ESSAY

Lipidomics: coming to grips with lipid diversity

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Abstract | Although lipids are biomolecules with seemingly simple chemical structures, the molecular composition of the cellular lipidome is complex and, currently, poorly understood. The exact mechanisms of how compositional complexity affects cell homeostasis and its regulation also remain unclear. This emerging field is developing sensitive mass spectrometry technologies for the quantitative characterization of the lipidome. Here, we argue that lipidomics will become an essential tool kit in cell and developmental biology, molecular medicine and nutrition.

The lipids are a class of biomolecules that for a long time remained in the shadow of the ongoing 'omics' trend in biology. The scientific community focused on DNA, RNA and proteins as studying these promised a shortcut to the mechanistic understanding of cell function and homeostasis. The progress in sequencing and annotating genomes, along with the rapid growth of knowledge databases, has led to the notion that the identification of a particular protein in a given biological context might provide a story on its own without follow-up experiments. Within this optimistic but rather myopic context, lipids were mainly seen as an uninteresting, heterogeneous set of molecules with few specific functions.

Most lipids reside in cell membranes where they form the lipid bilayer. This molecular model, which was formulated in 1925, is still valid. Lipids remained centre stage in membrane research until the 1980s, when the leading role was taken over by membrane proteins. The lipid bilayer was relegated to being thought of as a two-dimensional fluid phase in which proteins are embedded and carry out the important functions of cell membranes. Although structurally lipids are seemingly composed of not many 'building blocks', they have the potential to generate 9,000–100,000 different molecular species[1,2] (BOX 1; FIG. 1). A considerable part of our genome is required to synthesize, metabolize and regulate this lipid diversity. Nevertheless, we are far from understanding the biological significance of this compositional complexity.

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It is the intention of this Essay that, in studies of cellular function, lipid compositional diversity receives the attention that it deserves. The emerging scientific discipline of studying the full lipid complement of cells, tissues and organisms is termed lipidomics[3,4]. Although lipids have multiple functions, as, for example, storage compounds, energy sources and signalling molecules, here we mainly focus on membrane lipids. Lipids behave as collectives in cell membranes and therefore lipid species diversity will only be understood by including lipidomics technologies in the ongoing 'omics' (r)evolution.

The diversity of lipid function

Lipids not only form the matrix of the cell membrane but are directly involved in membrane trafficking, regulating membrane proteins, cellular architecture and creating specific subcompartments in membranes that contribute to cellular function. Each of these broadly defined roles is becoming an exciting target of lipidomics, as explained below.

Cellular architecture. Although some of the lipid constituents do not form bilayers by themselves, the natural mix of lipids in cell membranes tends to form stable lamellar bilayers. The bilayers can collectively fold into periodic 2-dimensional (2D) and 3D structures, as revealed by Luzzati in the 1960s[5,6]. This property of different lipid species is part of the tool kit that cells use during morphogenesis. Understanding the folding mechanisms requires exact knowledge of the membrane's molecular composition and is an upcoming area of lipidomics.

The lipid bilayer of the plasma membrane is remarkably asymmetric[7]. Its outer leaflet contains mostly phosphatidylcholines and sphingolipids, whereas the cytosolic leaflet is occupied by phosphatidylethanolamines, phosphatidylserines and phosphoinositides, with cholesterol residing in both leaflets. A membrane translocation machinery, which involves P-type ATPases, consumes large amounts of ATP to actively maintain this asymmetry[8]. Most of the work on bilayer asymmetry was done with conventional lipid analytics and focused on erythrocyte membranes and viruses. The time is now right to revisit this issue to understand how individual lipid species are distributed over the two leaflets and why lipid asymmetry is so important for cellular physiology.

Lipid rafts. One topic that stirred up the membrane field was the introduction of the raft concept of membrane subcompartmentalization. The concept came from our early work on epithelial cells, which polarize their cell surface into apical and basolateral plasma membrane domains[9]. The controversies due to crude biochemical methods, such as detergent insolubility and cholesterol depletion, are being overcome by the influx of new advanced spectroscopic, imaging and lipidomics technologies[10]. Membrane rafts are currently defined as dynamic, nanometre-sized, sterol- and sphingolipid-enriched protein assemblies.

