Zurich Open Repository and Archive
University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2010

Lipidomics: new tools and applications.

Wenk, Markus R

DOI: https://doi.org/10.1016/j.cell.2010.11.033

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-79015
Journal Article

Originally published at:
Wenk, Markus R (2010). Lipidomics: new tools and applications. Cell, 143(6):888-95.
DOI: https://doi.org/10.1016/j.cell.2010.11.033

Lipidomics: New Tools and Applications

Markus R Wenk[1,2,*]
[1]National University of Singapore, Yong Loo Lin School of Medicine, Department of Biochemistry and Faculty of Science, Department of Biological Sciences, Centre for Life Sciences (CeLS), 28 Medical Drive, Singapore 117456
[2]Swiss Tropical and Public Health Institute and the University of Basel, Switzerland
*Correspondence: markus_wenk@nuhs.edu.sg
DOI 10.1016/j.cell.2010.11.033

Once viewed simply as a reservoir for carbon storage, lipids are no longer cast as bystanders in the drama of biological systems. The emerging field of lipidomics is driven by technology, most notably mass spectrometry, but also by complementary approaches for the detection and characterization of lipids and their biosynthetic enzymes in living cells. The development of these integrated tools promises to greatly advance our understanding of the diverse biological roles of lipids.

Lipids are not genetically encoded. Instead, like other small molecules they are generated and metabolized by enzymes that are influenced by the environment of a given biological system, for instance by diet and temperature. Although still poorly defined, some estimates have placed the number of distinct chemical entities within the lipid sphere between 10,000 and 100,000. Although it is unclear how and why nature generates this staggering diversity, there is an increasing awareness across many disciplines of the critical importance of lipids in all aspects of life.

First, coordinated lipid anabolism and catabolism is a key molecular integrator of energy homeostasis, membrane structure and dynamics, and signaling (Figure 1) with imbalances in lipid metabolism contributing to diverse phenotypes and disease states. Second, there is an expanding number of drugs that target lipid metabolic and signaling pathways, including the well-known and profitable cholesterol-lowering agents (statins) and cyclooxygenase inhibitors. For therapeutic intervention in diseases ranging from inflammation and cancer to metabolic diseases, lipid researchers are seeking specific regulators of numerous targets, including phosphatidylinositol (PI) 3-kinases, nuclear hormone receptors (for instance, liver X receptor, LXR; peroxisome proliferator-activated receptors, PPARs), sphingosine, and ceramide kinases. A recent example is FTY720, approved for the treatment of multiple sclerosis in October 2010, an immunosuppressant that targets sphingosine-1-phosphate receptors (but interestingly does not inhibit serine palmitoyl transferase, unlike its mother compound myriocin, a natural product).

The scarcity of pertinent tools has led to investments in programs to develop new approaches for lipid research. Collectively, these efforts have added momentum to the field (reflected in part by the increasing number of publications and conferences dedicated to lipids), which promises to address fundamental questions of lipid function and to meet practical demands in the applied sciences. The aim of this Primer is to introduce the basic concepts behind biochemical (mass spectrometry-based) lipidomics, to discuss how these approaches are being integrated with complementary techniques, and to offer a view on the future of the field.

Mass Spectrometry-Based Lipidomics

The first reports of mass spectrometric (MS) analysis of complex lipid mixtures via soft ionization techniques (matrix-assisted laser desorption ionization, MALDI, and electrospray ionization, ESI) date back to the 1990s (Han and Gross, 1994; Kim et al., 1994). A large number of methods have been developed since then, and many biologically important lipids can now be analyzed on a fairly routine basis. However, unlike genomics and proteomics, which are well represented in various forms at leading research institutions worldwide, this is not yet the case for lipidomics (Figure 2).

A major difference in mass spectrometry of lipids (as opposed to proteins) is the large chemical diversity found in these molecules (Figure 1 and Figure 3A) (Fahy et al., 2005). As a consequence, it is currently not possible to comprehensively measure the lipidome of a cell or tissue in a single experiment. Furthermore, one often does not know what precise alteration in lipids to expect in any given case. Thus, first surveys are often exploratory, which is to say they often have "untargeted" readouts (Figure 3B). Such methods should have high mass accuracy and resolution, a characteristic of time of flight and Orbitrap mass spectrometry. Fragmentation of an ion of interest is then used for identification (Figure 3C). Analysis of fragmentation pathways has led to a detailed understanding of "bonding" between the different building blocks found in lipids (such as fatty acids, sphingoid bases, and head groups). It has also formed a basis for "shotgun" lipidomics in which precursor lipids are determined based on characteristic fragment ions. Other targeted approaches based on tandem mass spectrometry are now available for analysis of many different classes of lipids and in complex mixtures (Wenk, 2005; Blanksby and Mitchell, 2010).

The coordinated efforts of LIPID MAPS (http://www.lipidmaps.org) have laid the groundwork for standardization (for example, in protocols and in the nomenclature relevant to databases) in the field and to foster the commercial availability of many pure and synthetic lipid standards. These standards are deuterated versions or close chemical analogs of naturally occurring lipids that are used to quantify ion responses. They are used in a rapidly increasing number of lipidomic programs around the world (LIPID MAPS, Kansas Lipidomics Research Center, COBRE,

