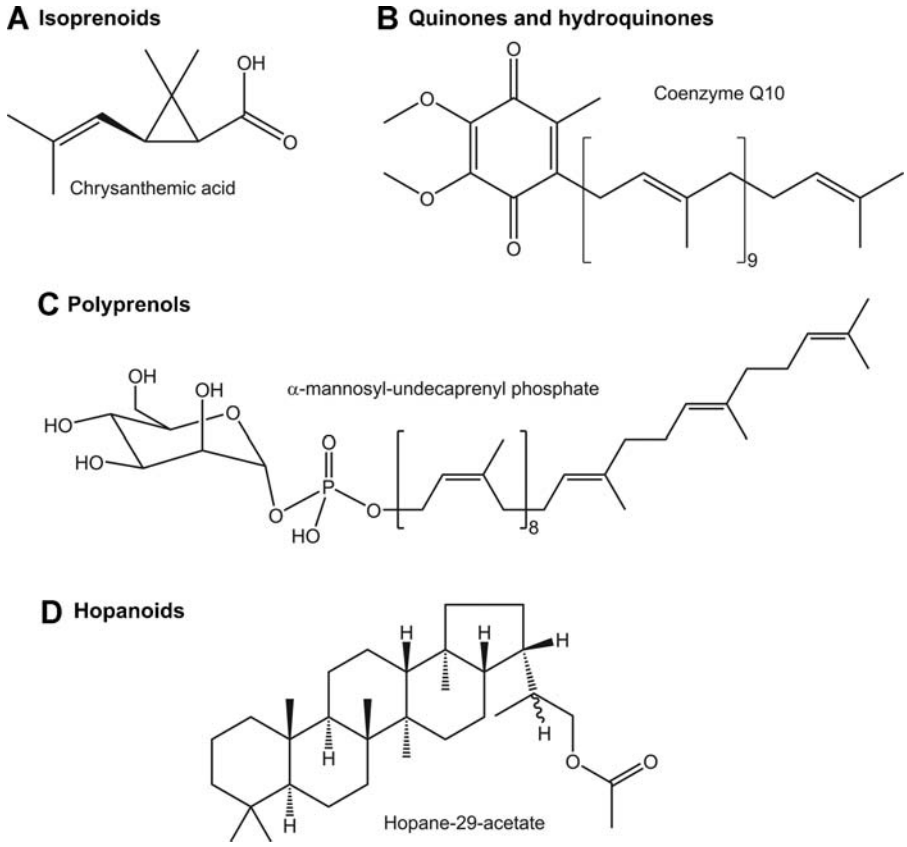


FIGURE 8. Representative structures of prenol lipids. There are five classes of prenol lipids: isoprenoids, Quinones and hydroquinones, polyprenols, hopanoids, and other prenols.

phosphorylceramide). The cerebrosides have one hexose sugar group, most commonly glucose or galactose. The esterification of a sulfur containing group to these sugar residues forms the negatively charged sulfatides. Sphingolipids with complex oligosaccharides and sialic residues form the gangliosides. These are oligoglycosylceramides containing *N*-acetyl-neuraminic acid (sialic acid) or less commonly, *N*-glycolyl-neuraminic acid, and are joined *via* glycosidic linkages to one or more of the monosaccharides or to another sialic acid residue. The sialic residues of gangliosides carry a net negative charge at neutral pH (LipidLibrary).

B. Hydrocarbon Chains

Membrane polar lipids contain unbranched fatty acyl chains with even carbon numbers (C14–C26) and *cis*–unsaturated double bonds located at specific positions. As a general rule, the position of the unsaturated double bond is determined with respect to the carboxyl functional group (Δ classification) or terminal methyl group (ω classification). The desaturation process of the fatty acyl chain involves insertion of the first double bond in the middle of a C-18 chain ($\Delta/\omega 9$). Subsequent desaturations occur sequentially in $\omega 6$ and then $\omega 3$ positions of the chain in plants and phytoplanktons (Wolf & Quinn, 2008). Rotation around the *cis*-unsaturated bonds is restricted, which produces a kink in the fatty acyl chains and impairs tight packing in the bilayer matrix. This results in reduced melting temperature of the lipid (biophysical trait) and a liquid-crystalline or fluid disordered bilayer arrangement. Thus, individual isoforms of given categories and classes will display unique properties and have different impacts on the cell.

The lipid matrix of biological membranes is fluid, with the notable exception of lipids in the purple membrane of *Halobacterium*, which adopt more or less crystal-like structures. Lipid molecules are relatively mobile in the bilayer, and constraints on this motion occur by interactions with the proteins and other membrane constituents. Lipids exhibit lateral diffusion and translational/rotational motion in the membrane bilayer, as well as internal motion within the lipid molecule. The latter involves rotational motion of the polar group and *trans-gauche* isomerisation of the hydrocarbon chains (Wolf & Quinn, 2008).

The presence of cholesterol in the cell membrane exerts a considerable ordering effect on the surrounding lipids. The depletion of cholesterol from the membrane was observed to have little effect on the orientation of the molecules in the surface region, but was associated with a marked transition to a more disordered environment in the hydrocarbon chains domain (Benninger et al., 2005). Polar lipids also exhibit spontaneous movement from one leaflet of the bilayer to the other. This movement is relatively slow because of the energy requirement to move the hydrated polar headgroup from an aqueous environment into the hydrocarbon domain in the center of the bilayer. This factor is responsible for maintaining the asymmetric

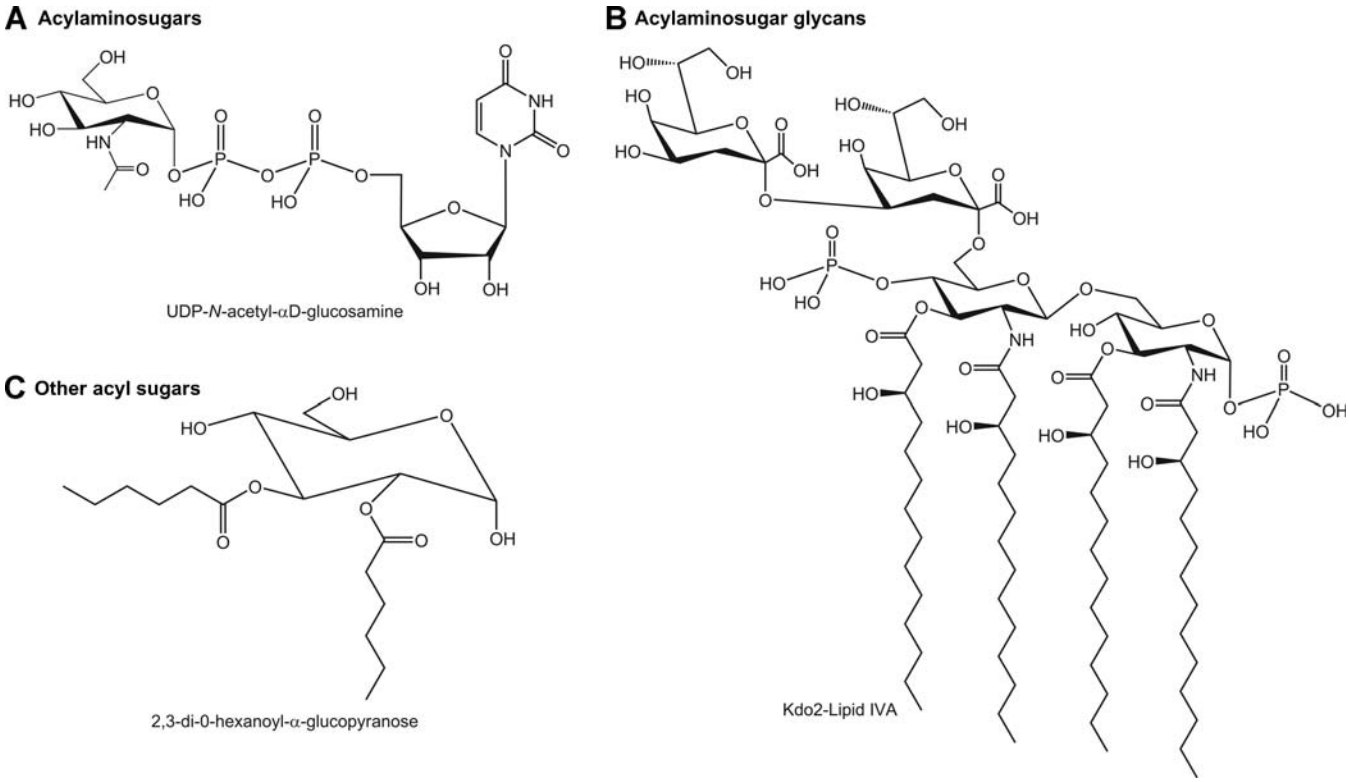


FIGURE 9. Representative structures of saccharolipids. Six classes of saccharolipids have been defined: acylaminosugars, acylaminosugar glycans, acyltrehaloses, acyltrehalose glycans, other acyl sugars, and other saccarolipids.

distribution of polar lipids across biological membranes. The creation of phospholipid asymmetry is an enzymatic, energy-requiring process and the dissipation of the asymmetry mediated by scramblase enzymes is associated with physiological processes such as apoptosis. Active bi-directional translocation of phospholipids across the inner and outer leaflet of the plasma membrane has been demonstrated for PS and PE (from the cytoplasmic leaflet to the outer surface of the membrane) and PC (from the outer leaflet to the cytoplasmic surface) (Wolf & Quinn, 2008).

IV. BIOCHEMICAL HOMEOSTASIS

Complex lipids are subject to enzyme hydrolysis and remodeling/turnover. Unfortunately, our ability to profile these changes at the molecular level and across molecular species is limited. For instance, glycerolipids and glycerophospholipids are subject to acylation-deacylation reactions. The superfamily of phospholipase A_2 (PLA_2) enzymes hydrolyzes glycerophospholipids at the *sn*-2 ester bond (Fig. 11A) (Burke & Dennis, 2009). Acyltransferases catalyze the exchange of esterified fatty acids from one lipid to another (Kerkhoff et al., 2000). The phospholipase C (PLC) family hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) to form the second messengers diacylglycerol (DAG) and inositol-1,4,5-trisphosphate ($InsP_3$) (Fig. 11A), leading to a cascade of intracellular responses that result in cell growth, cell differentiation, and gene expression. Modifications of the headgroup of phospholidpids are carried out by phospholipase D (PLD)-catalyzed transphosphatidylation. The PLD family of enzymes hydrolyses glycerophospholipids to generate PA and the respective headgroup (Fig. 11A). Sphingomyelinases catalyze the cleavage of the phosphocholine moiety from sphingomyelin to generate ceramide (Fig. 11B) (Wolf & Quinn, 2008). Lipids containing polyunsaturated fatty acyl chains are also subject to reversible or irreversible modifications as a result of oxidative stress (Leitinger, 2008; Vejux, Malvitte, & Lizard, 2008).

A detailed overview of the enzymatic and non-enzymatic modifications of lipids is beyond the scope of this review. Lipid analyses, characterization, and quantification are important to understand lipid metabolic turnover and the role of lipids in various industrial/occupational, agricultural, and drug developmental processes, and disease etiology.

V. CELLULAR FUNCTIONS OF LIPIDS

Cellular membranes consist of complex arrangements of lipids and proteins. Lipids fulfill diverse functions in the cell (Fig. 12) (Dowhan, 2009). They facilitate compartmentalization of cellular membranes and organelles by defining permeability barriers with respect to their surrounding milieu. Lipids are essential components of multi-subunit protein complexes, and they provide the matrix within which membrane proteins fold. Lipids also mediate membrane trafficking events essential for

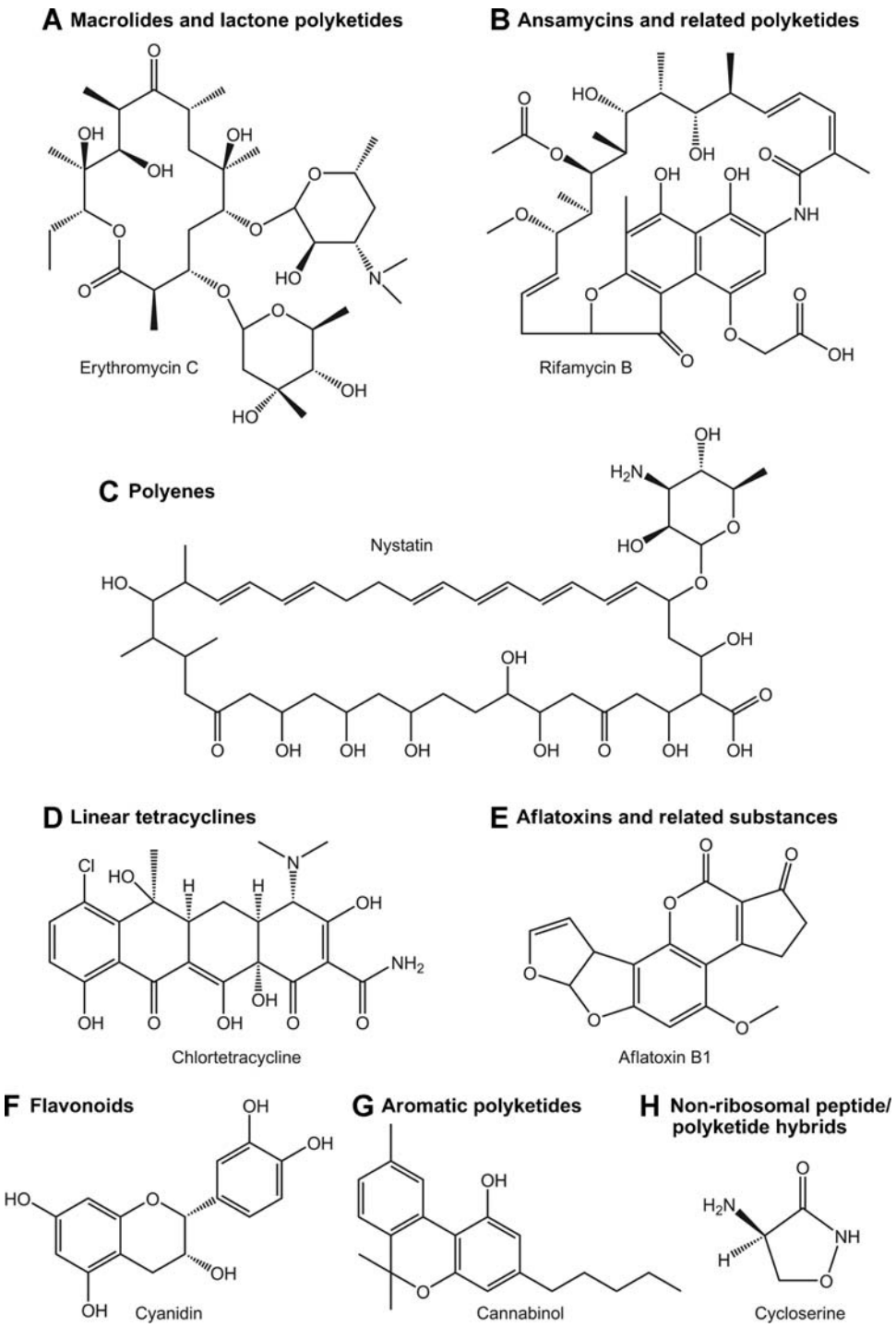


FIGURE 10. Representative structures of polyketides. Fourteen classes of polyketides have been defined: linear polyketides, halogenated acetogenins, ammonaceae acetogenins, macrolides and lactones, ansamycins and related polyketides, polyenes, linear tetracyclines, angucyclines, polyether antibiotics, aflatoxins and related substances, cytochalasins, flavonoids, aromatic polyketides, non-ribosomal peptide/ polykeytide hybrids, and other.

many key biological processes, and specific lipid molecular species are involved in signaling processes by defining membrane domains and by acting as first and second messengers. This section will focus on these vital cellular functions of lipids and will highlight several new findings.

A. Structural Integrity of Cellular Membranes

Cell membranes contain more than 1,000 different lipid species (van Meer, 2005). The multicomponent assemblage of cell membranes exhibits complex phase behavior, with regions of

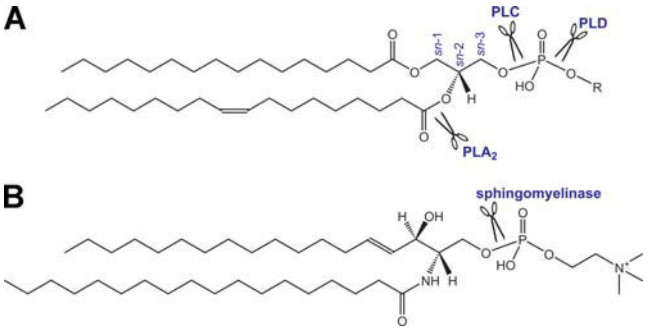


FIGURE 11. Phospholipases and sphingomyelinases cleavage sites on glycerophospholipids and sphingomyelin, respectively.

structural and compositional heterogeneity. Glycerophospholipids (i.e., PC, PE, PS, PI, and PA (Fig. 5)) represent the major structural lipid class in eukaryotic membranes. More than 50% of glycerophospholipids in most eukaryotic membranes consists of PC (van Meer, Voelker, & Feigenson, 2008). The relative abundance of 1,2-diacyl; 1-acyl, 2-alkyl; 1-alkyl, 2-acyl, and di-alkyl species that account for this 50% is not fully known, even though the name ''PC'' generally refers to the di-acyl species. PC molecular species generally have one *cis*-unsaturated fatty acyl chain which makes these species fluid at room temperature. Due to the relatively large-sized headgroup, PC is somewhat cylindrical and spontaneously forms planar bilayers (van Meer, 2005).

Mammalian cell membranes also contain sphingolipids such as sphingomyelin, glucosylceramide, galactosylceramide, and gangliosides (van Meer & Lisman, 2002). These mainly possess saturated (or *trans*-unsaturated) carbon chains, forming longer and narrower cylinders than corresponding PC species. Sphingolipids present in the membrane bilayer exhibit tight packing which is further strengthened by interfacial hydrogen bonding. The interaction of sphingolipids (e.g., sphingomyelin, gangliosides) and saturated glycerophospholipids with cholesterol (the major non-polar lipid of cell membranes) favors the formation of liquid-ordered microdomains or lipid rafts, which are segregated from the remaining more fluid membrane (Silvius, 2003; Simons & Vaz, 2004). A recent simulation modeling study assessed the molecular nature of lipid rafts at the nanoscale level. This study revealed spontaneous separation of saturated PC/unsaturated PC/cholesterol mixtures into liquid-ordered and liquid-disordered phases. Specifically, this study predicted the existence of surface tension between the monolayer leaflets that drives the registration of distinct domains (Risselada & Marrink, 2008).

The lipid composition of the plasma membrane and intracellular organelle membranes are uniquely and specifically functionally tailored (van Meer, Voelker, & Feigenson, 2008). The plasma membrane contains high amounts of sphingolipids, sterols, and saturated glycerophospholipids (Fig. 13), which pack at high densities and promote bilayer rigidity and impermeability, as well as the compartmentalization of specific proteins involved in signaling and cell–cell adhesion (see Section V.B). On the other hand, the ER membrane shows a symmetric lipid distribution and contains primarily unsaturated glycerophospholipids that make the membrane more fluid to facilitate the incorporation of newly synthesized proteins and fusogenic events (Holthuis & Levine, 2005). The involvement of the mitochondria in the synthesis of PA and PG (precursors of CL, unique to mitochondria, and PE) serves organelle-specific functions. It has been proposed that the presence of PG, the high proportion of CL in the inner mitochondrial membrane, and a high PE/PC ratio, optimize oxidative phosphorylation (van Meer, Voelker, & Feigenson, 2008).

Along these lines, a recent lipidomic study characterized the lipidome and electron transport chain activities in purified non-synaptic and synaptic mitochondria from the cerebral cortex of C57BL/6J mice (Kiebish et al., 2008). The activities of specific electron transport chain complexes were found to be lower in synaptic, compared to non-synaptic, mitochondria. Parallel ''shotgun lipidomic'' analyses revealed that the levels of CL were lower, whereas the levels of ceramide and PS were higher in synaptic mitochondria, as compared with non-synaptic mitochondria. The distribution of CL molecular species was similar in both populations and formed a unique pattern that consisted of seven major molecular species groups when arranged according to *m/z* ratios. The findings of this study imply that non-synaptic and synaptic mitochondrial lipidomic heterogeneity could influence energy metabolism, which may contribute to metabolic compartmentalization of the brain. The effects of lipid composition on mitochondrial electron transport chain activity point to a general concept of specialized lipid domains, the most well-known of which are lipid rafts. The following sections will outline the functional properties of lipid raft domains and the lipid messengers produced by the signaling-induced hydrolysis of structural lipids.

B. Signal Transduction

Regulated enzymatic processing by phospholipases (Fig. 11), as well as specific and non-specific oxidative modifications, convert structural lipids into intra- and extra-cellular first and second messenger signaling molecules of low molecular weight, or ''lipid mediators,'' with significant physiological and pathophysiological consequences. Examples of lipid mediators include eicosanoids such as prostaglandins and leukotrienes (Fig. 4) which are lipid metabolites derived from arachidonic acid, and also lysophospholipids and their metabolites which include platelet-activating factor (PAF), LPA, and sphingosine-1-phosphate (Bosetti, 2007; Shimizu, 2008). These lipid mediators can elicit physiological and pathophysiological responses independently or through specific G-protein coupled receptors. For instance, PLA_2 generates lipid mediators from structural glycerophospholipids by liberating fatty acids and producing lysophospholipids. LPC species activate specific G-protein coupled receptors to regulate the cytoskeleton and cellular Ca^{2+} homoeostasis, proliferation, survival, migration, and adhesion (Meyer zu Heringdorf & Jakobs, 2007; Shimizu, 2008). Ether LPC, also known as LPAF, is further modified by specific acyl-transferases to generate potent lipid signaling molecules (PAF). Specifically, 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (C16-PAF) is upregulated during PC12 cell differentiation (Whitehead et al., 2007). Inflammatory conditions such as stroke and various neurodegenerative diseases, in which PLA_2 activity is aberrantly increased cause LPC and PAF overproduction (Kita et al., 2006; Shimizu, 2008). PAF has long been known to be upregulated in ischemia and neurodegeneration. However, little was known about the role of

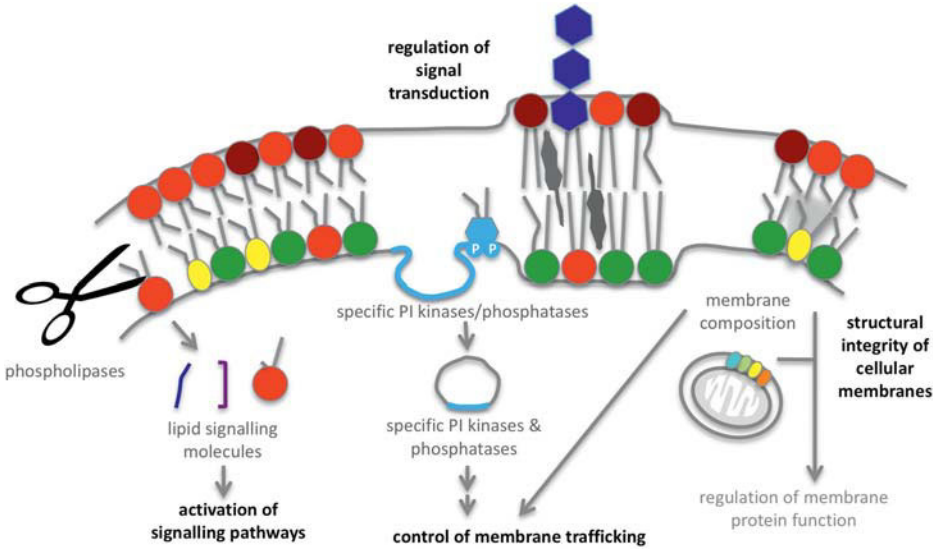


FIGURE 12. Biological functions of lipids. **A**: Compartmentalization: lipids provide a dynamic and flexible barrier from the extracellular environment, and compartmentalize biological functions within the cell in terms of organelles. **B**: Membrane trafficking: membrane lipid composition and the combinatorial PI kinase/phosphatase system of organelle-specific lipid/membrane trafficking effector protein recognition mediate membrane trafficking. **C**: Signal transduction: lipids are involved in signaling (i) by defining specific membrane domains, and (ii) by acting as first and second messengers.

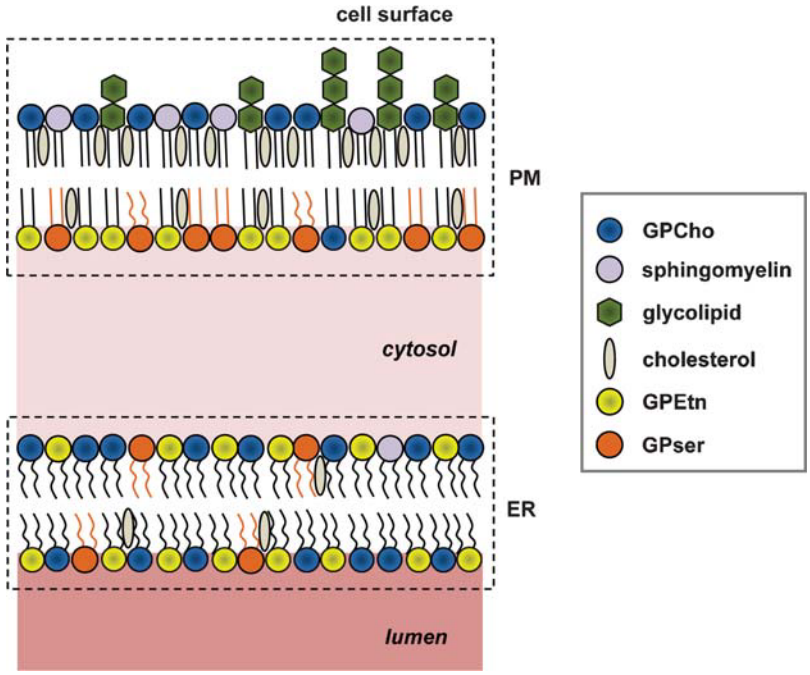


FIGURE 13. Non-random lipid composition of the plasma membrane (PM) and the endoplasmic reticulum (ER) membrane. The plasma membrane is rich in sterols, sphingolipids, and saturated glycerophospholipids. The plasma membrane has an asymmetric lipid arrangement with the aminoglycerophospholipids concentrated in the cytosolic leaflet and the sphingolipids concentrated in the exoplasmic leaflet. The membrane topology of cholesterol is not known, but its location is probably determined by its high affinity for sphingolipids and saturated glycerophospholipids (McConnell & Vrljic, 2003; Puri et al., 2003; Malathi et al., 2004). The ER membrane, on the other hand, has a symmetric lipid distribution, and contains primarily unsaturated glycerophospholipids to endow the membrane fluid characteristics to facilitate the incorporation of newly synthesized proteins and fusion related events. This figure was adapted from Holthuis and Levine (2005).

individual PAF isoforms in regulating neuronal survival until recently (Ryan et al., 2008). Both C16-PAF and C18-PAF cause PAF receptor-independent death through distinct signaling pathways. C16-PAF activates caspase-7, whereas C18-PAF triggers caspase-independent death in PAF receptor-deficient neurons. Interestingly, PAF receptor signaling is either pro- or anti-apoptotic, depending upon the identity of the *sn*-1 fatty acid of the PAF ligand. The activation of the PAF receptor by C16-PAF is anti-apoptotic, and it inhibits caspase-dependent death. The activation of the PAF receptor by C18-PAF, however, is pro-apoptotic. This PAF isoform-specific regulation of the PAF receptor dictates PAF signaling and cellular fate (apoptosis, neurodegeneration, or neuroprotection).

A large body of evidence implicates lipid rafts in cell signaling (Fig. 12) (Mishra & Joshi, 2007). Lipid rafts serve as concentration platforms of lipids (Iwabuchi, Handa, & Hakomori, 2000; Marmor & Julius, 2001; Nagafuku et al., 2003) and proteins (Moffett, Brown, & Linder, 2000; Galbiati, Razani, & Lisanti, 2001; Harris & Siu, 2002; Foster, De Hoog, & Mann, 2003) that are involved in cell adhesion and signaling. These molecules include monosialogangliosides GM_1 and GM_3, PIP_2, doubly acylated tyrosine kinases of the Src family, small GTP binding proteins, and regulatory subunits of heterotrimeric G proteins. Lipid rafts also harbor glycosylphosphatidylinositol (GPI)-anchored proteins and transmembrane proteins such as G-protein coupled receptors, whose activity is controlled by their localization to lipid rafts (Bari et al., 2005; Patel, Murray, & Insel, 2008; Zheng et al., 2008). Mo and co-workers demonstrated that GM_1 is involved in the phosphorylation and activation of the extracellular-regulated protein kinases (ERKs) in the striatum, hippocampus, and frontal cortex of male rats *in situ* or *in vivo* (Mo et al., 2005) A recent study suggests that modulation of GM_1 levels in lipid rafts regulates membrane partitioning of the platelet-derived growth factor receptor (PDGFR) and mitogenic signaling *via* the Src family of protein tyrosine kinases (Veracini et al., 2008).

The implication of intact subsets of lipid rafts and their lipid components in nerve growth factor signaling is also documented (Dobrowsky, 2000). Ceramide generation in lipid rafts is instrumental for Fas signaling and for the induction of apoptosis in B- and T-lymphocytes (Kirschnek et al., 2000). Specifically, Fas signaling triggers the translocation of acid sphingomyelinase to lipid rafts. The downstream accumulation of ceramide induces coalescence of raft units into large platforms in which oligomerisation of downstream effectors (i.e., FADD/MORT-1 and pro-caspase-8) occurs, thus leading to Fas apoptotic signals (Cremesti, Goni, & Kolesnick, 2002). In primary afferent neurons, neuronal excitability and synaptic transmission is modulated following the binding of endogenous cannabinoids, that is, *N*-arachidonoyl ethanolamine and 2-arachidonoyl glycerol, to the cannabinoid receptors CB1 and CB2. The synthesis of 2- arachidonoyl glycerol *via* the DAG pathway occurs within lipid rafts. Interestingly DAG, often perceived to act as a single lipid second messenger, is in fact, a family of over 50 isoforms (Callender et al., 2007). Evidence indicates that each isoform controls distinct cellular processes (Deacon et al., 2002). Moreover, the segregation of *N*-arachidonoyl ethanolamine and 2-arachidonoyl glycerol in lipid rafts along with the cannabinoid receptor CB1 suggests that intrinsic-autocrine signaling and/or retrograde-paracrine signaling occur within lipid rafts (Rimmerman et al., 2008).

C. Regulation of Membrane Trafficking

Lipids function in concert with specific proteins to regulate membrane trafficking events within the cell. The phosphoinositides (PI) control cell motility and membrane trafficking by regulating the recruitment, assembly and disassembly of specific protein complexes (Lemmon, 2008; Vicinanza et al., 2008). These complexes are targeted to sites of action by binding to specific PI species. These species are spatially and temporally regulated through various PI kinases and phosphatases localized to specific intracellular compartments. PI–protein interactions are characterized by relatively low binding affinities, and they require auxiliary stabilizing binding sites to recruit either membrane-resident proteins or specific-organelle-associated small GTPases. This combinatorial nature of the PI system enhances organelle recognition specificity. The accumulation of PIP_2 in lipid rafts has been proposed as the mechanism underlying its involvement in signaling to the actin cytoskeleton and in vesicle trafficking (Caroni, 2001). Specifically, PIP_2-dependent lipid rafts capture microtubules to promote and control cell motility, polarity, and organization (Golub & Caroni, 2005).

