Published in final edited form as:

Chem Phys Lipids. 2019 July ; 221: 93–107. doi:10.1016/j.chemphyslip.2019.03.012.

“Redox lipidomics technology: Looking for a needle in a haystack”

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Abstract

Aerobic life is based on numerous metabolic oxidation reactions as well as biosynthesis of oxygenated signaling compounds. Among the latter are the myriads of oxygenated lipids including a well-studied group of polyunsaturated fatty acids (PUFA) - octadecanoids, eicosanoids, and docosanoids. During the last two decades, remarkable progress in liquid-chromatography-mass spectrometry has led to significant progress in the characterization of oxygenated PUFA-containing phospholipids, thus designating the emergence of a new field of lipidomics, redox lipidomics. Although non-enzymatic free radical reactions of lipid peroxidation have been mostly associated with the aberrant metabolism typical of acute injury or chronic degenerative processes, newly accumulated evidence suggests that enzymatically catalyzed (phospho)lipid oxygenation reactions are essential mechanisms of many physiological pathways. In this review, we discuss a

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The authors have declared that no conflict of interest exists.

variety of contemporary protocols applicable for identification and quantitative characterization of different classes of peroxidized (phospho)lipids. We describe applications of different types of LC-MS for analysis of peroxidized (phospho)lipids, particularly cardiolipins and phosphatidylethanolamines, in two important types of programmed cell death - apoptosis and ferroptosis. We discuss the role of peroxidized phosphatidylserines in phagocytotic signaling. We exemplify the participation of peroxidized neutral lipids, particularly tri-acylglycerides, in immuno-suppressive signaling in cancer. We also consider new approaches to exploring the spatial distribution of phospholipids in the context of their oxidizability by MS imaging, including the latest achievements in high resolution imaging techniques. We present innovative approaches to interpretation of LC-MS data, including their audio-representation analysis. Overall, we emphasize the role of redox lipidomics as a communication language, unprecedented in diversity and richness, through the analysis of peroxidized (phospho)lipids.

Keywords

Redox lipidomics; peroxidized phospholipids; oxidatively truncated lipids; lipid mediators; mass spectrometry

Structural and functional/signaling competence of membranes as essential elements of cell organization is achieved, to a large extent, through a remarkable diversification of phospholipids (PLs), their major constitutive components. Appreciation of this huge molecular assortment of PLs has come with the development of contemporary liquid chromatography mass spectrometry (LC-MS)-based lipidomics and its part dealing with the characterization of oxidatively modified (phospho)lipids, redox lipidomics (1). While detailed information has been obtained with regards to phospholipid composition of biomembranes, the approaches to identification and quantitative analysis of peroxidized PLs are only beginning to emerge. Among the major challenges are the extreme low abundance of these oxidatively modified compounds combined with the inherent transient nature of their presence in membranes and difficulties in obtaining standards for accurate LC-MS-based analyses. In spite of this, significant progress in redox (phospho)lipidomics has been made and the major advancements are the focus of this review presented along with many technological issues that still remain to be resolved.

Diversity of polyunsaturated phospholipids and their vulnerability to oxidation.

PLs are lipids composed of fatty acid(s), an alcohol, a phosphate and a polar group (2). Glycerophospholipids are the major phospholipid class in which one or two fatty acids are attached at the *sn*-1, *sn*-2 positions of the glycerol backbone through an ester or ether bond. A polar group occupies the *sn*-3 position via a phosphodiester bond. Glycerophospholipids are further classified based on the nature of their polar groups (3). Saturated and mono-unsaturated fatty acids are usually present at both *sn*-1 and *sn*-2 positions, whereas polyunsaturated fatty acids (PUFA) preferably occupy the *sn*-2 position (Fig. 1). PUFA contain two or more methylene-interrupted *cis*-double bonds. Uncommon PUFA can have ethylene or other poly-methylene interrupted *cis*-double bonds (4). In eukaryotic cells,

PUFA are usually synthesized from saturated fatty acids by two major classes of enzymes, elongases and desaturases. Elongases add an ethylene group and desaturases insert a double bond in the fatty acids (5, 6). There are 7 classes of each of the elongases and desaturases reported in the literature (5, 7). A combination of these enzymes produces multiple PUFAs with lengths between 16–28 carbons and 2 to 6 double bonds. The KEGG's pathway for the biosynthesis of unsaturated fatty acids lists a total of 30 PUFA (7). Combined with the other possible saturated and unsaturated fatty acids with at least 12 carbons, each subclass of “two-legged” PLs can have 1980 species with at least one PUFA. These numbers are much greater (about 5.2 million) for cardiolipins (CL) in which two diacylglyceryl-groups are connected by a 1,3-bis-phosphoglyceryl-group, thus having 4 acyl chains. PUFA are highly susceptible to oxidation due to the presence of a weaker C-H bond at the *bis*-allelic position. These hydrogen atoms are easily abstracted to form the lipid radical (8) - the first intermediate of enzymatic and non-enzymatic lipid peroxidation (9).

Oxidized lipids as signals – diversity of lipid mediators (oxPUFA vs oxPL)

PUFA-containing lipids of ω -6 and ω -3 series and their oxidation products are essential signaling molecules, lipid mediators, orchestrating many metabolic processes and cell responses, including inflammation (10–15). LC-MS protocols are widely used for identification, quantification and monitoring of multiple different PUFA as well as their oxidatively modified products (10, 13, 16, 17). By using electrospray MS coupled with reverse phase liquid chromatography (LC) many of these lipid mediators – oxygenated derivatives of PUFA (eg, leukotrienes, lipoxins, hepxilins, protectins, resolvins, maresins) - have been identified and their specific roles in recruitment of innate immune cells (eg, neutrophils and macrophages) and resolution of inflammation is well established (13, 18–20).

The role of oxygenated PLs (oxPLs) in the development and resolution of inflammation as well as in the pathogenesis of different diseases is widely discussed (21–28). OxPLs include a highly diversified group of oxygenated PUFA-lipids and their structure is essential for their biological role, as determined by the polar head, the length of the fatty acid, the number of double bonds and oxygen-containing functional groups such as hydroperoxy-, hydroxy-, epoxy- and keto- that are positioned on the fatty acid residues (Fig. 1). OxPLs can be formed non-enzymatically (21) and enzymatically via reactions catalyzed by different metalloproteins such as iron-containing lipoxygenases (LOXs) (29, 30), cytochromes P450 (31) and cytochrome c (cyt c)/CL peroxidase complex (32). Some of the phospholipid oxidation products generated in these enzymatic reactions have been identified and their signaling roles established. For example, oxygenated CL (oxCL) has been documented to act as a required mitochondrial signal for the execution of the intrinsic apoptotic program (33). In ferroptosis, oxygenated phosphatidylethanolamine (oxPE) is generated as a predictive biomarker of cell death (34). Oxygenated phosphatidylserine (oxPS) is a potent enhancer of phagocytosis of apoptotic cells by macrophages (35). Finally, oxygenated phosphatidylcholine (oxPC) formation has been associated with signaling in chronic inflammation (21).

Low abundance of lipid oxidation products; spreading/diversification of oxygenated products over numerous PUFA.

Different polar (phospho)lipids as well as neutral lipids with esterified PUFA-residues can be enzymatically metabolized to yield the majority of lipid mediators (36–38). Oxygenated PUFA are known to play multi-functional roles as essential signals coordinating metabolism and physiology (10, 39–41). Although reactions of lipid peroxidation are widespread and found in most of tissues in a variety of organisms including humans, the absolute amounts/concentrations of lipid oxidation products detectable *in vivo* are quite low, on the order of 0.03–3.0 mol% of total non-oxidized lipids (21, 33–35, 42). Oxidation of lipids produces oxygenated derivatives of PUFA residues as well as secondary products with shortened hydrocarbon chains (21, 33, 34, 42–46). One of the important characteristic features of these oxidatively-truncated products is their high electrophilicity and reactivity towards nucleophilic targets in essential macromolecules (43, 45, 47). Consequently, peroxidized lipids may form adducts with many intracellular molecules of non-lipidic nature resulting in very low steady-state concentrations of peroxidized lipids *per se* (see below) (Fig. 2.)

Enzymatic vs non-enzymatic lipid oxidation mechanisms and their products – selectivity and specificity of lipid oxidation.

There are two schools of thought about the mechanisms involved in the production of oxPLs. In the 40–50s, an aggressive transfer of the chemical concepts on free radical mechanisms of liquid-phase oxidation of organic hydrocarbons into biology had occurred leading to the formulation of new disciplines of free radical biology and medicine (48–50). It has been assumed that under conditions of the so-called oxidative stress, non-enzymatic reactions of free radical oxidation overwhelm the well-controlled low molecular weight antioxidants and proteins with antioxidant action processes, thus leading the initiation of cell and tissue injury (51, 52). Following this chain of logic, significant research efforts have concentrated on the search for new natural and synthetic free radical scavengers capable of maintaining and/or re-establishing control over free radical oxidation reactions, particularly as they relate to lipid peroxidation (53). Based on the established role of transition metals and their interactions with intermediates of univalent oxygen reduction – reactive oxygen species (ROS) - in the catalysis of chemical oxidations, the ideas of dis-coordinated metabolism of iron and copper along with excessive ROS production were introduced as the major mechanisms of tissue injury in biomedicine (54–56). In spite of the initial enthusiasm and encouraging results (57–59), subsequent detailed experimental studies and clinical trials of a variety of antioxidants were disappointing (60–62).

One of the major features of these free radical oxidation reactions is the strong dependence of their rates on the number of double bonds and *bis*-allylic sites with readily abstractable hydrogens in PUFA-lipids rather than selectivity and specificity (63, 64). In parallel with these explorations of random free radical reactions in bio-systems, traditional biochemistry revealed a number of enzymatic processes where “caged” free radical mechanisms were engaged in highly selective as well as regio- and stereo-specific oxidation of PUFA lipids (65, 66). These studies have identified several families of oxygenases and (per)oxidases

utilizing PUFA-lipids as their substrates. Among them are three major groups represented by: i) di-oxygenases and LOXs common for prokaryotic and eukaryotic cells (30), ii) monooxygenases, CYP450 (31), and iii) peroxidases, cyclooxygenases (COXs) (67). In the context of this review, the important feature of these enzymes is their involvement in the production of signaling oxygenated free PUFA, lipid mediators, acting via specialized receptors (10, 68–70). These signaling molecules include a variety of octadecanoids, eicosanoids, docosapentanoids and docosahexanoids - oxygenated derivatives of PUFA with 18, 20, and 22 carbons (10). In addition to these well explored lipid mediators described over the last six decades (10–12, 15, 20, 71, 72), a significant focus has been lately placed on enzymatic peroxidation of different classes of glycerophospholipids (23, 34, 42, 73). Because of the huge variety of different positional, regio- and stereo-isomers of PUFA PLs they may represent a signaling language with essentially unlimited diversification encompassing millions of individual molecular species (42, 73). As much as this may be important for the myriads of their signaling functions, these same features represent huge analytical difficulties in their characterization.

Technological challenges of redox lipidomics.