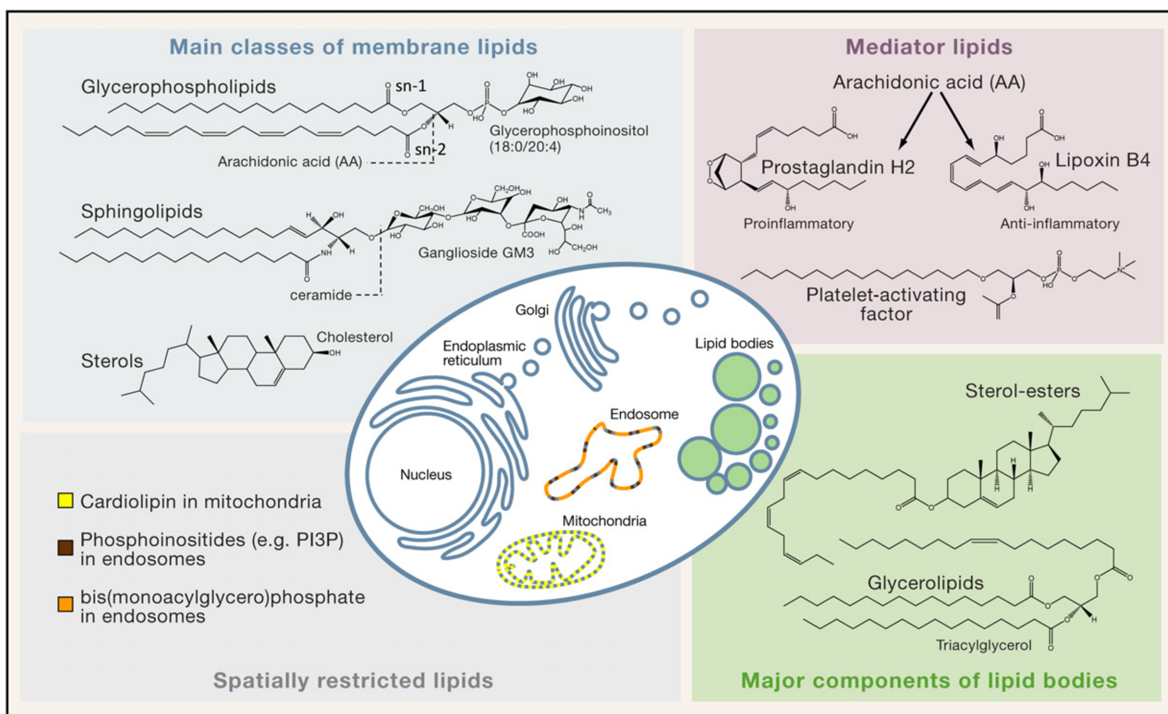

Figure 1. The Cellular Compartments of Common Biological Lipids

Lipids are small molecules of enormous chemical diversity. Unlike other major biomolecules (i.e., nucleic acids, polysaccharides, and proteins), they are not polymers of relatively small numbers of chemically distinct building blocks. Instead, they are the result of anabolic and catabolic reaction pathways that are under complex dietary and physiological control. It is thus difficult to define, name, and categorize lipids in a coherent and comprehensive fashion. Lipids of different chemical structures are highly organized within a typical eukaryotic cell. The lipid portion of biological membranes is to a large extent made up of glycerophospholipids, sterols, and sphingolipids (blue box, structures of three representative lipids from the different classes are shown). These are all examples of amphiphilic lipids, which have both hydrophilic and hydrophobic portions. The membrane-associated lipids are not evenly distributed. Some organelles are enriched with certain lipids (for instance, cardiolipin in mitochondria and lysobisphosphatidic acid/bis(monoacylglycero)phosphate in endosomes), and lateral distribution within membranes leads to functional domains. Metabolism of membrane lipids generates highly active signaling molecules (red box). These lipids, often much more soluble and diffusible than their membrane-associated parent, control organismal physiology. Very nonpolar lipids, such as sterol-esters and triglycerides, are assembled in the endoplasmic reticulum and stored in lipid bodies within cells.

WUSTL, Southampton Lipidomics Research Group, Lipidomics. Net, LipidX, Lipidomics Research Center Graz, LipidProfiles). In addition to these centers that harbor substantial analytical capabilities, individual laboratories are increasingly engaging in the analysis of specific metabolites and lipid pathways. The latter development can be explained, at least in part, by lowered costs and easier handling of modern mass spectrometers.

Two technical characteristics, high sensitivity and high specificity (mass resolution), account for the success of mass spectrometry in lipid analysis. For example, mass spectrometry has provided a detailed knowledge of the chemical (lipid) composition of highly purified vesicles or viruses, preparations in which sample amounts are limited. These "organelles" stay largely intact during preparation and are thus biochemically more accessible than other membrane fractions. Studies such as these provide evidence for segregation of specific sterol and sphingolipid species during formation of secretory vesicles at the trans-Golgi (Klemm et al., 2009) or enrichment of certain membrane lipids during formation of viruses at donor membranes of the host cell (Brügger et al., 2006; Chan et al., 2008). Sensitivity is also required for lipid metabolites that occur at low and transient levels. Phosphoinositides or fatty acyl derivatives have all been characterized by mass spectrometric methods and in complex lipid extracts, a task that would otherwise require laborious (and often indirect) techniques for detection. It should however be noted that, even with the major advances made by MS approaches, the detection of lipid species of very low abundance is still a major challenge (discussed below).

High-resolution mass spectrometry aids in identification of previously uncharacterized lipids and discrimination between lipids with similar mass and chemical structures. It has also provided evidence for the presence of isomeric species (which have the same chemical formula but different structures) and isobaric species (ions with the same mass) in cellular lipidomes. For example, ether phospholipids are often isomeric with other abundant cellular phospholipids (Yang et al., 2007).

There are several analytical challenges that cannot be addressed satisfactorily by mass spectrometry alone. These include unequivocal assignment of structures: double bond configurations are difficult to determine and cannot be readily assigned based on tandem mass spectrometry (Thomas et al., 2009); chemical derivatization and/or nuclear magnetic resonance might be required for structure determination of complex glycolipids.

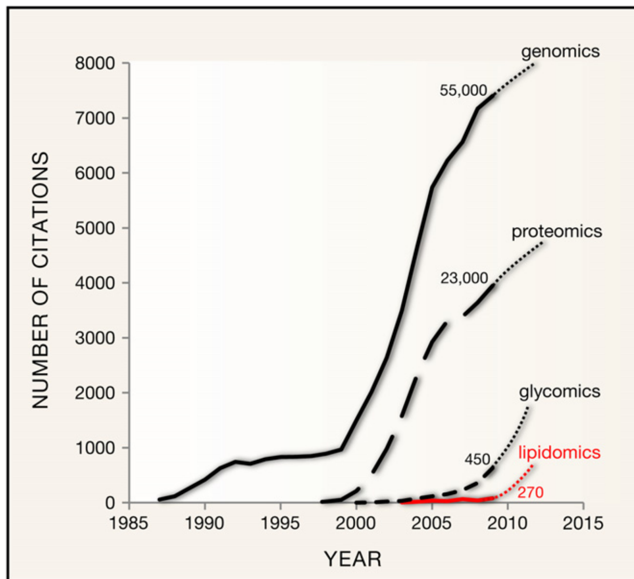

Figure 2. Lipidomics Is an Emerging Field

The sequencing of the human genome in the year 2000 sparked interest and investment in technologies and programs for the systematic analysis of genetic variation. As a result, the study of genomes and proteomes has produced large numbers of findings reported in the scientific literature (measured here as the cumulative numbers of citations in PubMed over time). Complete genomes can now be sequenced (and annotated) in a matter of days or weeks, and current development is primarily focused on lowering the cost per sequenced base. Many commercial products are available for sample preparation, analysis, and interpretation. This is also true for protein analysis, though it is still challenging to determine whole proteomes. Proteomics has gained tremendously from mass spectrometry for peptide detection and quantification. The boundaries for experimental measurements (such as number of proteins) are reasonably well established based on genetic information. None of the above is the case for lipidomic analysis. Currently, most of the mass spectrometric measurements are conducted by a few consortia and laboratories. The community is growing very rapidly, however, and these activities have led to interest in many disciplines. The first studies combining genomics and lipidomics have just been published. Given the central role of lipids as key metabolites with remarkably diverse biological roles, the field of lipidomics may follow a trajectory comparable to the developments seen in genomics and proteomics over the past decade.