Several enzymes involved in glycerophospholipid metabolism have long been implicated in membrane trafficking, including PLA_2, PLC, and PLD (Darios, Connell, & Davletov, 2007). PLA_2 activity, which mediates the release of long chain polyunsaturated fatty acids and LPC, has been shown to play a role in vesicle fusion in many biological systems (Freeman, Terrian, & Dorman, 1990; Nagao et al., 1995; Almeida, Cunha, & Ribeiro, 1999; Juhl et al., 2003; Staneva, Angelova, & Koumanov, 2004). Vesicle fusion is known to be directly modified by fatty acids. For example, palmitoylation of the SNARE proteins SNAP-23 and SNAP-25 stimulates their accumulation in membrane domains and membrane fusion (Washbourne et al., 2001; Koticha, McCarthy, & Baldini, 2002; Salaun, Gould, & Chamberlain, 2005). Recent studies have also highlighted an important role for ω6 and ω3 polyunsaturated fatty acids in activating one of SNAP-25's SNARE partners, syntaxin (Darios & Davletov, 2006; Connell et al., 2007). Important insights into the role of LPC have been gained from studies on snake presynaptic PLA_2 neurotoxins (SPANs), demonstrating that LPC induces exocytosis of neurotransmitters and subsequent paralysis.

Recent mass spectrometry studies have shown that SPAN activity in cerebellar neurons generates myristoyl LPC and oleic acid as the major hydrolytic products (Rigoni et al., 2005). The treatment of cerebellar neurons with a mixture of myristoyl LPC and oleic acid or with myristoyl LPC on its own replicates SPAN-induced exocytosis. Although the mechanism by which myristoyl LPC stimulates exocytosis remains elusive, the authors proposed that the preferential localization of LPC to the external presynaptic leaflet (Kalin et al., 2004) and its inverted cone shape could alter membrane curvature to enhance exocytosis. Thereby, it paralyzes the neuromuscular junction. Curvature is an important factor for membrane fusion because a high ''curvature strain'' can provide the driving force required to overcome the energetic barrier of fusion. Thus, the variation of lipid composition partly dictates the ability of membranes to undergo fusion. Plasma membranes in fusogenic contexts (synaptic vesicles, synaptic membranes, viral membranes) have a ratio of PC to PE headgroups measured

between 0.9 and 2.0 (Breckenridge et al., 1973; Aloia, Tian, & Jensen, 1993). PE (Emoto & Umeda, 2000), cholesterol (Marsh, 2006; Churchward et al., 2008), and DAG (Churchward et al., 2008) have been identified as critical factors for fusion. Specifically, the ability of PE to induce membrane fusion events may be governed by its headgroup which promotes negative membrane curvature (Haque, McIntosh, & Lentz, 2001).

In summary, lipids play highly specialized roles in cells. New technological advances in mass spectrometry are being used to characterize the specific lipids involved in each of the processes described above. Moreover, these new technologies will allow assessment of dynamic physiological and pathophysiological lipid changes, providing further insight into lipid control in cellular biology in health and disease.

VI. SAMPLE PREPARATION

Elucidation of the biochemical and biophysical properties of membrane lipids and their cellular functions is highly dependent upon the preparation of high purity, morphologically distinct membranes or subcellular membrane fractions. To achieve optimal separation the homogenization steps must break down cells into their subcellular organelles while preserving the integrity of vesicular organelles that encapsulate hydrolases and other enzymes capable of altering the composition of lipids. In addition, sample preparation should be quick and performed at low temperatures in the presence of antioxidants and inhibitors of hydrolytic enzymes and proteases/phosphatases.

A. Homogenization

The homogenization step is highly influenced by the operational criteria which differ among tissues. This step must be optimized in such a way as to result in a high relative yield of specific organelles with intact composition and functional integrity. The method of choice is influenced by the presence of tough extracellular matrices or cell walls, in which case pretreatment with collagenase or cellulase may be required. It is also crucial to employ homogenization buffers that mimic the *in vivo* pH and solute composition of the membranes.

A variety of homogenization methods has been employed based on mechanical disruption (Potter-Elvehjem, rotating blade devices such as Waring blendor and Ultraturax, glass beads) or other shear forces (pressure/cavitation, freeze-thaw). Homogenization may result in artefactual reshuffling of membrane subfractions/components or rupturing of organelles, and chimerical fusion of naturally separated membrane domains. It is thought that mixing can only be partially circumvented. However, application of ion beam- or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry imaging (Jackson, Wang, & Woods, 2005a; Schiller et al., 2007) may allow more precise determination of the excessive mechanical stress on tissues and whether cells produce artefactual membrane subfractions. Homogenization using a loosely fitted Potter homogenizer results in the formation of clumps which represent associations of different organelle membranes. Using buffers of high ionic strength and/or chelating agents like EDTA will reduce the propensity for clumping. Such buffers most likely cause desorption of peripheral membrane proteins. Moreover, the ionic strength and divalent cations present in buffers have a profound influence on lipid composition of detergent resistant membrane (DRM) domains prepared from lipid-rich tissues such as rat brain (Chen et al., 2007). Divalent cations appear to participate in the creation of a delicate scaffold network of lipids held together by binding and bridging polar headgroups. Together, this combination modulates the activity of the detergent and prevents the micellization of the cytosolic membrane leaflet. Furthermore, the temperature at which the detergent treatment is performed changes the compactness of the lipid phase, thereby influencing the penetration of the detergent into the hydrophobic bilayer core. Various detergents with distinct hydrophilic/hydrophobic balance also produce different effects that cannot readily be predicted from standard physicochemical parameters. For example, membrane treatment with detergents of different critical micellar concentrations (i.e., Triton X-100 and Brij98) produces DRM fractions that differ in lipid composition in the inner and outer leaflets of the original membrane (Pike, Han, & Gross, 2005).

B. Membrane Fractionation

Density gradient sequential centrifugation steps are routinely employed to isolate membrane fractions with distinct densities and lipid/protein compositions. Purity and authenticity of these membrane fractions is usually assessed based on their enrichment in different biological markers using immunoblotting and enzymatic assays. Marker enzymes are intrinsic membrane proteins (with the exception of enzymes located within lysosomes and peroxysomes), and they can be conveniently assayed. Lipidomics can also be helpful in characterization of membrane fractions, for example, to establish the cholesterol/sphingomyelin ratio in lipid raft domains (Koumanov, Wolf, & Quinn, 2004; Koumanov et al., 2005).

Subcellular fractionation can also be achieved by affinity chromatographic and polymer phase separation methods. In the latter polyethylene glycol and dextran, two structurally different polymers, are mixed in aqueous media. As a result, two distinct phases are formed with an upper layer enriched in polyethylene glycol and a lower layer enriched in dextran (Persson & Jergil, 1995). Membranes of different subcellular origin partition between the two phases based upon their surface charge or hydrophilic character. Polymer partition has been successfully employed for the separation of plasma membranes from rat liver (Cao et al., 2006) and crude rat brain microsomal membrane preparations (Schindler et al., 2006). In addition this two-phase partitioning method, in conjunction with affinity to agarose beads coated with nickel-nitrilotriacetic acid, proved successful in the isolation of *Escherichia coli* inner membranes (Everberg et al., 2006).

Membrane fractions can also be purified *via* immunoaffinity purification using antibodies specific for markers enriched in these fractions. This method proved successful for the purification of peroxisomes (Luers et al., 1998; Kikuchi et al., 2004), tubulovesicles (Calhoun & Goldenring, 1997), Golgi apparatus membranes (Henley & McNiven, 1996), microsomal membranes (Srinivasan et al., 2001), plasma membranes (Morciano et al., 2005; Lawson et al., 2006), and brain DRM fractions (Madore et al., 1999). Immunoaffinity purification has been successfully used in tandem with density gradient centrifugation for the

purification of plasma membranes from mouse liver (Zhang et al., 2007).

C. Lipid Extraction

Ideally a lipid extraction procedure should quantitatively extract cellular lipids without degradation and contamination with non-lipid components such as free sugars and amino acids. The effectiveness of the lipid extraction procedure will, to a large extent, depend on the chemical nature of the lipid components and the kind of complexes or associations in which they occur in the cell. Lipids in hydrophobically associated forms may be extracted with relatively non-polar solvents such as ethyl ether, chloroform or benzene. Membrane associated lipids require polar solvents such as ethanol or methanol to disrupt the hydrogen bonding networks or electrostatic forces between lipids and proteins. Covalently bound lipids cannot be extracted directly by any solvents, and must first be cleaved from the complex by acid or alkaline hydrolysis. This criterion is particularly useful when studying protein–lipid interactions as lipids covalently bound with proteins can be purified by protein immunoprecipitation and subsequently identified by mass spectrometry (Wislet-Gendebien et al., 2008). Therefore the chemical nature of lipids must be taken into consideration during lipid extraction.

In general, to avoid oxidation of double bonds all solvent destined for lipid extraction should be freshly distilled and peroxide free. For highly unsaturated lipids, solvents should be de-aerated by bubbling nitrogen through them and the extraction and subsequent operation steps should be carried out under nitrogen. It is desirable to not bring the lipid extract to complete dryness nor to leave it in this state for lengthy periods. Lipid extraction should be performed at room temperature (or below if necessary) to prevent/reduce lipid peroxidation and hydrolysis. To improve lipid recovery, extraction must be performed under experimental conditions that avoid rapid protein denaturation. The clumping of denaturated proteins into aggregates affects the separation of lipids from proteins and hence the extraction of lipids into non-polar solvents.

Lipids are typically extracted into glass tubes. It is therefore essential that all lipid extraction glassware is silanized to avoid the lipids sticking to glass, especially when working with polar lipids such as the phosphoinositides. A comprehensive description of lipid extraction protocols and a comparison of solvents used for lipid extraction are outlined on the Cyberlipid website (www.cyberlipid.org). Briefly, an aqueous suspension of biological material is mixed with a polar solvent such as methanol and non-polar solvents such as chloroform to allow the formation of a one-phase system. In this environment proteins form a fine precipitate that does not hinder lipid extraction. Lipidomics studies by mass spectrometry-based approaches may be conducted directly from this solvent mixture. However, it may be useful to adopt a subsequent step involving generation of a two immiscible-phase system (Folch, Lees, & Sloane Stanley, 1957; Bligh & Dyer, 1959) following the addition of aqueous buffer. This step helps to separate water soluble constituents (proteins, sugar, and salts) that act as ESI suppressors (Section VIII.A.5) into an upper aqueous phase separated from the lipid-containing water-insoluble lower phase. Proteins accumulate at the aqueous/chloroform interface. Ionic strength and acidic buffer pH favor the hydrolysis of anionic lipids (e.g., phosphoinositides and gangliosides). These lipids tend to partition into the aqueous-alcoholic layer at pH greater than 4. Excessive acidification results in the cleavage of vinylether bonds of plasmalogens, and this effect can be exploited to distinguish between akyl ether and vinyl ether isoforms.

In tissues where both acid-resistant and plasmenyl lipids are to be examined, two separate extracts should be prepared taking into consideration the preceding factors. If a biological sample is enriched in proteins or amphiphiles, the separation of the upper/lower phases may prove difficult. Thus, protocols derived from the Folch procedure (Folch, Lees, & Sloane Stanley, 1957) have been developed to allow an efficient separation of layers in a timely manner. These protocols may include centrifugation, cooling, alteration of chloroform/methanol ratio, or addition of saline buffers. When the lower chloroform layer is turbid, barium oxide or anhydrous sodium sulfate is added to the extract to remove water. However, contamination by water jeopardizes the integrity of lipid extracts. Specifically, a residue of acidic water remains in the lipid extract following solvent evaporation, and this results in the hydrolysis of vinyl ether bonds that again can be exploited through parallel extracts to distinguish between PC subclasses. Lipid oxidation also occurs at the air interface because evaporation of water is slow.

An automated robot with solid phase extraction protocols has been developed for the analysis of lipid classes (Ruiz-Gutierrez & Perez-Camino, 2000). Solid phase extraction reduces the volume of solvent required and lipid oxidation, and allows simultaneous treatment of numerous lipid samples. Stationary phases are designed for quantitative extraction of phospholipids on reverse phase, or separation of neutral lipids/phospholipids on normal phase (Persson et al., 2007).

D. Sample Storage

Sample storage is of paramount importance to protect lipids from chemical alterations. In living organisms intrinsic protective mechanisms and associations of lipids with natural compounds protect double bonds of vinyl ether and polyunsaturated fatty acids against attack by reactive oxygen species. The lipid isolation and extraction procedure eliminates these natural lines of defense. As a result, the addition of antioxidants, for example, butylated hydroxy-toluene (BHT, 2,6-di-tert-butyl-p-cresol) and/or tocopherol, to the lipid extracts is required. These radical scavengers are soluble in solvents of low polarity and are easily added to the lipid extract. Moreover, their oxidation can be used as a potential indicator of damage to the biological lipids. In general, antioxidants must be added to the lipid extracts with caution and at concentrations below 0.00001 (w/w) since BHT is known to act as pro-oxidant at high concentrations (Christie, 1989). Potential for oxidation also increases following sample contamination with heavy metals such as copper and/or iron which catalyse the production of free radicals in Fenton-type reactions. To circumvent such contamination, single-use vessels with polytetrafluoroethylene (PTFE) or Teflon fittings can be employed. In fact, it is recommended to utilize Teflon fittings for all pre-analytical steps in lipidomics reasearch. In addition, the exposure of lipid extracts to UV light can be particularly deleterious. Elevated storage temperatures also favor double-bond migration and diene conjugation, thus resulting in extensive peroxidation especially in concentrated solutions.

Lipid extracts are ideally stored at low temperatures ($\leq 20^{\circ}C$) in solvent-diluted solutions contained in opaque vessels

purged with nitrogen gas and sealed by PTFE caps and tape. Polyunsaturated glycerophospholipids remain unchanged for up to 6 months upon storage under these conditions. Sphingolipids are expected to be stable for much longer periods of time since they are generally more saturated. Sterols, steroids, and bile acids are also innately resistant to chemical alterations (Sobolik et al., 1996; Lin & Connor, 2001). In contrast, dehydrocholesterol and ergosterol, which contain double bonds in the B-ring, are less stable. They must be analyzed as soon as possible after isolation, and handled under low illumination and inert atmosphere. Evaporation of the solvent must be performed prior to analysis under a stream of nitrogen at temperatures not exceeding 40°C or using a SpeedVac.

VII. NON-MASS SPECTROMETRY BASED EXPERIMENTAL APPROACHES IN LIPIDOMICS RESEARCH

The separation and analysis of lipids have been traditionally carried out on gels (Vitic, Nikolic, & Nikolic, 1981) or paper (Curt Boeckel, 1962) in one- or two-dimensional electrophoresis. Other common lipid analyses methods include thin-layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry-based techniques (Otieno & Mwongela, 2008; Wenk, 2005). Specifically, metabolic labeling of lipids in tissues and cells using radioactive precursors (e.g., ^{3}H-, ^{14}C-, and ^{32}P-labeled carbon sources or phospholipid headgroups) and fluorescent precursors (e.g., *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl), NBD) has been employed to quantify lipids at the mass level if the label is exposed to the cell/tissue long enough to ensure steady-state incorporation (under the assumption of equilibration into all metabolic pools). For low abundance lipids such labeling regimes are coupled to chromatographic separation techniques for quantification. The following sections summarize the non-mass spectrometry based experimental approaches employed in lipidomics, emphasizing their shortcomings and advantages.

A. Thin Layer Chromatography

Thin layer chromatography (TLC) (Geiss, 1987; White et al., 1998) has been routinely used for lipid analyses. This method does not require sophisticated instrumentation and allows rapid screening of lipid extracts. The solvent systems employed in TLC are very well established for most lipid classes. Lipids are detected by iodine vapor and class specific dyes/radioactivity (Wenk, 2005). However, TLC based experiments are time consuming and lack resolution power and specificity. This generally limits the resolution power of TLC to the main classes of lipids present in a biological sample. Other problems associated with TLC that limit lipidomics applications are prolonged exposure of the sample to air, which can lead to sample degradation particularly if acids or bases are used in the developing phase, and the destructive charring of the plate for lipid identification prevents further analysis of the lipids.

B. Gas Chromatography

With the advent of GC the separation of lipids by TLC followed by their hydrolysis and derivatization made possible the identification of individual fatty acid molecular species (Gutnikov, 1995), TAG and sterols (Wenk, 2005). Detection methods generally include mass spectrometry. However, GC methods are very sensitive to compound polarity, and the need for multiple derivatization steps to improve volatility renders lipid analyses problematic. Sample recovery can also pose an issue during the derivatization procedures since low abundance lipid species may not be detected. GC based methods are time consuming (especially for long-chain fatty acids), and a major drawback of these methods is the large amount of lipids required for the derivatization. Although GC is sensitive, the analysis is conducted at high temperatures that may result in analyte isomerization and/or decomposition. Also, the GC-derived analyses yield information on the hydrolysis products of lipids and not on the parent compounds.

C. High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) has been applied for ascertaining the fatty acid profiles of lipid mixtures using ultraviolet, fluorescence, flame ionization, refractive index or mass spectrometric detection methods. HPLC is versatile for the separation of glycerophospholipids, sterols, neutral lipids (TAG and DAG), fatty acids, and lipid headgroup derivatives (Wenk, 2005). HPLC is an easily automated method with quantification potential, and both reverse- and normal-phase conditions have been developed and are available for lipid analyses. Normal-phase HPLC generally separates glycerophospholipids based on the polarity of their headgroups, whereas reverse-phase HPLC separates phospholipids based on the hydrophobicity of their fatty acyl chains. In reverse-phase HPLC separation the order of elution in a class of lipid is related to the length of the fatty acyl chain. Lipids containing shorter fatty acyl chains elute faster than those with longer ones. Furthermore, the higher the degree of unsaturation, the faster the lipid elutes (Houjou et al., 2005). Mobile phase gradients are also easily established in HPLC, thus allowing the collection of different lipid fractions for further examination.

Caution should be exercised during HPLC analyses at temperatures lower than 10°C since this may lead to the precipitation of lipids insoluble in the solvent system used, resulting in the splitting of elution peaks into micellar and monomeric compounds. In addition, the application of lipids in chloroform/methanol mixtures (1:1 or 2:1, v/v) on the column can interfere with retention on the silica gel of the stationary phase, and the metal tubing of the equipment may deteriorate due to exposure to HCl formed from chloroform. Recent studies have employed HPLC coupled to evaporative light-scattering to analyze and quantify neutral and acidic lipids (e.g., monogalactosyldiacylglycerol, digalactosyldiacylglycerol, and sulfoquinovosyldiacylglycerol) of photosynthetic organisms (Yunoki et al., 2009). HPLC has been also used in conjunction with secondary chromatographic systems and mass spectrometers for enhanced lipid detection and analysis performance (Altria, 2000; Vissers et al., 1996). Over the years, interest in microcolumns has been pursued. This approach has allowed working with small sample sizes and low volumetric flow rates (Vissers, 1999). However, a limitation of using microcolumns is the lower sample capacity, which is proportional to the surface area of the stationary phase.

D. Capillary Electrophoresis

The most common capillary electrophoresis modes used for lipid analyses include capillary zone electrophoresis, capillary electrochromatography, electrokinetic chromatography or micellar electrokinetic chromatography, microemulsion electrokinetic chromatography, and microchip capillary electrophoresis. Capillary electrophoresis coupled to indirect UV detection is preferable over direct UV detection which suffers from limited sensitivity. However, indirect ultraviolet absorbance detection also suffers from a moderate dynamic range and detection limits in the micromolar range. Capillary zone electrophoresis has been used to characterize saturated and unsaturated fatty acids, gangliosides, and CL (Otieno & Mwongela, 2008). Capillary electrophoresis has been employed in conjunction with mass spectrometry for the characterization of bacterial lipopolysaccharides (Li & Richards, 2007). Capillary electrochromatography has been utilized for the separation of triglycerides (Dermaux & Sandra, 1999), sterols and sterol derivatives (Abidi, 2004) in vegetable oils, and for the separation of retinyl esters (lipid vitamins) (Roed, Lundanes, & Greibrokk, 1999, 2000). Microchip capillary electrophoresis has been employed for the separation of PI, PIP, and PIP_2 (Lin et al., 2003). Difficulties encountered with the use of capillary electrophoresis for lipid separation include lipid aggregation especially at concentrations above their critical micellar concentrations, as well as reduced separation selectivity between successive homologs. Moreover, most capillary electrophoresis methods use aqueous electrolyte separation media, which limits determination of lipids due to their poor solubility in aqueous buffers. Although the presence of unsaturated groups such as carbonyl, carboxyl and phosphate allows the ultraviolet detection of glycerophospholipids at 200–214 nm, many lipids are difficult to detect due to the lack of conjugated double bonds and unreactive aliphatic function groups in their structures. Additionally, solvents, buffer additives and various analytes absorb in the region of 190–220 nm (Otieno & Mwongela, 2008). The coupling of capillary electrophoresis to mass spectrometry may provide a useful means for the rapid identification of low-abundance lipids, and for the structural characterization of complex lipids (Li & Richards, 2007; Gaspar et al., 2008). However, the difficulty of establishing a universally optimized buffer system that would allow sufficient solubilization of the entire cross-section of lipids seems to be insurmountable at present.

E. Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) has also been commonly used for the structural analyses and quantification of lipid species. For example, proton NMR was utilized to analyze lipid profiles of human erythrocytes (Adosraku et al., 1994). High resolution ^{31}P-NMR spectroscopy was also employed to determine the phospholipid composition of tissues and body fluids (Schiller & Arnold, 2002). Two-dimensional ^{1}H–^{13}C heteronuclear single quantum coherence NMR (2D HSQC NMR) was recently reported for the global profiling of mycobacteria lipids (Mahrous, Lee, & Lee, 2008).

Both ^{1}H and ^{31}P NMR can directly analyze lipids in a non-destructive manner. However, NMR suffers from low sensitivity which ultimately requires very long NMR signal accumulation times. In addition, NMR spectra are dominated by very abundant lipids such as phosphocholine and cholesterol (Wenk, 2005), which compromises the ability of this technique to resolve low-abundance lipids.

F. Biochemical Methods

Many lipids in pure form or mixtures have been analyzed by biochemical assays such as monolayer adsorption, Langmuir–Blodgett films, immobilized lipid biosensors and lipid blots/beads (Wenk, 2005). These approaches are sensitive and permit the determination of the identity of potential lipid interaction partners. The functional immobilization of lipids, however, complicates the technical feasibility, and automation and throughput are generally limited. Optical, calorimetric, and radiometric approaches using liposomes or micelles have been used for binding studies and enzymatic assays. Lipid antibodies and reactive lipids have been employed in cell biology studies to elucidate the subcellular localization of lipids and to identify lipid-binding proteins, respectively. However, availability and specificity of lipid antibodies and reactive probes pose technical challenges yet to be resolved.

VIII. MASS SPECTROMETRY IN LIPIDOMICS RESEARCH

The analysis of lipids poses a constant challenge owing to their large number and structural diversity. It was estimated that the theoretical number of distinct lipid isoforms is close to 200,000 (Oresic, Hanninen, & Vidal-Puig, 2008; Yetukuri et al., 2008). Although it is not yet possible to analyze all these lipids at once, new mass spectrometry-based technological innovations are expanding the number and types of lipids that can be analyzed (Han & Gross, 2003; Pulfer & Murphy, 2003; Guan & Wenk, 2006; Wolf & Quinn, 2008; Gross & Han, 2009). Mass spectrometry offers high sensitivity and resolution for the characterization of global lipid profiles in a given cell or organism. A schematic diagram of an overall lipidomics approach is presented in Figure 14.

Modern mass spectrometry methods include ionization by electrospray (ESI), fast atom bombardment (FAB), atmospheric pressure chemical-ionization (APCI), atmospheric pressure photo-ionization (APPI), MALDI, and tandem mass spectrometry (MS/MS or MS^n). Choosing a particular mass spectrometry method strongly depends on the lipid class to be analyzed. It is generally accepted that ESI is best suited for the analysis of phospholipids (Han et al., 2006b; Brugger et al., 1997), whereas APCI works best for relatively non-polar compounds like TAG and wax esters. However, APCI has been equally successful in detecting various glycerophospholipids in HPLC-MS experiments with human meibomian lipids (Butovich et al., 2007; Butovich, Uchiyama, & McCulley, 2007). The following sections will introduce the scanning and ionization modes, as well as the different types of mass spectrometers employed in lipidomics research.

A. Ionization Modes

Mass spectrometry is an analytical technique that separates charged analyte ions in the gas-phase based on their mass-to-charge ratios (m/z values). Creating these gas phase analytes is the role of the ionization method. The ionization methods

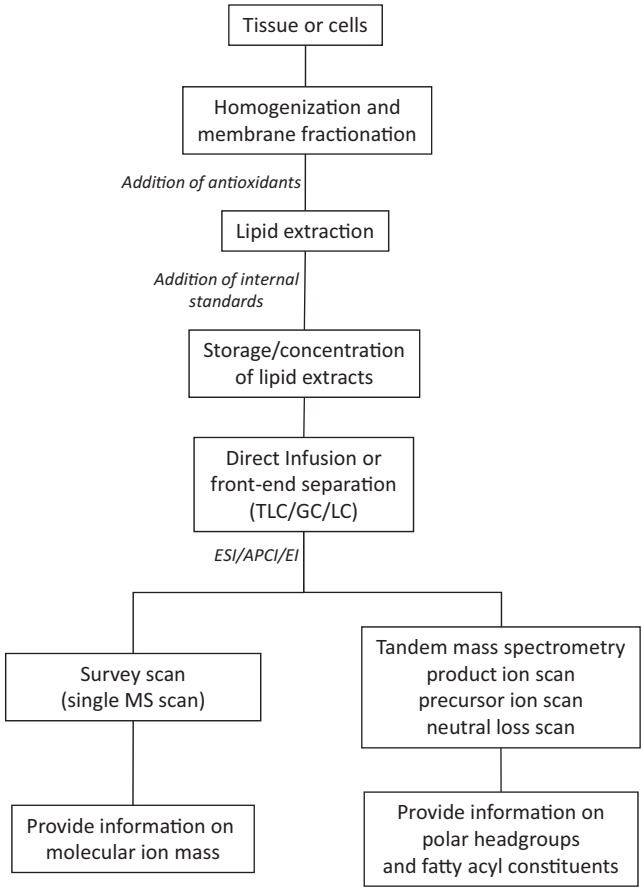


FIGURE 14. A schematic diagram of the overall lipidomics approach.

commonly used in lipidomics research are summarized in the following sections.

1. Electron Ionization

Electron ionization (EI), the most popular ionization method for organic molecules when applicable, was first described by Dempster (1918). Conventionally, the sample molecules vaporized in the gas phase are bombarded with a beam of energetic electrons (~70 eV) at low pressure (10^{-5} to 10^{-6} torr) (Dass, 2007b). During this process positive ions are formed by the ejection of an electron from the target molecules. EI is simple to use, and compatible with volatile lipid molecules. However, the non-volatile lipid molecules have to be converted to methyl, trimethylsilyl, or t-butyldimethylsilyl (TBDMS) derivatives prior to EI-MS analysis, which results in additional sample-handling steps and yields side products from incomplete derivatizations. Furthermore, the lipid molecules are not stable under high-energy electron-beam bombardment, resulting in ions produced by extensive fragmentation and double bond migration (Kawai, Takeda, & Terao, 2007; Minami et al., 2007; Oursel et al., 2007; Bicalho et al., 2008; Long et al., 2008).

2. Chemical Ionization and Atmospheric Pressure Chemical Ionization

Chemical ionization (CI) is a less energetic ionization process than electron ionization. As usually practiced it is based upon gas-phase acid-base reactions between the sample molecules and ions produced by EI of a reagent gas (Munson & Field, 1966). In the ion source the reagent gas (such as methane, ammonia, and isobutane) is present in large excess compared to the sample molecules. CI involves the following three steps: (i) the high-energy electron beam preferentially ionizes the reagent gas; (ii) the reagent gas ions (e.g., $CH_4^{+\bullet}$) initially formed interact with other reagent gas molecules, and produce the more stable reagent ion (e.g., CH_5^+ and $C_2H_5^+$) *via* ion-molecule reactions; (iii) the sample ions are formed by ion-molecule reactions (e.g., proton transfer) between the sample molecules and the stable reagent ions (Dass, 2007b).

Atmospheric pressure chemical ionization (APCI) is a type of chemical ionization performed at atmospheric pressure, and can be used for the analysis of liquid chromatography effluents. The LC effluents containing analytes are simultaneously heated to relatively high temperatures (the mobile phase solvent absorbs most of the heat) and atomized (sprayed) with high flow rates of nitrogen, creating an aerosol of fine droplets that readily evaporate. This mixture of gas-phase analytes, solvent and atomizing gas is then subjected to a corona discharge created by raising a sharp metal needle to several thousand volts. The primary ions (e.g., N_2^+) are produced by EI resulting from the electrons from the corona discharge and the excess atomizing gas. These primary ions react with solvent molecules (such as H_2O, methanol and acetonitrile) in a complex sequence to ultimately form the solvated ions of highest proton affinity (e.g., $H_3O^+(H_2O)_n$). Subsequently the analyte ions are formed through gas-phase ion-molecule reactions between the analyte molecules and these solvated ions (Dass, 2007b). APCI-MS is used for qualitative and/or quantitative analyses of complex mixtures of many lipid classes, including fatty acyls, TAG, phospholipids, ceramides, carotenoids, and steroids (Byrdwell, 2001, 2005). CI and APCI-MS are also used for locating double bonds in unsaturated lipids (Moneti et al., 1996; Van Pelt & Brenna, 1999; Lawrence & Brenna, 2006; Xu & Brenna, 2007).

3. Atmospheric Pressure Photo-Ionization

Atmospheric pressure photo-ionization (APPI) is a relatively new technique suitable for the analysis of PC (Delobel, Touboul, & Laprevote, 2005), fatty acyls and glycerololipids (Cai & Syage, 2006a), sphingolipids (Delobel et al., 2006; Roy et al., 2006; Munoz-Garcia et al., 2008) and oils (Gomez-Ariza, Arias-BorregoA, & Garcia-Barrera, 2006; Lerma-Garcia et al., 2008). In this approach, which is a modification of APCI, the liquid effluent is vaporized in a heated nebulizer to generate gas-phase analytes. Ionization is triggered by a beam of photons emitted from a UV lamp, usually a krypton discharge lamp emitting photons at 10 eV, as a replacement for the corona discharge in APCI. Since most lipid molecules have ionization energies higher than 10 eV, the ionization mechanism only rarely involves direct APPI of analyte molecules as in EI. In the more common dopant-assisted APPI mode two steps are involved. In the first step the ionization region is filled with the high-concentration dopant with a low ionization energy (e.g., acetone, benzene, toluene, and xylene), and the dopant radical cations are produced by absorption of photons. In the second step the analytes are then ionized by direct charge transfer from dopant radical cations or by the solvent-mediated ionization. During the latter, the solvent molecule S is ionized by a proton transfer from the dopant radical

cation, forming an SH^+ ion, which in turn ionizes the analyte molecule by a second proton transfer reaction (Raffaelli & Saba, 2003; Cai et al., 2007; Dass, 2007b). Compared to APCI (Section VIII.A.2) and ESI (Section VIII.A.5), APPI provides lower detection limits, generally the highest signal intensities, and the highest S/N ratio in analyzing lipid molecular species separated by normal-phase liquid chromatography (Cai & Syage, 2006b).