The analysis of oxidized lipids has become a formidable task in mass spectrometry. This is primarily due to the plethora and heterogeneity of oxidized products that can be formed. Oxidized lipids are present in low abundance and in some cases, their chemical instability and thermolabile nature make them susceptible to degradation. While hydroperoxides are the primary products of lipid oxidation, secondary products such as hydroxy, aldehyde, ketone and epoxide products add to the complexity/heterogeneity of the analysis. In addition, one has to consider not only full-length oxidation products but those with potential cyclization/rearrangement reactions, truncated PLs and fragments of oxidized fatty acyl chains as well (21, 74–77).

A variety of upfront biochemical methods aimed at derivatization of oxidized products have been used but many of the assays for hydroxy-derivatives, aldehydes and ketones are not specific for individual oxidized products (78–83). Immunoassays and ELISA assays for oxidized lipids have been reported, and while more specific, depend on available antibodies, which are limited (84, 85). While all of the above assays are helpful, most cannot identify specific oxidized species of PLs and are only valid for classes of oxidized lipid products allowing for only a generalized overview of the oxidation process.

Many different methods have been developed for the separation and quantification of different lipid classes and their oxidation products starting with specific protocols for sample preparation, time of analysis, type of detectors, ability to quantitate with increased sensitivity, including solid phase extraction (SPE), high performance thin-layer chromatography (HPTLC)-phosphorus analysis, HPLC-UV, light scattering analysis, gas chromatography-mass spectrometry (GC-MS) analysis, etc (86–88). However, these methods are not suitable for the simultaneous analysis of complex lipid mixtures due to their cumbersome procedures as well as insufficient sensitivity and specificity. For example, HPTLC as well as SPE, were frequently applied for lipid analyses in the past, but at the present time, these approaches are mostly used for the preparative separation of selected

lipids classes (86, 89). GC-MS analysis provides sufficient information about oxygenated products of PUFA but information about the intact lipid moiety is lost. In addition, GC-MS usually requires derivatization of samples (20).

Over the past three decades, LC-ESI-MS/MS has emerged as the premier method to structurally characterize and quantitate complex lipids, including lipid oxidation products, from living cells and tissues (90–96). These new methodologies gave birth to a new sub-discipline, oxidative (or redox) lipidomics, a rapidly growing field dealing with the identification and characterization of the totality of oxygenated lipids and understanding their role under normal physiological and disease conditions (10, 22, 33, 34, 37, 45, 71, 90–94, 97).

Various types of mass-spectrometry (MS) have become by far the most sensitive, accurate and quantitative methodology for studies of lipid peroxidation products. Both matrix assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) have been widely used for the analysis of PLs and their oxidized products (18,19). Recent technological improvements in mass spectrometric platforms allow for enhanced sensitivity, resolution and scan speed of mass ions. Both ESI and MALDI are considered soft ionization techniques. These platforms have been used for many years for the studies of intact biomolecules including lipids. However, more often than not, the use of ESI is chosen over MALDI due to its ability to be interfaced directly to a LC system. Coupled with LC and using a targeted analysis approach with confirmation by fragmentation, ESI techniques allow for further sensitivity and specificity. However, even with these improvements, some oxidized species may be isobaric, making structural identification and quantitation more difficult. In addition, standards for the large abundance of oxidized lipids species are scarce. While MS allows the direct detection of oxidized lipid species, in some cases, such as in oxygenated derivatives of arachidonic acid, further fragmentation (MS^n) or hydrolysis of oxPLs and further analysis of the hydrolyzed fatty acids is needed for distinguishing the position of oxygenated groups. LC-MS and LC-MS/MS can be set up for quantitative analysis using internal standards and calibration curves established with reference standards (98). LC-ESI-MS/MS strategies have been developed and optimized for the species-selective analysis of multiple oxidized species of PLs and triglycerides (TAGs) using normal-phase and reversed-phase chromatography.

Both normal phase, hydrophilic interaction liquid chromatography (HILIC) and reverse-phase have been used for the separation of lipids, with the latter being the most popular. Normal phase separation is based on adsorption and polarity of the lipids to the stationary surface whereas in reverse-phase, hydrophobicity plays the major role. Both techniques have their advantages and disadvantages. Normal phase chromatography allows separation of lipid classes although both oxidized and non-oxidized species may overlap and the signals for the low abundant (oxidized) species may be suppressed. While reverse- phase separation allows for the separation of individual molecular species, multiple lipid classes may overlap. However, any type of up-front chromatographic separation can dramatically reduce the complexity of the lipid sample, which aids in the identification and quantitation of oxidized lipids, including the low abundant oxidized species. Some groups have utilized supercritical fluid chromatography for the separation of peroxy-, hydroxy-, and epoxy-lipid species, however, specialized equipment, software and personalized databases are needed for this

type of approach (21). We and others have utilized a two-dimensional chromatography approach whereby lipids are separated by class in the first dimension under normal phase or HILIC conditions with further separation of oxidized and non-oxidized lipid species by reverse-phase in the second dimension (22,23). While this procedure is somewhat labor intensive and some sample loss may occur it offers a powerful alternative to a single chromatographic run when low-level oxidized lipid species are sought.

Accurate identification of oxPLs can be achieved by using recently developed high resolution MS orbitrap instruments such as Thermo's Q-Exactive and Fusion Lumos. Unlimited fragmentation capacities in the ion-trap part of the Fusion Lumos spectrometer combined with the high mass accuracy of its Orbitrap platform and coupled with the reverse phase liquid chromatography as well as increased speed of spectral acquisition permits detection, unequivocal identification and quantitative characterization of oxPLs in cells as well as in tissues *in vivo* as exemplified by the data shown in Fig. 3.

General approaches to analysis of oxidized lipids – shotgun lipidomics vs LC-MS.

The analysis of lipids by mass spectrometry has been conducted on a variety of mass spectrometry platforms including GC-MS, MALDI-MS and ESI-MS. While a variety of ionization techniques have been used in the field of lipidomics (including ESI, APCI, APPI, DESI and SIMS), ESI is most widely utilized (99). ESI-MS employs two approaches, a direct infusion approach (shotgun lipidomics) and a LC-MS approach. Both types of analyses have their own inherent advantages and disadvantages.

Liquid Chromatography-Mass Spectrometry (LC-MS).

A mass spectrometer coupled to an up-front liquid chromatography system separates lipids based on polarity (normal phase and HILIC columns) or hydrophobicity (reverse-phase columns) and thereby greatly reduces the complexity of the lipid sample which generally increases sensitivity (100). This may aid in the detection of low abundance lipids whereby the chances of suppression from other highly abundant lipids is greatly reduced (101). LC provides the important parameter of retention time, which aids in the structural identification of the lipid. Normal phase and HILIC columns have the ability to separate lipids by class based on polarity and this selectivity carries over to the standards/internal standards used. Thus, the standards will have a similar retention time aiding in the quantification of specific lipid species. However, separation of fatty acyl-chain isomers by HILIC and normal phase chromatography is not usually possible although some exceptions may exist.

Reverse phase chromatography on the other hand, separates lipid species based on their hydrophobicity that is reflected mostly in their hydrocarbon chains. Therefore, this chromatographic method has the ability to separate lipids based on acyl chain length and number of double bonds (102, 103). This method also allows the separation of isomeric lipid species with different acyl chains such as PE (18:0/18:2) and PE (18:1/18:1). Unlike normal phase chromatography, standards and internal standards may potentially elute at different retention times. This could complicate quantification, since the lipids of interest and

standards may be subjected to different matrix effects and solvent mixtures, affecting their overall ionization (104). On the other hand, each lipid species is usually associated with a specific retention time window for a given solvent system, which aids in the identification of a particular lipid species. A number of different scanning routines (see below) may also be used in conjunction with LC-MS/MS analyses, which increases confidence in lipid species identification.

Shotgun Lipidomics.

In shotgun lipidomics, no prior up-front separation of lipids is performed and the lipid extract is infused directly into the mass spectrometer in an appropriate solvent. The identification of lipids is carried out utilizing specific scanning routines (for example, product ion, neutral loss and precursor ion scanning) making this type of analysis simple and rapid (99, 105, 106). In addition, since standards/internal standards “see” the same solvent system as the lipid extracts, matrix and ion suppression effects are the same for all the lipid species. This makes quantification relatively straightforward and comprehensive. Low abundance ions, however, may suffer in this approach, due to the suppressive effects of highly abundant ions. In addition, shotgun lipidomics is limited in its ability to separate isomeric lipid species as well as in sensitivity as compared to LC-MS. Structural analysis may also be complicated by the fact that MS/MS or MSⁿ analysis may involve fragmentation of more than one precursor ion in the case of isobaric species.

Simplifying the analytical task – hydrolyzing phospholipids to reduce the vast number of oxidized species.

LC-MS analysis of oxPLs is still a difficult task mainly due to their low abundance, huge diversification and absence of appropriate standards. In contrast, the LC-MS protocols for detection and identification of fatty acids and their oxidation products are very well developed (101, 107, 108) and standards for many oxygenated fatty acids and their metabolites are commercially available. Given that the diversity of phospholipid oxidation products is 2–3 orders of magnitude greater than that of oxygenated PUFA, enzymatic hydrolysis of oxPLs by phospholipases A₂ have been utilized which significantly reduces the diversification and simplifies the initial analysis of oxPL species (Fig. 4). Clearly, this approach is associated with a loss of information regarding the phospholipid origin of the oxygenated PUFA. Porcine pancreatic phospholipase A₂ was successfully used to liberate identifiable hydrolysis products – oxygenated and non-oxygenated PUFA occupying the *sn*-2 position of oxCL and non-oxidized CL (36, 109). However, its low specificity for *sn*-2 oxPUFA-residues is associated with high yields of non-oxygenated fatty acids vs orders of magnitude lower concentrations of oxygenated fatty acids (36). This complication can be avoided by using phospholipases A₂ with a higher specificity towards oxygenated PUFA-residues. Several phospholipases A₂ have been identified as enzymes that easily release oxidatively modified fatty acids such as low-density lipoprotein-associated phospholipase A₂ (LpPLA₂VIIA or PAF-acetylhydrolase), calcium independent iPLA₂γ (110) and iPLA₂β (111). LpPLA₂ releases PAF or PAF-like oxidatively truncated fatty acids from phosphatidylcholine (PC). It can also hydrolyze oxPUFA from CL (112) and phosphatidylserine (PS) (35, 113). iPLA₂γ predominantly liberates oxygenated PUFA from

CL (110). Using an enzymatic digestion protocol followed by MS analysis, the major oxygenated molecular species of CL formed in a model system (36) as well as in dysfunctional mitochondria from rotenone exposed human lymphocytes have been identified, characterized and quantitatively assessed (114).

Redox phospholipidomics of cell death signaling – enzymatic nature, selectivity, specificity.

Oxidation of cardiolipins in apoptosis.