Box 1 | **Molecular composition of eukaryotic lipidomes**

Depending on the organism, eukaryotic lipidomes might contain a dozen major lipid classes (see REF. 1 for chemical formulas), each of which might comprise hundreds of individual molecular species (see FIG. 1). Lipids are first extracted from cells or tissues by a mixture of chloroform and methanol[33,51,52] or by methyl *t*-butyl ether[53], by partitioning into the organic phase. Total extracts are either directly infused into a tandem mass spectrometer (an approach termed 'shotgun lipidomics'), or separated by liquid chromatography coupled to a mass spectrometer. A typical analysis encompasses all major classes of glycerophospholipids (together with the corresponding lysolipids and ether species), sphingolipids, ceramides, cerebrosides, triacylglycerols, diacylglycerols, monoacylglycerols, cholesterol and cholesterol esters. Other lipids, such as phosphatidylinositol monophosphates, bisphosphates and trisphosphates[24], and sterols[54], are determined in separate analyses.

The metastable resting state of rafts can be stimulated to coalesce into larger, more stable raft domains by specific lipid–lipid, protein–lipid and protein–protein interactions[10]. When clustered, bilayer constituents are thought to be laterally stabilized according to their underlying affinity for pre-existing raft assemblies. In other words, clustering enhances the inclusion of proteins that partition into rafts and excludes those that segregate away. To understand how lipid diversity is used to generate dynamic rafts we need sensitive and quantitative lipidomics and proteomics techniques to define the lipid and protein specificity governing raft assembly[11].

Regulating membrane proteins by protein–lipid interactions. Cell membranes are crowded with membrane proteins that could specifically organize the distribution of lipids[10,12]. The lipid species that are best adapted to match the length of transmembrane domains have an increased probability of being close to the protein–lipid interface. Many membrane proteins are modified by glycosyl phosphatidylinositol anchors or sterol, myristoyl, palmitoyl or prenyl moieties, prompting their association with membranes. Additionally, an increasing number of proteins are reported to exhibit specific lipid-binding and/or lipid-interaction capacities, with possible structural and regulatory implications. Several proteins interact with cholesterol by direct binding, with caveolin and the β-adrenergic receptors being prime examples[13,14], and some proteins are regulated by gangliosides[15].

Although the number of documented protein–lipid interactions is increasing steadily, the precise structural mechanisms underlying specific binding and receptor regulation in membranes remain uncharacterized. Is there a strong binding preference towards a few specific species in a lipid class[12,16]? How do lipid–protein interactions dynamically alter the protein function? Do transmembrane proteins become 'raftophilic' by being lubricated by raft lipids[10]? To answer these questions, we have to determine which individual lipid molecules or their collectives interact with specific membrane proteins in the bilayer.

> "although proteins have been the focus of membrane trafficking studies, lipids are co-sorted with proteins during the formation of transport carriers."

Lipid synthesis and distribution

Another important question that could be addressed by lipidomics is how lipids are synthesized and distributed in cells[7]. Stable isotope labelling and mass spectrometry can be used to characterize the kinetics of lipid biosynthesis and turnover[17]. Most glycerolipids and sterols are synthesized in the endoplasmic reticulum (ER). Mitochondria, peroxisomes and a few other organellar membranes also contribute to the generation of the lipid spectrum in cells, but not many lipids are made in these organelles. Most lipids are moved from the site of synthesis thoughout the cell by membrane trafficking or other transfer mechanisms involving, for example, the formation of membrane contacts. How cells sort and distribute newly synthesized lipid species to their destinations are areas that remain largely unexplored.