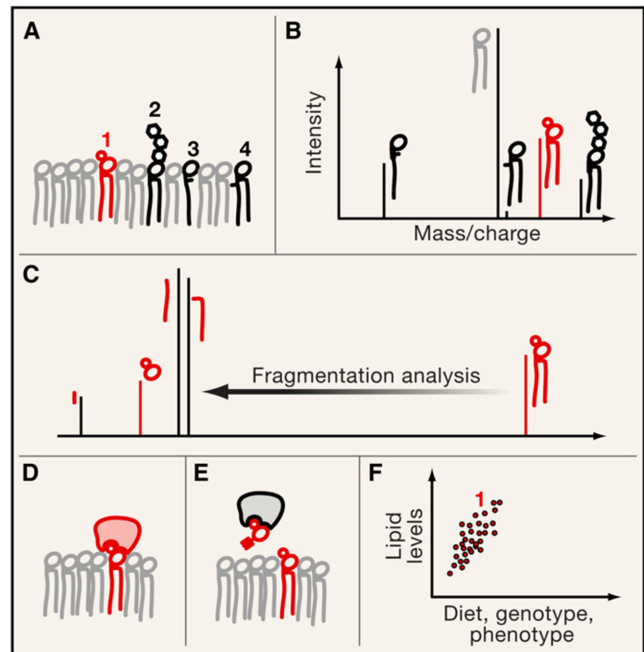

Figure 3. New Research Tools for Lipidomics

The precise size and dynamics of a cellular lipidome remains poorly understood both on theoretical as well as on experimental grounds. Hundreds to thousands of different chemical entities are recovered in an organic extract from a biological specimen where lipids are assembled in a coordinated fashion.

(A) An assembly of fatty acyl-containing membrane lipids with different head group decorations, for example phosphorylated (1) or glycosylated (2) forms, is depicted. Lipases hydrolyze lipids at various positions. Phospholipases A2 generate lysolipids (3), which have profound structural effects on lipid assemblies as well as signaling functions via G protein-coupled receptors. Less well understood are other modifications such as hydroxylations or methylations and oxidations or nitrosylation introduced via enzymatic and chemical reactions, respectively (4).

(B) Single stage and tandem mass spectrometry (C) have yielded tremendous insight into chemical details of cellular lipids. An ion with a mass/charge (m/z) ratio corresponding to the expected structure shown in red (structure 1 in panel A) can be fragmented and characterized based on product ions, which in the case of glycerophospholipids and negative mode ionization are fatty acyls, head group, and backbone-derived moieties.

(D) Complementary technologies that are currently being developed include chemical-biological approaches to probe lipid-protein interactions. For example, lipid-binding domains are used to visualize lipids in living cells or to locally interfere with lipid metabolism.

(E) Analogues of lipids can be introduced into cells to interfere with protein-lipid interactions, to inhibit enzymes, or for biochemical isolation of lipid-binding factors.

(F) Finally, bioinformatic tools will need to be further developed to support these experimental technologies (panels B–E) to facilitate combinations of genomics and lipidomics, compare between biological species, and identify clinically relevant biomarkers.

Current Challenges

Integration

Lipids and their metabolites serve as integrators of many cellular functions. Energy homeostasis is tightly coupled to fatty acid metabolism, and fatty acids are key building blocks of many cellular lipids (Figure 1). Thus, it seems evident that lipid metabolism must follow a very coordinated program during the cell cycle and proliferation. Given that cancer cells are dependent on fatty acids for the synthesis of membranes and signaling lipids, fatty acid synthase (FAS) is considered a potential therapeutic target. Recent work using cell biological approaches (Kurat et al., 2009) and functional proteomics (Nomura et al., 2010) discovered that breakdown of glycerolipids via lipases is a key mechanism for the generation of free fatty acids during cell proliferation, thus metabolically coupling lipid bodies with membrane synthesis (Figure 1) (Singh et al., 2009). Similar metabolic coupling, for instance, between membrane lipids and soluble lipid mediators, is likely to be discovered for specific phenotypes other than growth (Patwardhan et al., 2010). The known lipid "signaling" network is thus poised for a great expansion, in particular in the context of human disease (Wymann and Schneiter, 2008).

These are recent examples of integrated experimental approaches involving experiments that combine lipid biochemistry (via mass spectrometry or other means) and functional readouts. The first challenge in such endeavors is defining the

sample. Whole-cell extracts are often used, and as a consequence all information on spatial distribution is lost (van Meer, 2005). In the future, ensembles of protein markers will allow for better identification of subcellular organelles (Andreyev et al., 2010) and thus aid in preparations of lipid fractions related to specific cellular functions. The generation of lipid extracts prior to analysis is a critical aspect that currently attracts only moderate attention. Biochemical fractionation has inherent limitations in terms of the resulting purity and integrity of the samples. Furthermore, recovery rates during partitioning in organic solvents strongly depend on the lipid class, and nonquantitative recovery during extraction introduces variability.

Natural Variation

Metabolites can vary substantially between individuals and on a day-to-day basis (Assfalg et al., 2008), which complicates comparative studies. Often, the degree of natural variation of a metabolite/lipid in an individual or population is not known. In mice, some lysolipids display remarkable circadian patterns with up to 2-fold differences in their levels (Minami et al., 2009). Whereas modern mass spectrometers provide linear outputs over several orders of magnitude (linear dynamic range), the biologically relevant dynamic range is lipid specific, varying from 2- to 3-fold for abundant membrane lipids to 10- to 100-fold in extreme cases (such as mediator lipids). Importantly, lipids are also found at very different basal concentrations and have distinct temporal dependencies. Cellular fractionation, liquid chromatographic separation prior to MS (LC-MS), time course experiments, as well as selective capture of lipids will be required to overcome analytical challenges to resolving lipid species of interest when they are of low abundance. In cellular studies, metabolic labeling with chemical isotopes of lipid precursors followed by mass spectrometry is an elegant and powerful way to study kinetics of incorporation and turnover of some classes of lipids (Postle and Hunt, 2009).