Enzymatic oxidation of CL by cyt c in mitochondria is one of the early stages of intrinsic apoptosis (33). Redistribution of CLs from the inner mitochondrial membrane (IMM) to the outer mitochondrial membrane (OMM) creates conditions for the physical contact between the phospholipid facing the intermembrane space and cyt c resulting in the formation of cyt c/CL complex. This complex has a peroxidase activity and utilizes ROS generated by the disordered ETC to generate superoxide radicals and their dismutation product, H₂O₂. The latter can be used as a source of oxidizing equivalents for the cyt c/CL complexes to catalyze oxidative modifications of PUFA-CLs that can act as apoptotic signals (33). By using mass spectrometry, several species of hydroperoxy-CL and hydroxy-CL have been identified as major oxidation products formed by the complexes *in vitro* and in *in vivo* studies (93, 115–120). However, the low level of oxCL, less than 0.01% of all PLs, and a wide number of generated oxygenated species in cells and tissues (33, 115, 121) make identification and quantitative assessments of individual oxCL molecular species difficult and requires specific efforts. The huge diversification of oxCL species can be reduced by enzymatic hydrolysis with phospholipases such as PLA₁ plus PLA₂. The hydrolysis yields a limited number of identifiable oxygenated and non-oxygenated fatty acids as well as lyso-CLs (36). Recently, a new redox opto-lipidomics approach using acridine 10-nonyl bromide (nonyl acridine orange, NAO) to detect CL and its oxygenated species has been developed (122). Mono-oxygenated derivatives of C18:2-containing CLs in cells were identified as pro-apoptotic cell death signals.

Oxidation of phosphatidylserine in apoptosis and its role in clearance of apoptotic cells.

The presentation of essential “eat-me” signals, PS and oxPS, on the cell surface is a unique and critical signal for the engulfment of apoptotic or damaged cells by professional phagocytes - macrophages and microglia (35, 123–125). Early accumulation of oxCL in mitochondria results in the release of pro-apoptotic factors, including cyt c, into the cytosol (33), where the peroxidase function of released cyt c can be realized via the interaction with PS localized in the inner leaflet of plasma membrane (126). The apoptotic program includes externalization of PS and oxPS to the cell surface mainly due to inhibition of the aminophospholipid translocase (APT) (127, 128) (Fig. 5).

An elegant LC/MS study in combination with cell biology demonstrated that this action resulted in a dramatic increase in oxPUFA-containing PS species and their externalization on cell surface that enabled phagocytic clearance of apoptotic cells by macrophages. It has been demonstrated that oxygenated molecular species of PS containing two oxygens (hydroperoxy-, dihydroxy-, and hydroxy-epoxy-) in the PUFA chain of PS were the

dominant PS oxidation products (35). Additionally, the relevance of oxPS to phagocytosis was demonstrated by using Lp-PLA₂ – an enzyme with documented selectivity towards hydrolysis of oxidatively modified (but not of non-oxidized) PS species (35, 113). OxPS species were detected in conditions associated with apoptotic cell death *in vivo* and *in vitro* (115, 116, 118–120). The presence of oxPS on the surface of apoptotic cells is important for the interaction with the receptors (129, 130). Thus, acyl chains of oxPS can be recognized by different receptors including CD36, scavenger receptor A (SRA, MSR1, CD204), and SR-oxPS (scavenger receptor for PS and oxidized lipoproteins, CXCL16) (131–134).

Oxidation of phosphatidylethanolamines in ferroptosis.

Phosphatidylethanolamines (PE) are the second most abundant class of glycerophospholipids and the major lipid class containing ω -3 arachidonic acid (AA) and adrenic acid (AdA) residues (66, 135). During ferroptosis, the preformed 15-LOX/PEBP1 complexes selectively oxidize AA-PE and AdA-PE (34, 39). Binding of PEBP1 allosterically modifies 15-LOXes to modify their substrate selectivity from *free* AA and AdA towards AA/AdA-PE. Three independent factors: 1) relative abundance of AA containing PE, 2) hexagonal phase membrane structure and 3) 15LOX/phosphatidylethanolamine binding protein 1 (PEBP1) complex synergistically act towards the selective and specific production of 15-hydroperoxyeicosatetraenoic acid-PE (HpETE-PE) (66). A decrease in the phospholipid hydroperoxide reduction capacity due to either inactivation/low expression of glutathione peroxidase 4 (GPX4) or by the decline in glutathione (GSH) levels leads to the accumulation of HpETE-PE and induction of ferroptosis (136, 137). Notably, manipulations of acyl CoA synthase-4 (ACSL4) expression are associated with markedly decreased sensitivity of the cells to pro-ferroptotic stimulation (138). The amazing feature of this pro-ferroptotic reaction is its remarkable selectivity in preferring AA-PE out of thousands of other oxidizable PUFA-PLs along with the regio-specificity of the oxidation products - 15-HpETE-PE. It should be noted, however, that these particular propensities of 15-LOX/PEBP1 complexes have been established in cells highly enriched with AA-PLs, due to the growing of cells in the presence of AA (39). It is possible that other PUFA-PLs may undergo peroxidation yielding pro-ferroptotic signals in cells and tissues enriched with other oxidizable PLs.

Proximate death signals - adducts of oxidized lipids with proteins - how much do we know about them.

PUFA and PUFA-containing PLs are extremely vulnerable to oxidative attack by reactive oxygen species. Once oxidized, either by enzymatic or non-enzymatic means, a variety of reactive electrophilic oxidation products can be formed (139–142). While the initial oxidation product is a hydroperoxide, further decomposition can lead to a reactive electrophile, resulting in reactive fragments of PUFAs, reactive PUFA fragments from PLs or a reactive, truncated phospholipid (139–145). By far, the most widely studied lipid derived electrophiles are the reactive fragments from PUFAs or PUFA containing PLs (Fig. 2). These include α , β -unsaturated aldehydes, malondialdehyde as well as hydroxy-, oxo- and epoxy-alkenals and γ -ketoaldehydes (144, 146). Several species that have been studied in detail include the well-known lipid-derived electrophiles such as 4-hydroxynonenal

(HNE), 4-oxononenal (ONE), 4-hydroxyhexenal (HHE) and 4-oxohexenal (OHE) (116, 145–147).

These electrophiles are chemically reactive, can covalently modify protein targets and their small size makes them readily diffusible. The amino acids Cys, His and Lys are typically modified by the lipid-derived electrophiles although direct oxidation of a protein's amino acids (carbonylation of Pro, Arg, Lys and Thr) can also occur (143, 148). The abundance, reactivity and half-life of these products usually determines the extent of the protein modification event. Since their half-life tends to be short, the electrophiles usually react with proteins at or near the site of formation. Most lipid-adducted proteins result in deleterious/cytotoxic effects on the cell resulting in a modification of proteins leading to decreased gene expression and cell death (40, 148–150). However, some may modify a protein's function in an adaptive way by stimulating gene expression and cell survival (148, 150).

Lipid-derived protein adducts have been associated with various processes affected by oxidative stress, including inflammation, degenerative diseases and tumor formation. Indeed, lipid-derived protein adducts have been detected in Alzheimer's, Huntington's and Parkinson's disease, Amyotrophic lateral sclerosis, Down's Syndrome, cancer, atherosclerosis and various autoimmune diseases (148, 150). Since these adducts tend to be in low abundance, a variety of enrichment methods coupled to mass spectrometry have been devised. These include biotin hydrazide affinity capture as well as various click chemistry approaches (146, 151).

In addition to the well-known, short-chain lipid derived electrophiles, lipid aldehydes and their truncated products can also act as potential protein adducts. The majority of oxPL aldehydes studied thus far appear to be associated with choline PLs and display a complex reactivity toward proteins via Schiff base formation and Michael addition and can covalently modify lipoproteins and various proteins in cells (147).

We have identified various oxidized species of PEs as proximate death signals in ferroptosis (34). In addition, other studies have identified forms of oxPE as potential signals in exacerbating the effects of IL-13-induced secretion of mucins in asthmatics (152). A recent study has described a proteomic method for the detection of protein carbonylation produced by lipid-derived electrophiles (mostly HNE and ONE) during ferroptotic death (151). Several protein targets with potential links to ferroptosis have been identified. Truncated, reactive phospholipid aldehydes, if they participate in the ferroptotic death pathway, still remain to be determined. What remains unclear for all reactive oxidized lipid species is whether or not the adducted oxidized lipid products (either the small lipid-derived electrophiles or truncated phospholipid products) that covalently modify proteins are truly associated with the pathogenic/signaling process or generated as a consequence of the particular signaling/death pathway.

Oxidized neutral lipids in immune cells of the tumor micro-environment.

During cell-to-cell communications, tumor cells constantly modify their microenvironment that includes cancer-associated fibroblasts, endothelial cells and cells of the immune system

(T cell, leukocytes, macrophages, neutrophils etc.) with a variety of secretory factors released from all cellular components (153). Immuno-surveillance plays a critical role in the control of tumor progression whereby dendritic cells (DC) are the most potent antigen presenting cells responsible for the development of immune responses. Accumulation of cells with immune suppressive propensities, particularly myeloid-derived suppressor cells (MDSCs), and expansion of immature DCs with aberrant cross-presentation capacities – are important immunological abnormalities in cancer (154). Interactions between these types of cells are recognized as essential contributors to the failed anti-tumor immunity (155).

Excessive formation of lipid droplets (LDs) - neutral lipid storage organelles - has been identified as a potent regulator of DC functions (156). DCs isolated from tumor-bearing mice and cancer patients or treated with tumor explant supernatants (EL4, MC38) contained considerable amounts of oxygenated neutral lipids: TAGs, cholesterol esters (CEs) and free fatty acids (FFAs). LC-ESI-MS/MS analysis revealed increased amounts of mono- and di-oxygenated TAGs molecular species containing linoleic (LA) and arachidonic acid (AA) residues (16:0/18:2+[O]/18:1; 16:0/20:4+[O]/18:1; 18:1/18:2+2[O]/18:1; 16:0/20:4+2[O]/18:1) (Fig. 6). Moreover, oxygenated TAGs were represented by a wide spectrum of truncated molecular species 16:0/9-ONA/16:0; 16:0/9-ONA/18:2; 16:0/9-ONA/18:1; 18:1/9-ONA/18:1, containing mostly 9-oxo-nonanoic acid (41, 45, 157). Additionally, the content of oxygenated CE 18:2+[O] molecular species, containing hydroxy-, epoxy- groups and oxidatively truncated CE 9-ONA was detected (45). Oxidative truncation of LA in TAGs/CEs leads to the formation of electrophilic products, which may form adducts with -SH, -NH₂ groups of heat shock protein 70 (Hsp70) that is critically important for DC cross-presentation (157, 158). Accumulation of oxFFAs and oxTAGs/oxCEs in DCs, mediated via the Msr1 receptor, may be responsible for the loss of their immune-regulatory functions in cancer. While supplementation of DCs with non-oxidized LA revealed the accumulation of TAG/CEs, no oxygenated TAG/CE species were found under these conditions. Notably, the transfer of peptide-MHC class I complexes to the DC surface was not affected (41). In contrast, a lipid-soluble azo-initiator of peroxy radicals, AMVN, added to LA supplemented DCs caused the accumulation of oxidized lipids in LDs and blocked antigen cross-presentation by inhibiting the transfer of peptide-MHC class I complexes to the DC surface (41).

Imaging mass-spectrometry of lipids and its potential in redox lipidomics.