The concentration of sterols and sphingolipids increases from the ER to the cell surface[10,18]. Sphingomyelin and glycosphingolipids are mainly synthesized in the Golgi complex, and sterols are continuously moved from their site of synthesis in the ER to the Golgi and the plasma membrane. As was shown by an early lipidomics study[19], this gradient of sphingolipids and sterols towards the cell surface is augmented by the exclusion of sterols and sphingomyelin from the coat protein I (COPI) vesicles that transport proteins and lipids retrogradely in the Golgi complex and back to the ER.

By contrast, sphingolipids and sterols are sorted from the *trans*-Golgi network into vesicular carriers for transport to the plasma membrane. Mass spectrometry analysis of immuno-isolated carriers delivering raft proteins to the cell surface in yeast showed an enrichment of ergosterol and the most complex yeast sphingolipid, mannosyl-diinositolphosphoceramides[20]. In epithelial cells, sorting of proteins to the apical and basolateral cell surfaces is linked to lipid sorting, and glycolipids are preferentially routed towards the apical surface[9]. These findings show that, although proteins have been the focus of membrane trafficking studies, lipids are co-sorted with proteins during the formation of transport carriers. Therefore, quantifying lipids as individual molecular species rather than measuring the total abundances of lipid classes, as was done before, will be essential for understanding the mechanisms involved.

The organization of the biosynthetic and endocytic compartments are aided by the specific distribution of phosphoinositides[21,22]. These provide tags for peripheral proteins with specific phosphoinositide-binding domains to facilitate targeting to dynamically assemble the protein machinery involved in membrane trafficking and signalling. Mass spectrometry has the potential for analysing the molecular composition of phosphoinositides[23,24]; however, the field is still in need of more sensitive and, most importantly, robust and structure-specific analytical methodologies.

The lipid composition of cellular compartments has to be tightly regulated. The classical work of Brown and Goldstein has unravelled the fine-tuned regulation of cholesterol levels in cells[25]. As sterols and sphingolipids function together in membrane raft assemblies, it is not surprising that their levels are regulated together[26,27]. Many factors will come into play, such as bilayer thickness and the length of transmembrane domains of the membrane proteins, to avoid hydrophobic mismatching — the unfavourable exposure of hydrophobic protein surfaces to the aqueous environment. Specific lipid–protein interactions could regulate the interplay between protein and lipids that make up the membrane. Saturation and chain length of the acyl chain moieties in lipids have to be fine-tuned to match the requirements for maintaining cellular physiology[28].