In population studies, efforts to combine mass spectrometry-based lipidomics with genomics have been guided by the technical feasibility of measuring lipids on a large scale, the popularity of genome-wide association studies (GWAS), and human diseases associated with aberrant lipid metabolism. Strong associations are found between the levels of some polyunsaturated fatty acids (measured as fatty acid methyl esters by gas chromatography-MS) and lipid desaturases (Tanaka et al., 2009). GWAS with larger numbers of metabolic traits, measured via MS methods introduced above, have been conducted and published recently. In one study following 33 metabolic traits, several circulating sphingolipids were found to be under strong genetic control (Hicks et al., 2009). In another study with 163 metabolites (including major glycerophospholipids as well as acyl-CoAs and amino acids), ratios of substrate-product concentrations, rather than single metabolite levels, reduced variance and improved statistical significance (Illig et al., 2010).

Sequencing of candidate genes in individuals at the extremes of the population distribution with respect to lipoprotein levels led to the discovery of nonsynonymous sequence variants in enzymes involved in cholesterol metabolism (Fahmi et al., 2008). Targeted genomics of lipid metabolic pathways in combination with biochemical lipid analysis is an area of great future potential. The link between genetic variation and changes in lipid levels will be relevant not only for population-based studies but also at the level of individuals.

Data Analysis and Interpretation

Arguably, proteomics was transformed by the development of search algorithms that enabled assignment of protein sequences by comparisons of experimental and theoretical MS fragmentation patterns of tryptic peptides. In the case of lipids, the bioinformatic needs are different and to a substantial extent remain unmet. Biological lipids are small, nonpolymeric molecules (with molecular weights less than 2000 Da). Typical analytical readouts in "untargeted" approaches include retention time (in the case of LC separation), mass-to-charge ratio, (m/z, ideally with high mass accuracy), and information on fragment ions (in the case of tandem MS). "Targeted" analysis delivers a matrix of lipid identities (including precursors to fragment ions) and their intensities. Typical informatic frameworks include data processing (peak integration, identification, and normalization), statistics (univariate or multivariate), and integration into pathways (e.g., the Kyoto Encyclopedia of Genes and Genomes, KEGG) or other datasets (see above). Open source and commercial software packages are now becoming available to support some of these functions (Wheelock et al., 2009; Blanksby and Mitchell, 2010). Building databases for lipids follows closely related efforts for other small molecule metabolites (Fahy et al., 2007; Kind et al., 2009). Appropriate data processing and validation will be a particularly critical element in biomarker discovery where many hundreds of different lipids are measured in human body fluids such as blood plasma (Quehenberger et al., 2010).

These examples illustrate the benefits of data integration at all levels and across scientific disciplines. Biochemical analysis of lipids by mass spectrometry is only one element in such interdisciplinary projects but will be a key tool in many fields including cell and developmental biology, molecular medicine, and nutrition (Shevchenko and Simons, 2010).

Future Developments and Prospects

New features and functions will undoubtedly be introduced to augment those currently used in the MS analysis of lipids. For instance, ion mobility mass spectrometry (IM-MS), which combines information of molecular shape (the collisional cross-section) with the mass/charge, has not yet been extensively applied to the analysis of lipids. Biophysical studies have shown that the double bond configuration of fatty acyls determines the conformation of lipids in bilayers, and this structural characteristic might also affect the collisional cross-section. It is also conceivable that ion mobility is affected by head group geometry (which is impacted by phosphorylation and glycosylation). Thus, it is likely that IM-MS will provide valuable information that is otherwise difficult to obtain. IM-MS has been successfully used for detection of lipids directly from tissue sections via MALDI (Ridenour et al., 2010). The resulting "image" containing mass spectral data yields spatial information on lipid distribution (Murphy et al., 2009).

Many lipids bind to cations, such as Ca^{2+} and Mg^{2+}, via their charged head groups. These reactions regulate assembly of lipids in biological as well as cell-free systems. Lipid oxidation on the other hand is in part coupled to free radical chemistry. Thus, elemental composition of lipid preparations (metal ions in

particular) could yield important additional information related to biomarker discovery. Such information can be determined by inductively coupled plasma (ICP) mass spectrometry (Becker and Jakubowski, 2009), a method that is amenable to imaging.

An interesting new technique for imaging of lipids is coherent anti-stokes Raman scattering (CARS) microscopy. Images are generated based on the vibrational states of molecules, such as the CH2 bonds found in fatty acyls. Thus, CARS does not require external labels. It is rapid (1 s/frame) and can be used for live imaging. Currently, CARS works well in applications with high signal-to-noise ratios, for example lipid bodies that harbor many CH2 segments (Volkmer, 2005; Müller and Zumbusch, 2007). Future developments might lead to CARS spectroscopy, moving the technique beyond the monitoring of a single frequency such that C-C double bonds (lipid unsaturation), or ester bonds could also be imaged. Such refinements should also help overcome background problems.

Molecular Recognition of Lipids

Substrate Specificities

Progress has been made toward ascertaining the determinants of specificity for lipid enzymes and protein effectors. For instance, mammalian FAS generates mainly palmitic acid (C16:0,16 carbons and no double bonds between them) and to a lesser extent produces C14:0 and C18:0. This specificity is determined, at least in part, by the thioesterase domain of FAS and the geometry of its catalytic cavity (Pemble et al., 2007). Phospholipases and lipid kinases are other well-studied examples. Both require interfacial targeting and specific recognition of their substrates for catalysis (Manford et al., 2010). Phosphoinositides, an important class of cellular signaling lipids, are recognized by a large number of protein effectors that have vastly different folds. Sophisticated technology based on inducible formation of protein-protein complexes (Suh et al., 2006) or peptide sensors has helped to monitor (using optical imaging) the distributions of phosphoinositides and associated protein factors within cells (Fairn et al., 2009).

Despite these advances, it is clear that recognition of lipids at the atomic level remains poorly understood (Manford et al., 2010; Ernst et al., 2010). It is becoming increasingly evident that highly specific lipid-lipid and lipid-protein interactions regulate cell physiology (Guan et al., 2009; Shevchenko and Simons, 2010). It will therefore be a challenge to understand and therapeutically target such interactions. Lipid enzymes are an interesting case to consider given that they produce mediators that have closely related structures but opposing functions (Figure 1). Cyclooxygenase 2 (COX-2) is involved in the generation of both inflammatory compounds (e.g., prostaglandins) as well as anti-inflammatory compounds from similar, albeit chemically distinct, substrates (glycerophospholipids with omega-6 and omega-3 fatty acyl, respectively) (Groeger et al., 2010). Acetyl-salicylic acid (aspirin), a natural compound that targets COX-2, decreases production of proinflammatory mediators and increases production of anti-inflammatory compounds. This shift in COX activity is not achieved by synthetic and selective inhibitors of COX that are designed based on active site catalysis. Chemically synthesized derivatives of natural products are therefore promising tools for probing enzyme cavities and for identifying new lipid-binding factors and off-targets (Yang et al., 2010).