While soft ionization methods such as ESI-MS drastically improve structural quantitative lipidomic analysis, lipid extraction *en bloc* loses spatial localization information. There are several soft ionization techniques that allow direct mass spectrometric analysis of locations on the surface of a native sample (Mass Spectrometry Imaging or MSI) (159, 160). Samples are usually cultured cells or thin slices of frozen tissue, but other kinds are possible such as insects (161, 162) and plants (163). Many locations (“pixels”) are analyzed in a grid pattern to generate an image based on the MS data. The size of a single pixel can range from tens of microns to tens of nanometers, and is dependent on the instrument type, analyte abundance and sample preparation (164). MSI is different from fluorescent microscopy and immunohistochemistry (IHC) in that it is an untargeted approach that does not require custom antibodies or other probes. One MSI experiment can potentially detect hundreds of

different lipid molecular species from a single sample. Comparison of MSI images with other histology techniques (165, 166) allows lipid abundances to be mapped to specific features (167–169).

There are many soft ionization techniques for MSI, and two of the commonly used ones are MALDI (Matrix-Assisted Laser Desorption/Ionization) and SIMS (Secondary Ion Mass Spectrometry) (159, 160, 164, 170). MALDI is excellent not just for lipids but for proteins and other analytes. SIMS is capable of much higher spatial resolution than MALDI, although sub-cellular resolution of some lipids is possible with MALDI (171–174). The high beam energies of most SIMS primary ion sources cause extensive fragmentation of analytes larger than 1000 Da (175, 176). However, Gas Cluster Ion Beam SIMS (GCIB-SIMS) uses a lower energy primary ion beam and allows improved analysis of lipids above 1000 Da (177–181). Since SIMS sources ablate (“sputter”) the surface, they can provide depth profiling information by repeated analyses on the same region (178).

MS Imaging of oxPLs is challenging for many of the same reasons as ESI-MS, such as the low abundance and broad diversity of oxidized products (182, 183). A single lipid precursor could generate numerous primary and secondary oxidation products, each of which could be in very low abundance. These signals might be suppressed by more abundant ions. LC-MS gives a separation step prior to analysis, which helps to mitigate signal suppression, but this is not possible with MSI. Therefore, alternative approaches can be used for redox MSI, such as using chemical or enzymatic methods to remove suppressing signals (167–169). For example, MALDI detection and analysis of molecular species of CL - which are present in very low abundance - until recently was not practically possible. However, treatments of the samples with: i) cross-linking reagents, (to eliminate the signals from amino-containing PLs, PE and PS) and ii) phospholipase C (to eliminate the signals from most abundant and suppressive PC) “unmasks” CLs and allows for quantitative analysis of its distribution and relative presence in tissues and cells (167). This protocol, applied to freshly frozen tissue sections from brains of control rats and those after traumatic injury, revealed significant depletion of PUFA-containing CL species in the area of direct impact as well as other parts of the brain where the spreading lesion was reliably detectable (168, 169) (Fig. 7). These results are interpretable in terms of lower amounts of oxidizable PUFA-CL species disappearing as a result of their peroxidative modification (184). In other words, if the primary oxidation product is not directly detectable, then measuring the loss of its precursor material is an alternative (168, 169, 185–188). Chemical modification of the primary oxidation product into a derivative that is more easily detectable is another approach (189). Sometimes the short chain truncated product or various lyso-products from the redox reactions are detectable (183). Of note, contemporary protocols of MALDI and GCIB-SIMS imaging provide high spatial resolution (slightly over 1 micron) thus permitting single cell and subcellular imaging of individual lipids (190).

Audio-representation of redox lipidomics data – “rancid sounds” of oxidized lipids

Sonification is the auditory counterpart of data visualization, and one of the earliest examples is the Geiger counter, which detects the presence of radioactive decay (alpha particles) via an “auditory readout” (the characteristic clicking sound) (191, 192). There are many examples of audio-representation or sonification of data in the physical sciences (193). Sonification can provide new insights into data, such as discerning similarities or differences when comparing protein folding sequences (194). It also can ease the difficulty of visualizing multivariate, time-series datasets (195). MS data is a series of peaks representing the m/z and intensity of each ion, and high-resolution MS can analyze hundreds or thousands of different ions in a single experiment. Musical information is a series of pitches (frequencies) and silences organized in the framework of time (196). MS data would seem to be excellent for being depicted by sonification to musical serialism. However there has been very little work in sonifying mass spectral data (197). Depicting redox lipidomic data by musical serialism could complement the visual display and provide new insights in understanding the differences between two datasets.

To this end, we embarked on a project using the Nyquist programming language (158) to sonify CL mass spectral data from naïve rat brains and those from a traumatic brain injury (TBI)-induced animal model¹. We also utilized the web-based resource Music Algorithms², which allows any type of data to be sonified (198). The intensities from the mass spectra were converted into whole number integers as a prerequisite. For Music Algorithms, we utilized the intensities from the m/z range of 1500 to 1600 from control and TBI samples, since this is the region of the spectrum where the PUFA-CL substrates (and potentially their oxidation products) are detected. The TBI and control data each produced a separate one-voice audio file using the custom pitch input option. In order to set a minimum/maximum dynamic range for the pitch input, a value of 0 (no pitch) and the value 14320 (the maximum intensity from the two data sets) was placed at the beginning of each control and trauma data set. The pitch mapping was set to division, 1–88. The pitch input was then modified using the inversion function. This allows the higher intensity values to be displayed/sonified as lower notes and the lower intensity values as higher notes on a piano keyboard. For the duration input, the data were entered in the same way as for the pitch input, but without modification by inversion. The duration mapping was set to division, 1–9. A chromatic scale was chosen for both control and TBI samples, using 180 beats per minute as the tempo. The piano was chosen as the instrument, and the audio outputs were saved as MIDI files and converted to MP3 format.

When comparing the two audio files (see supplementary information), it becomes apparent that the TBI samples resulted in a greater number of lower tones/notes (higher intensity peaks) in the data from the 1500–1600 m/z region, indicating more oxidizable (or oxidized) CL species. The limitations of this program are tonal dynamics, (i.e. each note is played at the same volume) and dynamic range (for example, a data set may have more values than

¹www.cs.cmu.edu/~rbd/algocompbook/sonification-examples/sonification.html Accessed on 1 December 2018

²www.musicalgorithms.org/3.2/ Accessed on 1 December 2018

notes available on a piano keyboard). If data are mapped entirely arbitrarily, the result can be unpleasant and lacking in context (191). By its nature, mapping data by arbitrary musical serialism removes the context of the data. Much as examining a spectrum for key information (such as +16 Da mass differences indicative of oxygen addition), applying a small degree of “color” to the sonification can help highlight important information in the data³.

Integration of redox lipidomics data into bioinformatics and systems biomedicine.

The ultimate effectiveness of redox phospholipidomics analysis depends on the translation of obtained lipidomics data into the corresponding metabolic and other normal biological or aberrant disease-associated responses. Similar to other omics, redox lipidomics also depends on bioinformatics and system biology approaches to translate and interpret the data obtained. These packages primarily focus on building an oxidized lipid database and automatically identifying them in the LC-MS data after pre-processing for peak alignment and integration. LipidMatch includes 214 potential oxidized fatty acids, phospholipid species that have 126 oxidized fatty acids and 88 potential short chain oxidized fatty acids in its *in silico* library (199). LipidPioneer, an interactive template developed for the generation of lipid libraries can generate oxidized and oxidatively truncated lipid species (200). LipidFinder: a computational lipidomics work-flow was used to identify oxidized phosphatidylinositol in platelets (201, 202). LOBSTAHS exploits the unique tendency of adduct ions of lipids, which remain relatively consistent across samples, to discover and identify redox oxPLs and oxylipins (203). LPPtiger incorporates algorithms to predict the redox lipidome, generate spectral and fragmentation libraries and identify oxPLs (204). The continuous improvements in the understanding of redox lipidomics and analytical methods prompted the development of various bioinformatics packages for redox lipidomics data analysis. However, the bioinformatics-based interpretation of the redox lipidomics results is still limited to specific areas and has not been translated globally for use in all research fields. As a result, there are only a few examples of the use of custom in-house pipelines for redox lipidomic analysis (eg, in platelets and in ferroptosis) (34, 39, 205).

The complexity of the redox alterations in the lipidome and its involvement in biological processes necessitates the need for more detailed system biology approaches. At present, many studies used simple mathematical correlations and multivariate analysis for the data integration. Attempts have been made to integrate the redox biology data into pathway analysis. Currently, the KEGG pathway contains information on the role of various oxidized products in various pathways (34). These pathways include, but are not limited to: fatty acid oxidation products in inflammation, lipid peroxidation in nonalcoholic fatty liver disease, linoleic acid metabolism, arachidonic acid metabolism, PPAR signaling pathway, AA/AdA-PE in ferroptosis (206). Using the oxPL data we have constructed a model for predicting ferroptotic events using a simplified network of metabolic reactions. This model reproduced the major ferroptotic reaction pathways and the elements involved in such pathways.

³www.cs.cmu.edu/~rbd/algocompbook/sonification-examples/sonification.html Accessed on 1 December 2018

However, more detailed models and network analyses are needed for a better understanding and translation of redox lipidomics data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH U19AI068021, HL114453-06, CA165065-06, NS076511 and by Russian academic excellence project “5-100”.

Abbreviations:

Cyt c	cytochrome c
PUFA	polyunsaturated fatty acids
oxPUFA	oxygenated polyunsaturated fatty acids
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PS	phosphatidylserine
PG	phosphatidylglycerol
PA	phosphatidic acid
CL	cardiolipin
LPC	lyso-phosphatidylcholine
LPE	lyso-phosphatidylethanolamine
LPI	lyso-phosphatidylinositol
LPS	lyso-phosphatidylserine
LPG	lyso-phosphatidylglycerol
LPA	lyso-phosphatidic acid
mCL	mono-lyso-cardiolipin
oxPLs	oxygenated phospholipids
oxCL	oxygenated cardiolipin
oxPE	oxygenated phosphatidylethanolamine
OxPS	oxygenated phosphatidylserine

oxPC	phosphatidylcholine
TAG	triglyceride
CE	cholesterol ester
FFA	free fatty acids
LA	linoleic acid
AA	arachidonic acid
AdA	adrenic acid
HpETE-PE	15-hydroperoxyeicosatetraenoic acid-PE
HNE	4-hydroxynonenal
ONE	4-oxononenal
HHE	4-hydroxyhexenal and OHE, 4-oxohexenal
PEBP1	phosphatidylethanolamine binding protein 1
GPX4	glutathione peroxidase 4
GSH	glutathione
ASCL4	acyl-CoA synthase 4
APT	aminophospholipid translocase
ROS	reactive oxygen species
COX	cyclooxygenase
LOX	lipoxygenase
MS	mass spectrometry
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
SPE	solid phase extraction
HPTLC	high performance thin-layer chromatography
GC-MS	gas chromatography-mass spectrometry
MALDI	matrix assisted laser desorption ionization
ESI	electrospray ionization
HILIC	hydrophilic interaction liquid chromatography
APCI	atmospheric pressure chemical ionization

APPI	atmospheric pressure photoionization
DESI	desorption electrospray ionization
SIMS	secondary ion mass spectrometry
GCIB-SIMS	gas cluster ion beam secondary ion mass spectrometry
MSI	mass spectrometry imaging
IHC	immunohistochemistry
TBI	traumatic brain injury
IMM	inner mitochondrial membrane
OOM	outer mitochondrial membrane
ALS	amyotrophic lateral sclerosis
ETC	electron transport chain
NAO	nonenyl acridine orange
DC	dendritic cells
LD	lipid droplet
AMVN	2,2'-Azobis(2,4-dimethylvaleronitrile)