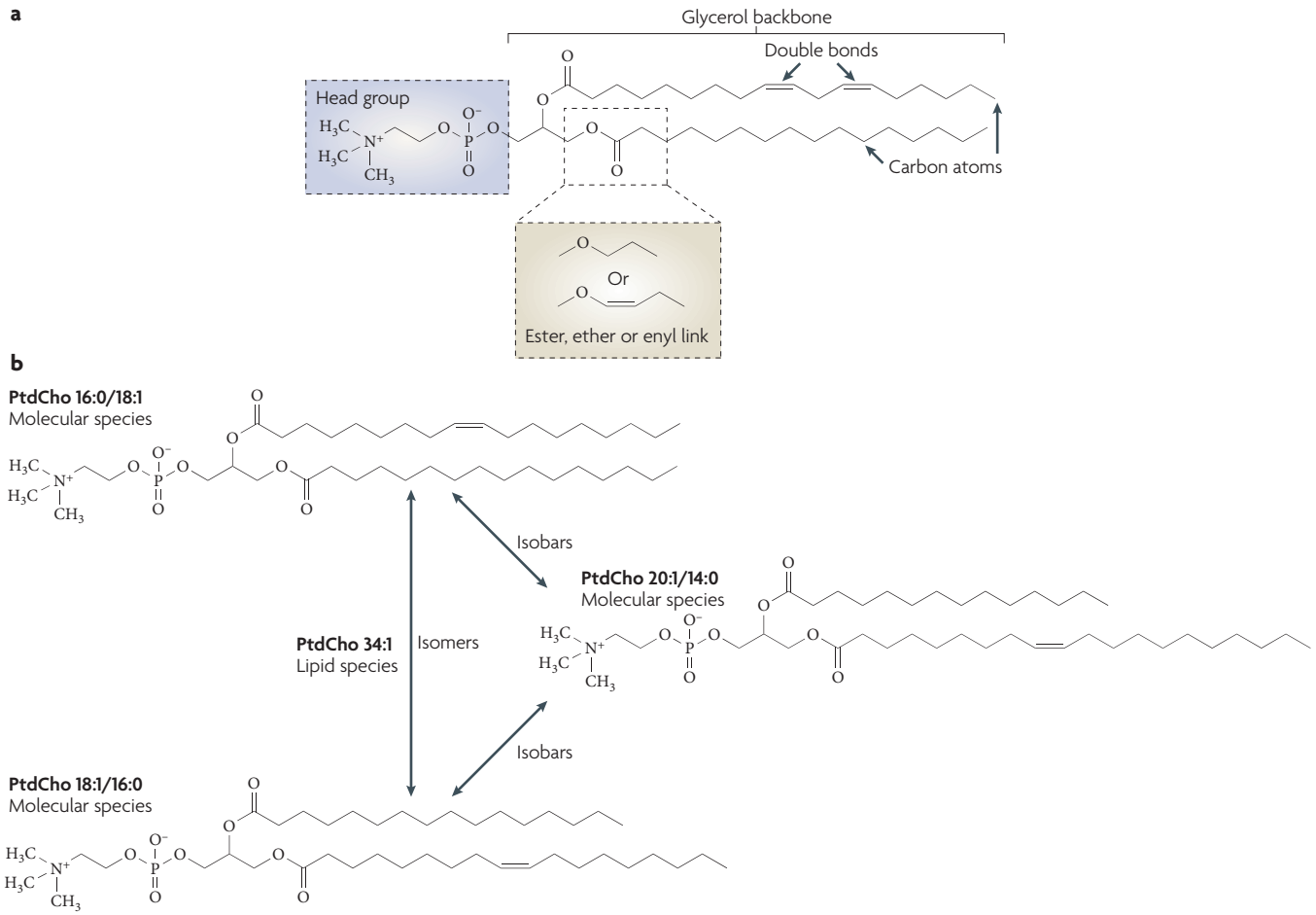

Figure 1 | **The molecular diversity of lipid species.** Phosphatidylcholines (PtdChos), are a major class of structural lipids. **a** | All PtdChos share the same phosphorylcholine head group attached at the *sn-3* position of the glycerol backbone. Individual species might differ by the number of carbon atoms (typically 12–22) and double bonds (typically 0–6) in their hydrocarbon moieties. The moieties are attached to the glycerol backbone by ester or, at the *sn-1* position, ether or enyl bonds. **b** | PtdChos comprising exactly the same fatty acid moieties might be present as positional isomers that only differ by the location of the moieties at the glycerol backbone. Direct mass spectrometric analysis only recognizes molecules with the same total number of carbon atoms and double bonds, such as the lipid species PtdCho 34:1 (34 carbon atoms and 1 double bond in both fatty acid moieties). However, each of these signals might represent a cluster of isobaric PtdChos (that is, they have the same nominal masses) with different fatty acids, and some of them might also have positional isomers. Identification and quantification of particular molecular species (PtdCho 16:0/18:1 (where 16:0 is at the *sn-1* position and 18:1 is at the *sn-2* position), PtdCho 18:1/16:0 and PtdCho 20:1/14:0) requires tandem mass spectrometry (MS/MS) experiments that detect individual fatty acid moieties and determine their location[60].

New methodologies are required to understand how lipid distribution and composition are regulated. Fluorescent analogues of fatty acids or lipid precursors that closely resemble the physicochemical properties of endogenous compounds have been used to localize the site of intracellular biosynthetic reactions and product storage[29,30], whereas some lipids can be directly imaged in tissues by mass spectrometry[31].