Enzymatic versus Chemical Modification of Lipids

Unlike the generation of "lipid mediators" (Serhan, 2009), oxidation of intact glycerophospholipids can be mediated by reactive oxygen species in addition to enzymes such as lipoxygenases. Typically, oxidation of polyunsaturated fatty acyls (PUFAs) in glycerophospholipids by reactive oxygen species leads to a variety of different products including hydroxyls, hemiacetals, and furans. Oxidized forms of membrane phospholipids are short-lived, reactive species that undergo fatty acyl chain shortening or covalent adduct formation with nearby proteins. Furthermore, such "damaged" lipids occur in very low abundance compared to their parent lipid thus complicating analytical capture (Zemski Berry et al., 2010). These lipids might exert their effects via receptor activation (for instance via G protein-coupled receptors, nuclear receptors, and/or innate immune receptors; Greenberg et al., 2006) and other mechanisms due to their reactivity and biophysical properties (Deigner and Hermetter, 2008).

The proportion of fatty acyls differs dramatically between organs. The brain, for example, is very rich in polyunsaturated fatty acyls (such as arachidonic acid, C20:4, and docosahexaenoic acid, C22:6), whereas the liver contains primarily saturated and monounsaturated fatty acyls. It is thus conceivable that oxidative stress might produce different lipid reaction products depending on the precise organ and/or cell type affected. This would influence downstream reactions such as activation of cell surface or nuclear lipid receptors and elevation of antibodies directed against lipids (discussed below). This characteristic is also relevant for biomarker development, which would require careful inspection and understanding of chemical versus enzymatic oxidations as well as an appreciation of the potential for selective transport as in the case of oxidized sterols.

Antibodies Directed against Lipids

With the important exception of glycolipids, relatively few antibodies that recognize specific lipids have been described. This cannot be ascribed solely to an inherent lack of antigenicity on the part of lipids. Certain glycosphingolipids, which are present in normal cells, are more abundant in tumor cells and elicit an antibody response (Hakomori and Zhang, 1997). In many cases, the precise chemical nature of the antigens remains unclear and is dependent on cell type and experimental conditions. Heteromeric glycolipid complexes, rather than an individual glycolipid, modulate (auto)antibody responses (Rinaldi et al., 2010), meaning that the antigenic determinant consists of a combination of two (or more) glycans. One explanation for this might be the different surface arrangement and presentation of glycosphingolipids on tumor cells. Indeed, it is becoming increasingly accepted that "local topography" influences antigenicity and immunogenicity of glycosphingolipids. Another explanation is that anti-lipid antibodies (of a limited range of isotypes) against cardiolipin and other phospholipids might be present at considerable frequencies but in hidden forms, for example, as circulating immune complexes, and therefore unable to engage normal tissues or cells (Alving, 2006). Lipids from external sources are likely to produce immune responses. Such lipids come from the diet or pathogens or are derivatives of endogenous lipids, such as oxidized lipids and their adducts.

Indeed, there is increasing evidence for the presence of anti-lipid antibodies, for example in individuals with HIV infections and autoinflammatory conditions such as multiple sclerosis (Kanter et al., 2006). Synthetic forms of lipid A have been used to raise monoclonal antibodies that can be utilized in vivo to target gram-negative bacteria (Syed et al., 1992). Antibodies against lipid components of mycobacteria have been in development for a number of years as a way of controlling *M. tuberculosis* and *M. leprae* infections. These include antibodies specific for lipoteichoic acid and lipoarabinomannan (Hamasur et al., 2004).

Relatively little is known about the precise molecular requirements for successful generation of antibodies against lipids either in terms of their presentation during immunization in vivo or their selection in vitro. In an interesting example, liposomes with very high content of cholesterol (71%) were used to generate monoclonal antibodies that recognized membranes with high cholesterol (as well as crystalline cholesterol in vitro) but not liposomes with 40% cholesterol (Swartz et al., 1988). Thus, there is reason to the hope that it will be possible to generate new and specific lipid antibodies with improved technologies for presentation and selection. Production of pure, synthetic, and stable lipids is one prerequisite. A second, more complicated issue is the selection of the lipid species that acts as the antigen. Such antibodies, if successful, would be entirely new tools for basic research in membrane trafficking with applications in immunohistochemistry, cytochemistry, and biochemistry. If proven highly specific, such antibodies could be used for clinical applications, including for diagnostics or potentially for therapeutic purposes.

Chemical Biology of Lipids

Small-molecule chemical probes (so-called activity-based or affinity-based probes) have in recent years become increasingly popular for the study of kinases, phosphatases, and hydrolytic enzymes (hydrolases and proteases). To date relatively little has been done to engineer lipid-based probes capable of detecting or capturing lipid-interacting proteins. "Click chemistry" is a recently developed approach in which small molecules can be joined selectively and has been used for selective chemical remodeling of cell-surface glycoproteins (Mahal et al., 1997). The technique builds on the assumption that biosynthetic enzymes are promiscuous enough to allow incorporation of precursors that have a chemically reactive "molecular handle" (a bio-orthogonal reporter) that subsequently can be used to form a covalent bond with a fluorophore for visualization or a solid resin for biochemical isolation. Such approaches should in principle be applicable to lipids. Indeed, palmitoylation (Martin and Cravatt, 2009; Yount et al., 2010) and myristoylation (Martin et al., 2008) of proteins can be successfully studied using such approaches. Alkyne-derivatized fatty acid incorporation into cells, followed by solid-phase sequestration and release, is a promising new method for unequivocally monitoring individual glycerophospholipids (Milne et al., 2010). Bio-orthogonal chemistry is not limited to the use of one reporter at a time. For example, it can be combined with photoaffinity labeling. Such strategies open new avenues for investigation of lipid-protein interactions (Gubbens and de Kroon, 2010) or asymmetry across a lipid bilayer. Fluorophosphonate derivates of phosphatidylcholines have been used to target phospholipases in protein extracts with the proteins then identified via alkyne-azide-based click chemistry (Tully and Cravatt, 2010).