4. Secondary Ion Mass Spectrometry and Fast Atom Bombardment Ionization

Secondary ion mass spectrometry (SIMS) is a surface characterization technique that is used for the analysis of samples from solid surfaces or thin films (Handa et al., 1983). In this approach a primary high-energy (several keV) ion beam bombards the surface of the sample directly, resulting in sputtering of secondary ions. The ejected secondary ions are then collected and analyzed by a mass spectrometer. Fast atom bombardment (FAB) may be considered as an improved version of SIMS for organic analysis, discovered by Barber et al. (1981, 1982). The sample to be analyzed is mixed with a low-volatility and inert liquid matrix (e.g., glycerol), and is bombarded with a beam of high-energy (usually several keV) atoms under vacuum. Both SIMS and FAB are suitable for lipid analysis after TLC separation. The differences between SIMS and FAB lie in the following: (i) SIMS is performed directly on the surface of the sample and the secondary ions produced during the sputtering process are measured, whereas FAB uses a liquid phase as a matrix and the sample is mixed with the matrix prior to ionization; (ii) while SIMS uses a cation beam such as Ar^+ or Xe^+, FAB uses an electrically neutral atom beam such as Ar° or Xe° (Matsubara & Hayashi, 1991). SIMS can be a powerful technique in imaging mass spectrometry for lipid distributions in biological specimens. However, a common drawback of SIMS is the difficulty to desorb ions with *m/z* over 500, which makes the detection of large lipid molecules very difficult (Walker, 2008). FAB-MS can reveal valuable information on the structures of complex lipids (such as homogeneity, molecular weight, molecular species, composition of fatty acid, fatty acid position in the glycerol backbone, etc.) (Matsubara & Hayashi, 1991). FAB has nowadays been largely replaced by APCI and ESI, but recently FAB-MS in the negative ion mode has been employed to unambiguously distinguish between *cis* and *trans* isomers of monounsaturated fatty acids (Ji et al., 2007a,b).

5. Electrospray Ionization

Electrospray ionization (ESI), a ''soft'' ionization technique (i.e., little or no molecular fragmentation occurs during ionization), was developed by Fenn et al. (1989). The sample solution (usually from liquid chromatography) is sprayed at the end of a fine capillary needle held at a high electric potential (several kV), forming small charged droplets. As the solvent vaporizes the sample ions are produced *via* either the charge-residue model (CRM, most likely valid for biopolymer analytes) or the ion-desorption model (IDM, believed to dominate for small-molecule analytes). ESI yields gas-phase ions directly from molecules in a solution, and thus can ionize effluents from liquid chromatography. Currently, it is the most frequently used ionization technique for lipid analysis. The principles and mechanisms of ESI for lipids have been well summarized in many review articles and books (Fenn et al., 1989; Duffin, Henion, & Shieh, 1991; Hsu, Bohrer, & Turk, 1998a; Hsu & Turk, 2003; Pulfer & Murphy, 2003; Isaac et al., 2007; Postle et al., 2007; Dass, 2007b). Here we emphasize the newer developments.

In positive ion mode ESI most commonly yields ions that are protonated molecules. However, ionization *via* adduction of doped-in metal cations was introduced to ESI for lipid structure identification, forming the sodiated, lithiated and copper-containing ions (Hsu & Turk, 2000e, 2003, 2006, 2008a,b; Afonso et al., 2005; Simoes et al., 2008; Smith et al., 2008). Another innovation of ESI in lipid analysis is ozone electrospray (OzESI). In this approach ozone is introduced into the ion source together with the desolvation gas, permitting the analysis of double-bond positions in a broad range of common unsaturated lipids including acidic and neutral glycerophospholipids, sphingomyelins, and TAG (Thomas, Mitchell, & Blanksby, 2006; Thomas et al., 2007).

''ESI with intrasource separation'' is used in shotgun lipidomics for direct quantification of lipids from ''crude'' extracts (Han & Gross, 2005). The intra-source separation strategy enables comprehensive profiling of the cellular lipidome by direct infusion of crude lipid extracts without front-end chromatographic separation (Han & Gross, 2003, 2005; Han et al., 2004). By employing both positive and negative ion modes, as well as adjusting the pH of the lipid extract, it is possible to preferentially ionize anionic lipids, weak anionic lipids and neutral lipids under different ESI experimental conditions. The use of intrasource separation with its exploitation of selective ionization methods is expected to simplify the requirements for lipid purification prior to mass spectrometry in such a shotgun survey approach, and indeed this is found to be the case (Adibhatla, Hatcher, & Dempsey, 2006).

6. Matrix-Assisted Laser Desorption/Ionization

MALDI is another ''soft'' ionization technique (Karas & Hillenkamp, 1988; Tanaka et al., 1988) successfully used for lipid analysis. During the MALDI process samples are first embedded in a dry crystalline matrix. A pulsed laser beam (most commonly a nitrogen laser) is then used to irradiate the resulting sample-matrix crystals, triggering vaporization and ionization of the target sample and matrix molecules. Unlike ESI, MALDI can ionize the analyte directly from the solid phase. MALDI is easily coupled with TLC for the analysis of different lipid classes (e.g., phosphatidylcholines, sphingomyelins, neutral glycosphingolipids and gangliosides) (Guittard, Hronowski, & Costello, 1999; Lessig et al., 2004; Dreisewerd et al., 2005; Nakamura et al., 2006; Rohlfing et al., 2007; Fuchs et al., 2007a; Distler et al., 2008). MALDI is also largely used in imaging mass spectrometry for investigating the distribution of lipids through the direct analysis of thin tissue sections (Rujoi, Estrada, & Yappert, 2004; Jackson, Wang, & Woods, 2005b; Cornett et al., 2007; Isaac et al., 2007; McDonnell & Heeren, 2007; Schiller et al., 2007; Chen et al., 2008; Hou et al., 2008). However, the use of this technology is still considered better suited to analysis of high molecular weight protein targets rather than low molecular weight lipids (Schiller et al., 2004). MALDI has been applied for the analysis of glycerophospholipids (e.g., PC, PE, PS, and CL) since they produce $[M+H]^+$, $[M+Na]^+$, and $[M+Li]^+$ ions in the positive-ion mode (Li, Gross, & Hsu, 2005; Fuchs et al.,

2007b; Hayasaka et al., 2008; Stubiger, Pittenauer, & Allmaier, 2008). The headgroup and the identity/position of individual fatty acyl chains of glycerophospholipids could be identified by MALDI coupled to post-source decay (PSD) fragmentation in a time-of-flight mass spectrometer (Fuchs et al., 2007b; Stubiger, Pittenauer, & Allmaier, 2008).

B. Mass Spectrometers

A mass spectrometer has three essential components: (i) an ion source that converts the sample molecules into charged ions in the gas phase; (ii) a mass analyzer employs electric and/or magnetic fields to sort ions according to their m/z values; and (iii) a detector that measures the signal of each m/z-resolved ion. Mass spectrometers with one type of mass analyzer, and hybrid mass spectrometers (e.g., quadrupole linear ion trap, quadrupole TOF and linear ion trap-orbitrap) combining different types of mass analyzers, are frequently used in lipid identification and quantification. For example, quadrupole-linear ion trap hybrids combine the functions of multistage MS^n in the ion trap with precursor ion scanning and neutral loss scanning as well as MRM for quantification using the triple quadrupole (Nakagawa et al., 2005; McDonald et al., 2007; Shaner et al., 2009; Zhao et al., 2008). The mass spectrometers commonly employed in lipidomics are described below.

1. Time-of-Flight Mass Spectrometer

In the TOF mass spectrometer ions are accelerated to the same high kinetic energy (Ek) and thus ions of different m/z have different velocities (v). The m/z values of ions are deduced from their flight time (t) through a tube with a fixed length (L) under high vacuum. The kinetic energy of a specific ion is $Ek = \mathrm{m} \times v^2/2$ where m is the actual mass of the ion (in kg in the SI system of units). Note that $\mathrm{m} = m \times m_\mathrm{u}$ where $\mathrm{m_u}$ is the atomic mass constant (1/12 of the mass in kg of a single atom of the isotope ^{12}C) and m is the mass of a single atom or molecule on the corresponding atomic mass scale. The time of arrival of an ion at the detector is then $t = L/v$, or $v = \mathrm{L}/t$. The potential energy (Ep) before acceleration of the ion is related to its charge ($q = z \times e$, where e is the fundamental unit of charge) and to the electrical potential U at the start of the ion's acceleration, as $Ep = q \times U = ze \times U$. Theoretically, in the absence of any distorting collisions all the potential energy is converted to kinetic energy, so that $Ek = Ep$, that is, $\mathrm{m} \times v^2/2 = ze \times U$. If we now replace m with $m \times m_\mathrm{u}$ and v with L/t we obtain the fundamental equation for the time-of-flight mass spectrometer: $m/q = (m/z) \times (m_\mathrm{u}/e) = (2U/L^2) \times t^2$. Since m_u and e are fundamental constants of nature and U and L are fixed parameters of the particular time-of-flight instrument, measurement of ion arrival time t amounts to measurement of m/z. Usually the accurate relationship between the two is established by calibration using ions of known m/z values rather than by direct calculation using values of U and L.

Time-of-flight analyzers are well suited for combination with MALDI ion sources that are intrinsically pulsed in nature, and as a result the majority of commercially available TOF instruments are equipped with MALDI device as ionization sources. TOF and hybrid TOF instruments, such as ion mobility-TOF, TOF-TOF, Qq-TOF, and QIT-TOF, have all been used for lipid analysis (Woods et al., 2004; Jackson et al., 2005c; Trimpin, Clemmer, & McEwen, 2007; Shvartsburg & Smith, 2008; Shimma et al., 2008).

2. Ion Trap Mass Spectrometer

The three-dimensional ion trap instrument is composed of a doughnut-shaped central ring electrode and two end-cap electrodes, all with hyperbolic geometry. The trapping electric field is formed by applying direct current (dc) and radio-frequency (rf) potentials to the ring electrode and ground potential to the end-cap electrodes. By manipulation of the ratios of the dc and rf field strengths, the analyte ions can be captured and subjected to various techniques of mass spectrometry including straightforward m/z analysis, MS/MS, and even multi-stage MS^n analyses. These tandem MS experiments are achieved by manipulating ions' motion in time rather than in space as is done in triple-quadrupole instruments for example (Dass, 2007a). Ion trap mass spectrometers are cost-friendly and offer good sensitivity and high throughput. These instruments have been used for glycerophospholipid characterization using MS^n up to MS^4 (Larsen et al., 2001). However, accurate mass determinations and highest-quality quantification are not possible with ion trap mass spectrometers since these instruments suffer from low dynamic range, limited resolving power, and space-charge effects that severely limit the number of ions that can be stored at any one time without significant degradation of resolving power, m/z accuracy, etc. Higher resolution and expanded dynamic range have been made possible with the development of ''linear'' or ''two-dimensional'' ion trap (LTQ) instruments (Schwartz, Senko, & Syka, 2002).

The orbitrap is a newer member of the ion-trap family of mass analyzers. It consists of an axial spindle-like central electrode and a coaxial barrel-like outer electrode. The trapped ions undergo rotation and harmonic oscillations along the central electrode. The m/z values of the trapped ions are related to the frequencies of their harmonic oscillations along the central axis. Mass analysis is performed by measuring the image current that is induced in the outer electrode by the motions of the ions and converting the time-domain signal into mass spectra using fast Fourier transforms (Dass, 2007a). Orbitrap mass spectrometers offer high mass accuracy and resolving power. Hybrid LIT-orbitrap instruments have been employed for high-quality lipidome identification and quantification (Schwudke et al., 2007a,b).

3. Triple-Quadrupole Mass Spectrometer

The advantages of triple-quadrupole mass spectrometers in lipidomics research include their ability to perform precursor ion scanning and neutral loss scanning and their unequalled ability to provide quantitative analyses of high precision and accuracy using the multiple-reaction monitoring (MRM) mode. These characteristics are suitable for the analysis of various lipid classes (Larsen et al., 2001; Bielawski et al., 2006; Isaac et al., 2007; Shaner et al., 2009).

A linear quadrupole consists of four precisely matched parallel metal rods. The dc and rf potentials are applied between the two opposing pairs of these electrodes to produce a high-frequency oscillating electric field. Mass separation is thus made possible by the oscillatory motions of ions in this electric field, rather similar to the selective ion trapping conditions in an ion

trap. Ions of a specific *m/z* value pass through the quadrupole rods only with specific values of the dc and rf potentials. A mass spectrum is usually obtained by changing both the dc and rf potentials while keeping their ratio constant (Dass, 2007a).

In triple-quadrupole instruments, three quadrupoles are arranged sequentially. While the first (Q1) and third (Q3) quadrupoles are operated in the mass-selective mode using both dc and rf potentials, the second quadrupole (Q2) is operated with only the rf potential. Therefore, Q2 allows all ions above a minimum cut-off *m/z* value to pass through, and serves as a total ion containment region and a gas collision cell in which ion-molecule collisions can induce ion fragmentations that provide chemical information.

Triple-quadrupole instruments are well suited for tandem mass spectrometry. Since ion transmission through quadrupoles is normally limited to ions with kinetic energies in the range 0–100 eV, the MS/MS fragmentations induced in Q2 are said to be in the low-energy range (Dass, 2007c). Triple-quadrupole instruments can perform tandem mass spectrometry in space (compare the case of ion traps that do so ''in time'' within a fixed spatial region). In product ion scanning, precursor ions with particular *m/z* values are selectively transmitted by Q1, fragmented in Q2 (collision cell), and the resulting product ions are analyzed by scanning Q3. Triple-quadrupole mass spectrometers can also perform precursor ion scanning (Q3 set to transmit ions of selected *m/z* while Q1 scans the precursor ions) and neutral loss scanning (Q1 and Q3 are scanned together with a selected fixed *m/z* difference between them). They are most often operated in MRM mode that provides the currently best available performance in quantitative analyses. In MRM mode Q1 is set to transmit a selected precursor ion and Q3 is also set for a selected product ion. After a sufficient time for enough ions to be counted to provide a statistically meaningful measurement, both Q1 and Q3 can be rapidly switched to different values for another analyte or for an internal standard used for quantification. This approach provides large dynamic range and impressive accuracy and precision with measurement capability compatible with even fast chromatography timescales, together with a significant improvement in detection selectivity (less interfence from co-eluting compounds) compared with simple monitoring of only selected precursor ions.

The main disadvantages of all quadrupole instruments arise from their low resolving power and thus limited precision and accuracy in *m/z* measurement for unambiguous identification of lipids. It is also essentially impossible to perform MS^n experiments with $n > 2$, unlike the trap instruments. Moreover, as scanning instruments they have very poor efficiencies of ion utilization in all scanning modes (so-called low duty-cycle), a disadvantage that is much less important in the ''peak jumping'' MRM mode. Some of these disadvantages are overcome by various hybrid instruments. The so-called Qq-TOF instruments, in which Q3 is replaced by a TOF (Ekroos et al., 2002; Rainville et al., 2007), have been employed to improve resolving power for product ions in MS/MS mode and allows more precise *m/z* determinations for lipids. The QQ-LIT (or QTRAP) mass spectrometers, in which the third quadrupole (Q3) is replaced with a LIT that can also be operated as a conventional Q3, can perform precursor and neutral loss scans and MRM analyses as well as multiple-stage MS/MS (MS^n).

4. Fourier Transform Ion Cyclotron Resonance Spectrometer

A Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, also known as Fourier transform mass spectrometer, allows the determination of *m/z* values of ions based on their cyclotron frequency in a fixed magnetic field. The essential part of FT-ICR is a cell that is composed of three pairs of opposing plates that permit trapping, excitation, and detection. The cell is placed in a strong magnetic field. Ions are confined laterally in this trap by a static magnetic field and axially by a static electric field applied using one of the pairs of opposing plates. Ions are excited to coherent orbital motions with frequencies that are *m/z*-dependent by a broadband rf pulse applied *via* a second pair of opposing plates, and are detected by measuring the image current induced in the third pair of opposing plates by the ions' orbital motions. The application of a Fourier transform converts this complex time-domain signal into a mass spectrum (Dass, 2007a). To date, the highest resolution and mass accuracy for lipids have been obtained using FT-ICR mass spectrometers and hybrid instruments that use them as product analyzer (e.g., LIT-FT) (Schwudke et al., 2007a,b). The further coupling of these instruments to HPLC-ESI makes possible the analysis of complex lipid mixtures (Ivanova et al., 2001; Yu et al., 2006; Hu et al., 2008). FT-ICR instruments, however, are expensive and quite laborious when it comes to operation and maintenance.

5. Imaging Mass Spectrometry

Imaging mass spectrometry (IMS) allows the acquisition of spatial distributions (maps) of *m/z*-analyzed lipids from tissue sections. The most commonly used ion formation techniques used in IMS are MALDI and SIMS. Applications of MALDI in imaging mass spectrometry have usually employed N_2 UV lasers (McDonnell & Heeren, 2007) and infrared laser beams (Woods et al., 2006) rastered over the tissue section, together with matrices composed of 2,5-dihydroxybenzoic acid (DHB) (Rujoi, Estrada, & Yappert, 2004; Jackson, Wang, & Woods, 2005a; Garrett & Yost, 2006; Woods et al., 2006; McLean, Ridenour, & Caprioli, 2007; Chen et al., 2008; Shimma et al., 2008), 2,4,6-trihydroxyacetophenone (THAP) (Stubiger & Belgacem, 2007) and 2-mercaptobenzothiazole (MBT) (Astigarraga et al., 2008). To optimize matrix homogeneity a new matrix coating system called the oscillating capillary nebulizer (OCN) has been recently developed (Chen et al., 2008). Though MALDI can generate ions of intact lipid molecules from tissue sections, it suffers from the following drawbacks: (i) limited lateral resolution (20–50 μm) due to fundamental physical limitations and disturbances induced by the application of the matrix; (ii) chemical noise at low mass, and (iii) long acquisition times (Carado et al., 2008).

In SIMS applied to IMS, the primary ion beam has evolved from Bi_3^+, Au_3^+, and Ga^+ (Woods & Jackson, 2006; Richter et al., 2007) to C_{60}^+ (Cheng et al., 2007; Carado et al., 2008; Piehowski et al., 2008). Compared to MALDI, SIMS yields cleaner spectra at low mass ($<m/z$ 1,000), and offers sub-micrometer lateral resolution without the use of matrix. However, SIMS cannot resolve total structural information due to fragmentation of analytes during the ionization process. To overcome the drawbacks of each method, Carado et al. (2008) combined MALDI

and SIMS in a single mass spectrometer, which might offer a promising tool for lipid imaging analyses.

C. Tandem Mass Spectrometry

Tandem mass spectrometry (MS/MS), or the study of specific ion fragmentations, is a powerful analytical approach for lipid structure elucidation and quantification in lipidomics research and was briefly described above. The most commonly used approach for ion fragmentation in lipid analysis by MS/MS is collision-induced dissociation (CID), also termed collisionally activated dissociation (CAD). In this process the mass-selected precursor ions gain high kinetic energy *via* acceleration through an electric potential drop. The accelerated ions are then caused to collide with neutral gas molecules (helium in ion traps and usually nitrogen or argon in tandem-in-space instruments such as triple-quadrupoles). Part of the kinetic energy is thus converted into internal energy, resulting in fragmentation of the precursor ions and hence in production of fragment ions whose *m*/*z* values contain chemical information. The CID process can be performed at low (eV range) and high (keV) collision energies (Sleno & Volmer, 2004). Triple-quadrupole and ion trap spectrometers (Afonso et al., 2005; Simoes et al., 2008) employ low energy CID, tandem sector and TOF instruments (Trimpin, Clemmer, & McEwen, 2007) employ high collision energy, and FT-ICR instruments (Mirgorodskaya, O'Connor, & Costello, 2002; Vukelic et al., 2005; Cui et al., 2008) are used at both low and high collision energy.

A significant disadvantage of MS/MS experiments conducted using CID in a three-dimensional ion trap is the low mass cut-off feature, the so-called ''1/3 Rule,'' whereby fragment ions with *m*/*z* values lower than approximately 1/3 of that of the precursor ion cannot be trapped and detected without special operating procedures. The same principle applies to linear quadrupoles, for example, the low-mass cut-off of a RF-only quadrupole used as a collision cell, but the practical effects are much less serious because the three quadrupoles are operated independently and the RF field conditions in one do not affect those in the others unlike the ''tandem-in-time'' approach used in an ion trap. This advantage also applies to other hybrid instruments. For example, in the LTQ Orbitrap XL^{TM}, high-energy CID can be performed on the accelerated ions from C-trap in the nitrogen-filled collision cell. The resulting fragment ions re-enter the C-trap, and are detected in the orbitrap without low mass cut-off. Both the low and high energy CID mass spectra can be obtained in parallel acquisitions, a feature which has been successfully employed for protein identification and quantification (Olsen et al., 2007), and holds tremendous potential for lipid structural elucidation.

Tandem mass spectrometry can also include multiple stages of mass analysis, the so-called MS^n approach that is practical only in ion trap instruments. MS^n experiments enable the study of sequential multi-stage fragmentations of the precursor ions within the same run to characterize the structures of complex analytes such as lipids (Larsen et al., 2001; Hsu & Turk, 2008a,b; Smith et al., 2008). On the other hand, tandem-in-space instruments such as triple-quadrupoles permit investigation of fragmentations of lipid species using four different scan modes. These include product ion scanning (as in trap instruments), precursor ion scanning, neutral loss scanning and MRM, described briefly above for triple-quadrupole instruments. Applications of product ion scanning to lipid analysis include sphingomyelins (Hsu & Turk, 2000e) and sulfatides (Hsu, Bohrer, & Turk, 1998a). The detection in complex mixtures of compounds containing specific functional groups, for example, different lipid subclasses, may be performed using precursor ion scanning if the functional group in question readily yields a fragment ion with a characteristic *m*/*z* value. The resulting spectrum contains all precursor ions that yield the characteristic fragment ion upon CID. Neutral loss scanning mode provides information similar to that of precursor ion scanning, but for cases in which the chosen functional group yields a characteristic neutral fragment rather than a fragment ion. Precursor ion and neutral loss scans have been employed to analyze glycerophospholipids and their functional groups (Ekroos et al., 2002; Han et al., 2005, 2006a). Applications of MRM in the quantification of specific lipid molecules, as opposed to lipid classes, have been described (Bielawski et al., 2006; Taguchi, Nishijima, & Shimizu, 2007).

Electron capture dissociation (ECD) is a fragmentation method that can be used with multiply charged ions as an alternative to CID for tandem mass spectrometry analysis (Zubarev, Kelleher, & McLafferty, 1998). A multiply charged even-electron positive ion can capture a low-energy electron (usually <0.2 eV) forming an odd-electron ion. For technical reasons ECD is most readily conducted in FT-ICR instruments. Subsequent ion fragmentations of the radical-cations formed by electron capture follow radical ion chemistry, which can be quite different from the fragmentation mechanisms of the even-electron precursor ions activated in CID. ECD tandem mass spectrometry has been successfully in revealing structural information about the headgroup, and the identity and position of the acyl chains of PC (James, Perugini, & O'Hair, 2008). Extensive structural information on monosialoganglioside GM_1, including the identification of the sphingoid base and fatty acyl entity of the ceramide backbone, as well as the cleavage of the acetyl moiety of the *N*-acetylated sugars, has been also possible with ECD mass spectrometry (McFarland et al., 2005). ECD is not practical in ion traps because the electrons are excited by the RF field and it is not possible to maintain their kinetic energies at the low values required for electron capture. Various problems prevent practicable application of ECD in ion beam (tandem-in-space) instruments. More recently (Syka et al., 2004), this problem was overcome by the development of a method of transferring an electron to an even-electron multiply charged analyte ion from suitable donor ions that can be trapped simultaneously in an ion trap. For example, ETD tandem MS has been used for PC analysis by ion–ion reactions between doubly-sodiated PC cations and azobenzene radical anions in a linear ion trap mass spectrometer (Liang et al., 2007). This approach proved useful for revealing information on the number of carbons and the degree of unsaturation of each fatty acyl chain, along with its stereospecific position on the glycerol backbone.

D. Direct Infusion or Front-End Chromatographic Separation

Lipid species are routinely analyzed by ESI mass spectrometry *via* direct infusion or integrated with front-end liquid chromatographic separations such as normal- and reverse-phase HPLC. Compared to direct infusion, coupling liquid chromatography to mass spectrometry significantly reduces the complexity of the

lipid mixture presented to the mass spectrometer for analysis. An advantage of this approach is that the front-end chromatographic separation limits high abundant lipid species to a relatively narrow chromatographic window, and thus allows low-abundance lipid species to be identified with less interference and higher confidence. In addition, parameters of the chromatographic separation itself provide additional information for lipid structure elucidation. Normal-phase HPLC, in which the stationary phase is more polar than the mobile phase, is capable of separating glycerophospholipids based on their polar headgroups. Reverse-phase HPLC, in which the mobile phase is the more polar, can distinguish glycerophospholipids based on their fatty acyl moities. However, due to the great diversity of the physicochemical properties of lipid species, front-end chromatographic separation is generally customized for the analysis of certain types of lipid species. As a result, for comprehensive lipid identification and quantification, multiple chromatographic separation techniques are normally required. Also, there are concerns over sample loss during liquid chromatographic separation and oxidative degradation during pre-fractionation using TLC for example (DeLong et al., 2001).

Mass spectrometric studies of crude lipid extracts using direct infusion have been reported in the literature (Abidi, 2004; Ejsing et al., 2006, 2009; Gross & Han, 2009; Yang et al., 2009b). The advantages of direct infusion over front-end chromatographic separation include the simpler experimental setup and high throughput. Crude lipid extracts can be analyzed by mass spectrometry without any extensive sample processing steps. Unlike aqueous protein extracts, where high amount of salts are present and desalting is a necessary step before analysis by mass spectrometry, lipid extracts in the organic phase are clean and almost free of salts. This minimizes the possibility of ionization suppression by inorganic salt ions. As a result, direct infusion is capable of providing useful analyses of complex mixtures of lipid species that could have a dynamic range of several orders of magnitude. It is acknowledged that different classes of lipid species are preferentially ionized under different experimental conditions, which leads to ionization suppression of other lipid species since an ESI source has an upper limit on the amount of total ionization that it can provide at any one instant. This intrinsic problem is addressed in the ''intra-source separation'' approach (Han et al., 2004), described previously in the section on ESI. Sequentially changing experimental conditions such as adjusting pH, in combination with switching the mode of ionization (positive or negative), can result in the preferential ionization of different types of lipid species each time. The combination of all the results obtained for the crude extract under different experimental conditions can result in a comprehensive lipid identification map for the crude extract. Intra-source separation in combination with multiple precursor ion scans and neutral loss scans has enabled the unambiguous identification of many components in complex lipid extracts (Han et al., 2004; Han & Gross, 2005; Yang et al., 2009a,b).

IX. LIPID STRUCTURE ELUCIDATION

To delineate structure characteristics of individual lipid molecules, the lipidomics field has benefited from the technical developments established in proteomics. However, protein identification has mostly involved a ''matching'' process in which the amino acid sequences of some peptide fragments, or other information related to the amino acid sequence of a protein, are determined by mass spectrometry and the information thus obtained is used to search against a library that contains all the possible protein sequences predicted by the genomic information of the biological system. The sole exception to this generalization is *de novo* protein sequencing that is generally used only when the shotgun method fails to provide an unambiguous result. Proteomic strategies thus capitalize on sequence-based nucleotide and peptide signatures.

Lipid second messengers lack such easily definable molecular signatures. As reviewed above, isoforms can vary only by one double bond or one methylene unit. Thus, in sharp contrast to protein analysis, lipid identification is inherently a ''*de novo*'' process where the structural elucidation of a lipid species is based on the interpretation of mass spectrometry-derived structural information. It is also a ''top-down'' approach, that is, the identification process requires intact lipid species. (Shotgun proteomics as usually practiced is a ''bottom-up'' process that requires first decomposing the proteins into much smaller constituent peptides that are amenable to analysis by tandem mass spectrometry.) In the case of lipids, structural information on all parts of the molecule (e.g., including both the polar headgroup and fatty acyl chains in glycerophospholipids) are required for a complete elucidation of the lipid structure. Therefore, it is crucial to have the lipids present in their native unmodified forms to allow accurate structural elucidation of the lipid species of interest. However, lipid species are subject to modifications or degradation (i.e., oxidation and hydrolysis) prior to mass spectrometry analyses as indicated above. As a result, the lipidomics field currently faces the challenge of decoding as much information as possible about the different lipid moieties to allow a non-biased and true identification of the original structures of lipids. The following sections will highlight the use of mass spectrometry-based approaches for structure elucidation of glycerophospholipids predominantly, as well as fatty acyls, glycerolipids, and sphingolipids.

A. Structure Elucidation of Glycerophospholipids

A generic strategy for the study of glycerophospholipids consists of coupling HPLC to ESI-MS/MS. As stated above, ESI is best suited for glycerophospholipids analysis. A survey scan is employed to determine the molecular weight of each lipid species. Greater mass accuracy translates into more accurate prediction of the atomic composition of the lipid. In a second stage, individual lipids are subjected to dissociations by ion activation. Although low-energy CID has been extensively applied for lipid studies, high-energy CID and electron transfer dissociation (ETD) have been also employed (Harvey, 2005; Liang et al., 2007). The fragmentation patterns normally reveal information on the lipid polar headgroup and its acyl chain moieties. Depending on the electrical propensity of a lipid species, it can be ionized more efficiently under either positive (protonated) or negative (deprotonated) electrospray ionization. A lipid can also be adducted by a cation (e.g., Na^+) or an anion (e.g., Cl^-) and then studied under positive or negative ion mode, respectively. Precursor ion scan and neutral loss scan enable the identification of specific lipid species from a complex mixture by monitoring charged and neutral loss ion fragments, respectively. The characteristic fragment can be associated with either a polar headgroup or a fatty acyl moiety.

1. ESI-MS/MS of Protonated Glycerophospholipids

Glycerophospholipids (i.e., PC, PE, PS, and their lyso derivatives) have been analyzed by positive ESI-MS/MS (Brugger et al., 1997; Retra et al., 2008). The fragmentation of protonated PC and LPC ions yield a peak at *m/z* 184, the diagnostic fragment for the phosphocholine headgroup (Hsu & Turk, 2003). The precursor ion scan of *m/z* 184 highlights phosphocholine-containing lipids (PC and sphingomyelin) out of all the lipids present in a survey scan. Fragmentation of protonated PE ($[M+H]^+$) yields a peak at $[M+H-141]^+$ corresponding to the neutral loss of the phosphoethanolamine headgroup. Similarly, a peak at $[M+H-185]^+$ in a tandem mass spectrum can be used to confirm the presence of protonated PS ($[M+H]^+$), which loses its phosphoserine headgroup during CID. Overall, strategies that combine survey scans, precursor ion scans and neutral loss scans can help in the unambiguous determination of the molecular masses and headgroup compositions of phospholipids. Although positive ESI-MS/MS has been widely used for lipid studies, one of its limitations is the limited information about the fatty acyl constituents that are revealed from the fragmentation patterns. Alternatively, negative ESI-MS/MS and positive ESI-MS/MS with metal ion adduction, have been employed to elucidate lipid structures.

2. ESI-MS/MS of Deprotonated Glycerophospholipids

Glycerophospholipids such as PE, PI, PS, PG, PA, and their lyso variants can all be detected by negative ESI-MS/MS (Kerwin, Tuininga, & Ericsson, 1994; Smith, Snyder, & Harden, 1995; Brugger et al., 1997; Hsu & Turk, 2000b,c,d, 2001b, 2005; Taguchi et al., 2000; Ivanova et al., 2001). This approach is very powerful in elucidating the structure of glycerophospholipids. Compared to its positive counterpart, negative ESI-MS/MS yields fragmentation patterns with a wealth of structural information on the polar headgroups and the fatty acyl constituents of phospholipids. Fragmentation of PE anions ($[M-H]^-$) generates fragments at *m/z* 140 and 196, both of which correspond to the polar headgroup of PE (Hsu & Turk, 2000d). As deprotonated PI ($[M-H]^-$) are subjected to CID, a peak corresponding to the dehydrated PI headgroup is observed at *m/z* 241. Similarly, PtdInsP and PtdInsP$_2$ can be identified by precursor ion scans of *m/z* 321 and 401, respectively, under negative ion mode (Hsu & Turk, 2000b; Wenk et al., 2003). The fragmentation of deprotonated PG yields a headgroup specific fragment at *m/z* 171 (Hsu & Turk, 2001b). PS anions ($[M-H]^-$) are identified by a peak at $[M-H-87]^-$ that corresponds to the neutral loss of the serine headgroup (Hsu & Turk, 2005). A fragment at *m/z* 153 ([Glycerophosphate-H_2O]$^-$) is detected in the fragmentation spectra of all phospholipid species under negative ion mode.