Emerging lipid analytics

Lipid analysis by mass spectrometry has undergone a big change in the past decade. Several developments in instrumentation and analytical methodology have pushed the field forwards (FIG. 2). First, progress in tandem mass spectrometry (BOX 2) has enabled high-throughput quantitative profiling of the lipidome from minute amounts of samples and the identification of lipids as individual molecular species[32,33]. Second, lipidomics initiatives (for example, the LIPID metabolites and pathways strategy (LIPID MAPS)) have improved the availability of internal standards and the scope of lipid classes that they represent[34], which has enabled absolute quantification of lipid species in moles or grams, as opposed to relative (fold) changes. Third, high-resolution instruments[35] have improved the identification of glycolipids such that they can be analysed in a common high-throughput lipidomics pipeline[36].

Lipidomics is in many ways developing in a similar manner to its sister 'omic' sciences. The initial enthusiasm of being able to acquire biologically interesting compositional data is being gradually replaced by a demand for higher analytical precision and better consistency. It is not uncommon for lipidomics data from independent laboratories to not corroborate. Efforts to harmonize lipid extraction protocols are ongoing. At the same time, sticking to a single 'golden standard' mass spectrometer is impossible or impractical. Therefore, lipidomics software should provide the statistical means to estimate the false discovery rate of lipid species identification[37] independently of the instrumentation platform.

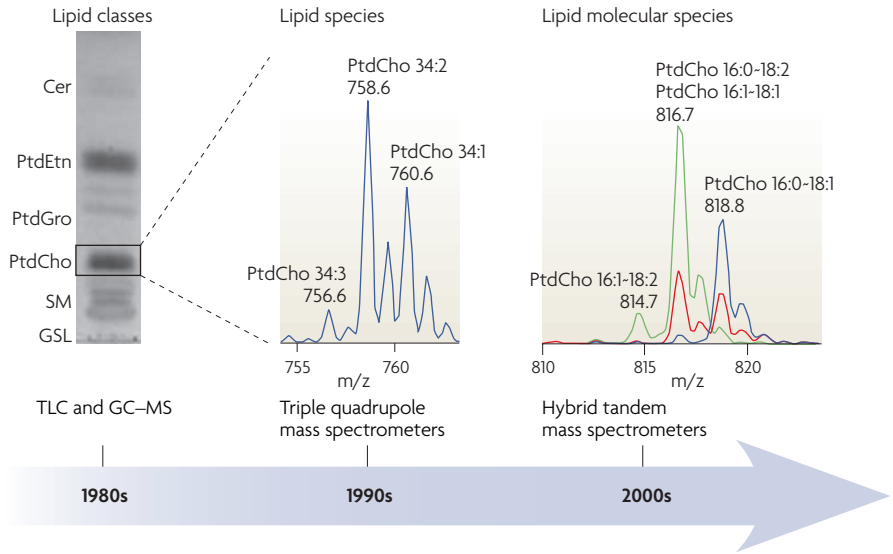

Figure 2 | **Analytical technologies for understanding the lipidome molecular complexity.** Since the 1950s, lipids have been extracted from cells or tissues and total lipid extracts analysed by chromatography and mass spectrometry. In the 1980s, the extracts were mostly analysed by thin layer chromatography (TLC) to determine the content of entire lipid classes. The upper panel shows a part of the TLC plate separating glycosphingolipids (GSLs), sphingomyelins (SMs), phosphatidylcholines (PtdChos), phosphatidylglycerols (PtdGros), phosphatidylethanolamines (PtdEtns) and ceramides (Cers). Total fatty acid composition can be determined by gas chromatography–mass spectrometry (GC–MS). By analysing total extracts using tandem mass spectrometry in the late 1990s, triple quadruple mass spectrometers quantified species with the same number of carbon atoms and double bonds (such as PtdCho 34:2 (34 carbon atoms and 2 double bonds in both fatty acid moieties) and PtdCho 34:1) in each lipid class. The middle panel shows a part of the precursor ion spectrum for the phosphorylcholine head group fragment (mass-to-charge ratio (*m/z*)184.07), which helps to distinguish PtdCho and sphingomyelin species in crude lipid mixtures. Since the 2000s, hybrid tandem mass spectrometers (quadruple time-of-flight or linear ion trap–Orbitrap) have enabled lipidome-wide quantification of individual molecular species (such as PtdCho 16:0/18:2 (where 16:0 is at the *sn-1* position and 18:2 is at the *sn-2* position) and PtdCho 16:1/18:1), although the exact location of the fatty acid moieties is not always possible to determine unequivocally (in these cases, species are annoted as PtdCho 16:0~18:2 and PtdCho 16:1~18:1, respectively). The right panel shows an overlay of several precursor ion spectra acquired for acyl anion fragments of fatty acid moieties: trace in red, *m/z* 255.2 for palmitic acid (16:0); trace in green, *m/z* 279.2 for linoleic acid (18:2); trace in blue, *m/z* 281.2 for oleic acid (18:1). All spectra were acquired in parallel on a quadruple time-of-flight mass spectrometer from molecular anions of acetate adducts of PtdCho precursors.