References

1. Maguire JJ, Tyurina YY, Mohammadyani D, Kapralov AA, Anthonymuthu TS, Qu F, Amoscato AA, Sparvero LJ, Tyurin VA, Planas-Iglesias J, He RR, Klein-Seetharaman J, Bayir H, Kagan VE. 2017 Known unknowns of cardiolipin signaling: The best is yet to come. *Biochim Biophys Acta Mol Cell Biol Lipids* 1862: 8–24 [PubMed: 27498292]
2. Gordon MH. 2003 FATS | Classification In *Encyclopedia of Food Sciences and Nutrition* (Second Edition), ed. Caballero B, pp. 2287–92. Oxford: Academic Press
3. Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH Jr, Murphy RC, Raetz CR, Russell DW, Seyama Y, Shaw W. 2005 A comprehensive classification system for lipids. *European journal of lipid science and technology* 107: 337–64
4. Zakhartsev M, Naumenko N, Chelomin V. 1998 Non-methylene-interrupted fatty acids in phospholipids of the membranes of the mussel *Crenomytilus grayanus*. *Russian Journal of Marine Biology* 24: 183–6
5. Jakobsson A, Westerberg R, Jacobsson A. 2006 Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog Lipid Res* 45: 237–49 [PubMed: 16564093]
6. Zhang JY, Kothapalli KS, Brenna JT. 2016 Desaturase and elongase-limiting endogenous long-chain polyunsaturated fatty acid biosynthesis. *Curr Opin Clin Nutr Metab Care* 19: 103–10 [PubMed: 26828581]
7. Kanehisa M, Goto S. 2000 KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research* 28: 27–30 [PubMed: 10592173]
8. Pratt DA, Mills JH, Porter NA. 2003 Theoretical calculations of carbon-oxygen bond dissociation enthalpies of peroxy radicals formed in the autoxidation of lipids. *J Am Chem Soc* 125: 5801–10 [PubMed: 12733921]

9. Yin H, Xu L, Porter NA. 2011 Free Radical Lipid Peroxidation: Mechanisms and Analysis. *Chemical Reviews* 111: 5944–72 [PubMed: 21861450]
10. Dennis EA, Norris PC. 2015 Eicosanoid storm in infection and inflammation. *Nat Rev Immunol* 15: 511–23 [PubMed: 26139350]
11. Levy BD. 2005 Lipoxins and lipoxin analogs in asthma. *Prostaglandins Leukot Essent Fatty Acids* 73: 231–7 [PubMed: 16046112]
12. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. 2001 Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* 2: 612–9 [PubMed: 11429545]
13. Serhan CN. 2014 Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 510: 92–101 [PubMed: 24899309]
14. Serhan CN. 2017 Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. *Mol Aspects Med* 58: 1–11 [PubMed: 28263773]
15. Serhan CN, Chiang N, Dalli J. 2018 New pro-resolving n-3 mediators bridge resolution of infectious inflammation to tissue regeneration. *Mol Aspects Med* 64: 1–17 [PubMed: 28802833]
16. Harkewicz R, Dennis EA. 2011 Applications of mass spectrometry to lipids and membranes. *Annu Rev Biochem* 80: 301–25 [PubMed: 21469951]
17. Dumlao DS, Buczynski MW, Norris PC, Harkewicz R, Dennis EA. 2011 High-throughput lipidomic analysis of fatty acid derived eicosanoids and N-acyl ethanolamines. *Biochim Biophys Acta* 1811: 724–36 [PubMed: 21689782]
18. Lewis RA, Austen KF, Soberman RJ. 1990 Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N Engl J Med* 323: 645–55 [PubMed: 2166915]
19. Levy BD, Vachier I, Serhan CN. 2012 Resolution of inflammation in asthma. *Clin Chest Med* 33: 559–70 [PubMed: 22929102]
20. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M. 2009 Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med* 206: 15–23 [PubMed: 19103881]
21. Bochkov VN, Oskolkova OV, Birukov KG, Levonen AL, Binder CJ, Stockl J. 2010 Generation and biological activities of oxidized phospholipids. *Antioxid Redox Signal* 12: 1009–59 [PubMed: 19686040]
22. Spickett CM, Pitt AR. 2015 Oxidative lipidomics coming of age: advances in analysis of oxidized phospholipids in physiology and pathology. *Antioxid Redox Signal* 22: 1646–66 [PubMed: 25694038]
23. O'Donnell VB, Murphy RC. 2012 New families of bioactive oxidized phospholipids generated by immune cells: identification and signaling actions. *Blood* 120: 1985–92 [PubMed: 22802337]
24. Anthonymuthu TS, Kenny EM, Bayir H. 2016 Therapies targeting lipid peroxidation in traumatic brain injury. *Brain Res* 1640: 57–76 [PubMed: 26872597]
25. Balasubramanian K, Maeda A, Lee JS, Mohammadyani D, Dar HH, Jiang JF, St Croix CM, Watkins S, Tyurin VA, Tyurina YY, Kloditz K, Polimova A, Kapralova VI, Xiong Z, Ray P, Klein-Seetharaman J, Mallampalli RK, Bayir H, Fadeel B, Kagan VE. 2015 Dichotomous roles for externalized cardiolipin in extracellular signaling: Promotion of phagocytosis and attenuation of innate immunity. *Sci Signal* 8: ra95
26. Dar HH, Tyurin VA, Mikulska-Ruminska K, Shrivastava I, Ting HC, Tyurin VA, Krieger J, St Croix CM, Watkins S, Bayir E, Mao G, Armbruster CR, Kapralov A, Wang H, Parsek MR, Anthonymuthu TS, Ogunsola AF, Flitter BA, Freedman CJ, Gaston JR, Holman TR, Pilewski JM, Greenberger JS, Mallampalli RK, Doi Y, Lee JS, Bahar I, Bomberger JM, Bayir H, Kagan VE. 2018 *Pseudomonas aeruginosa* utilizes host polyunsaturated phosphatidylethanolamines to trigger theft-ferroptosis in bronchial epithelium. *J Clin Invest* 128: 4639–53 [PubMed: 30198910]
27. Salomon RG. 2012 Structural identification and cardiovascular activities of oxidized phospholipids. *Circ Res* 111: 930–46 [PubMed: 22982874]
28. Thomas CP, O'Donnell VB. 2012 Oxidized phospholipid signaling in immune cells. *Curr Opin Pharmacol* 12: 471–7 [PubMed: 22445283]
29. Chaitidis P, Schewe T, Sutherland M, Kuhn H, Nigam S. 1998 15-Lipoxygenation of phospholipids may precede the sn-2 cleavage by phospholipases A2: reaction specificities of secretory and

cytosolic phospholipases A2 towards native and 15-lipoxygenated arachidonoyl phospholipids. *FEBS Lett* 434: 437–41 [PubMed: 9742970]

30. Kuhn H, Banthiya S, van Leyen K. 2015 Mammalian lipoxygenases and their biological relevance. *Biochim Biophys Acta* 1851: 308–30 [PubMed: 25316652]
31. Spector AA. 2009 Arachidonic acid cytochrome P450 epoxygenase pathway. *J Lipid Res* 50 Suppl: S52–6 [PubMed: 18952572]
32. Kapralov AA, Kurnikov IV, Vlasova II, Belikova NA, Tyurin VA, Basova LV, Zhao Q, Tyurina YY, Jiang J, Bayir H, Vladimirov YA, Kagan VE. 2007 The hierarchy of structural transitions induced in cytochrome c by anionic phospholipids determines its peroxidase activation and selective peroxidation during apoptosis in cells. *Biochemistry* 46: 14232–44 [PubMed: 18004876]
33. Kagan VE, Tyurin VA, Jiang J, Tyurina YY, Ritov VB, Amoscato AA, Osipov AN, Belikova NA, Kapralov AA, Kini V, Vlasova II, Zhao Q, Zou M, Di P, Svistunenko DA, Kurnikov IV, Borisenko GG. 2005 Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat Chem Biol* 1: 223–32 [PubMed: 16408039]
34. Kagan VE, Mao G, Qu F, Angeli JP, Doll S, Croix CS, Dar HH, Liu B, Tyurin VA, Ritov VB, Kapralov AA, Amoscato AA, Jiang J, Anthonymuthu T, Mohammadyani D, Yang Q, Proneth B, Klein-Seetharaman J, Watkins S, Bahar I, Greenberger J, Mallampalli RK, Stockwell BR, Tyurina YY, Conrad M, Bayir H. 2017 Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat Chem Biol* 13: 81–90 [PubMed: 27842066]
35. Tyurin VA, Balasubramanian K, Winnica D, Tyurina YY, Vikulina AS, He RR, Kapralov AA, Macphee CH, Kagan VE. 2014 Oxidatively modified phosphatidylserines on the surface of apoptotic cells are essential phagocytic ‘eat-me’ signals: cleavage and inhibition of phagocytosis by Lp-PLA2. *Cell Death Differ* 21: 825–35 [PubMed: 24464221]
36. Tyurina YY, Poloyac SM, Tyurin VA, Kapralov AA, Jiang J, Anthonymuthu TS, Kapralova VI, Vikulina AS, Jung MY, Epperly MW, Mohammadyani D, Klein-Seetharaman J, Jackson TC, Kochanek PM, Pitt BR, Greenberger JS, Vladimirov YA, Bayir H, Kagan VE. 2014 A mitochondrial pathway for biosynthesis of lipid mediators. *Nat Chem* 6: 542–52 [PubMed: 24848241]
37. Tyurina YY, Lou W, Qu F, Tyurin VA, Mohammadyani D, Liu J, Huttemann M, Frasso MA, Wipf P, Bayir H, Greenberg ML, Kagan VE. 2017 Lipidomics Characterization of Biosynthetic and Remodeling Pathways of Cardiolipins in Genetically and Nutritionally Manipulated Yeast Cells. *ACS Chem Biol* 12: 265–81 [PubMed: 27982579]
38. Burke JE, Dennis EA. 2009 Phospholipase A2 structure/function, mechanism, and signaling. *J Lipid Res* 50 Suppl: S237–42 [PubMed: 19011112]
39. Wenzel SE, Tyurina YY, Zhao J, St Croix CM, Dar HH, Mao G, Tyurin VA, Anthonymuthu TS, Kapralov AA, Amoscato AA, Mikulska-Ruminska K, Shrivastava IH, Kenny EM, Yang Q, Rosenbaum JC, Sparvero LJ, Emler DR, Wen X, Minami Y, Qu F, Watkins SC, Holman TR, VanDemark AP, Kellum JA, Bahar I, Bayir H, Kagan VE. 2017 PEBP1 Wardens Ferroptosis by Enabling Lipoxygenase Generation of Lipid Death Signals. *Cell* 171: 628–41 e26 [PubMed: 29053969]
40. Hauck AK, Bernlohr DA. 2016 Oxidative stress and lipotoxicity. *J Lipid Res* 57: 1976–86 [PubMed: 27009116]
41. Ramakrishnan R, Tyurin VA, Veglia F, Condamine T, Amoscato A, Mohammadyani D, Johnson JJ, Zhang LM, Klein-Seetharaman J, Celis E, Kagan VE, Gabrilovich DI. 2014 Oxidized lipids block antigen cross-presentation by dendritic cells in cancer. *J Immunol* 192: 2920–31 [PubMed: 24554775]
42. Tyurina YY, Shrivastava I, Tyurin VA, Mao G, Dar HH, Watkins S, Epperly M, Bahar I, Shvedova AA, Pitt B, Wenzel SE, Mallampalli RK, Sadovsky Y, Gabrilovich D, Greenberger JS, Bayir H, Kagan VE. 2018 “Only a Life Lived for Others Is Worth Living”: Redox Signaling by Oxygenated Phospholipids in Cell Fate Decisions. *Antioxid Redox Signal* 29: 1333–58 [PubMed: 28835115]
43. Salomon RG, Gu X. 2011 Critical insights into cardiovascular disease from basic research on the oxidation of phospholipids: the gamma-hydroxyalkenal phospholipid hypothesis. *Chem Res Toxicol* 24: 1791–802 [PubMed: 21870852]