Low-energy CID of glycerophospholipids under negative ion mode also yields fragment ions providing structural information on the fatty acyl constituents at the *sn*-1/*sn*-2 positions. These fragments include the carboxylate anions ($[R_xCOO]^-$ ($x=1, 2$)), ions arising from the neutral loss of fatty acids ($[M-H-R_xCOOH]^-$ ($x=1, 2$)), and ions due to neutral loss of the fatty moieties as ketenes ($[M-H-R'_xCH=C=O]^-$ ($x=1, 2$)) (Hsu & Turk, 2000b,c,Hsu & Turk, 2000d, 2001b, 2005).

It has been found that, in a low-energy CID process, the loss of fatty acyl chains from the *sn*-2 position is sterically more favorable than from the *sn*-1 position. As a result, the abundances of the $[M-H-R_2COOH]^-$ and $[M-H-R'_2CH=C=O]^-$ ions are greater than those of the $[M-H-R_1COOH]^-$ and $[M-H-R'_1CH=C=O]^-$ ions. However, as the major fragmentation pathways differ among different lipid species, the relative abundance of the carboxylate anion $[R_1COO]^-$ with respect to $[R_2COO]^-$ changes. For PE and PG, the abundances of $[R_2COO]^-$ ions are greater than $[R_1COO]^-$ ions (Hsu & Turk, 2000a, 2001b). For PI, the carboxylate anions $[R_1COO]^-$ and $[R_2COO]^-$ are generated due to direct dissociation from the glycerol backbone, and also due to the further dissociation of fragment ions $[M-H-R_xCOOH]^-$, $[M-H-R'_xCH=C=O]^-$, and $[M-H-R_xCOOH-\text{inositol}]^-$, which are affected by the collision energy applied. Thus, the relative abundances of the carboxylate anions $[R_1COO]^-$ and $[R_2COO]^-$ for PI depend on the collision energy applied (Hsu & Turk, 2000b). For PA, the major pathway that leads to the formation of the carboxylate anions $[R_1COO]^-$ and $[R_2COO]^-$ is the fragmentation of $[M-H-R_2COOH]^-$ and $[M-H-R_1COOH]^-$, respectively. As a result, the higher abundance of $[M-H-R_2COOH]^-$ ions leads to higher abundance of $[R_1COO]^-$ ions (Hsu & Turk, 2000c). As PS is subjected to low-energy CID, it first loses the serine headgroup to generate $[M-H-87]^-$ ions. The fragmentation patterns of $[M-H-87]^-$ ions are virtually identical to those of the corresponding PA (Hsu & Turk, 2005).

3. ESI-MS/MS of Metal Ion Adducts of Glycerophospholipids

The fragmentation of lipids can be modified by changing the counterion present in solution before ESI. In particular, alkali metal ions (e.g., Li^+, Na^+, and K^+) have been commonly used for adduct formation with phospholipids (Kerwin, Tuininga, & Ericsson, 1994; Han & Gross, 1995; Hsu & Turk, 2000a; Ho, Huang, & Deng, 2003). When these metal ion adducts of glycerophospholipids are subjected to CID their fragmentation patterns reveal information on the polar headgroups and the fatty acyl constituents. The fragmentations of sodiated PC and sphingomyelins both generate a sodiated five-member cyclophosphane at *m/z* 147 and a product ion at $[M+Na-59]^+$ due to the neutral loss of trimethylamine $N(CH_3)_3$, both of which are diagnostic fragments of the phosphocholine headgroup. In the tandem mass spectra of lithiated PC, abundant product ions can be found at $[M+Li-59]^+$ (neutral loss of $N(CH_3)_3$), $[M+Li-183]^+$ (neutral loss of cyclophosphane), and $[M+Li-189]^+$(neutral loss of lithium cyclophosphane) (Hsu & Turk, 2003). It has been observed that the most prominent ion is $[M+Li-183]^+$ for lithiated diacyl-PC, whereas the most high abundance ion is $[M+Li-59]^+$ for lithiated plasmenyl-, plasmanyl-, and LPC (Hsu, Bohrer, & Turk, 1998b; Hsu et al., 2003). CID of lithiated PE yields fragment ions associated with the polar headgroup including ions at $[M+Li-43]^+$ (neutral loss of aziridine (CH_2CH_2NH)), $[M+Li-141]^+$ (neutral loss of phosphoethanolamine headgroup), $[M+Li-147]^+$ (neutral loss of lithium phosphoethanolamine headgroup), and *m/z* 148 (phosphoethanolamine headgroup) (Hsu & Turk, 2000a). The CID analyses of PS as lithiated adducts yield diagnostic fragments of phosphoserine as a lithium salt at *m/z* 192 (Hsu & Turk, 2005). In addition to structural information on the

polar headgroups, structural information pertaining to the two fatty acyl moieties can also be revealed from product ions like $[M-H+Met-R_xCOOH]^-$ and $[M-H+Met-R'_xCH=C=O]^-$ ($x=1, 2$), arising from the loss of fatty acyl moieties as fatty acids or alkenes, respectively.

Metal ions such as Sr^{2+}, Ba^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} have been also used to form adduct ions of phospholipids (Ho, Huang, & Deng, 2003). The fragmentation spectra of these ions also provide information on the polar headgroups of phospholipids, and their fatty acyl chain moieties ($[M-H+Met-R_xCOOH]^-$, $[M-H+Met-R'_xCH=C=O]^-$). Cobalt(II) ion complexes of PE and PS yield $[M-H+Met-R_1COOH]^-$ ions with higher abundance than $[M-H+Met-R_2COOH]^-$ ions, whereas cobalt(II) adducted PG fragmentation patterns generate $[M-H+Met-R_2COOH]^-$ ions with higher abundance than $[M-H+Met-R_1COOH]^-$ ions. The information on abundance ratios could be used to determine the stereospecific positions of the two fatty acid substituents. Chloride adducts have also been reported for the identification of the fatty acyl substituents of PC in negative ESI-MS/MS (Han & Gross, 1995; Lehmann et al., 1997).

In conclusion, different metal ion adducts of glycerophospholipids generate lipid-specific fragmentation signatures that allow unambiguous identification of the lipid species of interest.

4. Glycerophospholipid Identification by Multiple Precursor Ion Scans and Neutral Loss Scans

Strategies that combine survey scans (simple mass spectra), product ion scans, precursor ion scans, and neutral loss scans can be employed for the study of the lipidome. In this context, a product ion scan records all fragment ions generated during CID of an *m/z*-selected precursor ion, including fragment ions relevant to the polar headgroups and the fatty acyl moieties. Precursor ion and neutral loss scans each monitor a specific fragmentation reaction (i.e., defined by whether a specific diagnostic fragment is generated), and when applied to lipid analyses have the capability of identifying all lipid species in a complex mixture that contain a specific diagnostic moiety (e.g., a polar headgroup or a fatty acyl moiety). On the other hand, these particular scans do not make full use of many other fragment ions that are helpful in lipid structural elucidation. In practice the combination of multiple precursor ion scans and neutral loss scans can enable simultaneous monitoring of multiple fragmentation reactions, allowing the identification of multiple diagnostic fragments from a complex mixture of product ions for lipid structure elucidation. Broad attention has been drawn to this approach for comprehensive lipid analysis and profiling (Ekroos et al., 2002; Han & Gross, 2003, 2005; Ejsing et al., 2006; Han et al., 2006a; Schwudke et al., 2006). A two-dimensional ESI-MS/MS analysis of lipid extracts has been reported using multiple precursor ion scans and neutral loss scans under either positive or negative ion mode. This strategy made possible the identification of isobaric components with different structures, and the determination of the regiospecificity of individual lipid species. Both precursor ion and neutral loss scans can be performed on a triple-quadrupole mass spectrometer, but each scan must be performed separately because of the incompatible scanning conditions. However, if the multiple precursor ion scans are carried out on hybrid quadrupole-TOF mass spectrometers with ion trapping capability (e.g., QSTAR Pulsar), all ions can be simultaneously monitored due to the non-scanning acquisition of TOF mass analyzers. It has been reported that such hybrid quadrupole-TOF mass spectrometers allow the simultaneous acquisition of 41 precursor ion spectra which provide information on specific lipid classes and common fatty acyl chain moieties (Ejsing et al., 2006). Conventionally, neutral loss scans cannot be performed on a hybrid quadrupole-TOF mass spectrometer. However, as all fragment ions are recorded in the tandem mass spectrum of the specific precursor ions of interest, pseudo multiple neutral loss scans can be constructed post-acquisition by re-processing all combined information from product ion spectra of the multiple precursor ions selected (Schwudke et al., 2006). The high mass accuracy and high resolution of TOF analyzers are very helpful in determining the atomic composition and hence the structure of a lipid species. However, precautions must be taken when reviewing data from a TOF mass analyzer for the quantitative analysis of lipid species as the transfer of ions from the quadrupole to the TOF is done using a pulsed technique that can affect quantification (Chernushevich, Loboda, & Thomson, 2001; Ekroos et al., 2002; Han & Gross, 2005; Ejsing et al., 2006).

5. Glycerophospholipid Identification by Multiple Stage Tandem Mass Spectrometry

Structural information revealed by single-stage MS/MS alone cannot always provide adequate information to identify a lipid species unambiguously. In many cases the lipid species of interest are complex and/or the determination of very specific information on a fatty acyl chain is required (e.g., double bond location). In such cases, multiple stage MS^n can be applied to provide additional information. Hsu et al. (2005) characterized the structure of CL using negative ion mode with MS^n using an ion trap. The MS^3-spectra of the phosphatidic anions provided critical information on the identity of the fatty acyl substituents and their stereospecific positions. Hsu and Turk (2008b) also determined the double bond location in the fatty acyl chain of glycerophospholipids using multiple-stage mass spectrometry. In another work these authors employed multiple stage mass spectrometry to differentiate 1-*O*-alkyl-1′-enyl-2-acyl-, 1-*O*-alkyl-2-acyl- and diacyl-glycerophospholipid molecules from one another based on the MS^3 or MS^4 spectra of their $[M-H-R_2COOH-headgroup]^-$ ions. These ions arise from the consecutive losses of the fatty acid substituents at the *sn*-2 position and their respective polar headgroups from the $[M-H]^-$ ions (Hsu & Turk, 2007). It has been also reported that isobaric PAF (Fig. 15) and LPC can be effectively identified by MS^3 using a QTRAP mass spectrometer within complex cellular lysates and tissue extracts (Smith et al., 2008). Sodiated lipid species were fragmented to produce radical cations which lost successive methylene groups upon further collisional activation to reveal the identity of the parent molecule.

6. Characterization of the Fatty Acyl Constituents of Glycerophospholipids

Under both positive and negative ESI-MS/MS, glycerophospholipids fragment to yield product ions allowing relatively easy elucidation of their polar headgroups. The characterization of the two fatty acyl moieties at the *sn*-1/*sn*-2 positions, however, poses challenges. The structural information relevant to the fatty acyl chains can only be revealed under negative ion mode ESI or

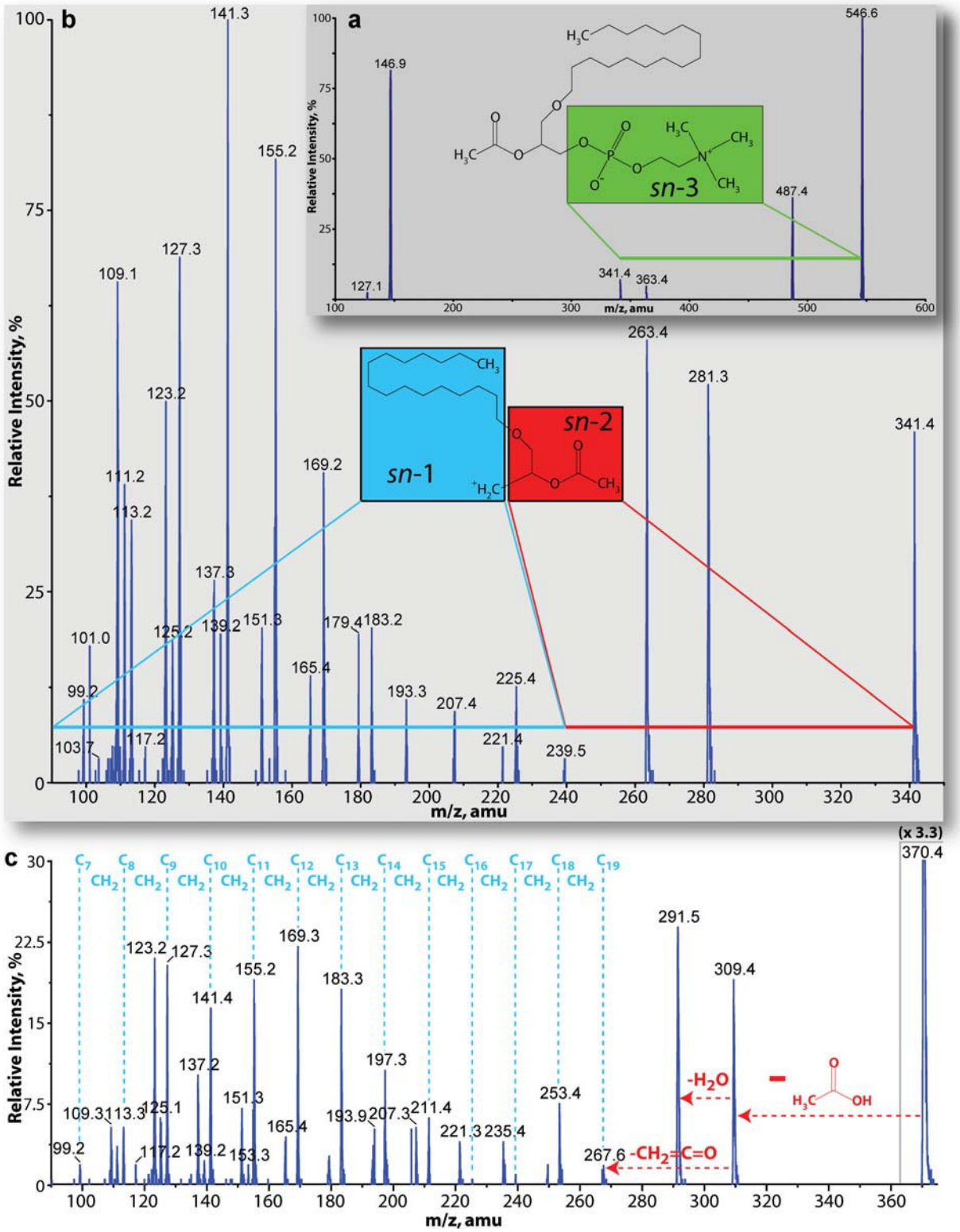


FIGURE 15. MS^2 and MS^3 analysis of PAF. **a**: MS^2 spectrum of sodiated C16:0 PAF (structure indicated in inset) revealing a peak at *m/z* 341. The mass difference between *m/z* 341 and the molecular ion is consistent with the loss of neutral sodiated phosphocholine (highlighted in green). **b**: Further fragmentation of the *m/z* 341 radical cation produced a spectrum revealing structural characteristics of the *sn*-1 and *sn*-2 groups. The lower mass ions correspond to fragments of the fatty alcohol chain of the *sn*-1 group while the higher mass fragments represent an intact *sn*-1 group with fragmentation occurring on the *sn*-2 group and glycerol backbone. **c**: MS^3 spectrum of sodiated C18:0 PAF further illustrating how the low mass ions reveal the carbon chain connectivity of the *sn*-1 group fatty alcohol and the high mass ions reveal structural information pertaining to the *sn*-2 group and glycerol backbone. The lower mass peaks are separated by equal masses of 14 Da, representing successive losses of CH_2 from the *sn*-1 fatty alcohol, identifying the lipid subspecies with high confidence. Peaks corresponding to successive losses of 2 Da from each fragment peak arise from gas phase dehydrogenation, a commonly observed phenomenon. This figure is reproduced from Smith et al. (2008) by permission of John Wiley & Sons, Ltd. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

positive ion mode ESI with metal ion adduction. In eukaryotes the fatty acids at the *sn*-2 position of glycerophospholipids are always linked to the glycerol backbone through an ester bond. The fatty acids at the *sn*-1 position can be attached *via* an ester linkage (diacyl-GP), an alkyl ether linkage (1-alkyl, 2-acyl-GP), or a vinyl ether linkage (plasmologens). For the complete identification of a glycerophospholipid the following information pertaining to the fatty acyl chains is needed: (i) the chain lengths of the two fatty acyl moieties and the total number of carbon–carbon double bonds in each chain; (ii) the assignment of the two fatty acyl moieties to *sn*-1/*sn*-2 position (for diacyl-glycerophospholipids); and (iii) the location of the double bonds on each acyl moiety which will be discussed in Section X.

The molecular mass of a glycerophospholipid can be determined accurately by a survey scan (simple mass spectrum). The polar headgroup of a phospholipid can be identified by a precursor ion scan or a neutral loss scan as discussed above. The combined information for the two fatty acid chains (i.e., the combined lengths of the two fatty acyl chains at the *sn*-1 and *sn*-2 positions and the total number of double bonds) can thus be determined. However, a number of combinations of different fatty acyl moieties could add up to the same molecular mass, leaving the length of each fatty acyl chain and the number of double bonds on each fatty acyl moiety undetermined. Using negative ESI-MS/MS, a variety of fragment ions relevant to the two fatty acyl moieties can be observed. These include carboxylate anions $[R_xCOO]^-$, as well as $[M-H-R_xCOOH]^-$ and $[M-H-R'_xCH=C=O]^-$ ions. The latter two ions arise from the loss of one of the two fatty acyl moieties. These fragment ions reveal critical structural information relevant to the individual fatty acyl constituents, and allow the unambiguous determination of the length of each fatty acyl moiety and the total number of double bonds on each fatty acyl chain (Hsu & Turk, 2000b,c,d, 2001b, 2005; Houjou et al., 2005). Under positive ion mode ESI the fragmentations of metal adducts of glycerophospholipids also yield fragment ions corresponding to their polar headgroups and fatty acyl moieties ($[M-H+Met-R_xCOOH]^-$, $[M-H+Met-R'_xCH=C=O]^-$). This again permits the unequivocal characterization of the fatty acyl moieties (Han & Gross, 1995; Ho, Huang, & Deng, 2003).

The use of mass spectrometry to determine the regiospecificity of the two fatty acyl substituents of diacyl-glycerophospholipids has been attempted (Vernooij et al., 2002; Houjou et al., 2005). As discussed earlier it appears that under low-energy CID the $[R_1COO]^-/[R_2COO]^-$ abundance ratio follows certain rules for some diacyl-glycerophospholipids, and this information has been used to determine the positions of the two fatty acyl chains (Hsu & Turk, 2000b,2000c,d, 2001b). Such attempts have also been made on the basis of fragmentation patterns of glycerophospholipid adducts with certain metal ions, where the relative abundances of ions corresponding to the losses of the fatty acyl moieties at the *sn*-1/*sn*-2 positions have been used to assign the positions of the two fatty acyl substituents (Han & Gross, 1995; Ho & Huang, 2002; Ho, Huang, & Deng, 2003). However, it has been noticed that the $[R_1COO]^-/[R_2COO]^-$ abundance ratio recorded from the fragmentation of PI under negative ion mode is affected by the collision energy applied (Hsu & Turk, 2000b). Detailed studies on the effect of collision energy on the $[R_1COO]^-/[R_2COO]^-$ ratio for phospholipid species have been carried out by Hvattum et al. (1998). These results reveal that as the collision energy increases from 15 to 70 eV, the abundance of $[R_1COO]^-$ ions increases from lower to higher than the abundance of $[R_2COO]^-$ ions for PC, PE, and PI (Hvattum, Hagelin, & Larsen, 1998). It is therefore clear that the collision energy plays an important role during the CID of glycerophospholipids. Other factors, such as the chain length and the number of double bonds of the fatty acyl moieties should also be taken into consideration. It has been reported that the relative amounts of positional isomers could be accurately determined by MS^3 analysis using ion trap mass spectrometers (Ekroos et al., 2003).

B. Structure Elucidation of Fatty Acyls

Fatty acids normally form carboxylate anions under negative mode ESI. They can also form adducts with alkali-metal ions such as lithium (Hsu & Turk, 1999a; Kerwin, Wiens, & Ericsson, 1996). CID of unsaturated fatty acids, either deprotonated or alkali-metal cation adducts, generates abundant ions that are helpful in localizing the double bonds. However, as double-bond rearrangement can occur during fragmentation, the application of mass spectrometry to accurately determine the location of double bonds requires great caution.

Eicosanoids are the biological oxidative metabolites of arachidonic acid. Depending on the metabolic pathways, different metabolites such as prostaglandins and leukotrienes can be generated. Even though there are great similarities in the structures of different eicosanoids, it has been reported that various eicosanoids are successfully separated and determined by HPLC-coupled to mass spectrometry under negative ion mode, by either selective ion monitoring or multiple reaction monitoring (Murphy et al., 2005; Blewett et al., 2008).

C. Structure Elucidation of Neutral Glycerolipids

The neutral lipids DAG and TAG can form adduct ions with NH_4^+ and alkali-metal ions (e.g., Li^+, Na^+) through ESI (Duffin, Henion, & Shieh, 1991; Cheng, Gross, & Pittenauer, 1998; Hsu & Turk, 1999b; Callender et al., 2007). The ionization efficiency of DAG and TAG appears to be affected by the total number of carbons in the fatty acyl chains and the degree of unsaturation (Duffin, Henion, & Shieh, 1991; Callender et al., 2007). The CID of lithiated TAG has been reported to yield abundant ions including $[M+Li-R_nCOOH]^+$, $[M+Li-R_nCOOLi]^+$ and R_nCO^+, where n denotes the position where each fatty acid is esterified. Therefore structural information pertaining to each of the acyl chains, such as the total chain length and degree of unsaturation, can be ascertained (Hsu & Turk, 1999b). Fragment ions reflecting the combined losses of two of fatty acids have also been observed. However, such combined losses always involve the loss of fatty acid at the *sn*-2 position, providing a robust approach to determine the fatty acid attached at this position.

D. Structure Elucidation of Sphingolipids

ESI of ceramides yields abundant $[M+H]^+$ ions. The fragmentation patterns of these ions generate abundant product ions ($[M+H-H_2O]^+/[M+H-2H_2O]^+$) due to the neutral loss of H_2O, and less abundant product ions characteristic of ceramide subclasses, for example, *m/z* 264 and 282 for sphingosine and *m/z* 266 and 284 for dihydrosphingosine (Gu et al., 1997). The fragmentation of lithiated ceramides yields fragment ions specific to each ceramide subclass, in addition to those product

ions associated with fatty acid constituents and long chain bases (Hsu et al., 2002). Ceramides have also been studied with negative ESI-MS/MS, either as deprotonated molecules $[M-H]^-$ or adducted with $[CH_3CO_2]^-$. In both cases rich product ions referring to both the fatty acid moieties and long chain bases can be generated and hence the structures of ceramides can be identified (Han, 2002; Hsu & Turk, 2002).

Like PC, sphingomyelin readily generates abundant protonated ions ($[M+H]^+$) and alkali metal ion adducts (e.g., $[M+Li]^+/[M+Na]^+/[M+K]^+$) (Han & Gross, 1995; Hsu & Turk, 2000e; Merrill et al., 2005). CID of $[M+H]^+$ generates an abundant ion at *m/z* 184, corresponding to the phosphocholine polar headgroup. Protonated sphingomyelin ($[M+H]^+$) and sphingolipids molecules with zero or an even number of nitrogen atoms appear at odd *m/z* values (Brugger et al., 1997; Ivanova et al., 2001). Similarly to PC, CID of sphingomyelin adducted with alkali metal ions yields fragment ions revealing information on the fatty acyl moieties and long-chain bases, in addition to the phosphocholine polar headgroup. The CID of lithium, sodium, and potassium ion adducts of sphingomyelin yields very similar mass spectra. Lithiated, sodiated, and potassiated cyclophosphanes yield characteristic ions at *m/z* 131, 147, and 163, respectively, in addition to other abundant ions including $[M+Met-59]^+$, $[M+Met-183]^+$ and $[M+Met-189]^+$. Fragment ions providing information on the fatty acyl moieties and long-chain bases can also be observed (Hsu & Turk, 2000e). Glycosphingolipids adducted with lithium ions have also been studied using low-energy CID tandem mass spectrometry, where abundant fragment ions pertaining to long chain bases, fatty acids, and sugar moieties have been observed, and glycosphingolipids such as cerebrosides, di-, trihexosyl ceramides, and globosides can be unambiguously distinguished (Hsu & Turk, 2001a).

X. ANALYSIS OF UNSATURATED LIPIDS

The unsaturated fatty acyl chains of membrane lipids play a particularly important role in the overall structure, compartmentalization and stability of biological membranes (Quinn, Joo, & Vigh, 1989). The position of the double bond within the fatty acyl chains can perturb tight packing arrangements in the bilayer matrix due to induced changes in the dynamic motion and the melting point of lipids (Quinn, Joo, & Vigh, 1989). The analysis of unsaturated lipids and elucidation of the double bond position is thus a major challenge in the field of lipidomics. Traditionally, the analysis of unsaturated lipids has been performed by GC–MS. However, methyl ester derivatives of lipids must be prepared for GC-MS analysis, and the position of a double bond is very difficult to identify since double bonds tend to migrate along the aliphatic hydrocarbon chains in the electron ionization (EI) mode (Luthria & Sprecher, 1993; Mossoba et al., 1994). The conversion of methyl esters into 4,4-dimethyloxazoline (DMOX) derivatives facilitates the task of determining the location of double bonds along the fatty acyl chain of unsaturated lipids (Luthria & Sprecher, 1993; Mossoba et al., 1994). However, the use of GC-MS is limited to volatile analytes, and it is not used to elucidate the structure and double bond location of intact complex unsaturated lipids. The following sections summarize the most commonly employed mass spectrometry-based approaches for the analysis of unsaturated lipids and for the localization of their double bonds.

A. Acetonitrile Chemical Ionization

Moneti et al. (1996) found that acetonitrile could act as an effective reactant gas in positive-ion chemical ionization by GC-ion trap mass spectrometry for the identification of long-chain hydrocarbons. The resulting $C_3H_4N^+$ ions from acetonitrile CI can react with carbon–carbon double bonds, and can thus be used for the determination of the double bond position in unsaturated hydrocarbon chains (Moneti et al., 1997; Moneti et al., 1999; Oldham & Svatos, 1999; Van Pelt & Brenna, 1999; Van Pelt, Carpenter, & Brenna, 1999). The $C_3H_4N^+$ ions at *m/z* 54 correspond to (1-methyleneimino)-1-ethenylium ion (CH_2=C=N^+=CH_2), generated by self-reaction of acetonitrile under chemical ionization conditions, and form a charged covalent adduct across carbon–carbon double bonds in unsaturated fatty acid methyl esters. CID of the resulting $[M+54]^+$ ions yields two diagnostic ions formed by C–C bond cleavage at the position that is vinylic to each double bond (Michaud et al., 2002, 2003, 2005; Lawrence & Brenna, 2006). All of the above acetonitrile CI investigations were only performed on GC-MS platforms. Recently, Xu and Brenna (2007) developed a HPLC-compatible method named atmospheric pressure covalent adduct chemical ionization (APCACI), which uses acetonitrile as an APCI reagent. By this approach, structural information of TAG could be deciphered, including the location of double bonds along the fatty acyl groups and the position of acyl groups on the glycerol backbone.

B. Vicinal Hydroxylation

McCloskey and McClelland (1965) used osmium tetroxide (OsO_4) for vicinal di-hydroxylation of double bonds to determine olefinic sites in fatty acyls by GC-MS. More recently, Moe and Jensen (2004) developed a method using negative-ion ESI with low-energy CID to determine the position of the double bonds in unsaturated fatty acids after vicinal hydroxylation of the double bonds. The 1,2-dihydroxy derivatives of unsaturated lipids were generated in a mixture of chloroform/methanol/water (2:1:0.1, v/v) following the addition of OsO_4 and *N*-methylmorpholine-*N*-oxide under nitrogen (Moe & Jensen, 2004; Moe et al., 2004, 2005). In a triple-quadrupole instrument the resulting deprotonated fatty acids are obtained after α-cleavages relative to the hydroxyl groups under low-energy CAD, producing structurally informative ions called $[\alpha'_n]^-$ and $[\alpha_n]^-$ (where *n* indicates the position of the hydroxyl group and hence the double bond location) (Moe & Jensen, 2004). The $[\alpha'_n]^-$ ions are formed after cleavage distal to the hydroxyl groups. As a result the charge is retained at the carboxylate side, and the formed $[\alpha'_n]^-$ ions contain one or two hydroxyl groups and the carboxylate group. The $[\alpha_n]^-$ ions originate from the transfer of a hydroxyl proton to the carboxylate group with subsequent cleavages proximal to the hydroxyl groups. These ions contain one or two hydroxyl groups and the alkyl terminus (Moe & Jensen, 2004). This method is useful for the analysis of the double bond position of free fatty acids (Moe & Jensen, 2004) and phospholipids (Moe et al., 2004, 2005). OsO_4, as a catalyst for vicinal hydroxylation of double bonds in the olefins, reacts with virtually all olefins (Dupau et al., 2002). However, it also reacts slowly with other common organic functional groups (Dupau et al., 2002), which results in the formation of by-products. The efficiency of vicinal hydroxylation should be further evaluated.

C. Ozonolysis

Ozonolysis has been used to determine the double bond position of unsaturated natural products for many years (Hamberg, 1971). Ozone reacts with carbon–carbon double bonds to produce an initial 1,2,3-trioxolane (molozonide). The resulting 1,2,3-trioxolane can convert to the more stable 1,2,4-trioxolane (Criegee ozonide) *via* carbonyl oxide and aldehyde intermediates through two-step rearrangements (Criegee, 1975). As described by Harrison and Murphy (1996), unsaturated glycerophospholipids deposited onto a glass surface can produce almost quantitative conversion of double bonds to ozonides using an offline process by exposure to a stream of ozone. In the MS/MS spectra of these adducts in either positive or negative ion mode, ω-aldehyde and ω-carboxylate acyl species ions could be observed. These product ions are formed by homolytic cleavage of the peroxide bridge of the 1,2,4-trioxolane (Criegee ozonide) ring, followed by rearrangement (Harrison & Murphy, 1996). Those fragment ions could be used to uniquely identify the double bond position. Thomas, Mitchell, and Blanksby (2006) developed the online in-source corona discharge ozonolysis method by using oxygen as the electrospray nebulizing gas in combination with high electrospray voltages to initiate the formation of ozone-producing plasma. They further developed this method and named it ozone electrospray ionization mass spectrometry (OzESI-MS). This method could be used for the analysis of unsaturated lipids such as acidic and neutral glycerophospholipids, sphingomyelins, and TAG, and the conversion of olefinic bonds to ozonides could be observed regardless of the polarity, the number of charges, or the adduct ion (e.g., deprotonated, protonated, sodiated, and ammoniated ions) (Thomas et al., 2007). The ozone/oxygen mixture produced by an ozone generator is introduced into a linear ion trap, where unsaturated lipid ions can react with ozone vapor. This novel analytical technique is called ozone-induced dissociation (OzID) (Thomas et al., 2008). Two primary product ions from each carbon–carbon double bond within the unsaturated lipids, aldehyde and Criegee ions, are formed by gas-phase ion-molecule reactions between unsaturated lipid ions and ozone, which could be used for the elucidation of the unsaturation position within the precursor ion.