Monitoring the fold changes gives an idea of the relative enrichment of particular lipid species or entire lipid classes. The next logical step is to investigate the molecular architecture of complex assemblies of lipids and proteins by determining their stoichiometry[38], which would require their absolute quantification. This is being approached from both lipidomics and proteomics directions, and efforts towards absolute quantification of the proteome are already under way[39].

At this point in time we should remain cautiously optimistic. A generally accepted lipidomics workflow and data quality standards (similar to those being imposed in proteomics) have not yet emerged. Also, despite continuous efforts[40], quantifying the full lipidome is still impossible because, for several major lipid classes, particularly glycolipids, no reliable internal standards are available. However, recent advances in oligosaccharide synthesis[41] bring hope that at least some reference compounds might become available in the near future. What the field currently lacks is a systematic effort to develop basic sets of standards that cover the lipidomes of all the main model organisms. By providing absolute quantities of the molecular species, lipidomics is bound to become an integral part of systems analysis in the future.

> “Improved lipid analysis should also benefit molecular medicine and nutritional research”

Lipidomics in health and disease

Improved lipid analysis should also benefit molecular medicine and nutritional research as our health is clearly dependent on our diet. To take one striking example, the mortality of males in the eastern Finnish population used to be unusually high because of cardiovascular disease due to a high intake of saturated lipids[42]. By changing the eating habits of the population, the life expectancy of males is becoming normalized to reach the European average. Evidence for the beneficial effects of a diet with fish and omega 3 fatty acid-rich products on coronary disease is equally compelling[43]. However, little is known about how the lipid content of the diet affects the lipid composition of cell membranes. The methodology used so far has been to determine the levels of most common lipid classes, such as triacylglycerols, cholesterol, cholesterol esters, major glycerophospholipids and ceramides. Because the fatty acid moieties in individual lipid species were not resolved, they were hydrolysed off their glycerol and sphingosine backbones and analysed separately, irrespective of their molecular distribution in the lipidome. This outdated approach was similar to determining the amino acid composition in protein analysis. Clinicians had to operate with integral indices of lipid homeostasis (for example, total triacylglycerols and total cholesterol) determined in blood plasma, even though they are bound to reflect only the main changes in bulk lipid content. However, accurate profiling of the molecular composition of the plasma lipidome suggests that the relationship between the lipid composition and several diet-related disorders is subtle and might only involve a few species of relatively low-abundant lipid classes[44–46]. Therefore, the time is now right to broadly introduce mass spectrometry analyses of lipidomes into all aspects of nutritional research.

It will also be important to understand how to sample lipidomes. In blood, lipids are in the erythrocyte plasma membranes, membranes of the white blood cells and in the serum lipoproteins[47]. The solubility of most lipid molecules is so low that their concentration in lipoprotein-free serum is negligible. This means that one has to determine which source reflects the nutritional status of each individual most accurately. It would not be surprising to find that many dietary superstitions vanished into thin air by accurate and biologically relevant measurements.