44. Gugu BG, Mesaros CA, Sun M, Gu X, Crabb JW, Salomon RG. 2006 Identification of oxidatively truncated ethanolamine phospholipids in retina and their generation from polyunsaturated phosphatidylethanolamines. *Chem Res Toxicol* 19: 262–71 [PubMed: 16485902]
45. Veglia F, Tyurin VA, Mohammadyani D, Blasi M, Duperret EK, Donthireddy L, Hashimoto A, Kapralov A, Amoscato A, Angelini R, Patel S, Alicea-Torres K, Weiner D, Murphy ME, Klein-Seetharaman J, Celis E, Kagan VE, Gabrilovich DI. 2017 Lipid bodies containing oxidatively truncated lipids block antigen cross-presentation by dendritic cells in cancer. *Nat Commun* 8: 2122 [PubMed: 29242535]
46. Marathe GK, Harrison KA, Murphy RC, Prescott SM, Zimmerman GA, McIntyre TM. 2000 Bioactive phospholipid oxidation products. *Free Radic Biol Med* 28: 1762–70 [PubMed: 10946218]
47. Barayeu U, Lange M, Mendez L, Arnhold J, Shadyro OI, Fedorova M, Flemmig J. 2018 Cytochrome c auto-catalyzed carbonylation in the presence of hydrogen peroxide and cardiolipins. *J Biol Chem*
48. Radi R 2018 Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. *Proc Natl Acad Sci U S A* 115: 5839–48 [PubMed: 29802228]
49. Levonen AL, Hill BG, Kansanen E, Zhang J, Darley-USmar VM. 2014 Redox regulation of antioxidants, autophagy, and the response to stress: implications for electrophile therapeutics. *Free Radic Biol Med* 71: 196–207 [PubMed: 24681256]
50. Gutteridge JM, Halliwell B. 2000 Free radicals and antioxidants in the year 2000. A historical look to the future. *Ann N Y Acad Sci* 899: 136–47 [PubMed: 10863535]
51. Sies H 2015 Oxidative stress: a concept in redox biology and medicine. *Redox Biol* 4: 180–3 [PubMed: 25588755]
52. Harman D 2006 Free radical theory of aging: an update: increasing the functional life span. *Ann N Y Acad Sci* 1067: 10–21 [PubMed: 16803965]
53. Halliwell G B, M.C J 2015 *Free Radicals in Biology and Medicine*, fifth ed Clarendon Press, Oxford, UK
54. Halliwell B, Gutteridge JM. 1984 Role of iron in oxygen radical reactions. *Methods Enzymol* 105: 47–56 [PubMed: 6203010]
55. Halliwell B, Gutteridge JM. 1986 Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Arch Biochem Biophys* 246: 501–14 [PubMed: 3010861]
56. Halliwell B, Gutteridge JM. 1984 Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* 219: 1–14 [PubMed: 6326753]
57. Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Palmgren J, Freedman LS, Haapakoski J, Barrett MJ, Pietinen P, Malila N, Tala E, Liippo K, Salomaa ER, Tangrea JA, Teppo L, Askin FB, Taskinen E, Erozan Y, Greenwald P, Huttunen JK. 1996 Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst* 88: 1560–70 [PubMed: 8901854]
58. Wilson CW, Loh HS, Foster FG. 1973 The beneficial effect of vitamin C on the common cold. *Eur J Clin Pharmacol* 6: 26–32 [PubMed: 4587514]
59. Lohr JB, Cadet JL, Lohr MA, Larson L, Wasli E, Wade L, Hylton R, Vidoni C, Jeste DV, Wyatt RJ. 1988 Vitamin E in the treatment of tardive dyskinesia: the possible involvement of free radical mechanisms. *Schizophr Bull* 14: 291–6 [PubMed: 2904696]
60. Ashor AW, Brown R, Keenan PD, Willis ND, Siervo M, Mathers JC. 2019 Limited evidence for a beneficial effect of vitamin C supplementation on biomarkers of cardiovascular diseases: an umbrella review of systematic reviews and meta-analyses. *Nutr Res* 61: 1–12 [PubMed: 30683434]
61. Celik T, Yuksel C, Iyisoy A. 2010 Alpha tocopherol use in the management of diabetic cardiomyopathy: lessons learned from randomized clinical trials. *J Diabetes Complications* 24: 286–8 [PubMed: 19328015]
62. Dotan Y, Pinchuk I, Lichtenberg D, Leshno M. 2009 Decision analysis supports the paradigm that indiscriminate supplementation of vitamin E does more harm than good. *Arterioscler Thromb Vasc Biol* 29: 1304–9 [PubMed: 19286632]

63. Aliwarga T, Raccor BS, Lemaitre RN, Sotoodehnia N, Gharib SA, Xu L, Totah RA. 2017 Enzymatic and free radical formation of cis- and trans- epoxyeicosatrienoic acids in vitro and in vivo. *Free Radic Biol Med* 112: 131–40 [PubMed: 28734877]
64. Kanner J, German JB, Kinsella JE. 1987 Initiation of lipid peroxidation in biological systems. *Crit Rev Food Sci Nutr* 25: 317–64 [PubMed: 3304843]
65. Suardiaz R, Masgrau L, Lluch JM, Gonzalez-Lafont A. 2013 An insight into the regiospecificity of linoleic acid peroxidation catalyzed by mammalian 15-lipoxygenases. *J Phys Chem B* 117: 3747–54 [PubMed: 23496802]
66. Anthonymuthu TS, Kenny EM, Shrivastava IH, Tyurina YY, Hier ZE, Ting HC, Dar H, Tyurin VA, Nesterova A, Amoscato AA, Mikulska-Ruminska K, Rosenbaum JC, Mao G, Jinming Z, Conrad M, Kellum JA, Wenzel SE, VanDemark AP, Bahar I, Kagan VE, Bayir H. 2018 Empowerment of 15-lipoxygenase catalytic competence in selective oxidation of membrane ETE-PE to ferroptotic death signals, HpETE-PE. *J Am Chem Soc*
67. Marnett LJ. 2000 Cyclooxygenase mechanisms. *Curr Opin Chem Biol* 4: 545–52 [PubMed: 11006543]
68. Tilley SL, Coffman TM, Koller BH. 2001 Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest* 108: 15–23 [PubMed: 11435451]
69. Erridge C, Kennedy S, Spickett CM, Webb DJ. 2008 Oxidized phospholipid inhibition of toll-like receptor (TLR) signaling is restricted to TLR2 and TLR4: roles for CD14, LPS-binding protein, and MD2 as targets for specificity of inhibition. *J Biol Chem* 283: 24748–59 [PubMed: 18559343]
70. Hazen SL. 2008 Oxidized phospholipids as endogenous pattern recognition ligands in innate immunity. *J Biol Chem* 283: 15527–31 [PubMed: 18285328]
71. Dennis EA. 2016 Liberating Chiral Lipid Mediators, Inflammatory Enzymes, and LIPID MAPS from Biological Grease. *J Biol Chem* 291: 24431–48 [PubMed: 27555328]
72. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL. 2002 Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med* 196: 1025–37 [PubMed: 12391014]
73. Kagan VE, Tyurina YY, Tyurin VA, Mohammadyani D, Angeli JP, Baranov SV, Klein-Seetharaman J, Friedlander RM, Mallampalli RK, Conrad M, Bayir H. 2015 Cardiolipin signaling mechanisms: collapse of asymmetry and oxidation. *Antioxid Redox Signal* 22: 1667–80 [PubMed: 25566681]
74. Breusing N, Grune T, Andrisic L, Atalay M, Bartosz G, Biasi F, Borovic S, Bravo L, Casals I, Casillas R, Dinischiotu A, Drzewinska J, Faber H, Fauzi NM, Gajewska A, Gambini J, Gradinaru D, Kokkola T, Lojek A, Łuczaj W, Margina D, Mascia C, Mateos R, Meinitzer A, Mitjavila MT, Mrakovcic L, Munteanu MC, Podborska M, Poli G, Sicinska P, Skrzydlewska E, Vina J, Wiswedel I, Zarkovic N, Zelzer S, Spickett CM. 2010 An inter-laboratory validation of methods of lipid peroxidation measurement in UVA-treated human plasma samples. *Free Radical Research* 44: 1203–15 [PubMed: 20836662]
75. Niki E. 2009 Lipid peroxidation: Physiological levels and dual biological effects. *Free Radical Biology and Medicine* 47: 469–84 [PubMed: 19500666]
76. Niki E, Yoshida Y, Saito Y, Noguchi N. 2005 Lipid peroxidation: Mechanisms, inhibition, and biological effects. *Biochemical and Biophysical Research Communications* 338: 668–76 [PubMed: 16126168]
77. Reis A, Spickett CM. 2012 Chemistry of phospholipid oxidation. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1818: 2374–87 [PubMed: 22342938]
78. Jessup W, Dean RT, Gebicki JM. 1994 Iodometric determination of hydroperoxides in lipids and proteins. *Oxygen Radicals in Biological Systems*, Pt C 233: 289–303
79. Miyazawa T, Fujimoto K, Suzuki T, Yasuda K. 1994 Determination of phospholipid hydroperoxides using luminol chemiluminescence—high-performance liquid chromatography In *Methods in Enzymology*, pp. 324–32: Academic Press
80. Moore K, Roberts LJ 2nd. 1998 Measurement of lipid peroxidation. *Free Radic Res* 28: 659–71 [PubMed: 9736317]