However, both OzESI and OzID require additional special apparatus such as ozone generators and introduction connections, which are not readily interfaced with commercial mass spectrometers. Ozone is a toxic and corrosive gas, and ozone must not vent into the laboratory when it is introduced into the ion source or into the ion trap. Only ozone-compatible materials must be used in the connections for ozone introduction, and rubber is not suitable. The O-rings employed for sealing inside the mass spectrometer are made of rubber, and long-time ozone introduction might affect the O-rings. The reaction process of ozonidation is very fast, but incomplete, and the resulting by-products might make the MS or MS/MS spectra more complicated. It is in the best interests of the reader to be aware of the fact that the derivatization of lipids with corrosive reagents or highly reactive chemicals such as ozone may annul the warranty of most mass spectrometers.

D. Adduct Formation

Duffin, Henion, and Shieh (1991) have shown that acylglycerols, dissolved in chloroform/methanol (70:30, v/v) containing alkali-metal or ammonium salts, yield $(M + Na)^+$ or $(M + NH_4)^+$ ions during electrospray ionization (ESI). They observed that ion signals resulting from acylglycerols that contained unsaturated fatty acid chains were more abundant than those of the saturated chains. They also found that the dissociation energy required for MS/MS fragmentation of sodiated acylglycerols was greater than that of ammoniated ones in a triple quadrupole mass spectrometer (Duffin, Henion, & Shieh, 1991). The ammonium adduct ions could yield interesting fragmentation patterns suitable for the characterization of acylglycerols under low-energy CID conditions (∼50 eV) (Duffin, Henion, & Shieh, 1991; Zehethofer & Pinto, 2008). In the resulting MS/MS spectra of ammoniated TAG under low-energy CID, the major product ions were the protonated TAG formed by the loss of ammonia, and DAG ions formed by the loss of one fatty acid moiety. Under higher-energy CID (∼130 eV), monoglycerides and acylium ions and carbon–carbon bond cleavage could be observed, but the position of the double bond could not be established because of double bond migration (Duffin, Henion, & Shieh, 1991; Zehethofer & Pinto, 2008).

Although MS^3 spectra of sodiated lipids reveal some structural information, the product ions formed cannot provide the information of double bond location due to double bond migration (Hsu & Turk, 2006; Smith et al., 2008). Using multiple-stage mass spectrometry with a linear ion-trap instrument for the lithiated, dilithiated, or trilithiated adduct ions formed by ESI, the total structure of glycerophospholipids was characterized (i.e., the polar headgroup, the position of double-bond(s) along the fatty acyl substituents, and the positions of acyl groups on the glycerol backbone) (Hsu & Turk, 2008b). The position of double bond(s) of unsaturated long-chain fatty acids was also located using this approach for the dilithiated adduct ($[M - H + 2Li]^+$) ions (Hsu & Turk, 2008a). As previously reported, the copper-containing ions of fatty acids (e.g., $[M - H + Cu^{II}]^+$) are also used for double bond localization and *cis*/*trans* isomers distinction (Afonso et al., 2005).

E. Negative Fast Atom Bombardment

As described by Tomer, Crow, and Gross (1983), negative FAB-MS/MS was applied for the structural analysis of unsaturated fatty acids. It is also used to distinguish the *cis* and *trans* isomers of mono-unsaturated fatty acids because the definitive fragmentation is at the bond allylic to the double bond in the distal position on the chain (Jensen et al., 1990; Bryant et al., 1991; Matsubara & Hayashi, 1991). Recently, Ji et al. (2007a,b) exploited this technique to locate the double bond position and to distinguish the geometric isomers of long-chain monounsaturated fatty acids. Under negative FAB ionization the monounsaturated fatty acids produce spectra containing two intense clusters of peaks with three intervening weak ones *via* charge remote fragmentation. The pattern of the resulting two intense peaks corresponds to cleavage allylic to the double bond (Tomer, Crow, & Gross, 1983; Jensen et al., 1990; Matsubara & Hayashi, 1991; Ji et al., 2007a,b). This technique has the advantage of simultaneously finding the localization of double bond and diffentiating *cis* and *trans* isomers. However, the drawback of this approach is that it is limited to analysis of mono-unsaturated fatty acids.

XI. LIPIDOME QUANTIFICATION

In the lipidomics field mass spectrometry is employed not only for qualitative analyses (i.e., lipid structure elucidation) but also for quantitative purposes. The latter can imply measuring the

absolute concentrations of particular lipid species, or the relative abundances of particular lipid species formed in two similar organisms under different physiological conditions (e.g., ''normal healthy'' and ''diseased''), or the relative abundances of different molecular species within a lipid class, in complex mixtures (Kerwin, Tuininga, & Ericsson, 1994; Brugger et al., 1997; Lehmann et al., 1997; Liebisch et al., 2002; Zacarias, Bolanowski, & Bhatnagar, 2002). Unlike the field of quantitative proteomics, where several quantitative techniques have been established and broadly accepted, a general consensus is yet to be reached for the most appropriate quantification approach for lipids.

Quantitative analyses of lipids are mostly performed on a case-by-case basis. The quantitative analysis of a lipidome is generally divided into global and targeted lipidomics. Global lipidomics allows the identification and relative quantification of numerous molecular lipid species across multiple structural classes in total lipid extracts. This strategy includes shotgun lipidomics, which utilizes direct infusion of lipid extracts into a mass spectrometer (Han & Gross, 2005; Schwudke et al., 2007b), and liquid chromatography-mass spectrometry (LC-MS) methods (Houjou et al., 2005; Laaksonen et al., 2006). Targeted lipidomics, on the other hand, permits the quantitative analysis of a single or a few lipid species within a specific lipid class (Murphy et al., 2005; Krank et al., 2007).

It is important to note that the instrument response can vary, sometimes widely, between different classes of lipids (Koivusalo et al., 2001). In addition, identical lipid species exhibit different behaviors when analyzed using different types of mass spectrometers or when analyzed using the same mass spectrometer under different experimental parameters (DeLong et al., 2001; Larsen et al., 2001). Therefore, the relative intensities of signals corresponding to different lipid species in a mass spectrum do not directly reflect their molar abundances and concentrations. In particular, it is impractical to use mass spectrometry to directly estimate the relative abundances of different phospholipid subclasses due to the differences in the ionization efficiencies of their polar headgroups (Koivusalo et al., 2001). The gross estimation of the relative abundance of lipid species within a specific phospholipid subclass among different samples is less problematic, provided the responses of all lipid species are normalized with appropriate internal standards (DeLong et al., 1999; Rouzer et al., 2006).

It has been documented that the instrument response for glycerophospholipids decreases with increasing fatty acyl chain length (Brugger et al., 1997; Koivusalo et al., 2001). However, as the concentration is lowered to 5 pmol/μl, the differences between the short and long chain species are diminished (Koivusalo et al., 2001). It is also worth noticing that no isotope correction has been taken into consideration in these studies. When the $M+1$ and $M+2$ isotopologues of the molecular ions are included in the response, it has been confirmed that glycerophospholipids of the same class with different factty acyl chain length exibit similar responses when subjected to ESI-MS analysis (Han & Gross, 2005; Ejsing et al., 2006). It should be noted, however, that the ionization efficiency of TAG molecular species greatly relies on their fatty acyl chain, that is, the total number of carbon atoms of the three fatty acyl chains and the total number of double bonds. This is because of the absence of a dominant polar headgroup in their molecular structure (Han & Gross, 2001, 2005).

Generally quantitative comparisons between lipid species are carried out using the molecular ions. It has been demonstrated that accurate quantification can also been performed with respect to certain fragment pathways, which are recorded by neutral loss and precursor scans, provided the collisional energy is optimized (Han & Gross, 2005; Ejsing et al., 2006).

In general, when using direct infusion ESI-MS (including the intra-source separation approach), the peak intensity is proportional to the concentration of a lipid species in the particular mixture being analyzed. However, when front-end separation is applied (e.g., GC or liquid chromatography) so as to minimize matrix effects such as ionization suppression, the integrated peak area of a lipid species is generally considered to be proportional to its quantity present in complex mixtures of similar origin. As with most quantitative methods it is crucial to work in the linear range of the quantification methodology.

A. Absolute Lipid Quantification

The absolute quantification of specific lipid species relies on the availability of internal standards preferably as isotopically labeled forms although closely related compounds (homologues or simple structural analogues) can be used. This strategy is applicable to a limited number of lipids in complex mixtures (Liebisch et al., 2002; Whitehead et al., 2007). The absolute concentration of a lipid species is then determined by spiking in known amounts of lipid standards labeled with stable isotopes (stable-isotope dilution) (Haroldsen, Clay, & Murphy, 1987). The isotopically labeled lipid standard and its normal (''native'' or ''wild type'') counterpart exhibit identical responses in mass spectrometers as they have identical physicochemical properties if the isotopic labeling involves heavier atoms (usually ^{13}C). Then the original concentration of the target lipid species can be determined by comparing the peak heights or peak areas of the paired labeled and native lipids. However, if ^{2}H labeling is used together with MS/MS analysis, kinetic isotope effects on the fragmentation reactions can sometimes lead to differences in the responses of native and ^{2}H-labeled compounds, in which case complete calibration curves must be constructed.

The absolute concentrations of lipids can also be determined without internal standards using standard addition methods. In brief, known amounts of standards of the target lipid are sequentially spiked in the complex mixture. The respective mass spectrometry responses are used to determine by extrapolation the original concentration of the lipid species of interest (Fig. 16) (Liebisch et al., 2002; Whitehead et al., 2007).

B. Relative Lipid Quantification

In many cases, comprehensive lipid profiling comparison between wild type and mutant, or basal and stimulated, or normal and disease samples, is required (Ivanova et al., 2001; Ekroos et al., 2002; Rouzer et al., 2006). Mass spectrometry is the method of choice to fulfill this demanding task. This approach requires comparison of multiple mass spectrometric analyses using different scan modes. Uncontrolled fluctuations in signal intensity due to the lipid extraction and analytical processes can be an issue as each sample is individually processed. Fortunately, non-naturally occurring lipid standards can be spiked into the samples at different stages of sample processing, which facilitates the correction of signal intensity. As the ionization

efficiency of different glycerophospholipids differs significantly, normally at least one lipid standard belonging to each phospholipid subclass is spiked into samples to serve as internal standard for its respective subclass. When the analytical strategy includes both positive and negative ESI-MS, internal standards for normalization that can be detected in both positive and negative modes (e.g., PE and PS) must be included (Ivanova et al., 2001).

C. Stable Isotope Labeling through Metabolism

Isotopic labeling is also employed for the relative quantification of a total lipidome, and for tracking the fate of lipids *via* different metabolic pathways (Bleijerveld et al., 2006). Appropriate chemicals labeled with stable isotopes are introduced into the growth medium during cell culture and, depending on the target metabolic pathway, either all or a fraction of the lipids are labeled. For instance, Ekroos and co-workers cultured cells with ^{13}C-labeled glucose for 24 hr; the lipid extracts from these cells were a mixture of isotopically labeled endogenous lipids, useful as a comprehensive internal standard for quantitative profiling of phospholipids (Ekroos et al., 2002). Delong and co-workers introduced D_4-ethanolamine and D_9-choline chloride into the cell culture medium to distinguish the metabolic product PC from different metabolic pathways (DeLong et al., 1999). Metabolically labeling of mouse neuronal cells with [^{3}H]inositol allowed the profiling of lipids involved in intracellular signaling (Wenk et al., 2003). Hence, stable isotopic labeling through metabolism is a promising approach for the relative quantification of lipidomes. Current limitations, however, include the lack of a wide range of isotopically labeled standards that conform to the structure of lipids of interest.

XII. BIOINFORMATICS IN LIPIDOMICS

Mass spectrometry-based analytical methods are powerful for the qualitative and quantitative analysis of the entire spectrum of cellular lipids. The introduction of bioinformatics into lipidomics has revolutionized this research field and solved challenging problems associated with the management and analysis of the structurally diverse lipids. The tools for lipidomics, a subclass of the metabolomics, are still emerging. This section describes the contributions of bioinformatics, which entails the creation and advancement of databases, algorithms, computational and statistical techniques, to the analysis and interpretation of analytical lipidomics data.

A. Lipid Classification and Databases

The lack of a universally accepted lipid classification scheme has led to the creation of several lipid-oriented databases that differ in organization and mission. The LipidBank (Yasugi & Watanabe, 2002; Yetukuri et al., 2008), LIPIDAT (Caffrey & Hogan, 1992), Lipid Library (http://www.lipidlibrary.co.uk/), Cyberlipids (http://www.cyberlipid.org/) provide information on lipid structures and their chemical and biological functions. The LipidBank has classified lipids into 17 categories covering a wide variety of animals and plants. The LIPIDAT is composed mostly of phospholipids and their thermodynamic data. The Lipid Library concentrates on fatty acyls and their derivatives. In particular, the Cyberlipids database has a broader approach, and contains classification information on steroids and terpenes (Fahy et al., 2007a). Online resources for lipids classification databases are listed in Table 2.

A US-based consortium called the LIPID Metabolites and Pathway Strategy (LIPID MAPS) has taken a more chemistry-based approach and has proposed a classification scheme for lipids. This system classifies lipids into eight categories listed above (Fig. 1) (Schmelzer et al., 2007), and includes a unique alphanumeric 12-character lipid identifier that provides information on the source database and the lipid category, class, and subclass. In fact, the LIPID MAPS identifies and encodes information on 1.68 million lipids, and this system allows straightforward database storage/retrieval, and bioinformatics manageability. The LIPID MAPS consortium has developed two databases: the LIPID MAPS Structure Database (LMSD) and the LIPID MAPS Proteome Database (LMPD) (Sud et al., 2007). LMSD uses the LIPID MAPS scheme for drawing lipid structures, and contains information on more than 10,000 lipid structures compiled from the LipidBank and LIPIDAT, LIPID MAPS consortium, lipids identified by LIPID MAPS experiments, and computationally generated structures for lipid classes (Fahy et al., 2007b). The LMSD also maintains the original identification number of the lipid source to enable cross-references, and LMSD lipid structures are deposited onto the PubChem database with a link to the substance ID (SID). On the other hand, LMPD focuses on proteins and genes associated with all lipids (Sud et al., 2007), and contains information on more than 2,500 proteins associated with at least one of the eight lipid classes (Cotter et al., 2006). These associations are based on the GO/KEGG annotation, and information on gene association is obtained from NCBI EntrezGene, Uniprot accession and GO/KEGG annotation.

B. Data Processing and Identification

The increasing demand for better data processing has generated a number of software packages either from commercial sources or the open source community. Tables 3 and 4 provide non-exhaustive lists of commercially available and free software packages, respectively, that can be used to process lipidomics data. LipidNavigator (http://lipidsearch.jp/LipidNavigator.htm) is a free software used for lipid analyses. This program was developed by Mitsui Knowledge Industry in collaboration with the Taguchi laboratory from the University of Tokyo. LipidNavigator is a high-throughput web tool and automated system for the identification of glycerophospholipids and sphingolipids that uses as input various types of mass spectrometry raw data such as Q-TRAP4000™ from Applied Biosystems, Q-Tof micro™ from Micromass and Finnigan™ LTQ from Thermo. LipidNavigator consists of three components: a mass navigator (a visualization and data analysis module), a lipid search tool (the core module for lipids identification), and a lipid database (storing theoretical masses and lipid identification results). A beta version of the lipid search tool is also available on the LipidNavigator web page.

TriglyAPCI is another free program developed by Cvacka and associates using Microsoft Visual basic 6.0 to interpret mass spectrometry data of TAG (Cvacka et al., 2006). This software identifies each ion in the mass spectrum obtained from LC-MS analyses as a fragment or a molecular adduct, and then searches for a relationship among fragment ions to deduce possible TAG structures. One of the founding groups of the Lipid MAPS

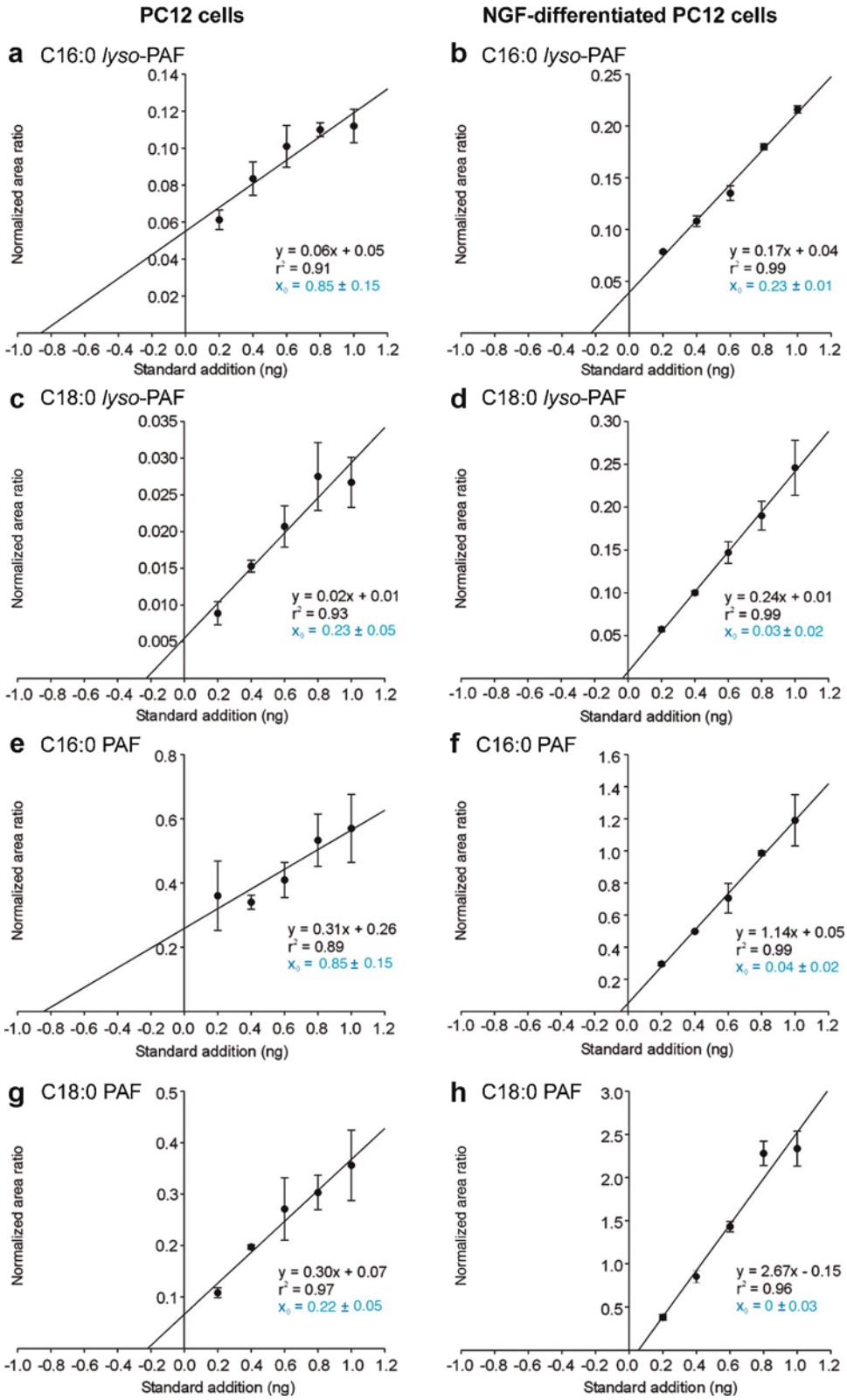


FIGURE 16. Quantification of PAF species from PC12 cells and PC12 cells differentiated to a neuronal phenotype with nerve growth factor (NGF). Lipid extracts from these cells were spiked with 200, 400, 600, 800, or 1,000 ng of C16:0, C18:0 PAF and C16:0, C18:0 lyso-PAF, and analyzed in positive ion mode followed by a precursor ion scan for masses having a *m/z* 184.0 MS/MS fragment. Each standard addition was repeated in triplicate. Corresponding peaks of interest were identified (*m/z* 482.4, 524.3, 510.4, 552.4) and normalized against the internal standard (496.5). Native PAF species from the cell samples were calculated from the x-intercept using linear regression. Data points depict mean of triplicate measurements ± standard deviation. This figure is adapted from Whitehead et al. (2007). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

TABLE 2. Online resources for lipid classification and databases

Resource	URL	Country	Comments
LIPID MAPS	http://www.lipidmaps.org/	U.S.	Lipid classification scheme
LipidBank	http://lipidbank.jp/	Japan	Covers lipids from animals to plant.
LIPIDAT	http://www.lipidat.ul.ie/	U.S.	Contains phospholipids and their thermodynamic data
LMSD	http://www.lipidmaps.org/data /structure/index.html	U.S.	Composed of structures and annotations
LMPD	http://www.lipidmaps.org/data /proteome/index.html	U.S.	proteins and genes associated with lipids
Cyberlipid	http://www.cyberlipid.org/	France	Lipid database that includes isoprenoid-derived molecules.
SphinGOMAP	http://sphingolab.biology.gatech.edu/	U.S.	Pathway map for sphingolipid biosynthesis
Lipid Library	http://www.lipidlibrary.co.uk/	U.K.	Composed of information about lipid chemistry, biology and analysis.
KEGG	http://www.genome.jp/kegg/pathway.html	Japan	Manually drawn pathway maps that included fatty acid biosynthesis and degradation, sterol metabolism, and phospholipids pathway.
GOLD	http://gold.uni-graz.at/index.html	Austria	Composed of annotated pathway and curated data set.

Consortium has developed software for analyzing large amounts of mass spectrometry data (Ivanova et al., 2004). This software makes use of the S-Plus version 3.3 for Windows programming suite, and employs algorithms for data normalization to allow statistical comparison of different spectral patterns from different replicates. To this end, the software converts signal intensities to standard units (-1, 0, $+1$) to sort and rank data. A Shewhart control chart is then constructed for each peak to ensure stability of analysis over a specific time frame. This software can compare mass spectrometry data collected under different conditions, and allows the construction of lipid arrays that highlight the changes in lipid profiles under various biological conditions.

The Spectrum Extraction from Chromatographic Data (SECD) and Lipid Mass Spectrum Analysis (LIMSA) represent another generation of free softwares used to process both positive and negative ion mode data, as well as to perform lipid identification based on MS/MS spectra (Haimi et al., 2006). SECD extracts LC-MS data, uses the NetCDF format for input data, and displays the results as a pseudo-3D map. In this map retention time, the *m/z* value and intensity are represented by the *x*-axis, *y*-axis and a gray scale, respectively. The SECD software allows the selection and analysis of subregions of the chromatogram. The LIMSA software is a dynamic library that can be used alone, for batch processing, or in combination with other softwares as it can use the SECD output as its input data. This software allows identification, deconvolution and quantification of lipids. Lu et al. (2005) introduced the cognoscitive-contrast-angle algorithm and databases (COCAD) to improve correct identifications of polyunsaturated lipid mediators using known standard MS/MS or LC-UV-MS/MS data. COCAD identification involves empirical fragmentation and peak intensity modification. Katajamaa et al. (Haimi et al., 2006; Katajamaa, Miettinen, & Oresic, 2006) disclosed a Java-based toolbox, platform independent software called MZmine. This data processing software implements algorithms for spectral filtering, peak picking detection, 2D plot visualization, chromatographic alignment, and normalisation. This team has recently released a new version of MZmine that implements automated processing, enhanced secondary peak picking, and two post-processing methods for non-linear mapping (Katajamaa, Miettinen, & Oresic, 2006). MZmine is capable of distributing the computation to multiple processors or computers, and supports import of NetCDF and mzXML raw data formats. Smith and co-workers introduced software packages called XCMS that implement a non-linear retention time alignment, matched filtration, peak detection, and peak matching (Smith et al., 2006). This software package also supports LC-MS data stored in NetCDF, mzXML, and mzData files. The XCMS allows data processing from various mass spectrometers, including Q-Tof micro™ from Waters, LTQ from Finnigan and 1100 LC/MSD from Agilent. Unlike metAlign that also includes peak selection and the option of non-linear, iterative alignment similar to XCMS, this software

TABLE 3. Commercially available softwares for lipidomics data processing

Name	Vendor	Main application field
Lipid Profiler	MDS Sciex	Lipidomics with LC-MS
Bluefuse	BlueGnome	Metabolomics with MS and NMR
MarkerLynx	Waters	Metabolomics with LC-MS
Metabolic Profiler	Bruker BioSpin	Metabolomics with MS
metAlign	Plant Research International	LC-MS and GC-MS
MS Resolver	Pattern Recognition Systems	LC-MS and GC-MS
Profile	Phenomenome Discoveries	Metabolomics with MS
MarkerView	Applied Biosystems	Metabolomics with LC-MS

TABLE 4. Free softwares for lipidomics data processing

Name	License	Main application field
MZmine	GNU General Public License	Metabolomics with LC-MS and GC-MS
LIMSA	GNU General Public License	Lipidomics with LC-MS and SECD data
SECD	Freely available	Metabolomics with LC-MS
TriglyAPCI	Freely available	Lipidomics with LC-MS
MSPECTRA	Freely available	Lipidomics with LC-MS
XCMS	GNU General Public License	Metabolomics with LC-MS and GC-MS

is not based on any published algorithms, and it is not open source (Smith et al., 2006) (i.e., not amenable to outside modification or inspection). MDS Sciex has developed a software called Lipid Profiler that has been used in several lipidomics studies (Ejsing et al., 2006; Linden et al., 2006; Lelliott et al., 2007). Lipid Profiler implements algorithms for isotope correction, identification, and quantification of lipid species detected by multiple precursor ion scanning (Ekroos et al., 2002; Ejsing et al., 2006). This software is written in Visual Basic and used a standalone Microsoft Access lipid database.

C. Tools for Lipidomics

The chemical structures of large and complex lipids are difficult to draw. To tackle this issue David Weininger initiated the Simplified Molecular Line Entry Specification (SMILES) project in the late 1980s (Weininger, 1998). In the SMILES format the lipid structure is represented as a compact graph with nodes as atoms and edges as bonds. Although this format is accurate in terms of connectivity, valence, and chirality (Fahy et al., 2007b), visual recognition and comparison of lipids are complicated since the SMILES format lacks a representation in 2D coordinates. The LIPID MAPS consortium developed multiple drawing programs and mass spectrometry prediction tools coupled to drawing tools to settle this issue (Fahy et al., 2007b). Specifically, the LIPID MAPS website includes information on the most commonly found lipids in mammalian cells, and presently contains six drawing programs that can precisely depict the structures of fatty acyls, glycerolipids, glycerophospholipids, cardiolipins, sphingolipids and sterols. The LIPID MAPS drawing tool is composed of a ''core'' structure, and several pull-down menus that allow the end-user to choose from a list of headgroups and *sn*-1/*sn*-2 fatty acyl side chains. After the end-user specifies the details of the desired structure, the lipid structure may be visualized in the web browser as a Java-based MarvinView applet, or with Chemdraw ActiveX/Plugin.

D. Bioinformatics Resources for Lipid-Related Pathways

The Kyoto Encyclopedia of Genes and Genomes (KEGG) as a bioinformatics resource links genomes to life and the environment. The KEGG consortium maintains one of the most commonly used reference databases for lipid pathways. This collection includes information on fatty acid biosynthesis and degradation, as well as information on sterols and phospholipids. In addition, the consortium has developed another collection called KEGG Brite that includes a section devoted to lipids where the user can select a lipid of interest and view reactions and pathways associated with that lipid. SphinGOMAP is another network database that contains information on more than 400 sphingolipid and glycosphingolipid species (Fahy et al., 2007a).

Bioinformatics resources are imperative in lipidomics to manage and integrate experimental data at several levels: (i) definition of lipid classification and ontologies, (ii) relational database design, (iii) capture and automated pipelining of experimental data, (iv) efficient management of metadata, (v) development of lipid-centric search tools, (vi) analysis and visual display of results, and (vii) integration of the lipid knowledge base into biochemical pathways and interactive maps.

XIII. LIPIDOMICS APPLICATIONS

The implication of lipidomics in various diseases has been rooted in the literature for over 20 years (Gross, 1984, 1985). Lipid imbalances are associated with the metabolic syndrome, lipodystrophies, neurological disorders (Alzheimer's, Parkinson's, Niemann-Pick, multiple sclerosis, Huntington, amyotrophic lateral sclerosis, schizophrenia, bipolar disorders, and epilepsy) and central nervous system injury (stroke, traumatic brain injury; and spinal cord injury). The studies of cellular lipidomes have provided insights into disease states through the comprehensive quantification of altered cellular lipids (e.g., lipid classes, subclasses, and individual molecular species) in response to gene deletions, gene replacements or gene mutations in model organisms, and through examination of the kinetics of lipid metabolism/signaling and the interactions of lipids with cellular proteomes (Hazen et al., 1993; Han et al., 1996, 2000, 2002; Han, Holtzman, & McKeel, 2001). Mass spectrometry has played an instrumental role in decoding such information, which is of paramount importance for the development of novel therapeutics for lipid-driven disorders.

A. Lipidomics and Cell Signaling

Mass spectrometry-based methods have been successfully used for the analysis of lipids implicated in signal transduction pathways (e.g., phosphoinositides, LPA, sphingosine 1-phosphate, DAG, and ceramide). Stable isotope labeling methods have been used to analyze glycerophospholipid synthesis, and to gain insights into the dynamics of signaling pathways in most cell types. Much of the lipidomic research in signaling revolves around phosphoinositides, eicosanoids, and steroids (Wakelam, Pettitt, & Postle, 2007). Mattila et al. (2008) have successfully decoded the identity of lipids and metabolites with relevance to adipose tissue metabolism and insulin signaling by ultra performance liquid chromatography (UPLC)-mass spectrometry and GCxGC-TOF mass spectrometry. Positive mode ESI has been successfully used for the quantification of 28 DAG molecular species in RAW 264.7 macrophage cells after stimulation of a G-protein coupled receptor with PAF (Callender et al., 2007). Mass spectrometric analyses revealed that purified iPLA$_2\gamma$ hydrolyzes saturated or monounsaturated aliphatic fatty

acyls from the *sn*-1 or *sn*-2 positions of glycerophospholipids, and also liberates arachidonic acid from the *sn*-2 position of plasmenylcholine substrates. In contrast, incubation of $iPLA_2\gamma$ with 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine resulted in the rapid release of palmitoyl and the selective accumulation of 2-arachidonoyl LPC, which was not metabolized further by $iPLA_2\gamma$. Furthermore, incubation of purified rat hepatic peroxisomes with $iPLA_2\gamma$ results in the selective accumulation of 2-arachidonoyl LPC, which was shown to be a natural product in human myocardium (a tissue exhibiting robust $iPLA_2\gamma$ expression) by tandem mass spectrometry. Since 2-arachidonoyl LPC represents a key branch point in eicosanoid signaling (e.g., arachidonic acid, 2-arachidonoylglycerol), these results reveal the usefulness of mass spectrometry-based approaches in tracking signalling pathways and second messengers production in response to various cellular stimuli (Yan et al., 2005).

Using ESI-MS, Milne et al. (2005) resolved and identified 28 PIP and PIP_2 species, and 8 PI trisphosphate (PIP_3) species in RAW 264.7 macrophages or in primary murine macrophage cell extracts. Moreover, analysis of PIP profiles after agonist stimulation of cells revealed the generation of differential PIP_3 species. The ability to analyze the fatty acyl chain composition of signaling lipids initiates new venues for investigation of phosphoinositide signal transduction cascades. ESI-MS is also efficient for the detection of elevated concentrations of PIP_2 in human fibroblasts from patients with Lowe syndrome, a genetic disorder that affects phosphoinositide metabolism (Wenk et al., 2003). Disrupted PIP_2 balance in the brain was also recently suggested to account for the amyloid-β peptide induced synaptic dysfunction, which is linked to cognitive deficits in Alzheimer's disease (Berman et al., 2008). Therefore, mass spectrometric analysis of phosphoinositides represents a powerful diagnostic tool for human diseases that involve defective phosphoinositide metabolism.