Lipidomics across Biological Species

Many lipid metabolic pathways are conserved in function from yeast to man. However, it is not trivial to search for lipid enzymes, modulators of enzymes, or even lipid effectors based on protein sequence information alone. Phosphatidylinositol transfer proteins (PITPs), for example, share some functional redundancy but almost no sequence similarity between yeast (Sec14p-like) and metazoans. They also adopt very different structural folds. Certain lipid classes differ substantially between biological species. The sphingolipids in yeast, mammals, and insects have very different head group decorations, hydroxylation patterns, and lengths of fatty acids and long chain bases. Thus, in addition to experimental methods (Guan et al., 2009; Ejsing et al., 2009), new in silico approaches (Fahy et al., 2007; Baker et al., 2008) are needed to tap the information stored in existing databases, such as gene ontologies and protein-protein interaction maps of model organisms.

Our appreciation of lipid heterogeneity, biosynthetic routes, and process engineering has been substantially bolstered by work coming out of the environmental and plant sciences. These developments are supported by the belief that whatever can be derived from fossil fuels can also be made from vegetable oils and the fact that the cost differential between these two sources of lipids has decreased over the past 20 years. Currently, 90% of fossil oil is converted to fuel and 10% is used by the petrochemical industry for production of plastics, detergents, etc. This presents numerous opportunities for lipidomic research and development, in addition to the obvious desire to generate biofuels via food crops or other feedstock.

Take for example spermaceti oil (cetyl-palmitate, a wax), which was harvested from the heads of sperm whales and used in lubricants until whale hunting bans mandated the search for alternative sources. It is indeed difficult to find a petroleum-based replacement. Likewise, a wax derived from the seed of the Jojoba plant is used in cosmetics and would also be a useful industrial lubricant were it not for its current cost of production. Several large-scale programs are currently addressing this need. These efforts will likely tap into lipidomic technologies at various levels. Ultra high-resolution mass spectrometry can be used to provide detailed chemical information of petroleum crude oils from different sources (Marshall and Rodgers, 2008). This molecular information can then be used to correlate and predict, using theoretical chemistry, their properties during the refining process (chemical cracking). Mathematical modeling is also applicable to enzymatic lipid metabolism (Miskovic and Hatzimanikatis, 2010). Identification of lipid enzymes and their cell biological and biochemical characterization will require additional tools, some of which can be taken from the current set that have proven successful in life sciences. New tools in bioinformatics are needed to address plant-specific pathways. For example, comparative deep sequencing of transcripts from multiple plant tissues aided in the identification of an acyltransferase that produces an unusual triacylglycerol in which one of the fatty acyls is an acetyl residue, rather than a fatty acid of C16 or C18 (Durrett et al., 2010). This particular lipid has

desirable cold temperature properties, and thus this finding might be readily translatable.

Concluding Remarks

Methods based on mass spectrometry are now available for qualitative and quantitative analysis of many major lipids in complex samples (such as tissue and cell extracts) and from several biological species (including yeast and mammals). The near future promises technical improvements stemming from cell isolation, sample fractionation and preparation, standardization and cross-validation, and automation as well as wider coverage of biochemical lipidomics from integration with imaging, databases, and inclusion of additional biological species. In parallel to these trends it can be anticipated that interdisciplinary programs will continue to integrate biochemical lipidomics with chemical biology, proteomics, and genomics to span the entire flow of information encoded in biological systems. These efforts will provide us with a better understanding of natural variation found within lipids and will likely lead to customized applications in life sciences, industrial settings, and medicine.

ACKNOWLEDGMENTS

Work in our laboratories (http://www.lipidprofiles.com) is supported by the National University of Singapore and by grants from the Singapore National Research Foundation under CRP Award No. 2007-04, the Biomedical Research Council of Singapore (R-183-000-211-305), the National Medical Research Council (R-183-000-224-213), as well as the SystemsX.ch RTD project LipidX.

REFERENCES

Alving, C.R. (2006). Antibodies to lipids and liposomes: immunology and safety. J. Liposome Res. *16*, 157–166.

Andreyev, A.Y., Shen, Z., Guan, Z., Ryan, A., Fahy, E., Subramaniam, S., Raetz, C.R., Briggs, S., and Dennis, E.A. (2010). Application of proteomic marker ensembles to subcellular organelle identification. Mol. Cell. Proteomics *9*, 388–402.

Assfalg, M., Bertini, I., Colangiuli, D., Luchinat, C., Schäfer, H., Schütz, B., and Spraul, M. (2008). Evidence of different metabolic phenotypes in humans. Proc. Natl. Acad. Sci. USA *105*, 1420–1424.

Baker, C.J., Kanagasabai, R., Ang, W.T., Veeramani, A., Low, H.S., and Wenk, M.R. (2008). Towards ontology-driven navigation of the lipid bibliosphere. BMC Bioinformatics *9* (*Suppl 1*), S5.

Becker, J.S., and Jakubowski, N. (2009). The synergy of elemental and biomolecular mass spectrometry: new analytical strategies in life sciences. Chem. Soc. Rev. *38*, 1969–1983.

Blanksby, S.J., and Mitchell, T.W. (2010). Advances in mass spectrometry for lipidomics. Annu Rev Anal Chem (Palo Alto Calif) *3*, 433–465.

Brügger, B., Glass, B., Haberkant, P., Leibrecht, I., Wieland, F.T., and Kräusslich, H.G. (2006). The HIV lipidome: a raft with an unusual composition. Proc. Natl. Acad. Sci. USA *103*, 2641–2646.

Chan, R., Uchil, P.D., Jin, J., Shui, G., Ott, D.E., Mothes, W., and Wenk, M.R. (2008). Retroviruses human immunodeficiency virus and murine leukemia virus are enriched in phosphoinositides. J. Virol. *82*, 11228–11238.

Deigner, H.P., and Hermetter, A. (2008). Oxidized phospholipids: emerging lipid mediators in pathophysiology. Curr. Opin. Lipidol. *19*, 289–294.

Durrett, T.P., McClosky, D.D., Tumaney, A.W., Elzinga, D.A., Ohlrogge, J., and Pollard, M. (2010). A distinct DGAT with sn-3 acetyltransferase activity that synthesizes unusual, reduced-viscosity oils in Euonymus and transgenic seeds. Proc. Natl. Acad. Sci. USA *107*, 9464–9469.

Ejsing, C.S., Sampaio, J.L., Surendranath, V., Duchoslav, E., Ekroos, K., Klemm, R.W., Simons, K., and Shevchenko, A. (2009). Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc. Natl. Acad. Sci. USA *106*, 2136–2141.