81. Ungvari Z, Bailey-Downs L, Gautam T, Sosnowska D, Wang M, Monticone RE, Telljohann R, Pinto JT, de Cabo R, Sonntag WE, Lakatta EG, Csiszar A. 2011 Age-Associated Vascular Oxidative Stress, Nrf2 Dysfunction, and NF- κ B Activation in the Nonhuman Primate *Macaca mulatta*. *The Journals of Gerontology: Series A* 66A: 866–75
82. Wolff SP. 1994 Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides In *Methods in Enzymology*, pp. 182–9: Academic Press
83. Yamamoto Y 1994 Chemiluminescence-based high-performance liquid chromatography assay of lipid hydroperoxides In *Methods in Enzymology*, pp. 319–24: Academic Press
84. Li P, Tong Y, Yang H, Zhou S, Xiong F, Huo T, Mao M. 2014 Mitochondrial translocation of human telomerase reverse transcriptase in cord blood mononuclear cells of newborns with gestational diabetes mellitus mothers. *Diabetes Research and Clinical Practice* 103: 310–8 [PubMed: 24480248]
85. Wang Z, Ciabattini G, Crminon C, Lawson J, Fitzgerald GA, Patrono C, MacLouf J. 1995 Immunological characterization of urinary 8-epi-prostaglandin F2 α excretion in man. *Journal of Pharmacology and Experimental Therapeutics* 275: 94 [PubMed: 7562601]
86. Jurowski K, Kochan K, Walczak J, Baranska M, Piekoszewski W, Buszewski B. 2017 Analytical Techniques in Lipidomics: State of the Art. *Crit Rev Anal Chem* 47: 418–37 [PubMed: 28340309]
87. Peterson BL, Cummings BS. 2006 A review of chromatographic methods for the assessment of phospholipids in biological samples. *Biomed Chromatogr* 20: 227–43 [PubMed: 16138296]
88. Barden A, Mori TA. 2018 GC-MS Analysis of Lipid Oxidation Products in Blood, Urine, and Tissue Samples. *Methods Mol Biol* 1730: 283–92 [PubMed: 29363082]
89. Fauland A, Trotschmuller M, Eberl A, Afuni-Zadeh S, Kofeler H, Guo X, Lankmayr E. 2013 An improved SPE method for fractionation and identification of phospholipids. *J Sep Sci* 36: 744–51 [PubMed: 23349108]
90. Pulfer M, Murphy RC. 2003 Electrospray mass spectrometry of phospholipids. *Mass Spectrometry Reviews* 22: 332–64 [PubMed: 12949918]
91. Hayakawa J, Okabayashi Y. 2004 Simultaneous analysis of phospholipid in rabbit bronchoalveolar lavage fluid by liquid chromatography/mass spectrometry. *J Pharm Biomed Anal* 35: 583–92 [PubMed: 15137983]
92. Ikeda K, Oike Y, Shimizu T, Taguchi R. 2009 Global analysis of triacylglycerols including oxidized molecular species by reverse-phase high resolution LC/ESI-QTOF MS/MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 2639–47
93. Tyurina YY, Polimova AM, Maciel E, Tyurin VA, Kapralova VI, Winnica DE, Vikulina AS, Domingues MR, McCoy J, Sanders LH, Bayir H, Greenamyre JT, Kagan VE. 2015 LC/MS analysis of cardiolipins in substantia nigra and plasma of rotenone-treated rats: Implication for mitochondrial dysfunction in Parkinson's disease. *Free Radic Res* 49: 681–91 [PubMed: 25740198]
94. Nakanishi H, Ogiso H, Taguchi R. 2009 Qualitative and quantitative analyses of phospholipids by LC-MS for lipidomics. *Methods Mol Biol* 579: 287–313 [PubMed: 19763482]
95. Lydic TA, Goo YH. 2018 Lipidomics unveils the complexity of the lipidome in metabolic diseases. *Clin Transl Med* 7: 4 [PubMed: 29374337]
96. Holcapek M, Liebisch G, Ekroos K. 2018 Lipidomic Analysis. *Anal Chem* 90: 4249–57 [PubMed: 29543437]
97. Xia W, Budge SM. 2018 Simultaneous quantification of epoxy and hydroxy fatty acids as oxidation products of triacylglycerols in edible oils. *J Chromatogr A* 1537: 83–90 [PubMed: 29370919]
98. Murphy RC, Gaskell SJ. 2011 New applications of mass spectrometry in lipid analysis. *J Biol Chem* 286: 25427–33 [PubMed: 21632539]
99. Yang K, Han X. 2016 Lipidomics: Techniques, Applications, and Outcomes Related to Biomedical Sciences. *Trends in Biochemical Sciences* 41: 954–69 [PubMed: 27663237]
100. Cajka T, Fiehn O. 2014 Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. *Trends in analytical chemistry : TRAC* 61: 192–206 [PubMed: 25309011]

101. Astarita G, Kendall AC, Dennis EA, Nicolaou A. 2015 Targeted lipidomic strategies for oxygenated metabolites of polyunsaturated fatty acids. *Biochim et Biophys Acta - Molecular and Cell Biology of Lipids* 1851: 456–68
102. Ovčáková M, Lísá M, Cífková E, Holápek M. 2016 Retention behavior of lipids in reversed-phase ultrahigh-performance liquid chromatography–electrospray ionization mass spectrometry. *Journal of Chromatography A* 1450: 76–85 [PubMed: 27179677]
103. Sandra K, Sandra P. 2013 Lipidomics from an analytical perspective. *Current Opinion in Chemical Biology* 17: 847–53 [PubMed: 23830914]
104. Krautbauer S, Büchler C, Liebisch G. 2016 Relevance in the Use of Appropriate Internal Standards for Accurate Quantification Using LC–MS/MS: Tauro-Conjugated Bile Acids as an Example. *Analytical Chemistry* 88: 10957–61 [PubMed: 27736066]
105. 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. *Proceedings of the National Academy of Sciences* 106: 2136
106. Han X, Yang K, Gross RW. 2012 Multi-dimensional mass spectrometry-based shotgun lipidomics and novel strategies for lipidomic analyses. *Mass Spectrometry Reviews* 31: 134–78 [PubMed: 21755525]
107. Massey KA, Nicolaou A. 2011 Lipidomics of polyunsaturated-fatty-acid-derived oxygenated metabolites. *Biochem Soc Trans* 39: 1240–6 [PubMed: 21936796]
108. Quehenberger O, Dahlberg-Wright S, Jiang J, Armando AM, Dennis EA. 2018 Quantitative determination of esterified eicosanoids and related oxygenated metabolites after base hydrolysis. *J Lipid Res* 59: 2436–45 [PubMed: 30323111]
109. Kim J, Hoppel CL. 2011 Monolysocardiolipin: improved preparation with high yield. *J Lipid Res* 52: 389–92 [PubMed: 20959418]
110. Liu GY, Moon SH, Jenkins CM, Li M, Sims HF, Guan S, Gross RW. 2017 The phospholipase iPLA2gamma is a major mediator releasing oxidized aliphatic chains from cardiolipin, integrating mitochondrial bioenergetics and signaling. *J Biol Chem* 292: 10672–84 [PubMed: 28442572]
111. Song H, Wohltmann M, Tan M, Ladenson JH, Turk J. 2014 Group VIA phospholipase A2 mitigates palmitate-induced beta-cell mitochondrial injury and apoptosis. *J Biol Chem* 289: 14194–210 [PubMed: 24648512]
112. Buland JR, Wasserloos KJ, Tyurin VA, Tyurina YY, Amoscato AA, Mallampalli RK, Chen BB, Zhao J, Zhao Y, Ofori-Acquah S, Kagan VE, Pitt BR. 2016 Biosynthesis of oxidized lipid mediators via lipoprotein-associated phospholipase A2 hydrolysis of extracellular cardiolipin induces endothelial toxicity. *Am J Physiol Lung Cell Mol Physiol* 311: L303–16 [PubMed: 27233995]
113. Tyurin VA, Yanamala N, Tyurina YY, Klein-Seetharaman J, Macphée CH, Kagan VE. 2012 Specificity of lipoprotein-associated phospholipase A(2) toward oxidized phosphatidylserines: liquid chromatography-electrospray ionization mass spectrometry characterization of products and computer modeling of interactions. *Biochemistry* 51: 9736–50 [PubMed: 23148485]
114. Tyurina YY, Winnica DE, Kapralova VI, Kapralov AA, Tyurin VA, Kagan VE. 2013 LC/MS characterization of rotenone induced cardiolipin oxidation in human lymphocytes: implications for mitochondrial dysfunction associated with Parkinson's disease. *Mol Nutr Food Res* 57: 1410–22 [PubMed: 23650208]
115. Tyurin VA, Tyurina YY, Feng W, Mnuskin A, Jiang J, Tang M, Zhang X, Zhao Q, Kochanek PM, Clark RS, Bayir H, Kagan VE. 2008 Mass-spectrometric characterization of phospholipids and their primary peroxidation products in rat cortical neurons during staurosporine-induced apoptosis. *J Neurochem* 107: 1614–33 [PubMed: 19014376]
116. Tyurin VA, Tyurina YY, Jung MY, Tunekar MA, Wasserloos KJ, Bayir H, Greenberger JS, Kochanek PM, Shvedova AA, Pitt B, Kagan VE. 2009 Mass-spectrometric analysis of hydroperoxy- and hydroxy-derivatives of cardiolipin and phosphatidylserine in cells and tissues induced by pro-apoptotic and pro-inflammatory stimuli. *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 2863–72