B. Lipidomics and Apoptosis

Oxidized phospholipids play an important role in execution of the mitochondrial stage of apoptosis and clearance of apoptotic cells by macrophages. ESI-MS and fluorescence high-performance liquid chromatography have been employed to successfully identify/quantify oxidized glycerophospholipids generated during apoptosis. This method revealed selective oxidation of CL in the mitochondria, and PS outside of the mitochondria. The application of lipidomics was performed under various conditions in both *in vitro* and *in vivo* models of apoptosis and/or inflammation. This approach proved powerful for the identification of oxidized CL and PS molecular species in the tested models (i) following cytochrome c/H_2O_2 induced oxidation or experimental traumatic injury of rat brain, (ii) in postmortem brain samples from patients with Alzheimer's disease, and (iii) in the small intestine of γ-irradiated mice (Tyurin et al., 2008; Tyurina et al., 2008). In particular, oxidized molecular species of CL and PS may be potential biomarkers of γ-irradiation-induced intestinal apoptosis *in vivo*.

C. Lipidomics and Disease Diagnosis

Lipid homeostasis is fundamental to health maintenance, and lipid defects are central to the pathogenesis of important and devastating diseases. Altered sulfatide metabolism, trafficking and homoeostasis are the earliest clinically recognizable pathological features of Alzheimer's disease. A recent shotgun lipidomics study investigated the effect of sulfatide supplementation on neuroblastoma cells. The authors showed time- and dose-dependent accumulations of sulfatide, ceramide and sphingosine in these cells. Combining shotgun lipidomics with subcellular fractionation revealed that sulfatide accumulated predominantly in the lysosomes, whereas the increased ceramide content was generated in the endosomes mainly *via* the action of β-galactosidase, which hydrolyses sulfatide to ceramide without a prior desulfation step. The accumulation of sulfatides and ceramides in neuroblastoma cells resulted in apoptosis as revealed by mitochondrial membrane depolarization, PS translocation and by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay (Zeng et al., 2008). These findings were also extended to primary neuron cultures, and provide a mechanistic link between abnormal sulfatide metabolism, cell apoptosis, and neuronal diseases. A recent lipidomic study revealed that higher levels of arachidonic acid and its metabolites, as a result of increased activity of group IV isoform of PLA_2, contribute to the pathogenesis of Alzheimer's disease (Sanchez-Mejia et al., 2008).

Static imaging time-of-flight secondary ion mass spectrometry (TOF-SIMS) has been successfully used to map lipid disorders in human muscles of a patient suffering from dystrophy (Brunelle & Laprevote, 2009). This method also proved useful for the study of skeletal muscle lipid accumulation associated with obesity, insulin resistance and type 2 diabetes (Magnusson et al., 2008), as well as for the detection of cholesterol in macrophages (Piehowski et al., 2008), which accumulate in atheromatous plaques (Schmitz & Grandl, 2008). Shotgun lipidomics has been recently employed to identify alterations in neutral lipid metabolism in diabetic cardiomyopathy. Substantial accumulations of TAG and altered myocardial eicosanoid metabolism and signaling have been successfully identified in the diabetic heart (Gross & Han, 2009).

Wheeler et al. (2008) have studied multiple sclerosis using ESI-MS/MS. They characterised the modification of sphingolipids, glycerophospholipid and sterol in normal appearing gray and white mater of multiple sclerosis brains during periods of disease activity or inactivity. Their results reveal that, the quantity of total lipids in gray and white matter is not affected in multiple sclerosis brains, a reduction in sphingolipids and a gain in glycerophospholipids content is prominent during the active state of the disease. The authors suggested that the cross talk between the sphingolipids and glycerophospholipid metabolic pathways, which results in the conversion of sphingolipids to ceramides to glycerophospholipids, is the underlying mechanism of multiple sclerosis. Schwarz et al. (2008) have studied schizophrenia and bipolar disorder, two conditions that share similar symptoms and, up to now, do not have distinct disease markers. Since lipid modifications are associated with both disorders, the authors profiled free fatty acyls, PC and ceramides in post-mortem brains from schizophrenia and bipolar patients in an attempt to find distinct disease biomarkers. Their findings demonstrated that white matter PC species increase in bipolar disorder, and decrease in schizophrenia. However, the observed lipid modifications in schizophrenic brains were not reproduced in red blood cells. Mass spectrometry, therefore, opens the way to an unbiased post-mortem diagnosis of schizophrenia and bipolar disorder.

Lipidomic studies play an essential role in defining the biochemical mechanisms of lipid-related diseases through identifying alterations in cellular lipid metabolism, trafficking and homeostasis. However, the lipidome varies with gender, age and lifestyle. In early childhood, multiple changes in circulating lipids composition reflect rapid developmental and environmental changes (Nikkila et al., 2008). In older individuals, the lipidome is characterized by a decrease in the proportion of antioxidant lipids, thus reflecting the increase in oxidative stress with age (Maeba et al., 2007). Therefore, the development of effective preventive medicine requires the identification of early diagnostic and prognostic biomarkers.

XIV. ACCOMPLISHMENTS AND CHALLENGES

Research in the lipidomics field is an integral part of the ''systems biology'' approach, which relies on collaborative multidisciplinary research networks comprising biologists, physicists, computational scientists, chemists, and mathematicians. The establishment of COBRE, Lipid MAPS Consortium, European initiatives for decoding lipids through genomic approaches, and Genomics Of Lipid-associated Disorder (GOLD) project have revolutionized the lipidomics research field, and provided universal platforms to provide a snapshot of the cell lipidomes, which is crucial for disease diagnosis, toxicity assessment, and treatment efficacy.

The tremendous success of the lipidomics field, in terms of structural analyses, profiling and quantification of complex lipids, is faced with the lack of a single uniform analytical platform for the analysis of all lipids. This challenge can be alleviated with the introduction of chip-type technologies into the lipidomics field to entail simultaneous analysis of different lipid classes. However, the immense structural diversity of lipids (i.e., all potential enantiomeric, stereoisomeric and regioisomeric lipid species that can be identified) is still a great challenge to overcome along with the availability of commercial lipid standards for identity authentication and quantification of lipid species.

The ability to localize lipids in cells is yet another challenge facing lipidomic studies that can be surmounted by the availability of sophisticated histochemical, cytochemical and physical imaging techniques, as well as cell fractionation protocols. Technologies must also be developed to enhance/enrich local concentrations of low abundance lipid species. This will allow thorough examination of various lipid biosynthetic, metabolic and signaling pathways, as well as explict characterization of lipids under normal and disease conditions.

Analytical lipidomics datasets often include data on large numbers of lipids representing multiple functional classes. These results are subject to four inherent problems of the omics sciences (i.e., bias, statistics, methodology, and method misuse) (Lay et al., 2006). Advanced statistical analysis tools and strict quality assurance regimens are, therefore, a must to provide reliable and meaningful lipidomic results. A key challenge to overcome following data analysis is the ability to establish a link between experimental outputs and specific lipid metabolic pathways or phenotypes.

The cataloguing and quantification of the lipidome reflect spatial and dynamic regulation, for example, global changes in cell membrane composition, systemic lipid metabolism, lipid trafficking, lipid oxidation, lipid–protein interactions or biochemical reactions. To understand lipid function and their biological/medical relevance, the impact of physicochemical properties of lipids on lipid–lipid and lipid–protein interactions must be explicitly elucidated. Knowledge of lipid networks, connections between lipid biosynthetic and metabolic pathways, as well as their regulation and interaction with non-lipid signaling cascades (e.g., protein-protein interactions, protein phosphorylation/dephosphorylation) is required to gain insight into the biology of these lipids. New mass spectrometry methodologies must be developed to delineate specificity of lipid–lipid interactions and covalent modifications of lipids. However, defining the topology of lipid–lipid interaction networks is not sufficient to define lipid-related cellular processes. As a result, quantitative kinetic models need to be developed and enforced in contemporary lipidomics research.

The identification and discovery of new lipid species in biological systems is another challenge the lipidomics field must conquer since currently available lipid databases do not archive and encompass information on all possible lipid species due to the structural diversity of lipids across different organisms, tissues and cells. Databases such as KEGG are limited to pathway representations of generic lipid classes, that is, including information on the headgroup but not the fatty acyl side chains of lipids. Such databases lack the level of detail that is becoming available by modern mass spectrometry-based approaches. In addition, due to common structural similarities among different lipid classes, which are often regulated by the same enzymes in class-specific manner, there is a large degree of co-regulation to be expected in lipid profiles. To elucidate and track changes of the lipidome, data analysis and interpretation therefore need to balance the analysis of global lipid pattern changes with the analysis of molecular species specific pathways.

The ultimate goal of the lipidomics field is to cross-link existing lipidomics research centres and integrate lipid databases worldwide to standardize lipidomic analyses, and to link technological advances to pressing scientific issues. This task is highly dependent upon (i) establishing an open access and central database containing lipid species maps, and (ii) integrating lipidomic data with lipid-related genetic, proteomic and metabolomic data. The combined efforts of bioinformaticians and scientists across the globe are instrumental to interconnect these multidisciplinary databases and cross-reference different types of datasets to generate new modeling concepts. Although cumbersome to maintain, such integrative database and infrastructure provide the foundation for bioinformatic tools to integrate all lipid-linked physicochemical, biological, metabolic, and medical information. This will lead to the discovery of novel lipid biomarkers and pathobiological-mechanisms for the diagnosis of lipid-related disorders.

The improvement of techniques employed in lipidomic research and analyses, that is, sample preparation, lipid synthesis, analytical techniques, and bioinformatics methods, demands the elaboration of standard operation procedures. This will facilitate the sorting of the literature into annotated pathways, the extraction of results from experimental data, as well as the management, processing and integration of experimental outputs to hold assembled information. This harmonization effort relies heavily on strong links between technology developers and the lipid scientific community, and on the establishment of interdisciplinary, educational training and research programs to understand lipid function with respect to their functions in health and disease.

XV. CONCLUDING REMARKS

Advances in genomics have enabled researchers to identify genetic determinants of early onset disease. Direct biochemical investigations have elucidated multiple signalling pathways altered by these mutant gene products. Further combination of genomics with proteomics is allowing researchers to map global gene and protein changes associated with progressive neurodegeneration. Together, these studies have provided remarkable insight into the molecular nature of multifactorial disease. However, despite these advances, we do not know why discrete subsets of cells are uniquely susceptible to early dysfunction nor can we protect vulnerable populations. Thus, the next major advance in rational therapeutic design will come from tying a cell's metabolome into genomic and proteomic maps of disease. Such insight will provide new therapeutic avenues designed to protect vulnerable neuronal populations in the face of ongoing insult. When combined with other high throughput technologies in genomics and proteomics, lipidomics offers the opportunity to elucidate the molecular signature and biological importance of lipid metabolism. Lipidomics-driven research is therefore of paramount importance for in depth understanding of pathophysiological events, as well as for the development of novel lipid-based drug targets.

XVI. ABBREVIATIONS

APCI	atmospheric pressure chemical-ionization
APCACI	atmospheric pressure covalent adduct chemical ionization
APPI	atmospheric pressure photo-ionization
CAD	collision-activated dissociation
CI	chemical ionization
CID	collision-induced dissociation
CL	cardiolipin
DAG	diacylglycerol
DRM	detergent resistant membrane
ECD	electron capture dissociation
EI	electron ionization
ER	endoplasmic reticulum
ESI	electrospray ionization
ETD	electron transfer dissociation
FAB	fast atom bombardment
FT-ICR	Fourier transform ion cyclotron resonance
GalNAc	*N*-acetylgalactosamine
GC	gas chromatography
GlcNAc	*N*-acetylglucosamine
PA	glycerophosphatidic acid
PC	glycerophosphocholine
PE	glycerophosphoethanolamine
PG	glycerophosphoglycerol
PI	glycerophosphoinositol
PS	glycerophosphoserine
HPLC	high performance liquid chromatography
IMS	imaging mass spectrometry
LC-MS	liquid chromatography-mass spectrometry
LPC	lyso-PC
LPA	lyso-PA
LPAF	lyso-PAF
MALDI	matrix-assisted laser desorption/ionization
MRM	multiple reaction monitoring
MS/MS or MS^n	tandem mass spectrometry
NMR	nuclear magnetic resonance
OCN	oscillating capillary nebulizer
OzESI	ozone electrospray ionization
OzID	ozone-induced dissociation
PAF	platelet activating factor
SIMS	secondary ion mass spectrometry
TAG	triacylglycerol
TLC	thin-layer chromatography
TOF	time-of-flight
UPLC	ultra performance liquid chromatography
sn	stereospecific number

ACKNOWLEDGMENTS

DF would like to acknowledge the support from the Canada Research Chairs Program in Proteomics and Systems Biology. Funding was provided by OMHF, CIHR MOP-89999, the University of Ottawa and the Jean-Louis Lévesque Foundation. The authors would also like to acknowledge Rachel Figeys for editorial help.

REFERENCES

Abidi SL. 2004. Capillary electrochromatography of sterols and related steryl esters derived from vegetable oils. J Chromatogr A 1059:199–208.

Adibhatla RM, Hatcher JF, Dempsey RJ. 2006. Lipids and lipidomics in brain injury and diseases. AAPS J 8:E314–E321.

Adosraku RK, Choi GT, Constantinou-Kokotos V, Anderson MM, Gibbons WA. 1994. NMR lipid profiles of cells, tissues, and body fluids: Proton NMR analysis of human erythrocyte lipids. J Lipid Res 35:1925–1931.

Afonso C, Riu A, Xu Y, Fournier F, Tabet JC. 2005. Structural characterization of fatty acids cationized with copper by electrospray ionization mass spectrometry under low-energy collision-induced dissociation. J Mass Spectrom 40:342–349.

Agrawal GK, Tamogami S, Han O, Iwahashi H, Rakwal R. 2004. Rice octadecanoid pathway. Biochem Biophys Res Commun 317:1–15.

Almeida T, Cunha RA, Ribeiro JA. 1999. Facilitation by arachidonic acid of acetylcholine release from the rat hippocampus. Brain Res 826:104–111.

Aloia RC, Tian H, Jensen FC. 1993. Lipid composition and fluidity of the human immunodeficiency virus envelope and host cell plasma membranes. Proc Natl Acad Sci USA 90:5181–5185.

Altria KD. 2000. Background theory and applications of microemulsion electrokinetic chromatography. J Chromatogr A 892:171–186.

Astigarraga E, Barreda-Gomez G, Lombardero L, Fresnedo O, Castano F, Giralt MT, Ochoa B, Rodriguez-Puertas R, Fernandez JA. 2008. Profiling and imaging of lipids on brain and liver tissue by matrix-assisted laser desorption/ionization mass spectrometry using 2-mercaptobenzothiazole as a matrix. Anal Chem 80:9105–9114.

Bach D, Wachtel E. 2003. Phospholipid/cholesterol model membranes: Formation of cholesterol crystallites. Biochim Biophys Acta 1610:187–197.

Barber M, Bordoli RS, Sedgwick RD, Tyler AN. 1981. Fast atom bombardment of solids (F.A.B.): A new ion source for mass spectrometry. J Chem Soc Chem Commun 325–327, DOI: 10.1039/C39810000325. Available; http://www.rsc.org/publishing/journals/C3/article.asp?doi=c39810000325.

Barber M, Bordoli RS, Elliott GJ, Sedgwick RD, Tyler AN. 1982. Fast atom bombardment mass spectrometry. Anal Chem 54:645A–657A.

Bari M, Battista N, Fezza F, Finazzi-Agro A, Maccarrone M. 2005. Lipid rafts control signaling of type-1 cannabinoid receptors in neuronal cells. Implications for anandamide-induced apoptosis. J Biol Chem 280:12212–12220.

Bazan NG. 1989. The metabolism of omega-3 polyunsaturated fatty acids in the eye: The possible role of docosahexaenoic acid and docosanoids in retinal physiology and ocular pathology. Prog Clin Biol Res 312:95–112.

Benninger RK, Onfelt B, Neil MA, Davis DM, French PM. 2005. Fluorescence imaging of two-photon linear dichroism: Cholesterol depletion disrupts molecular orientation in cell membranes. Biophys J 88:609–622.

Berman DE, Dall'Armi C, Voronov SV, McIntire LB, Zhang H, Moore AZ, Staniszewski A, Arancio O, Kim TW, Di Paolo G. 2008. Oligomeric amyloid-beta peptide disrupts phosphatidylinositol-4,5-bisphosphate metabolism. Nat Neurosci 11:547–554.

Bicalho B, David F, Rumplel K, Kindt E, Sandra P. 2008. Creating a fatty acid methyl ester database for lipid profiling in a single drop of human blood using high resolution capillary gas chromatography and mass spectrometry. J Chromatogr A 1211:120–128.

Bielawski J, Szulc ZM, Hannun YA, Bielawska A. 2006. Simultaneous quantitative analysis of bioactive sphingolipids by high-performance liquid chromatography-tandem mass spectrometry. Methods 39: 82–91.

Bleijerveld OB, Houweling M, Thomas MJ, Cui Z. 2006. Metabolipidomics: Profiling metabolism of glycerophospholipid species by stable isotopic precursors and tandem mass spectrometry. Anal Biochem 352:1–14.

Blewett AJ, Varma D, Gilles T, Libonati JR, Jansen SA. 2008. Development and validation of a high-performance liquid chromatography-electrospray mass spectrometry method for the simultaneous determination of 23 eicosanoids. J Pharm Biomed Anal 46:653–662.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917.

Bosetti F. 2007. Arachidonic acid metabolism in brain physiology and pathology: Lessons from genetically altered mouse models. J Neurochem 102:577–586.

Breckenridge WC, Morgan IG, Zanetta JP, Vincendon G. 1973. Adult rat brain synaptic vesicles II. Lipid composition. Biochim Biophys Acta 320:681–686.

Brennan PJ, Nikaido H. 1995. The envelope of mycobacteria. Annu Rev Biochem 64:29–63.

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 94:2339–2344.

Brunelle A, Laprevote O. 2009. Lipid imaging with cluster time-of-flight secondary ion mass spectrometry. Anal Bioanal Chem 393:31–35.

Bryant DK, Orlando RC, Fenselau C, Sowder RC, Henderson LE. 1991. Four-sector tandem mass spectrometric analysis of complex mixtures of phosphatidylcholines present in a human immunodeficiency virus preparation. Anal Chem 63:1110–1114.

Burke JE, Dennis EA. 2009. Phospholipase A(2) biochemistry. Cardiovasc Drugs Ther 23:49–59.

Butovich IA, Uchiyama E, Di Pascuale MA, McCulley JP. 2007. Liquid chromatography-mass spectrometric analysis of lipids present in human meibomian gland secretions. Lipids 42:765–776.

Butovich IA, Uchiyama E, McCulley JP. 2007. Lipids of human meibum: Mass-spectrometric analysis and structural elucidation. J Lipid Res 48:2220–2235.

Byrdwell WC. 2001. Atmospheric pressure chemical ionization mass spectrometry for analysis of lipids. Lipids 36:327–346.

Byrdwell WC. 2005. The bottom-up solution to the triacylglycerol lipidome using atmospheric pressure chemical ionization mass spectrometry. Lipids 40:383–417.

Caffrey M, Hogan J. 1992. LIPIDAT: A database of lipid phase transition temperatures and enthalpy changes. DMPC data subset analysis. Chem Phys Lipids 61:1–109.

Cai SS, Syage JA. 2006a. Atmospheric pressure photoionization mass spectrometry for analysis of fatty acid and acylglycerol lipids. J Chromatogr A 1110:15–26.

Cai SS, Syage JA. 2006b. Comparison of atmospheric pressure photoionization, atmospheric pressure chemical ionization, and electrospray ionization mass spectrometry for analysis of lipids. Anal Chem 78:1191–1199.

Cai SS, Short LC, Syage JA, Potvin M, Curtis JM. 2007. Liquid chromatography-atmospheric pressure photoionization-mass spectrometry analysis of triacylglycerol lipids—Effects of mobile phases on sensitivity. J Chromatogr A 1173:88–97.

Calhoun BC, Goldenring JR. 1997. Two Rab proteins, vesicle-associated membrane protein 2 (VAMP-2) and secretory carrier membrane proteins (SCAMPs), are present on immunoisolated parietal cell tubulovesicles. Biochem J 325(Pt 2):559–564.

Callender HL, Forrester JS, Ivanova P, Preininger A, Milne S, Brown HA. 2007. Quantification of diacylglycerol species from cellular extracts by electrospray ionization mass spectrometry using a linear regression algorithm. Anal Chem 79:263–272.

Cao R, Li X, Liu Z, Peng X, Hu W, Wang X, Chen P, Xie J, Liang S. 2006. Integration of a two-phase partition method into proteomics research on rat liver plasma membrane proteins. J Proteome Res 5:634–642.

Carado A, Passarelli MK, Kozole J, Wingate JE, Winograd N, Loboda AV. 2008. C60 secondary ion mass spectrometry with a hybrid-quadrupole orthogonal time-of-flight mass spectrometer. Anal Chem 80:7921–7929.

Caroni P. 2001. New EMBO members' review: Actin cytoskeleton regulation through modulation of PI(4,5)P(2) rafts. EMBO J 20:4332–4336.

Chen X, Morris R, Lawrence MJ, Quinn PJ. 2007. The isolation and structure of membrane lipid rafts from rat brain. Biochimie 89:192–196.

Chen Y, Allegood J, Liu Y, Wang E, Cachon-Gonzalez B, Cox TM, Merrill AH, Jr., Sullards MC. 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 80:2780–2788.

Cheng C, Gross ML, Pittenauer E. 1998. Complete structural elucidation of triacylglycerols by tandem sector mass spectrometry. Anal Chem 70:4417–4426.

Cheng J, Kozole J, Hengstebeck R, Winograd N. 2007. Direct comparison of Au(3)(+) and C(60)(+) cluster projectiles in SIMS molecular depth profiling. J Am Soc Mass Spectrom 18:406–412.

Chernushevich IV, Loboda AV, Thomson BA. 2001. An introduction to quadrupole-time-of-flight mass spectrometry. J Mass Spectrom 36:849–865.

Chester MA. 1998. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature of glycolipids-recommendations 1997. Eur J Biochem 257:293–298.

Christie WW. 1989. Gas chromatography and lipids: A practical guide. Ayr: The Oily Press.

Churchward MA, Rogasevskaia T, Brandman DM, Khosravani H, Nava P, Atkinson JK, Coorssen JR. 2008. Specific lipids supply critical negative spontaneous curvature—An essential component of native Ca2+-triggered membrane fusion. Biophys J 94:3976–3986.

Coleman RA, Lee DP. 2004. Enzymes of triacylglycerol synthesis and their regulation. Prog Lipid Res 43:134–176.

Connell E, Darios F, Broersen K, Gatsby N, Peak-Chew SY, Rickman C, Davletov B, Darios F, Davletov B. 2007. Mechanism of arachidonic acid action on syntaxin-Munc18 Omega-3 and omega-6 fatty acids stimulate cell membrane expansion by acting on syntaxin 3. EMBO Rep 8:414–419.

Cornett DS, Reyzer ML, Chaurand P, Caprioli RM. 2007. MALDI imaging mass spectrometry: Molecular snapshots of biochemical systems. Nat Methods 4:828–833.

Cotter D, Maer A, Guda C, Saunders B, Subramaniam S. 2006. LMPD: LIPID MAPS proteome database. Nucleic Acids Res 34:D507–D510.

Cremesti AE, Goni FM, Kolesnick R. 2002. Role of sphingomyelinase and ceramide in modulating rafts: Do biophysical properties determine biologic outcome? FEBS Lett 531:47–53.

Criegee R. 1975. Mechanism of ozonolysis. Angew Chem Int Ed Engl 14:745–752.

Cronan JE. 2003. Bacterial membrane lipids: Where do we stand? Annu Rev Microbiol 57:203–224.

Cui L, Isbell MA, Chawengsub Y, Falck JR, Campbell WB, Nithipatikom K. 2008. Structural characterization of monohydroxyeicosatetraenoic acids and dihydroxy- and trihydroxyeicosatrienoic acids by ESI-FTICR. J Am Soc Mass Spectrom 19:569–585.

Curt Boeckel CR. 1962. Study of the paper electrophoretic separation of lipids, phospholipids and proteins from normal serums. Sem Med 120:390–393.

Cvacka J, Krafkova E, Jiros P, Valterova I. 2006. Computer-assisted interpretation of atmospheric pressure chemical ionization mass spectra of triacylglycerols. Rapid Commun Mass Spectrom 20:3586–3594.

Darios F, Davletov B. 2006. Omega-3 and omega-6 fatty acids stimulate cell membrane expansion by acting on syntaxin 3. Nature 440:813–817.

Darios F, Connell E, Davletov B. 2007. Phospholipases and fatty acid signalling in exocytosis. J Physiol 585:699–704.

Dass C. 2007a. Mass analysis and ion detection. In: Fundamentals of contemporary mass spectrometry. Desiderio DM, Nibbering NM, Series Editors. Hoboken, New Jersey: John Wiley & Sons, Inc.

Dass C. 2007b. Modes of ionization. In: Fundamentals of contemporary mass spectrometry. Desiderio DM, Nibbering NM, Series Editors. Hoboken, New Jersey: John Wiley & Sons, Inc.

Dass C. 2007c. Tandem mass spectrometry instrumentation. In: Fundamentals of contemporary mass spectrometry. Desiderio DM, Nibbering NM, Series Editors. Hoboken, New Jersey: John Wiley & Sons, Inc.

Deacon EM, Pettitt TR, Webb P, Cross T, Chahal H, Wakelam MJ, Lord JM. 2002. Generation of diacylglycerol molecular species through the cell cycle: A role for 1-stearoyl, 2-arachidonyl glycerol in the activation of nuclear protein kinase C-betaII at G2/M. J Cell Sci 115:983–989.

Delobel A, Touboul D, Laprevote O. 2005. Structural characterization of phosphatidylcholines by atmospheric pressure photoionization mass spectrometry. Eur J Mass Spectrom (Chichester, Eng) 11:409–417.

Delobel A, Roy S, Touboul D, Gaudin K, Germain DP, Baillet A, Brion F, Prognon P, Chaminade P, Laprevote O. 2006. Atmospheric pressure photoionization coupled to porous graphitic carbon liquid chromatography for the analysis of globotriaosylceramides. Application to Fabry disease. J Mass Spectrom 41:50–58.

DeLong CJ, Shen YJ, Thomas MJ, Cui Z. 1999. Molecular distinction of phosphatidylcholine synthesis between the CDP-choline pathway and phosphatidylethanolamine methylation pathway. J Biol Chem 274: 29683–29688.

DeLong CJ, Baker PR, Samuel M, Cui Z, Thomas MJ. 2001. Molecular species composition of rat liver phospholipids by ESI-MS/MS: The effect of chromatography. J Lipid Res 42:1959–1968.

Demmig-Adams B, Adams WW III. 2002. Antioxidants in photosynthesis and human nutrition. Science 298:2149–2153.

Dempster AJ. 1918. A new method of positive ray analysis. Phys Rev 11:316.

Dermaux A, Sandra P. 1999. Applications of capillary electrochromatography. Electrophoresis 20:3027–3065.

Distler U, Hulsewig M, Souady J, Dreisewerd K, Haier J, Senninger N, Friedrich AW, Karch H, Hillenkamp F, Berkenkamp S, Peter-Katalinic J, Muthing J. 2008. Matching IR-MALDI-o-TOF mass spectrometry with the TLC overlay binding assay and its clinical application for tracing tumor-associated glycosphingolipids in hepatocellular and pancreatic cancer. Anal Chem 80:1835–1846.

Dobrowsky RT. 2000. Sphingolipid signalling domains floating on rafts or buried in caves? Cell Signal 12:81–90.

Dowhan W. 2009. Molecular genetic approaches to defining lipid function. J Lipid Res 50(Suppl):S305–S310.

Dreisewerd K, Muthing J, Rohlfing A, Meisen I, Vukelic Z, Peter-Katalinic J, Hillenkamp F, Berkenkamp S. 2005. Analysis of gangliosides directly from thin-layer chromatography plates by infrared matrix-assisted laser desorption/ionization orthogonal time-of-flight mass spectrometry with a glycerol matrix. Anal Chem 77:4098–4107.

Duffin KL, Henion JD, Shieh JJ. 1991. Electrospray and tandem mass spectrometric characterization of acylglycerol mixtures that are dissolved in nonpolar solvents. Anal Chem 63:1781–1788.

Dupau P, Epple R, Thomas AA, Fokin VV, Sharpless B. 2002. Osmium-catalyzed dihydroxylation of olefins in acidic media: Old process, new tricks. Adv Synth Cat 344:421–433.

Ejsing CS, Duchoslav E, Sampaio J, Simons K, Bonner R, Thiele C, Ekroos K, Shevchenko A. 2006. Automated identification and quantification of glycerophospholipid molecular species by multiple precursor ion scanning. Anal Chem 78:6202–6214.

Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, Simons K, Shevchenko A. 2009. Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc Natl Acad Sci USA 106:2136–2141.

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 74:941–949.

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 MS3 fragmentation. J Lipid Res 44:2181–2192.

Emoto K, Umeda M. 2000. An essential role for a membrane lipid in cytokinesis. Regulation of contractile ring disassembly by redistribution of phosphatidylethanolamine. J Cell Biol 149:1215–1224.

Everberg H, Clough J, Henderson P, Jergil B, Tjerneld F, Ramirez IB. 2006. Isolation of Escherichia coli inner membranes by metal affinity two-phase partitioning. J Chromatogr A 1118:244–252.

Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH, Jr., Murphy RC, Raetz CR, Russell DW, Seyama Y, Shaw W, Shimizu T, Spener F, van Meer G, VanNieuwenhze MS, White SH, Witztum JL, Dennis EA. 2005. A comprehensive classification system for lipids. J Lipid Res 46:839–861.

Fahy E, Cotter D, Byrnes R, Sud M, Maer A, Li J, Nadeau D, Zhau Y, Subramaniam S. 2007a. Bioinformatics for lipidomics. Methods Enzymol 432:247–273.

Fahy E, Sud M, Cotter D, Subramaniam S. 2007b. LIPID MAPS online tools for lipid research. Nucleic Acids Res 35:W606–W612.

Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CR, Shimizu T, Spener F, van Meer G, Wakelam MJ, Dennis EA. 2009. Update of the LIPID MAPS comprehensive classification system for lipids. J Lipid Res 50(Suppl):S9–14.

Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. 1989. Electrospray ionization for mass spectrometry of large biomolecules. Science 246:64–71.

Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509.

Forrester JS, Milne SB, Ivanova PT, Brown HA. 2004. Computational lipidomics: A multiplexed analysis of dynamic changes in membrane lipid composition during signal transduction. Mol Pharmacol 65:813–821.

Foster LJ, De Hoog CL, Mann M. 2003. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. Proc Natl Acad Sci USA 100:5813–5818.

Freeman EJ, Terrian DM, Dorman RV. 1990. Presynaptic facilitation of glutamate release from isolated hippocampal mossy fiber nerve endings by arachidonic acid. Neurochem Res 15:743–750.

Fuchs B, Schiller J, Suss R, Schurenberg M, Suckau D. 2007a. A direct and simple method of coupling matrix-assisted laser desorption and

ionization time-of-flight mass spectrometry (MALDI-TOF MS) to thin-layer chromatography (TLC) for the analysis of phospholipids from egg yolk. Anal Bioanal Chem 389:827–834.

Fuchs B, Schober C, Richter G, Suss R, Schiller J. 2007b. MALDI-TOF MS of phosphatidylethanolamines: Different adducts cause different post source decay (PSD) fragment ion spectra. J Biochem Biophys Methods 70:689–692.