Ernst, A.M., Contreras, F.X., Brügger, B., and Wieland, F. (2010). Determinants of specificity at the protein-lipid interface in membranes. FEBS Lett. *584*, 1713–1720.

Fahmi, S., Yang, C., Esmail, S., Hobbs, H.H., and Cohen, J.C. (2008). Functional characterization of genetic variants in NPC1L1 supports the sequencing extremes strategy to identify complex trait genes. Hum. Mol. Genet. *17*, 2101–2107.

Fahy, E., Subramaniam, S., Brown, H.A., Glass, C.K., Merrill, A.H., Jr., Murphy, R.C., Raetz, C.R., Russell, D.W., Seyama, Y., Shaw, W., et al. (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., and Subramaniam, S. (2007). Bioinformatics for lipidomics. Methods Enzymol. *432*, 247–273.

Fairn, G.D., Ogata, K., Botelho, R.J., Stahl, P.D., Anderson, R.A., De Camilli, P., Meyer, T., Wodak, S., and Grinstein, S. (2009). An electrostatic switch displaces phosphatidylinositol phosphate kinases from the membrane during phagocytosis. J. Cell Biol. *187*, 701–714.

Greenberg, M.E., Sun, M., Zhang, R., Febbraio, M., Silverstein, R., and Hazen, S.L. (2006). Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. J. Exp. Med. *203*, 2613–2625.

Groeger, A.L., Cipollina, C., Cole, M.P., Woodcock, S.R., Bonacci, G., Rudolph, T.K., Rudolph, V., Freeman, B.A., and Schopfer, F.J. (2010). Cyclooxygenase-2 generates anti-inflammatory mediators from omega-3 fatty acids. Nat. Chem. Biol. *6*, 433–441.

Guan, X.L., Souza, C.M., Pichler, H., Dewhurst, G., Schaad, O., Kajiwara, K., Wakabayashi, H., Ivanova, T., Castillon, G.A., Piccolis, M., et al. (2009). Functional interactions between sphingolipids and sterols in biological membranes regulating cell physiology. Mol. Biol. Cell *20*, 2083–2095.

Gubbens, J., and de Kroon, A.I. (2010). Proteome-wide detection of phospholipid-protein interactions in mitochondria by photocrosslinking and click chemistry. Mol. Biosyst. *6*, 1751–1759.

Hakomori, S., and Zhang, Y. (1997). Glycosphingolipid antigens and cancer therapy. Chem. Biol. *4*, 97–104.

Hamasur, B., Haile, M., Pawlowski, A., Schroder, U., Kallenius, G., and Svenson, S.B. (2004). A mycobacterial lipoarabinomannan specific monoclonal antibody and its F(ab') fragment prolong survival of mice infected with Mycobacterium tuberculosis. Clin. Exp. Immunol. *138*, 30–38.

Han, X., and Gross, R.W. (1994). Electrospray ionization mass spectroscopic analysis of human erythrocyte plasma membrane phospholipids. Proc. Natl. Acad. Sci. USA *91*, 10635–10639.

Hicks, A.A., Pramstaller, P.P., Johansson, A., Vitart, V., Rudan, I., Ugocsai, P., Aulchenko, Y., Franklin, C.S., Liebisch, G., Erdmann, J., et al. (2009). Genetic determinants of circulating sphingolipid concentrations in European populations. PLoS Genet. *5*, e1000672.

Illig, T., Gieger, C., Zhai, G., Römisch-Margl, W., Wang-Sattler, R., Prehn, C., Altmaier, E., Kastenmüller, G., Kato, B.S., Mewes, H.W., et al. (2010). A genome-wide perspective of genetic variation in human metabolism. Nat. Genet. *42*, 137–141.

Kanter, J.L., Narayana, S., Ho, P.P., Catz, I., Warren, K.G., Sobel, R.A., Steinman, L., and Robinson, W.H. (2006). Lipid microarrays identify key mediators of autoimmune brain inflammation. Nat. Med. *12*, 138–143.

Kim, H.Y., Wang, T.C., and Ma, Y.C. (1994). Liquid chromatography/mass spectrometry of phospholipids using electrospray ionization. Anal. Chem. *66*, 3977–3982.

Kind, T., Wohlgemuth, G., Lee, Y., Lu, Y., Palazoglu, M., Shahbaz, S., and Fiehn, O. (2009). FiehnLib: mass spectral and retention index libraries for

metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. Anal. Chem. *81*, 10038–10048.

Klemm, R.W., Ejsing, C.S., Surma, M.A., Kaiser, H.J., Gerl, M.J., Sampaio, J.L., de Robillard, Q., Ferguson, C., Proszynski, T.J., Shevchenko, A., and Simons, K. (2009). Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. J. Cell Biol. *185*, 601–612.

Kurat, C.F., Wolinski, H., Petschnigg, J., Kaluarachchi, S., Andrews, B., Natter, K., and Kohlwein, S.D. (2009). Cdk1/Cdc28-dependent activation of the major triacylglycerol lipase Tgl4 in yeast links lipolysis to cell-cycle progression. Mol. Cell *33*, 53–63.

Mahal, L.K., Yarema, K.J., and Bertozzi, C.R. (1997). Engineering chemical reactivity on cell surfaces through oligosaccharide biosynthesis. Science *276*, 1125–1128.

Manford, A., Xia, T., Saxena, A.K., Stefan, C., Hu, F., Emr, S.D., and Mao, Y. (2010). Crystal structure of the yeast Sac1: implications for its phosphoinositide phosphatase function. EMBO J. *29*, 1489–1498.

Marshall, A.G., and Rodgers, R.P. (2008). Petroleomics: chemistry of the underworld. Proc. Natl. Acad. Sci. USA *105*, 18090–18095.

Martin, B.R., and Cravatt, B.F. (2009). Large-scale profiling of protein palmitoylation in mammalian cells. Nat. Methods *6*, 135–138.

Martin, D.D., Vilas, G.L., Prescher, J.A., Rajaiah, G., Falck, J.R., Bertozzi, C.R., and Berthiaume, L.G. (2008). Rapid detection, discovery, and identification of post-translationally myristoylated proteins during apoptosis using a bio-orthogonal azidomyristate analog. FASEB J. *22*, 797–806.

Milne, S.B., Tallman, K.A., Serwa, R., Rouzer, C.A., Armstrong, M.D., Marnett, L.J., Lukehart, C.M., Porter, N.A., and Brown, H.A. (2010). Capture and release of alkyne-derivatized glycerophospholipids using cobalt chemistry. Nat. Chem. Biol. *6*, 205–207.