Box 2 | **Mass spectrometry in lipidomics**

Lipids can be identified by mass spectrometry in many ways[55,56]. Species of some lipid classes have unique elemental compositions and could therefore be directly identified, even in crude total extracts, solely by their masses determined and with higher than parts per million accuracy[57]. Such 'top-down' lipidomics approaches require high-resolution mass spectrometers, such as Orbitraps[35]. However, it is more common for lipid molecular ions to be subjected to tandem mass spectrometry analysis (MS/MS) to produce structure-specific fragment ions that help to distinguish the species — an approach termed 'bottom-up' lipidomics[58]. The most common types of tandem mass spectrometers are triple quadrupole, ion trap and quadrupole time-of-flight, among others[59]. They take advantage of different physical principles of selecting, fragmenting and detecting ions, and the machines differ by features such as sensitivity, speed of spectra acquisition, mass resolution, precursor isolation window and dynamic range. Importantly, even lipids of the same class might produce different fragment ions, depending on the type of tandem mass spectrometer.

Reflecting the central role of membranes and fat droplets, it is not totally unexpected that altered lipid metabolism might play an important part in disease pathogenesis as many major diseases involve lipids. Examples include atherosclerosis, Alzheimer's disease, diabetes, lipid storage disease and cancer[46,48,49]. The introduction of lipidomics into clinical diagnosis will probably open up new sources of information that were inaccessible before. In many cases, the alteration in lipidomes will be local and difficult to identify without biopsies. However, because of the improved analysis sensitivity, it is becoming possible to analyse minute slices of tissues[50]. In other cases it is likely that changes in the lipidome will become distributed such that they are also reflected in blood plasma samples. Only careful clinical screening of multiple lipidomes, not necessarily limited to blood plasma, which has so far received the most attention, will reveal how to proceed. It is important to find out how specific changes, for example those caused by the deficiency of a single metabolic step, will provoke 'ripple effects' through the whole lipidome. Lipidomics screens of patients are likely to deliver revealing information not only on the functions of the individual lipids themselves but also on the effects of particular lipid deficiencies on the whole organism.

Lipidomics of this type will be a special branch of metabolomics — a systematic study of the unique chemical fingerprints that specific cellular processes leave behind[48]. What, however, specifically distinguishes lipidomics within the entire metabolomics realm is that it targets a broad, but well-defined, subset of biomolecules that makes lipidomes amenable to hypothesis-driven interpretation. We anticipate that quantitative lipidomics measurements will become a standard clinical tool to ensure reliable diagnostics in the future.

Outlook

In this Essay we have emphasized the functional importance of lipid diversity in cell membranes and argued that a more comprehensive lipidomics approach to study lipid complexity is a timely move. Membranes are 'hotbeds' of activities in cells that support the interplay between lipids and proteins. As an example, structural compatibility of sphingolipids, sterols and raft proteins enable the formation of dynamic subcompartments that function in signalling, trafficking and modulation of membrane protein activity. Membrane lipids and proteins form a 2D fluid, the composition of which is fine-tuned to maintain its multitasking capabilities. The decisive change that will enable progress in membrane research will be to abandon the 'lipids-only' or 'proteins-only' approach, as it is the collective capability of lipids and proteins that make membrane research so fascinating. We have to unravel both the biophysics and chemistry that govern the collective behaviour in cell membranes. The enormous progress in biology in the 20th century was due to a powerful reductionist approach, but now the challenge will be to combine reductionist biology with systems approaches. By introducing quantitative lipidomics into ongoing research in different model systems, for example yeast, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Danio rerio* and mice, we will rapidly transform the now restricted niche of lipid research into a multidisciplinary systems biology platform. The most sophisticated model system in this respect is the study of humans. Here we can expect that our 'greasy' future will be full of surprises and that hopefully we will be lead away from the deadly mix of fast food and metabolic syndromes.

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Competing interests statement

The authors declare competing financial interests: see web version for details.

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