Galbiati F, Razani B, Lisanti MP. 2001. Emerging themes in lipid rafts and caveolae. Cell 106:403–411.

Garrett TJ, Yost RA. 2006. Analysis of intact tissue by intermediate-pressure MALDI on a linear ion trap mass spectrometer. Anal Chem 78:2465–2469.

Gaspar A, Englmann M, Fekete A, Harir M, Schmitt-Kopplin P. 2008. Trends in CE-MS 2005–2006. Electrophoresis 29:66–79.

Geiss F. 1987. Fundamentals of thin layer chromatography (planar Chromatography), huethig. In: Stahl E, editor. Chromatographic methods. Berlin: Huthig Verlag, Heidelberg. p. 257.

Ghangas GS, Steffens JC. 1993. UDPglucose: Fatty acid transglucosylation and transacylation in triacylglucose biosynthesis. Proc Natl Acad Sci USA 90:9911–9915.

Golub T, Caroni P. 2005. PI(4,5)P2-dependent microdomain assemblies capture microtubules to promote and control leading edge motility. J Cell Biol 169:151–165.

Gomez-Ariza JL, Arias-Borrego A, Garcia-Barrera T. 2006. Use of flow injection atmospheric pressure photoionization quadrupole time-of-flight mass spectrometry for fast olive oil fingerprinting. Rapid Commun Mass Spectrom 20:1181–1186.

Gross RW. 1984. High plasmalogen and arachidonic acid content of canine myocardial sarcolemma: A fast atom bombardment mass spectroscopic and gas chromatography-mass spectroscopic characterization. Biochemistry 23:158–165.

Gross RW. 1985. Identification of plasmalogen as the major phospholipid constituent of cardiac sarcoplasmic reticulum. Biochemistry 24:1662–1668.

Gross RW, Han X. 2009. Shotgun lipidomics of neutral lipids as an enabling technology for elucidation of lipid-related diseases. Am J Physiol Endocrinol Metab 297:E297–303.

Gu M, Kerwin JL, Watts JD, Aebersold R. 1997. Ceramide profiling of complex lipid mixtures by electrospray ionization mass spectrometry. Anal Biochem 244:347–356.

Guan XL, Wenk MR. 2006. Mass spectrometry-based profiling of phospholipids and sphingolipids in extracts from *Saccharomyces cerevisiae*. Yeast 23:465–477.

Guittard J, Hronowski XL, 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 13:1838–1849.

Gutnikov G. 1995. Fatty acid profiles of lipid samples. J Chromatogr B Biomed Appl 671:71–89.

Haimi P, Uphoff A, Hermansson M, Somerharju P. 2006. Software tools for analysis of mass spectrometric lipidome data. Anal Chem 78:8324–8331.

Hamberg M. 1971. Steric analysis of hydroperoxides formed by lipoxygenase oxygenation of linoleic acid. Anal Biochem 43:515–526.

Han X. 2002. Characterization and direct quantitation of ceramide molecular species from lipid extracts of biological samples by electrospray ionization tandem mass spectrometry. Anal Biochem 302:199–212.

Han X, Gross RW. 1995. Structural determination of picomole amounts of phospholipids via electrospray ionization tandem mass spectrometry. J Am Soc Mass Spectrom 6:1202–1210.

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.

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 44:1071–1079.

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.

Han X, Gubitosi-Klug RA, Collins BJ, Gross RW. 1996. Alterations in individual molecular species of human platelet phospholipids during thrombin stimulation: Electrospray ionization mass spectrometry-facilitated identification of the boundary conditions for the magnitude and selectivity of thrombin-induced platelet phospholipid hydrolysis. Biochemistry 35:5822–5832.

Han X, Abendschein DR, Kelley JG, Gross RW. 2000. Diabetes-induced changes in specific lipid molecular species in rat myocardium. Biochem J 352(Pt 1):79–89.

Han X, Holtzman DM, McKeel DW, Jr. 2001. Plasmalogen deficiency in early Alzheimer's disease subjects and in animal models: Molecular characterization using electrospray ionization mass spectrometry. J Neurochem 77:1168–1180.

Han X, Holtzman DM, McKeel DW, Jr., Kelley J, Morris JC. 2002. Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: Potential role in disease pathogenesis. J Neurochem 82:809–818.

Han X, Yang J, Cheng H, Ye H, Gross RW. 2004. Toward fingerprinting cellular lipidomes directly from biological samples by two-dimensional electrospray ionization mass spectrometry. Anal Biochem 330:317–331.

Han X, Yang K, Cheng H, Fikes KN, Gross RW. 2005. Shotgun lipidomics of phosphoethanolamine-containing lipids in biological samples after one-step in situ derivatization. J Lipid Res 46:1548–1560.

Han X, Yang K, Yang J, Cheng H, Gross RW. 2006a. Shotgun lipidomics of cardiolipin molecular species in lipid extracts of biological samples. J Lipid Res 47:864–879.

Han X, Yang K, Yang J, Fikes KN, Cheng H, Gross RW. 2006b. Factors influencing the electrospray intrasource separation and selective ionization of glycerophospholipids. J Am Soc Mass Spectrom 17:264–274.

Handa S, Kushi Y, Kambara H, Shizukuishi K. 1983. Secondary ion mass spectra of neutral sphingoglycolipids. J Biochem 93:315–318.

Haque ME, McIntosh TJ, Lentz BR. 2001. Influence of lipid composition on physical properties and peg-mediated fusion of curved and uncurved model membrane vesicles: ''Nature's own'' fusogenic lipid bilayer. Biochemistry 40:4340–4348.

Haroldsen PE, Clay KL, Murphy RC. 1987. Quantitation of lyso-platelet activating factor molecular species from human neutrophils by mass spectrometry. J Lipid Res 28:42–49.

Harris TJ, Siu CH. 2002. Reciprocal raft-receptor interactions and the assembly of adhesion complexes. Bioessays 24:996–1003.

Harrison KA, Murphy RC. 1996. Direct mass spectrometric analysis of ozonides: Application to unsaturated glycerophosphocholine lipids. Anal Chem 68:3224–3230.

Harvey DJ. 2005. A new charge-associated mechanism to account for the production of fragment ions in the high-energy CID spectra of fatty acids. J Am Soc Mass Spectrom 16:280–290.

Hayasaka T, Goto-Inoue N, Sugiura Y, Zaima N, Nakanishi H, Ohishi K, Nakanishi S, Naito T, Taguchi R, Setou M. 2008. Matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight (MALDI-QIT-TOF)-based imaging mass spectrometry reveals a layered distribution of phospholipid molecular species in the mouse retina. Rapid Commun Mass Spectrom 22:3415–3426.

Hazen SL, Hall CR, Ford DA, Gross RW. 1993. Isolation of a human myocardial cytosolic phospholipase A2 isoform. Fast atom bombardment mass spectroscopic and reverse-phase high pressure liquid chromatography identification of choline and ethanolamine glycerophospholipid substrates. J Clin Invest 91:2513–2522.

Heerklotz H, Epand RM. 2001. The enthalpy of acyl chain packing and the apparent water-accessible apolar surface area of phospholipids. Biophys J 80:271–279.

Helenius A, Aebi M. 2001. Intracellular functions of N-linked glycans. Science 291:2364–2369.

Henley JR, McNiven MA. 1996. Association of a dynamin-like protein with the Golgi apparatus in mammalian cells. J Cell Biol 133:761–775.

Hirschmann H. 1960. The nature of substrate asymmetry in stereoselective reactions. J Biol Chem 235:2762–2767.

Ho YP, Huang PC. 2002. A novel structural analysis of glycerophosphocholines as TFA/K(+) adducts by electrospray ionization ion trap tandem mass spectrometry. Rapid Commun Mass Spectrom 16:1582–1589.

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 17:114–121.

Holthuis JC, Levine TP. 2005. Lipid traffic: Floppy drives and a superhighway. Nat Rev Mol Cell Biol 6:209–220.

Hopwood DA. 2004. Cracking the polyketide code. PLoS Biol 2:E35.

Hou W, Zhou H, Elisma F, Bennett SA, Figeys D. 2008. Technological developments in lipidomics. Brief Funct Genomic Proteomic.

Houjou T, Yamatani K, Imagawa M, Shimizu T, Taguchi R. 2005. A shotgun tandem mass spectrometric analysis of phospholipids with normal-phase and/or reverse-phase liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom 19:654–666.

Hsu FF, Turk J. 1999a. Distinction among isomeric unsaturated fatty acids as lithiated adducts by electrospray ionization mass spectrometry using low energy collisionally activated dissociation on a triple stage quadrupole instrument. J Am Soc Mass Spectrom 10:600–612.

Hsu FF, Turk J. 1999b. Structural characterization of triacylglycerols as lithiated adducts by electrospray ionization mass spectrometry using low-energy collisionally activated dissociation on a triple stage quadrupole instrument. J Am Soc Mass Spectrom 10:587–599.

Hsu FF, Turk J. 2000a. Characterization of phosphatidylethanolamine as a lithiated adduct by triple quadrupole tandem mass spectrometry with electrospray ionization. J Mass Spectrom 35:595–606.

Hsu FF, Turk J. 2000b. Characterization of phosphatidylinositol, phosphatidylinositol-4-phosphate, and phosphatidylinositol-4,5-bisphosphate by electrospray ionization tandem mass spectrometry: A mechanistic study. J Am Soc Mass Spectrom 11:986–999.

Hsu FF, Turk J. 2000c. Charge-driven fragmentation processes in diacyl glycerophosphatidic acids upon low-energy collisional activation. A mechanistic proposal. J Am Soc Mass Spectrom 11:797–803.

Hsu FF, Turk J. 2000d. Charge-remote and charge-driven fragmentation processes in diacyl glycerophosphoethanolamine upon low-energy collisional activation: A mechanistic proposal. J Am Soc Mass Spectrom 11:892–899.

Hsu FF, Turk J. 2000e. Structural determination of sphingomyelin by tandem mass spectrometry with electrospray ionization. J Am Soc Mass Spectrom 11:437–449.

Hsu FF, Turk J. 2001a. Structural determination of glycosphingolipids as lithiated adducts by electrospray ionization mass spectrometry using low-energy collisional-activated dissociation on a triple stage quadrupole instrument. J Am Soc Mass Spectrom 12:61–79.

Hsu FF, Turk J. 2001b. Studies on phosphatidylglycerol with triple quadrupole tandem mass spectrometry with electrospray ionization: Fragmentation processes and structural characterization. J Am Soc Mass Spectrom 12:1036–1043.

Hsu FF, Turk J. 2002. Characterization of ceramides by low energy collisional-activated dissociation tandem mass spectrometry with negative-ion electrospray ionization. J Am Soc Mass Spectrom 13:558–570.

Hsu FF, Turk J. 2003. Electrospray ionization/tandem quadrupole mass spectrometric studies on phosphatidylcholines: The fragmentation processes. J Am Soc Mass Spectrom 14:352–363.

Hsu FF, 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 16:1510–1522.

Hsu FF, Turk J. 2006. Characterization of cardiolipin as the sodiated ions by positive-ion electrospray ionization with multiple stage quadrupole ion-trap mass spectrometry. J Am Soc Mass Spectrom 17:1146–1157.

Hsu FF, 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 18:2065–2073.

Hsu FF, Turk J. 2008a. Elucidation of the double-bond position of long-chain unsaturated Fatty acids by multiple-stage linear ion-trap mass spectrometry with electrospray ionization. J Am Soc Mass Spectrom 19:1673–1680.

Hsu FF, Turk J. 2008b. Structural characterization of unsaturated glycerophospholipids by multiple-stage linear ion-trap mass spectrometry with electrospray ionization. J Am Soc Mass Spectrom 19:1681–1691.

Hsu FF, Bohrer A, Turk J. 1998a. Electrospray ionization tandem mass spectrometric analysis of sulfatide. Determination of fragmentation patterns and characterization of molecular species expressed in brain and in pancreatic islets. Biochim Biophys Acta 1392:202–216.

Hsu FF, Bohrer A, Turk J. 1998b. Formation of lithiated adducts of glycerophosphocholine lipids facilitates their identification by electrospray ionization tandem mass spectrometry. J Am Soc Mass Spectrom 9:516–526.

Hsu FF, Turk J, Stewart ME, Downing DT. 2002. Structural studies on ceramides as lithiated adducts by low energy collisional-activated dissociation tandem mass spectrometry with electrospray ionization. J Am Soc Mass Spectrom 13:680–695.

Hsu FF, Turk J, Thukkani AK, Messner MC, Wildsmith KR, Ford DA. 2003. Characterization of alkylacyl, alk-1-enylacyl and lyso subclasses of glycerophosphocholine by tandem quadrupole mass spectrometry with electrospray ionization. J Mass Spectrom 38:752–763.

Hsu FF, Turk J, Rhoades ER, Russell DG, Shi Y, Groisman EA. 2005. Structural characterization of cardiolipin by tandem quadrupole and multiple-stage quadrupole ion-trap mass spectrometry with electrospray ionization. J Am Soc Mass Spectrom 16:491–504.

Hu C, van Dommelen J, van der Heijden R, Spijksma G, Reijmers TH, Wang M, Slee E, Lu X, Xu G, van der Greef J, Hankemeier T. 2008. RPLC-Ion-Trap-FTMS method for lipid profiling of plasma: Method validation and application to p53 mutant mouse model. J Proteome Res 7:4982–4991.

Hvattum E, Hagelin G, Larsen A. 1998. Study of mechanisms involved in the collision-induced dissociation of carboxylate anions from glycerophospholipids using negative ion electrospray tandem quadrupole mass spectrometry. Rapid Commun Mass Spectrom 12:1405–1409.

Isaac G, Jeannotte R, Esch SW, Welti R. 2007. New mass-spectrometry-based strategies for lipids. Genet Eng (NY) 28:129–157.

Israelachvili J. 1992. Relationship between chain protrusions and surfactant critical micelle concentration values. Langmuir 8:1501.

IUPAC-IUB-CBN. 1967. IUPAC-IUB Commission on Biochemical Nomenclature (CBN). The nomenclature of lipids. Eur J Biochem 2: 127–1131.

Ivanova PT, Cerda BA, Horn DM, Cohen JS, McLafferty FW, Brown HA. 2001. Electrospray ionization mass spectrometry analysis of changes in phospholipids in RBL-2H3 mastocytoma cells during degranulation. Proc Natl Acad Sci USA 98:7152–7157.

Ivanova PT, Milne SB, Forrester JS, Brown HA. 2004. LIPID arrays: New tools in the understanding of membrane dynamics and lipid signaling. Mol Interv 4:86–96.

Iwabuchi K, Handa K, Hakomori S. 2000. Separation of glycosphingolipid-enriched microdomains from caveolar membrane characterized by presence of caveolin. Methods Enzymol 312:488–494.

Jackson SN, Wang HY, Woods AS. 2005a. Direct profiling of lipid distribution in brain tissue using MALDI-TOFMS. Anal Chem 77:4523–4527.

Jackson SN, Wang HY, Woods AS. 2005b. In situ structural characterization of phosphatidylcholines in brain tissue using MALDI-MS/MS. J Am Soc Mass Spectrom 16:2052–2056.

Jackson SN, Wang HY, Woods AS, Ugarov M, Egan T, Schultz JA. 2005c. Direct tissue analysis of phospholipids in rat brain using MALDI-TOFMS and MALDI-ion mobility-TOFMS. J Am Soc Mass Spectrom 16:133–138.

James PF, Perugini MA, O'Hair RA. 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 19:978–986.

Jensen N, Lam K, Cody RB, Tamura J. 1990. Evidence for distinction of cis and trans isomers of mono-unsaturated fatty acids by fast-atom bombardment tandem mass spectrometric analysis. Rapid Commun Mass Spectrom 4:239–241.

Ji H, Morre J, Voinov VG, Deinzer ML, Barofsky DF. 2007a. Charge-remote metastable ion decomposition of free fatty acids under FAB MS: Evidence for biradical ion structures. Anal Chem 79:2822–2826.

Ji H, Voinov VG, Deinzer ML, Barofsky DF. 2007b. Distinguishing between cis/trans isomers of monounsaturated fatty acids by FAB MS. Anal Chem 79:1519–1522.

Jones G, Strugnell SA, DeLuca HF. 1998. Current understanding of the molecular actions of vitamin D. Physiol Rev 78:1193–1231.

Juhl K, Hoy M, Olsen HL, Bokvist K, Efanov AM, Hoffmann EK, Gromada J. 2003. cPLA2alpha-evoked formation of arachidonic acid and lysophospholipids is required for exocytosis in mouse pancreatic beta-cells. Am J Physiol Endocrinol Metab 285:E73–E81.

Kalin N, Fernandes J, Hrafnsdottir S, van Meer G. 2004. Natural phosphatidylcholine is actively translocated across the plasma membrane to the surface of mammalian cells. J Biol Chem 279:33228–33236.

Karas M, Hillenkamp F. 1988. Laser desorption ionization of proteins with molecular masses exceeding 10,000 Daltons. Anal Chem 60:2299–2301.

Katajamaa M, Miettinen J, Oresic M. 2006. MZmine: Toolbox for processing and visualization of mass spectrometry based molecular profile data. Bioinformatics 22:634–636.

Kawai Y, Takeda S, Terao J. 2007. Lipidomic analysis for lipid peroxidation-derived aldehydes using gas chromatography-mass spectrometry. Chem Res Toxicol 20:99–107.

Kerkhoff C, Trumbach B, Gehring L, Habben K, Schmitz G, Kaever V. 2000. Solubilization, partial purification and photolabeling of the integral membrane protein lysophospholipid:acyl-CoA acyltransferase (LAT). Eur J Biochem 267:6339–6345.

Kerwin JL, Tuininga AR, Ericsson LH. 1994. Identification of molecular species of glycerophospholipids and sphingomyelin using electrospray mass spectrometry. J Lipid Res 35:1102–1114.

Kerwin JL, Wiens AM, Ericsson LH. 1996. Identification of fatty acids by electrospray mass spectrometry and tandem mass spectrometry. J Mass Spectrom 31:184–192.

Khosla C, Gokhale RS, Jacobsen JR, Cane DE. 1999. Tolerance and specificity of polyketide synthases. Annu Rev Biochem 68:219–253.

Kiebish MA, Han X, Cheng H, Lunceford A, Clarke CF, Moon H, Chuang JH, Seyfried TN. 2008. Lipidomic analysis and electron transport chain activities in C57BL/6J mouse brain mitochondria. J Neurochem 106:299–312.

Kikuchi M, Hatano N, Yokota S, Shimozawa N, Imanaka T, Taniguchi H. 2004. Proteomic analysis of rat liver peroxisome: Presence of peroxisome-specific isozyme of Lon protease. J Biol Chem 279:421–428.

Kirschnek S, Paris F, Weller M, Grassme H, Ferlinz K, Riehle A, Fuks Z, Kolesnick R, Gulbins E. 2000. CD95-mediated apoptosis in vivo involves acid sphingomyelinase. J Biol Chem 275:27316–27323.

Kita Y, Ohto T, Uozumi N, Shimizu T. 2006. Biochemical properties and pathophysiological roles of cytosolic phospholipase A2s. Biochim Biophys Acta 1761:1317–1322.

Koga Y, Nishihara M, Morii H, Akagawa-Matsushita M. 1993. Ether polar lipids of methanogenic bacteria: Structures, comparative aspects, and biosyntheses. Microbiol Rev 57:164–182.

Koivusalo M, Haimi P, Heikinheimo L, Kostiainen R, Somerharju P. 2001. Quantitative determination of phospholipid compositions by ESI-MS: Effects of acyl chain length, unsaturation, and lipid concentration on instrument response. J Lipid Res 42:663–672.

Koticha DK, McCarthy EE, Baldini G. 2002. Plasma membrane targeting of SNAP-25 increases its local concentration and is necessary for SNARE complex formation and regulated exocytosis. J Cell Sci 115:3341–3351.

Koumanov KS, Wolf C, Quinn PJ. 2004. Lipid composition of membrane domains. Subcell Biochem 37:153–163.

Koumanov KS, Tessier C, Momchilova AB, Rainteau D, Wolf C, Quinn PJ. 2005. Comparative lipid analysis and structure of detergent-resistant membrane raft fractions isolated from human and ruminant erythrocytes. Arch Biochem Biophys 434:150–158.

Krank J, Murphy RC, Barkley RM, Duchoslav E, McAnoy A. 2007. Qualitative analysis and quantitative assessment of changes in neutral glycerol lipid molecular species within cells. Methods Enzymol 432:1–20.

Laaksonen R, Katajamaa M, Paiva H, Sysi-Aho M, Saarinen L, Junni P, Lutjohann D, Smet J, Van Coster R, Seppanen-Laakso T, Lehtimaki T, Soini J, Oresic M. 2006. A systems biology strategy reveals biological pathways and plasma biomarker candidates for potentially toxic statin-induced changes in muscle. PLoS ONE 1:e97.

Larsen A, Uran S, Jacobsen PB, Skotland T. 2001. Collision-induced dissociation of glycero phospholipids using electrospray ion-trap mass spectrometry. Rapid Commun Mass Spectrom 15:2393–2398.

Lawrence P, Brenna JT. 2006. Acetonitrile covalent adduct chemical ionization mass spectrometry for double bond localization in non-methylene-interrupted polyene fatty acid methyl esters. Anal Chem 78:1312–1317.

Lawson EL, Clifton JG, Huang F, Li X, Hixson DC, Josic D. 2006. Use of magnetic beads with immobilized monoclonal antibodies for isolation of highly pure plasma membranes. Electrophoresis 27:2747–2758.

Lay JO, Jr., Borgmann S, Liyanage R, Wilkins CL. 2006. Problems with the "omics". Trends Anal Chem 25:1046–1056.

Lehmann WD, Koester M, Erben G, Keppler D. 1997. Characterization and quantification of rat bile phosphatidylcholine by electrospray-tandem mass spectrometry. Anal Biochem 246:102–110.

Leitinger N. 2008. The role of phospholipid oxidation products in inflammatory and autoimmune diseases: Evidence from animal models and in humans. Subcell Biochem 49:325–350.

Lelliott CJ, Ljungberg A, Ahnmark A, William-Olsson L, Ekroos K, Elmgren A, Arnerup G, Shoulders CC, Oscarsson J, Linden D. 2007. Hepatic PGC-1beta overexpression induces combined hyperlipidemia and modulates the response to PPARalpha activation. Arterioscler Thromb Vasc Biol 27:2707–2713.

Lemmon MA. 2008. Membrane recognition by phospholipid-binding domains. Nat Rev Mol Cell Biol 9:99–111.

Lerma-Garcia MJ, Ramis-Ramos G, Herrero-Martinez JM, Simo-Alfonso EF. 2008. Classification of vegetable oils according to their botanical origin using sterol profiles established by direct infusion mass spectrometry. Rapid Commun Mass Spectrom 22:973–978.

Lessig J, Gey C, Suss R, Schiller J, Glander HJ, Arnhold J. 2004. Analysis of the lipid composition of human and boar spermatozoa by MALDI-TOF mass spectrometry, thin layer chromatography and 31P NMR spectroscopy. Comp Biochem Physiol B Biochem Mol Biol 137:265–277.

Li J, Richards JC. 2007. Application of capillary electrophoresis mass spectrometry to the characterization of bacterial lipopolysaccharides. Mass Spectrom Rev 26:35–50.

Li YL, Gross ML, Hsu FF. 2005. Ionic-liquid matrices for improved analysis of phospholipids by MALDI-TOF mass spectrometry. J Am Soc Mass Spectrom 16:679–682.

Liang X, Liu J, LeBlanc Y, Covey T, Ptak AC, Brenna JT, McLuckey SA. 2007. Electron transfer dissociation of doubly sodiated glycerophosphocholine lipids. J Am Soc Mass Spectrom 18:1783–1788.

Liebisch G, Drobnik W, Lieser B, Schmitz G. 2002. High-throughput quantification of lysophosphatidylcholine by electrospray ionization tandem mass spectrometry. Clin Chem 48:2217–2224.

Lin DS, Connor WE. 2001. Fecal steroids of the coprolite of a Greenland Eskimo mummy, AD 1475: A clue to dietary sterol intake. Am J Clin Nutr 74:44–49.

Lin S, Fischl AS, Bi X, Parce W. 2003. Separation of phospholipids in microfluidic chip device: Application to high-throughput screening assays for lipid-modifying enzymes. Anal Biochem 314:97–107.

Linden D, William-Olsson L, Ahnmark A, Ekroos K, Hallberg C, Sjogren HP, Becker B, Svensson L, Clapham JC, Oscarsson J, Schreyer S. 2006. Liver-directed overexpression of mitochondrial glycerol-3-phosphate acyltransferase results in hepatic steatosis, increased triacylglycerol secretion and reduced fatty acid oxidation. FASEB J 20:434–443.

LipidLibrary. http://www.lipidlibrary.co.uk/lipids.html.

LipidMapsConsortium. 2009. LipidMaps Consortium: Lipid metabolites and pathways strategy. http://wwwlipidmapsorg.

Long EK, Smoliakova I, Honzatko A, Picklo MJ, Sr. 2008. Structural characterization of alpha,beta-unsaturated aldehydes by GC/MS is dependent upon ionization method. Lipids 43:765–774.

Lu Y, Hong S, Tjonahen E, Serhan CN. 2005. Mediator-lipidomics: Databases and search algorithms for PUFA-derived mediators. J Lipid Res 46:790–802.

Luers GH, Hartig R, Mohr H, Hausmann M, Fahimi HD, Cremer C, Volkl A. 1998. Immuno-isolation of highly purified peroxisomes using magnetic beads and continuous immunomagnetic sorting. Electrophoresis 19:1205–1210.

Luthria DL, Sprecher H. 1993. 2-Alkenyl-4,4-dimethyloxazolines as derivatives for the structural elucidation of isomeric unsaturated fatty acids. Lipids 28:561–564.

Madore N, Smith KL, Graham CH, Jen A, Brady K, Hall S, Morris R. 1999. Functionally different GPI proteins are organized in different domains on the neuronal surface. EMBO J 18:6917–6926.

Maeba R, Maeda T, Kinoshita M, Takao K, Takenaka H, Kusano J, Yoshimura N, Takeoka Y, Yasuda D, Okazaki T, Teramoto T. 2007. Plasmalogens in human serum positively correlate with high-density lipoprotein and decrease with aging. J Atheroscler Thromb 14:12–18.

Magnusson YK, Friberg P, Sjovall P, Malm J, Chen Y. 2008. TOF-SIMS analysis of lipid accumulation in the skeletal muscle of ob/ob mice. Obesity 16:2745–2753.

Mahrous EA, Lee RB, Lee RE. 2008. A rapid approach to lipid profiling of mycobacteria using 2D HSQC NMR maps. J Lipid Res 49:455–463.

Malathi K, Higaki K, Tinkelenberg AH, Balderes DA, Almanzar-Paramio D, Wilcox LJ, Erdeniz N, Redican F, Padamsee M, Liu Y, Khan S, Alcantara F, Carstea ED, Morris JA, Sturley SL. 2004. Mutagenesis of the putative sterol-sensing domain of yeast Niemann Pick C-related protein reveals a primordial role in subcellular sphingolipid distribution. J Cell Biol 164:547–556.

Marmor MD, Julius M. 2001. Role for lipid rafts in regulating interleukin-2 receptor signaling. Blood 98:1489–1497.

Marsh D. 2006. Elastic curvature constants of lipid monolayers and bilayers. Chem Phys Lipids 144:146–159.

Matsubara T, Hayashi A. 1991. FAB/mass spectrometry of lipids. Prog Lipid Res 30:301–322.

Mattila I, Seppanen-Laakso T, Suortti T, Oresic M. 2008. Application of lipidomics and metabolomics to the study of adipose tissue. Methods Mol Biol 456:123–130.

McCloskey JA, McClelland MJ. 1965. Mass spectra of O-isopropylidene derivatives of unsaturated fatty esters. J Am Soc Mass Spectrom 87:5090–5093.

McConnell HM, Vrljic M. 2003. Liquid-liquid immiscibility in membranes. Annu Rev Biophys Biomol Struct 32:469–492.

McDonald JG, Thompson BM, McCrum EC, Russell DW. 2007. Extraction and analysis of sterols in biological matrices by high performance liquid chromatography electrospray ionization mass spectrometry. Methods Enzymol 432:145–170.

McDonnell LA, Heeren RM. 2007. Imaging mass spectrometry. Mass Spectrom Rev 26:606–643.

McFarland MA, Marshall AG, Hendrickson CL, Nilsson CL, Fredman P, Mansson JE. 2005. Structural characterization of the GM1 ganglioside by infrared multiphoton dissociation, electron capture dissociation, and electron detachment dissociation electrospray ionization FT-ICR MS/MS. J Am Soc Mass Spectrom 16:752–762.

McLean JA, Ridenour WB, Caprioli RM. 2007. Profiling and imaging of tissues by imaging ion mobility-mass spectrometry. J Mass Spectrom 42:1099–1105.

Meganathan R. 2001a. Biosynthesis of menaquinone (vitamin K2) and ubiquinone (coenzyme Q): A perspective on enzymatic mechanisms. Vitam Horm 61:173–218.

Meganathan R. 2001b. Ubiquinone biosynthesis in microorganisms. FEMS Microbiol Lett 203:131–139.

Merill AH Jr, Sandhoff K. 2002. Sphingolipids: Metabolism and cell signaling. In: Vance DE, Vance JE, editors. New comprehensive biochemistry: Biochemistry of lipids, lipoproteins and membranes. New York: Elsevier Science. pp. 373–407.

Merrill AH, Jr., Sullards MC, Allegood JC, Kelly S, Wang E. 2005. Sphingolipidomics: High-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry. Methods 36:207–224.

Meyer zu Heringdorf D, Jakobs KH. 2007. Lysophospholipid receptors: Signalling, pharmacology and regulation by lysophospholipid metabolism. Biochim Biophys Acta 1768:923–940.

Michaud AL, Diau GY, Abril R, Brenna JT. 2002. Double bond localization in minor homoallylic fatty acid methyl esters using acetonitrile chemical ionization tandem mass spectrometry. Anal Biochem 307:348–360.

Michaud AL, Yurawecz MP, Delmonte P, Corl BA, Bauman DE, Brenna JT. 2003. Identification and characterization of conjugated fatty acid methyl esters of mixed double bond geometry by acetonitrile chemical ionization tandem mass spectrometry. Anal Chem 75:4925–4930.

Michaud AL, Lawrence P, Adlof R, Brenna JT. 2005. On the formation of conjugated linoleic acid diagnostic ions with acetonitrile chemical ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 19:363–368.

Milne SB, Ivanova PT, DeCamp D, Hsueh RC, Brown HA. 2005. A targeted mass spectrometric analysis of phosphatidylinositol phosphate species. J Lipid Res 46:1796–1802.

Minami Y, Yokoi S, Setoyama M, Bando N, Takeda S, Kawai Y, Terao J. 2007. Combination of TLC blotting and gas chromatography-mass spectrometry for analysis of peroxidized cholesterol. Lipids 42:1055–1063.

Mirgorodskaya E, O'Connor PB, Costello CE. 2002. A general method for precalculation of parameters for sustained off resonance irradiation/collision-induced dissociation. J Am Soc Mass Spectrom 13:318–324.

Mishra S, Joshi PG. 2007. Lipid raft heterogeneity: An enigma. J Neurochem 103(Suppl. 1):135–142.

Mo L, Ren Q, Duchemin AM, Neff NH, Hadjiconstantinou M. 2005. GM1 and ERK signaling in the aged brain. Brain Res 1054:125–134.

Moe MK, Jensen E. 2004. Structure elucidation of unsaturated fatty acids after vicinal hydroxylation of the double bonds by negative electrospray ionisation low-energy tandem mass spectrometry. Eur J Mass Spectrom (Chichester, England) 10:47–55.