Minami, Y., Kasukawa, T., Kakazu, Y., Iigo, M., Sugimoto, M., Ikeda, S., Yasui, A., van der Horst, G.T., Soga, T., and Ueda, H.R. (2009). Measurement of internal body time by blood metabolomics. Proc. Natl. Acad. Sci. USA *106*, 9890–9895.

Miskovic, L., and Hatzimanikatis, V. (2010). Production of biofuels and biochemicals: in need of an ORACLE. Trends Biotechnol. *28*, 391–397.

Müller, M., and Zumbusch, A. (2007). Coherent anti-stokes raman scattering microscopy. ChemPhysChem *8*, 2156–2170.

Murphy, R.C., Hankin, J.A., and Barkley, R.M. (2009). Imaging of lipid species by MALDI mass spectrometry. J. Lipid Res. Suppl. *50*, S317–S322.

Nomura, D.K., Long, J.Z., Niessen, S., Hoover, H.S., Ng, S.W., and Cravatt, B.F. (2010). Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell *140*, 49–61.

Patwardhan, A.M., Akopian, A.N., Ruparel, N.B., Diogenes, A., Weintraub, S.T., Uhlson, C., Murphy, R.C., and Hargreaves, K.M. (2010). Heat generates oxidized linoleic acid metabolites that activate TRPV1 and produce pain in rodents. J. Clin. Invest. *120*, 1617–1626.

Pemble, C.W., 4th, Johnson, L.C., Kridel, S.J., and Lowther, W.T. (2007). Crystal structure of the thioesterase domain of human fatty acid synthase inhibited by Orlistat. Nat. Struct. Mol. Biol. *14*, 704–709.

Postle, A.D., and Hunt, A.N. (2009). Dynamic lipidomics with stable isotope labelling. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. *877*, 2716–2721.

Quehenberger, O., Armando, A.M., Brown, A.H., Milne, S.B., Myers, D.S., Merrill, A.H., Bandyopadhyay, S., Jones, K.N., Kelly, S., Shaner, R.L., et al. (2010). Lipidomics reveals a remarkable diversity of lipids in human plasma. J. Lipid Res. *51*, 3299–3305.

Ridenour, W.B., Kliman, M., McLean, J.A., and Caprioli, R.M. (2010). Structural characterization of phospholipids and peptides directly from tissue sections by MALDI traveling-wave ion mobility-mass spectrometry. Anal. Chem. *82*, 1881–1889.

Rinaldi, S., Brennan, K.M., and Willison, H.J. (2010). Heteromeric glycolipid complexes as modulators of autoantibody and lectin binding. Prog. Lipid Res. *49*, 87–95.

Serhan, C.N. (2009). Systems approach to inflammation resolution: identification of novel anti-inflammatory and pro-resolving mediators. J. Thromb. Haemost. *7* (*Suppl 1*), 44–48.

Shevchenko, A., and Simons, K. (2010). Lipidomics: coming to grips with lipid diversity. Nat. Rev. Mol. Cell Biol. *11*, 593–598.

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A.M., and Czaja, M.J. (2009). Autophagy regulates lipid metabolism. Nature *458*, 1131–1135.

Suh, B.C., Inoue, T., Meyer, T., and Hille, B. (2006). Rapid chemically induced changes of PtdIns(4,5)P2 gate KCNQ ion channels. Science *314*, 1454–1457.

Swartz, G.M., Jr., Gentry, M.K., Amende, L.M., Blanchette-Mackie, E.J., and Alving, C.R. (1988). Antibodies to cholesterol. Proc. Natl. Acad. Sci. USA *85*, 1902–1906.

Syed, S.A., Taylor, R.H., Crean, P.M., and Stewart, R.J. (1992). Successful use of monoclonal anti-lipid-A IgM in infant with meningococcal sepsis. Lancet *339*, 496.

Tanaka, T., Shen, J., Abecasis, G.R., Kisialiou, A., Ordovas, J.M., Guralnik, J.M., Singleton, A., Bandinelli, S., Cherubini, A., Arnett, D., et al. (2009). Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI Study. PLoS Genet. *5*, e1000338.

Thomas, M.C., Mitchell, T.W., and Blanksby, S.J. (2009). OnLine ozonolysis methods for the determination of double bond position in unsaturated lipids. Methods Mol. Biol. *579*, 413–441.

Tully, S.E., and Cravatt, B.F. (2010). Activity-based probes that target functional subclasses of phospholipases in proteomes. J. Am. Chem. Soc. *132*, 3264–3265.

van Meer, G. (2005). Cellular lipidomics. EMBO J. *24*, 3159–3165.

Volkmer, A. (2005). Vibrational imaging and microspectroscopies based on coherent anti-Stokes Raman scattering microscopy. J. Phys. D Appl. Phys. *38*, R59–R81.

Wenk, M.R. (2005). The emerging field of lipidomics. Nat. Rev. Drug Discov. *4*, 594–610.

Wheelock, C.E., Goto, S., Yetukuri, L., D'Alexandri, F.L., Klukas, C., Schreiber, F., and Oresic, M. (2009). Bioinformatics strategies for the analysis of lipids. Methods Mol. Biol. *580*, 339–368.

Wymann, M.P., and Schneiter, R. (2008). Lipid signalling in disease. Nat. Rev. Mol. Cell Biol. *9*, 162–176.

Yang, K., Zhao, Z., Gross, R.W., and Han, X. (2007). Shotgun lipidomics identifies a paired rule for the presence of isomeric ether phospholipid molecular species. PLoS ONE *2*, e1368.

Yang, P.Y., Liu, K., Ngai, M.H., Lear, M.J., Wenk, M.R., and Yao, S.Q. (2010). Activity-based proteome profiling of potential cellular targets of Orlistat—an FDA-approved drug with anti-tumor activities. J. Am. Chem. Soc. *132*, 656–666.

Yount, J.S., Moltedo, B., Yang, Y.Y., Charron, G., Moran, T.M., López, C.B., and Hang, H.C. (2010). Palmitoylome profiling reveals S-palmitoylation-dependent antiviral activity of IFITM3. Nat. Chem. Biol. *6*, 610–614.

Zemski Berry, K.A., Turner, W.W., VanNieuwenhze, M.S., and Murphy, R.C. (2010). Characterization of oxidized phosphatidylethanolamine derived from RAW 264.7 cells using 4-(dimethylamino)benzoic acid derivatives. Eur. J. Mass Spectrom. (Chichester, Eng.) *16*, 463–470.