Moe MK, Anderssen T, Strom MB, Jensen E. 2004. Vicinal hydroxylation of unsaturated fatty acids for structural characterization of intact neutral phospholipids by negative electrospray ionization tandem quadrupole mass spectrometry. Rapid Commun Mass Spectrom 18:2121–2130.

Moe MK, Anderssen T, Strom MB, Jensen E. 2005. Total structure characterization of unsaturated acidic phospholipids provided by vicinal di-hydroxylation of fatty acid double bonds and negative

electrospray ionization mass spectrometry. J Am Soc Mass Spectrom 16:46–59.

Moffett S, Brown DA, Linder ME. 2000. Lipid-dependent targeting of G proteins into rafts. J Biol Chem 275:2191–2198.

Moneti G, Pieraccini G, Dani FR, Catinella S, Traldi P. 1996. Acetonitrile as an effective reactant species for positive-ion chemical ionization of hydrocarbons by ion-trap mass spectrometry. Rapid Commun Mass Spectrom 10:167–170.

Moneti G, Pieraccini G, Dani FR, Turillazzi S, Favretto D, Traldi P. 1997. Ion-molecule reactions of ionic species from acetonitrile with unsaturated hydrocarbons for the identification of the double-bond position using an ion trap. J Mass Spectrom 32:1371–1373.

Moneti G, Pieraccini G, Favretto D, Traldi P. 1999. Reactions of ionic species from acetonitrile with long-chain saturated and unsaturated alcohols. J Mass Spectrom 34:1354–1360.

Morciano M, Burre J, Corvey C, Karas M, Zimmermann H, Volknandt W. 2005. Immunoisolation of two synaptic vesicle pools from synaptosomes: A proteomics analysis. J Neurochem 95:1732–1745.

Mossoba MM, Yurawecz MP, Roach JA, Lin HS, McDonald RE, Flickinger BD, Perkins EG. 1994. Rapid determination of double bond configuration and position along the hydrocarbon chain in cyclic fatty acid monomers. Lipids 29:893–896.

Munoz-Garcia A, Ro J, Brown JC, Williams JB. 2008. Cutaneous water loss and sphingolipids in the stratum corneum of house sparrows, Passer domesticus L., from desert and mesic environments as determined by reversed phase high-performance liquid chromatography coupled with atmospheric pressure photospray ionization mass spectrometry. J Exp Biol 211:447–458.

Munson MS, Field FH. 1966. Chemical ionization mass spectrometry. I. General introduction. J Am Soc Mass Spectrom 88:2621–2630.

Murphy RC, Smith WL. 2002. The eicosanoids: Cyclooxygenase, lipoxygenase, epoxygenase pathways. In: Vance DE, Vance JE, editors. New comprehensive biochemistry: Biochemistry of lipids, lipoproteins and membranes. New York: Elsevier Science. pp. 341–371.

Murphy RC, Barkley RM, Zemski Berry K, Hankin J, Harrison K, Johnson C, Krank J, McAnoy A, Uhlson C, Zarini S. 2005. Electrospray ionization and tandem mass spectrometry of eicosanoids. Anal Biochem 346:1–42.

Nagafuku M, Kabayama K, Oka D, Kato A, Tani-ichi S, Shimada Y, Ohno-Iwashita Y, Yamasaki S, Saito T, Iwabuchi K, Hamaoka T, Inokuchi J, Kosugi A. 2003. Reduction of glycosphingolipid levels in lipid rafts affects the expression state and function of glycosylphosphatidylinositol-anchored proteins but does not impair signal transduction via the T cell receptor. J Biol Chem 278:51920–51927.

Nagao T, Kubo T, Fujimoto R, Nishio H, Takeuchi T, Hata F. 1995. Ca(2+)-independent fusion of secretory granules with phospholipase A2-treated plasma membranes in vitro. Biochem J 307(Pt 2):563–569.

Nakagawa K, Oak JH, Higuchi O, Tsuzuki T, Oikawa S, Otani H, Mune M, Cai H, Miyazawa T. 2005. Ion-trap tandem mass spectrometric analysis of Amadori-glycated phosphatidylethanolamine in human plasma with or without diabetes. J Lipid Res 46:2514–2524.

Nakamura K, Suzuki Y, Goto-Inoue N, Yoshida-Noro C, Suzuki A. 2006. Structural characterization of neutral glycosphingolipids by thin-layer chromatography coupled to matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight MS/MS. Anal Chem 78:5736–5743.

Nikkila J, Sysi-Aho M, Ermolov A, Seppanen-Laakso T, Simell O, Kaski S, Oresic M. 2008. Gender-dependent progression of systemic metabolic states in early childhood. Mol Syst Biol 4:197.

Ohlrogge JB, Jaworski JG. 1997. Regulation of fatty acid synthesis. Annu Rev Plant Physiol Plant Mol Biol 48:109–136.

Oldham NJ, Svatos A. 1999. Determination of the double bond position in functionalized monoenes by chemical ionization ion-trap mass spectrometry using acetonitrile as a reagent gas. Rapid Commun Mass Spectrom 13:331–336.

Olsen JV, Macek B, Lange O, Makarov A, Horning S, Mann M. 2007. Higher-energy C-trap dissociation for peptide modification analysis. Nat Methods 4:709–712.

Oresic M, Hanninen VA, Vidal-Puig A. 2008. Lipidomics: A new window to biomedical frontiers. Trends Biotechnol 26:647–652.

Otieno AC, Mwongela SM. 2008. Capillary electrophoresis-based methods for the determination of lipids—A review. Anal Chim Acta 624:163–174.

Oursel D, Loutelier-Bourhis C, Orange N, Chevalier S, Norris V, Lange CM. 2007. Identification and relative quantification of fatty acids in Escherichia coli membranes by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 21:3229–3233.

Pahlsson P, Spitalnik SL, Spitalnik PF, Fantini J, Rakotonirainy O, Ghardashkhani S, Lindberg J, Konradsson P, Larson G. 2001. Characterization of galactosyl glycerolipids in the HT29 human colon carcinoma cell line. Arch Biochem Biophys 396:187–198.

Patel HH, Murray F, Insel PA. 2008. G-protein-coupled receptor-signaling components in membrane raft and caveolae microdomains. Handb Exp Pharmacol 186:167–184.

Pereto J, Lopez-Garcia P, Moreira D. 2004. Ancestral lipid biosynthesis and early membrane evolution. Trends Biochem Sci 29:469–477.

Persson A, Jergil B. 1995. The purification of membranes by affinity partitioning. FASEB J 9:1304–1310.

Persson E, Lofgren L, Hansson G, Abrahamsson B, Lennernas H, Nilsson R. 2007. Simultaneous assessment of lipid classes and bile acids in human intestinal fluid by solid-phase extraction and HPLC methods. J Lipid Res 48:242–251.

Piehowski PD, Carado AJ, Kurczy ME, Ostrowski SG, Heien ML, Winograd N, Ewing AG. 2008. MS/MS methodology to improve subcellular mapping of cholesterol using TOF-SIMS. Anal Chem 80:8662–8667.

Pike LJ, Han X, Gross RW. 2005. Epidermal growth factor receptors are localized to lipid rafts that contain a balance of inner and outer leaflet lipids: A shotgun lipidomics study. J Biol Chem 280:26796–26804.

Piomelli D, Astarita G, Rapaka R. 2007. A neuroscientist's guide to lipidomics. Nat Rev Neurosci 8:743–754.

Postle AD, Wilton DC, Hunt AN, Attard GS. 2007. Probing phospholipid dynamics by electrospray ionisation mass spectrometry. Prog Lipid Res 46:200–224.

Pulfer M, Murphy RC. 2003. Electrospray mass spectrometry of phospholipids. Mass Spectrom Rev 22:332–364.

Puri V, Jefferson JR, Singh RD, Wheatley CL, Marks DL, Pagano RE. 2003. Sphingolipid storage induces accumulation of intracellular cholesterol by stimulating SREBP-1 cleavage. J Biol Chem 278:20961–20970.

Quinn PJ, Joo F, Vigh L. 1989. The role of unsaturated lipids in membrane structure and stability. Prog Biophys Mol Biol 53:71–103.

Raetz CR, Whitfield C. 2002. Lipopolysaccharide endotoxins. Annu Rev Biochem 71:635–700.

Raffaelli A, Saba A. 2003. Atmospheric pressure photoionization mass spectrometry. Mass Spectrom Rev 22:318–331.

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 6:552–558.

Reeves CD. 2003. The enzymology of combinatorial biosynthesis. Crit Rev Biotechnol 23:95–147.

Retra K, Bleijerveld OB, van Gestel RA, Tielens AG, van Hellemond JJ, Brouwers JF. 2008. A simple and universal method for the separation and identification of phospholipid molecular species. Rapid Commun Mass Spectrom 22:1853–1862.

Ricciarelli R, Zingg JM, Azzi A. 2001. Vitamin E: Protective role of a Janus molecule. FASEB J 15:2314–2325.

Richter K, Nygren H, Malmberg P, Hagenhoff B. 2007. Localization of fatty acids with selective chain length by imaging time-of-flight secondary ion mass spectrometry. Microsc Res Tech 70:640–647.

Rigoni M, Caccin P, Gschmeissner S, Koster G, Postle AD, Rossetto O, Schiavo G, Montecucco C. 2005. Equivalent effects of snake PLA2 neurotoxins and lysophospholipid-fatty acid mixtures. Science 310:1678–1680.

Rimmerman N, Hughes HV, Bradshaw HB, Pazos MX, Mackie K, Prieto AL, Walker JM. 2008. Compartmentalization of endocannabinoids into lipid rafts in a dorsal root ganglion cell line. Br J Pharmacol 153:380–389.

Risselada HJ, Marrink SJ. 2008. The molecular face of lipid rafts in model membranes. Proc Natl Acad Sci USA 105:17367–17372.

Roche DM, Byers JT, Smith DS, Glansdorp FG, Spring DR, Welch M. 2004. Communications blackout? Do N-acylhomoserine-lactone-degrading enzymes have any role in quorum sensing? Microbiology 150:2023–2028.

Rodriguez-Concepcion M. 2004. The MEP pathway: A new target for the development of herbicides, antibiotics and antimalarial drugs. Curr Pharm Des 10:2391–2400.

Roed L, Lundanes E, Greibrokk T. 1999. Nonaqueous electrochromatography on C30 columns: Separation of retinyl esters. Electrophoresis 20:2373–2378.

Roed L, Lundanes E, Greibrokk T. 2000. Nonaqueous electrochromatography on continuous bed columns of sol-gel bonded large-pore C18 material: Separation of retinyl esters. J Chromatogr A 890:347–353.

Rohlfing A, Muthing J, Pohlentz G, Distler U, Peter-Katalinic J, Berkenkamp S, Dreisewerd K. 2007. IR-MALDI-MS analysis of HPTLC-separated phospholipid mixtures directly from the TLC plate. Anal Chem 79:5793–5808.

Rouzer CA, Ivanova PT, Byrne MO, Milne SB, Marnett LJ, Brown HA. 2006. Lipid profiling reveals arachidonate deficiency in RAW264.7 cells: Structural and functional implications. Biochemistry 45: 14795–14808.

Roy S, Delobel A, Gaudin K, Touboul D, Germain DP, Baillet A, Prognon P, Laprevote O, Chaminade P. 2006. Liquid chromatography on porous graphitic carbon with atmospheric pressure photoionization mass spectrometry and tandem mass spectrometry for the analysis of glycosphingolipids. J Chromatogr A 1117:154–162.

Ruiz-Gutierrez V, Perez-Camino MC. 2000. Update on solid-phase extraction for the analysis of lipid classes and related compounds. J Chromatogr A 885:321–341.

Rujoi M, Estrada R, Yappert MC. 2004. In situ MALDI-TOF MS regional analysis of neutral phospholipids in lens tissue. Anal Chem 76:1657–1663.

Russell DW. 2003. The enzymes, regulation, and genetics of bile acid synthesis. Annu Rev Biochem 72:137–174.

Ryan SD, Harris CS, Carswell CL, Baenziger JE, Bennett SA. 2008. Heterogeneity in the sn-1 carbon chain of platelet-activating factor glycerophospholipids determines pro- or anti-apoptotic signaling in primary neurons. J Lipid Res 49:2250–2258.

Salaun C, Gould GW, Chamberlain LH. 2005. Lipid raft association of SNARE proteins regulates exocytosis in PC12 cells. J Biol Chem 280:19449–19453.

Sanchez-Mejia RO, Newman JW, Toh S, Yu GQ, Zhou Y, Halabisky B, Cisse M, Scearce-Levie K, Cheng IH, Gan L, Palop JJ, Bonventre JV, Mucke L. 2008. Phospholipase A2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. Nat Neurosci 11:1311–1318.

Schenk B, Fernandez F, Waechter CJ. 2001. The ins(ide) and out(side) of dolichyl phosphate biosynthesis and recycling in the endoplasmic reticulum. Glycobiology 11:61R–70R.

Schiller J, Arnold K. 2002. Application of high resolution 31P NMR spectroscopy to the characterization of the phospholipid composition of tissues and body fluids—A methodological review. Med Sci Monit 8:MT205–MT222.

Schiller J, Suss R, Arnhold J, Fuchs B, Lessig J, Muller M, Petkovic M, Spalteholz H, Zschornig O, Arnold K. 2004. Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry in lipid and phospholipid research. Prog Lipid Res 43:449–488.

Schiller J, Suss R, Fuchs B, Muller M, Zschornig O, Arnold K. 2007. MALDI-TOF MS in lipidomics. Front Biosci 12:2568–2579.

Schindler J, Lewandrowski U, Sickmann A, Friauf E, Nothwang HG. 2006. Proteomic analysis of brain plasma membranes isolated by affinity two-phase partitioning. Mol Cell Proteomics 5:390–400.

Schmelzer K, Fahy E, Subramaniam S, Dennis EA. 2007. The lipid maps initiative in lipidomics. Methods Enzymol 432:171–183.

Schmitz G, Grandl M. 2008. Lipid homeostasis in macrophages—Implications for atherosclerosis. Rev Physiol Biochem Pharmacol 160:93–125.

Schwartz JC, Senko MW, Syka JE. 2002. A two-dimensional quadrupole ion trap mass spectrometer. J Am Soc Mass Spectrom 13:659–669.

Schwarz E, Prabakaran S, Whitfield P, Major H, Leweke FM, Koethe D, McKenna P, Bahn S. 2008. High throughput lipidomic profiling of schizophrenia and bipolar disorder brain tissue reveals alterations of free fatty acids, phosphatidylcholines, and ceramides. J Proteome Res 7:4266–4277.

Schwudke D, Oegema J, Burton L, Entchev E, Hannich JT, Ejsing CS, Kurzchalia T, Shevchenko A. 2006. Lipid profiling by multiple precursor and neutral loss scanning driven by the data-dependent acquisition. Anal Chem 78:585–595.

Schwudke D, Hannich JT, Surendranath V, Grimard V, Moehring T, Burton L, Kurzchalia T, Shevchenko A. 2007a. Top-down lipidomic screens by multivariate analysis of high-resolution survey mass spectra. Anal Chem 79:4083–4093.

Schwudke D, Liebisch G, Herzog R, Schmitz G, Shevchenko A. 2007b. Shotgun lipidomics by tandem mass spectrometry under data-dependent acquisition control. Methods Enzymol 433:175–191.

Shaner RL, Allegood JC, Park H, Wang E, Kelly S, Haynes CA, Sullards MC, Merrill AH Jr. 2009. Quantitative analysis of sphingolipids for lipidomics using triple quadrupole and quadrupole linear ion trap mass spectrometers. J Lipid Res 50:1692–1707.

Shimizu T. 2008. Lipid mediators in health and disease: Enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. Annu Rev Pharmacol Toxicol 3:3.

Shimma S, Sugiura Y, Hayasaka T, Zaima N, Matsumoto M, Setou M. 2008. Mass imaging and identification of biomolecules with MALDI-QIT-TOF-based system. Anal Chem 80:878–885.

Shvartsburg AA, Smith RD. 2008. Fundamentals of traveling wave ion mobility spectrometry. Anal Chem 80:9689–9699.

Silvius JR. 2003. Role of cholesterol in lipid raft formation: Lessons from lipid model systems. Biochim Biophys Acta 1610:174–183.

Simoes C, Simoes V, Reis A, Domingues P, Domingues MR. 2008. Determination of the fatty acyl profiles of phosphatidylethanolamines by tandem mass spectrometry of sodium adducts. Rapid Commun Mass Spectrom 22:3238–3244.

Simons K, Vaz WL. 2004. Model systems, lipid rafts, and cell membranes. Annu Rev Biophys Biomol Struct 33:269–295.

Sleno L, Volmer DA. 2004. Ion activation methods for tandem mass spectrometry. J Mass Spectrom 39:1091–1112.

Small DM. 1986. The physical chemistry of lipids. Handbook of lipid research. New York: Plenum Press.

Smith PB, Snyder AP, Harden CS. 1995. Characterization of bacterial phospholipids by electrospray ionization tandem mass spectrometry. Anal Chem 67:1824–1830.

Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. 2006. XCMS: Processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem 78:779–787.

Smith JC, Hou W, Whitehead SN, Ethier M, Bennett SA, Figeys D. 2008. Identification of lysophosphatidylcholine (LPC) and platelet activating factor (PAF) from PC12 cells and mouse cortex using liquid chromatography/multi-stage mass spectrometry (LC/MS(3)). Rapid Commun Mass Spectrom 22:3579–3587.

Sobolik KD, Gremillion KJ, Whitten PL, Watson PJ. 1996. Sex determination of prehistoric human paleofeces. Am J Phys Anthropol 101:283–290.

Spaink HP. 2000. Root nodulation and infection factors produced by rhizobial bacteria. Annu Rev Microbiol 54:257–288.

Spener F, Lagarde M, Géloên A, Record M. 2003. Editorial: What is lipidomics? Eur J Lipid Sci Technol 105:481–482.

Srinivasan S, Traini M, Herbert B, Sexton D, Harry J, Alexander H, Williams KL, Alexander S. 2001. Proteomic analysis of a developmentally regulated secretory vesicle. Proteomics 1:1119–1127.

Staneva G, Angelova MI, Koumanov K. 2004. Phospholipase A2 promotes raft budding and fission from giant liposomes. Chem Phys Lipids 129:53–62.

Stubiger G, Belgacem O. 2007. Analysis of lipids using 2,4,6-trihydroxyacetophenone as a matrix for MALDI mass spectrometry. Anal Chem 79:3206–3213.

Stubiger G, Pittenauer E, Allmaier G. 2008. MALDI seamless postsource decay fragment ion analysis of sodiated and lithiated phospholipids. Anal Chem 80:1664–1678.

Sud M, Fahy E, Cotter D, Brown A, Dennis EA, Glass CK, Merrill AH, Jr., Murphy RC, Raetz CR, Russell DW, Subramaniam S. 2007. LMSD: LIPID MAPS structure database. Nucleic Acids Res 35:D527–D532.

Syka JE, 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 101:9528–9533.

Taguchi R, Hayakawa J, Takeuchi Y, Ishida M. 2000. Two-dimensional analysis of phospholipids by capillary liquid chromatography/electrospray ionization mass spectrometry. J Mass Spectrom 35:953–966.

Taguchi R, Nishijima M, Shimizu T. 2007. Basic analytical systems for lipidomics by mass spectrometry in Japan. Methods Enzymol 432:185–211.

Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T. 1988. Protein and polymer analysis up to m/z 100.000 by laser ionisation time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 2:151–153.

Taniguchi N, Honke K, Fukuda M. 2002. Handbook of glycosyltransferases and related genes. Tokyo: Springer-Verlag.

Thomas MC, Mitchell TW, Blanksby SJ. 2006. Ozonolysis of phospholipid double bonds during electrospray ionization: A new tool for structure determination. J Am Chem Soc 128:58–59.

Thomas MC, Mitchell TW, Harman DG, Deeley JM, Murphy RC, Blanksby SJ. 2007. Elucidation of double bond position in unsaturated lipids by ozone electrospray ionization mass spectrometry. Anal Chem 79:5013–5022.

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 80:303–311.

Tieleman DP, Marrink SJ. 2006. Lipids out of equilibrium: Energetics of desorption and pore mediated flip-flop. J Am Chem Soc 128:12462–12467.

Tomer KB, Crow FW, Gross ML. 1983. Location of double bond position in unsaturated fatty acids by negative ion MS/MS. J Am Soc Mass Spectrom 105:5487–5488.

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 18:1967–1972.

Tsai MJ, O'Malley BW. 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486.

Tyurin VA, Tyurina YY, Kochanek PM, Hamilton R, DeKosky ST, Greenberger JS, Bayir H, Kagan VE. 2008. Oxidative lipidomics of programmed cell death. Methods Enzymol 442:375–393.

Tyurina YY, Tyurin VA, Epperly MW, Greenberger JS, Kagan VE. 2008. Oxidative lipidomics of gamma-irradiation-induced intestinal injury. Free Radic Biol Med 44:299–314.

UPAC-IUB-JCBN. 1999. IUPAC-IUB joint commission on biochemical nomenclature (JCBN) nomenclature of glycolipids recommendations 1997. J Mol Biol 286:963–970.

van Meer G. 2005. Cellular lipidomics. EMBO J 24:3159–3165.

van Meer G, Lisman Q. 2002. Sphingolipid transport: Rafts and translocators. J Biol Chem 277:25855–25858.

van Meer G, Voelker DR, Feigenson GW. 2008. Membrane lipids: Where they are and how they behave. Nat Rev Mol Cell Biol 9:112–124.

Van Pelt CK, Brenna JT. 1999. Acetonitrile chemical ionization tandem mass spectrometry to locate double bonds in polyunsaturated fatty acid methyl esters. Anal Chem 71:1981–1989.

Van Pelt CK, Carpenter BK, Brenna JT. 1999. Studies of structure and mechanism in acetonitrile chemical ionization tandem mass spectrometry of polyunsaturated fatty acid methyl esters. J Am Soc Mass Spectrom 10:1253–1262.

Vance DE, Vance JE. 2002. Biochemistry of lipids, lipoproteins and membranes. New York: Elsevier Science.

Vejux A, Malvitte L, Lizard G. 2008. Side effects of oxysterols: Cytotoxicity, oxidation, inflammation, and phospholipidosis. Braz J Med Biol Res 41:545–556.

Veracini L, Simon V, Richard V, Schraven B, Horejsi V, Roche S, Benistant C. 2008. The Csk-binding protein PAG regulates PDGF-induced Src mitogenic signaling via GM1. J Cell Biol 182:603–614.

Vernooij E, Brouwers J, Kettenes-van den Bosch J, Crommelin D. 2002. RP-HPLC/ESI MS determination of acyl chain positions in phospholipids. J Sep Sci 25:285–289.

Vicinanza M, D'Angelo G, Di Campli A, De Matteis MA. 2008. Function and dysfunction of the PI system in membrane trafficking. EMBO J 27:2457–2470.

Vissers JP. 1999. Recent developments in microcolumn liquid chromatography. J Chromatogr A 856:117–143.

Vissers JP, Hulst WP, Chervet JP, Snijders HM, Cramers CA. 1996. Automated on-line ionic detergent removal from minute protein/peptide samples prior to liquid chromatography-electrospray mass spectrometry. J Chromatogr B Biomed Appl 686:119–128.

Vitic J, Nikolic B, Nikolic V. 1981. A specific type of dislipidaemia—A complex between monotypic immunoglobulins and lipids. J Clin Lab Immunol 6:227–231.

Vukelic Z, Zamfir AD, Bindila L, Froesch M, Peter-Katalinic J, Usuki S, Yu RK. 2005. Screening and sequencing of complex sialylated and sulfated glycosphingolipid mixtures by negative ion electrospray Fourier transform ion cyclotron resonance mass spectrometry. J Am Soc Mass Spectrom 16:571–580.

Wakelam MJ, Pettitt TR, Postle AD. 2007. Lipidomic analysis of signaling pathways. Methods Enzymol 432:233–246.

Walker AV. 2008. Why is SIMS underused in chemical and biological analysis? Challenges and opportunities. Anal Chem 80:8865–8870.

Walsh CT. 2004. Polyketide and nonribosomal peptide antibiotics: Modularity and versatility. Science 303:1805–1810.

Washbourne P, Cansino V, Mathews JR, Graham M, Burgoyne RD, Wilson MC. 2001. Cysteine residues of SNAP-25 are required for SNARE disassembly and exocytosis, but not for membrane targeting. Biochem J 357:625–634.

Weininger D. 1998. SMILES, a chemical language and information system. 1. Introduction to methodology and encoding rules. J Chem Inf Comput Sci 28:31–36.

Wenk MR. 2005. The emerging field of lipidomics. Nat Rev Drug Discov 4:594–610.

Wenk MR, Lucast L, Di Paolo G, Romanelli AJ, Suchy SF, Nussbaum RL, Cline GW, Shulman GI, McMurray W, De Camilli P. 2003. Phosphoinositide profiling in complex lipid mixtures using electrospray ionization mass spectrometry. Nat Biotechnol 21:813–817.

Wheeler D, Bandaru VV, Calabresi PA, Nath A, Haughey NJ. 2008. A defect of sphingolipid metabolism modifies the properties of normal appearing white matter in multiple sclerosis. Brain 131:3092–3102.

White T, Bursten S, Federighi D, Lewis RA, Nudelman E. 1998. High-resolution separation and quantification of neutral lipid and phospholipid species in mammalian cells and sera by multi-one-dimensional thin-layer chromatography. Anal Biochem 258:109–117.

Whitehead SN, Hou W, Ethier M, Smith JC, Bourgeois A, Denis R, Bennett SA, Figeys D. 2007. Identification and quantitation of changes in the platelet activating factor family of glycerophospholipids over the course of neuronal differentiation by high-performance liquid chromatography electrospray ionization tandem mass spectrometry. Anal Chem 79:8539–8548.

Wislet-Gendebien S, Visanji NP, Whitehead SN, Marsilio D, Hou W, Figeys D, Fraser PE, Bennett SA, Tandon A. 2008. Differential regulation of wild-type and mutant alpha-synuclein binding to synaptic membranes by cytosolic factors. BMC Neurosci 9:92.

Wolf C, Quinn PJ. 2008. Lipidomics: Practical aspects and applications. Prog Lipid Res 47:15–36.

Woods AS, Jackson SN. 2006. Brain tissue lipidomics: Direct probing using matrix-assisted laser desorption/ionization mass spectrometry. AAPS J 8:E391–E395.

Woods AS, Ugarov M, Egan T, Koomen J, Gillig KJ, Fuhrer K, Gonin M, Schultz JA. 2004. Lipid/peptide/nucleotide separation with MALDI-ion mobility-TOF MS. Anal Chem 76:2187–2195.

Woods AS, Ugarov M, Jackson SN, Egan T, Wang HY, Murray KK, Schultz JA. 2006. IR-MALDI-LDI combined with ion mobility orthogonal time-of-flight mass spectrometry. J Proteome Res 5:1484–1487.

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 79:2525–2536.

Yan W, Jenkins CM, Han X, Mancuso DJ, Sims HF, Yang K, Gross RW. 2005. The highly selective production of 2-arachidonoyl lysophosphatidylcholine catalyzed by purified calcium-independent phospholipase A2gamma: Identification of a novel enzymatic mediator for the generation of a key branch point intermediate in eicosanoid signaling. J Biol Chem 280:26669–26679.

Yang K, Cheng H, Gross RW, Han X. 2009a. Automated lipid identification and quantification by multidimensional mass spectrometry-based shotgun lipidomics. Anal Chem 81:4356–4368.

Yang K, Zhao Z, Gross RW, Han X. 2009b. Systematic analysis of choline-containing phospholipids using multi-dimensional mass spectrometry-based shotgun lipidomics. J Chromatogr B Analyt Technol Biomed Life Sci 877:2924–2936.

Yasugi E, Watanabe K. 2002. LIPIDBANK for Web, the newly developed lipid database. Tanpakushitsu Kakusan Koso 47:837–841.

Yetukuri L, Ekroos K, Vidal-Puig A, Oresic M. 2008. Informatics and computational strategies for the study of lipids. Mol Biosyst 4:121–127.

Yu S, Cho K, Kim YH, Park S, Kim J, Oh HB. 2006. Identification of phospholipid molecular species in porcine brain extracts using high mass accuracy of 4.7Tesla Fourier transform ion cyclotron resonance mass spectrometry. Bull Korean Chem Soc 27:793–796.

Yunoki K, Sato M, Seki K, Ohkubo T, Tanaka Y, Ohnishi M. 2009. Simultaneous quantification of plant glyceroglycolipids including sulfoquinovosyldiacylglycerol by HPLC-ELSD with binary gradient elution. Lipids 44:77–83.

Zacarias A, Bolanowski D, Bhatnagar A. 2002. Comparative measurements of multicomponent phospholipid mixtures by electrospray mass spectroscopy: Relating ion intensity to concentration. Anal Biochem 308:152–159.

Zehethofer N, Pinto DM. 2008. Recent developments in tandem mass spectrometry for lipidomic analysis. Anal Chim Acta 627:62–70.

Zeng Y, Cheng H, Jiang X, Han X. 2008. Endosomes and lysosomes play distinct roles in sulfatide-induced neuroblastoma apoptosis: Potential mechanisms contributing to abnormal sulfatide metabolism in related neuronal diseases. Biochem J 410:81–92.

Zhang L, Wang X, Peng X, Wei Y, Cao R, Liu Z, Xiong J, Ying X, Chen P, Liang S. 2007. Immunoaffinity purification of plasma membrane with secondary antibody superparamagnetic beads for proteomic analysis. J Proteome Res 6:34–43.

Zhao S, Jia L, Gao P, Li Q, Lu X, Li J, Xu G. 2008. Study on the effect of eicosapentaenoic acid on phospholipids composition in membrane microdomains of tight junctions of epithelial cells by liquid chromatography/electrospray mass spectrometry. J Pharm Biomed Anal 47:343–350.

Zheng H, Chu J, Qiu Y, Loh HH, Law PY. 2008. Agonist-selective signaling is determined by the receptor location within the membrane domains. Proc Natl Acad Sci USA 105:9421–9426.

Zubarev RA, Kelleher NL, McLafferty FW. 1998. Electron capture dissociation of multiply charged protein cations—A nonergodic process. J Am Chem Soc 120:3265–3266.

Maroun Bou Khalil is a research associate at the Ottawa Institute of Systems Biology of the University of Ottawa focusing on lipidomics and proteomics of lipid metabolism and disorders.

Weimin Hou is a PhD candidate in the Chemistry Department at the University of Ottawa focusing on lipidomics and proteomics technologies.

Hu Zhou is a postdoctoral fellow at the Ottawa Institute of Systems Biology of the University of Ottawa focusing on proteomics and lipidomics technologies.

Fred Elisma is a research bioinformatician at the Ottawa Institute of Systems Biology of the University of Ottawa focusing on the development of tools for proteomics and lipidomics.

Leigh Anne Swayne is a postdoctoral fellow in the Biochemistry, Microbiology and Immunology Department and Ottawa Institute of Systems Biology at the University of Ottawa studying lipid mediators of neurodegenerative disease.

Alexandre P. Blanchard is a PhD candidate at the Neuroscience department at the University of Ottawa studying lipid mediators of neurodegenerative disease.

Zemin Yao is a professor and chairman at the Biochemistry, Microbiology and Immunology Department at the University of Ottawa focusing on lipids/lipoprotein metabolism and disease.

Steffany A.L. Bennett is an associate professor in the Biochemistry, Microbiology and Immunology Department and Ottawa Institute of Systems Biology at the University of Ottawa focusing on mechanistic assessment of lipid mediators of neurodegenerative disease.

Daniel Figeys is a Canada Research Chair in Proteomics and Systems Biology, a professor in the Biochemistry, Microbiology and Immunology Department at the University of Ottawa, and the director of the Ottawa Institute of Systems Biology of the University of Ottawa focusing on proteomics and lipidomics technologies and application.