117. Tyurina YY, Kisin ER, Murray A, Tyurin VA, Kapralova VI, Sparvero LJ, Amoscato AA, Samhan-Arias AK, Swedin L, Lahesmaa R, Fadeel B, Shvedova AA, Kagan VE. 2011 Global phospholipidomics analysis reveals selective pulmonary peroxidation profiles upon inhalation of single-walled carbon nanotubes. *ACS Nano* 5: 7342–53 [PubMed: 21800898]
118. 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 [PubMed: 18215738]
119. Tyurina YY, Tyurin VA, Kapralova VI, Wasserloos K, Mosher M, Epperly MW, Greenberger JS, Pitt BR, Kagan VE. 2011 Oxidative lipidomics of gamma-radiation-induced lung injury: mass spectrometric characterization of cardiolipin and phosphatidylserine peroxidation. *Radiat Res* 175: 610–21 [PubMed: 21338246]
120. Tyurina YY, Tyurin VA, Kaynar AM, Kapralova VI, Wasserloos K, Li J, Mosher M, Wright L, Wipf P, Watkins S, Pitt BR, Kagan VE. 2010 Oxidative lipidomics of hyperoxic acute lung injury: mass spectrometric characterization of cardiolipin and phosphatidylserine peroxidation. *Am J Physiol Lung Cell Mol Physiol* 299: L73–85 [PubMed: 20418384]
121. Bayir H, Tyurin VA, Tyurina YY, Viner R, Ritov V, Amoscato AA, Zhao Q, Zhang XJ, Janesko-Feldman KL, Alexander H, Basova LV, Clark RS, Kochanek PM, Kagan VE. 2007 Selective early cardiolipin peroxidation after traumatic brain injury: an oxidative lipidomics analysis. *Ann Neurol* 62: 154–69 [PubMed: 17685468]
122. Mao G, Qu F, St Croix CM, Tyurina YY, Planas-Iglesias J, Jiang J, Huang Z, Amoscato AA, Tyurin VA, Kapralov AA, Cheikhi A, Maguire J, Klein-Seetharaman J, Bayir H, Kagan VE. 2016 Mitochondrial Redox Opto-Lipidomics Reveals Mono-Oxygenated Cardiolipins as Pro-Apoptotic Death Signals. *ACS Chem Biol* 11: 530–40 [PubMed: 26697918]
123. Kagan VE, Gleiss B, Tyurina YY, Tyurin VA, Elenstrom-Magnusson C, Liu SX, Serinkan FB, Arroyo A, Chandra J, Orrenius S, Fadeel B. 2002 A role for oxidative stress in apoptosis: oxidation and externalization of phosphatidylserine is required for macrophage clearance of cells undergoing Fas-mediated apoptosis. *J Immunol* 169: 487–99 [PubMed: 12077280]
124. Tyurina YY, Kawai K, Tyurin VA, Liu SX, Kagan VE, Fabisiak JP. 2004 The plasma membrane is the site of selective phosphatidylserine oxidation during apoptosis: role of cytochrome C. *Antioxid Redox Signal* 6: 209–25 [PubMed: 15025923]
125. Tyurina YY, Serinkan FB, Tyurin VA, Kini V, Yalowich JC, Schroit AJ, Fadeel B, Kagan VE. 2004 Lipid antioxidant, etoposide, inhibits phosphatidylserine externalization and macrophage clearance of apoptotic cells by preventing phosphatidylserine oxidation. *J Biol Chem* 279: 6056–64 [PubMed: 14630936]
126. Jiang J, Kini V, Belikova N, Serinkan BF, Borisenko GG, Tyurina YY, Tyurin VA, Kagan VE. 2004 Cytochrome c release is required for phosphatidylserine peroxidation during Fas-triggered apoptosis in lung epithelial A549 cells. *Lipids* 39: 1133–42 [PubMed: 15726829]
127. Tyurina YY, Tyurin VA, Zhao Q, Djukic M, Quinn PJ, Pitt BR, Kagan VE. 2004 Oxidation of phosphatidylserine: a mechanism for plasma membrane phospholipid scrambling during apoptosis? *Biochem Biophys Res Commun* 324: 1059–64 [PubMed: 15485662]
128. Tyurina YY, Basova LV, Konduru NV, Tyurin VA, Potapovich AI, Cai P, Bayir H, Stoyanovsky D, Pitt BR, Shvedova AA, Fadeel B, Kagan VE. 2007 Nitrosative stress inhibits the aminophospholipid translocase resulting in phosphatidylserine externalization and macrophage engulfment: implications for the resolution of inflammation. *J Biol Chem* 282: 8498–509 [PubMed: 17229723]
129. Arroyo A, Modriansky M, Serinkan FB, Bello RI, Matsura T, Jiang J, Tyurin VA, Tyurina YY, Fadeel B, Kagan VE. 2002 NADPH oxidase-dependent oxidation and externalization of phosphatidylserine during apoptosis in Me2SO-differentiated HL-60 cells. Role in phagocytic clearance. *J Biol Chem* 277: 49965–75 [PubMed: 12376550]
130. Tyurina YY, Tyurin VA, Liu SX, Smith CA, Shvedova AA, Schor NF, Kagan VE. 2002 Phosphatidylserine peroxidation during apoptosis. A signaling pathway for phagocyte clearance. *Subcell Biochem* 36: 79–96 [PubMed: 12037991]
131. Fadeel B, Quinn P, Xue D, Kagan V. 2007 Fat(al) attraction: oxidized lipids act as “eat-me” signals. *HFSP J* 1: 225–9 [PubMed: 19404423]

132. Shimaoka T, Kume N, Minami M, Hayashida K, Kataoka H, Kita T, Yonehara S. 2000 Molecular cloning of a novel scavenger receptor for oxidized low density lipoprotein, SR-PSOX, on macrophages. *J Biol Chem* 275: 40663–6 [PubMed: 11060282]
133. Zhang L, Liu HJ, Li TJ, Yang Y, Guo XL, Wu MC, Rui YC, Wei LX. 2008 Lentiviral vector-mediated siRNA knockdown of SR-PSOX inhibits foam cell formation in vitro. *Acta Pharmacol Sin* 29: 847–52 [PubMed: 18565283]
134. Greenberg ME, Sun M, Zhang R, Febbraio M, Silverstein R, Hazen SL. 2006 Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. *J Exp Med* 203: 2613–25 [PubMed: 17101731]
135. Kenny EM, Fidan E, Yang Q, Anthonymuthu TS, New LA, Meyer EA, Wang H, Kochanek PM, Dixon CE, Kagan VE, Bayir H. 2018 Ferroptosis Contributes to Neuronal Death and Functional Outcome After Traumatic Brain Injury. *Critical Care Medicine Online First*
136. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascon S, Hatzios SK, Kagan VE, Noel K, Jiang X, Linkermann A, Murphy ME, Overholtzer M, Oyagi A, Pagnussat GC, Park J, Ran Q, Rosenfeld CS, Salnikow K, Tang D, Torti FM, Torti SV, Toyokuni S, Woerpel KA, Zhang DD. 2017 Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell* 171: 273–85 [PubMed: 28985560]
137. Angeli JPF, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, Herbach N, Aichler M, Walch A, Eggenhofer E. 2014 Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nature cell biology* 16: 1180 [PubMed: 25402683]
138. Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmeler M, Beckers J, Aichler M, Walch A, Prokisch H, Trumbach D, Mao G, Qu F, Bayir H, Fullekrug J, Scheel CH, Wurst W, Schick JA, Kagan VE, Angeli JP, Conrad M. 2017 ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol* 13: 91–8 [PubMed: 27842070]
139. Esterbauer H, Schaur RJ, Zollner H. 1991 Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine* 11: 81–128 [PubMed: 1937131]
140. Fritz KS, Petersen DR. 2011 Exploring the Biology of Lipid Peroxidation-Derived Protein Carbonylation. *Chemical Research in Toxicology* 24: 1411–9 [PubMed: 21812433]
141. Fritz KS, Petersen DR. 2013 An overview of the chemistry and biology of reactive aldehydes. *Free Radical Biology and Medicine* 59: 85–91 [PubMed: 22750507]
142. Schopfer FJ, Cipollina C, Freeman BA. 2011 Formation and Signaling Actions of Electrophilic Lipids. *Chemical Reviews* 111: 5997–6021 [PubMed: 21928855]
143. Ayala A, Muñoz MF, Argüelles S. 2014 Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Medicine and Cellular Longevity* 2014: 31
144. Pizzimenti S, Ciamporcero E, Daga M, Pettazzoni P, Arcaro A, Cetrangolo G, Minelli R, Dianzani C, Lepore A, Gentile F, Barrera G. 2013 Interaction of aldehydes derived from lipid peroxidation and membrane proteins. *Frontiers in Physiology* 4
145. Stemmer U, Hermetter A. 2012 Protein modification by aldehydophospholipids and its functional consequences. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1818: 2436–45 [PubMed: 22450235]
146. Maier CS, Chavez J, Wang J, Wu J. 2010 Chapter 16 - Protein Adducts of Aldehydic Lipid Peroxidation Products: Identification and Characterization of Protein Adducts Using an Aldehyde/Keto-Reactive Probe in Combination with Mass Spectrometry In *Methods in Enzymology*, ed. Cadenas E, Packer L, pp. 305–30: Academic Press
147. Domingues RM, Domingues P, Melo T, Pérez-Sala D, Reis A, Spickett CM. 2013 Lipoxidation adducts with peptides and proteins: Deleterious modifications or signaling mechanisms? *Journal of Proteomics* 92: 110–31 [PubMed: 23770299]
148. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. 2003 Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta* 329: 23–38
149. Catalá A, Díaz M. 2016 Editorial: Impact of Lipid Peroxidation on the Physiology and Pathophysiology of Cell Membranes. *Frontiers in Physiology* 7

150. Zhong H, Yin H. 2015 Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: Focusing on mitochondria. *Redox Biology* 4: 193–9 [PubMed: 25598486]
151. Chen Y, Liu Y, Lan T, Qin W, Zhu Y, Qin K, Gao J, Wang H, Hou X, Chen N, Friedmann Angeli JP, Conrad M, Wang CW. 2018 Quantitative Profiling of Protein Carbonylations in Ferroptosis by an Aniline-Derived Probe. *Journal of the American Chemical Society* 140: 4712–20 [PubMed: 29569437]
152. Zhao J, Maskrey B, Balzar S, Chibana K, Mustovich A, Hu H, Trudeau JB, O'Donnell V, Wenzel SE. 2009 Interleukin-13-induced MUC5AC is regulated by 15-lipoxygenase 1 pathway in human bronchial epithelial cells. *Am J Respir Crit Care Med* 179: 782–90 [PubMed: 19218191]
153. Gupta S, Roy A, Dwarakanath BS. 2017 Metabolic Cooperation and Competition in the Tumor Microenvironment: Implications for Therapy. *Front Oncol* 7: 68 [PubMed: 28447025]
154. Gabrilovich DI. 2017 Myeloid-Derived Suppressor Cells. *Cancer Immunol Res* 5: 3–8 [PubMed: 28052991]
155. Ostrand-Rosenberg S, Sinha P, Beury DW, Clements VK. 2012 Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. *Semin Cancer Biol* 22: 275–81 [PubMed: 22313874]
156. Herber DL, Cao W, Nefedova Y, Novitskiy SV, Nagaraj S, Tyurin VA, Corzo A, Cho HI, Celis E, Lennox B, Knight SC, Padhya T, McCaffrey TV, McCaffrey JC, Antonia S, Fishman M, Ferris RL, Kagan VE, Gabrilovich DI. 2010 Lipid accumulation and dendritic cell dysfunction in cancer. *Nat Med* 16: 880–6 [PubMed: 20622859]
157. Mohammadyani D, Tyurin VA, O'Brien M, Sadovsky Y, Gabrilovich DI, Klein-Seetharaman J, Kagan VE. 2014 Molecular speciation and dynamics of oxidized triacylglycerols in lipid droplets: Mass spectrometry and coarse-grained simulations. *Free Radic Biol Med* 76: 53–60 [PubMed: 25110833]
158. Dannenberg RB. 1997 Machine Tongues XIX: Nyquist, a Language for Composition and Sound Synthesis. *Computer Music Journal* 21: 50–60
159. Spengler B. 2015 Mass Spectrometry Imaging of Biomolecular Information. *Analytical Chemistry* 87: 64–82 [PubMed: 25490190]
160. Norris JL, Caprioli RM. 2013 Analysis of Tissue Specimens by Matrix-Assisted Laser Desorption/Ionization Imaging Mass Spectrometry in Biological and Clinical Research. *Chemical Reviews* 113: 2309–42 [PubMed: 23394164]
161. Khalil SM, Römpf A, Pretzel J, Becker K, Spengler B. 2015 Phospholipid Topography of Whole-Body Sections of the *Anopheles stephensi* Mosquito, Characterized by High-Resolution Atmospheric-Pressure Scanning Microprobe Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging. *Analytical Chemistry* 87: 11309–16 [PubMed: 26491885]
162. Phan NTN, Munem M, Ewing AG, Fletcher JS. 2017 MS/MS analysis and imaging of lipids across *Drosophila* brain using secondary ion mass spectrometry. *Analytical and Bioanalytical Chemistry*: 1–10 [PubMed: 27837266]
163. Qin L, Zhang Y, Liu Y, He H, Han M, Li Y, Zeng M, Wang X. 2018 Recent advances in matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) for in situ analysis of endogenous molecules in plants. *Phytochemical Analysis* 29: 351–64 [PubMed: 29667236]
164. Bodzon-Kulakowska A, Suder P. 2016 Imaging mass spectrometry: Instrumentation, applications, and combination with other visualization techniques. *Mass Spectrometry Reviews* 35: 147–69 [PubMed: 25962625]
165. Porta Siegel T, Hamm G, Bunch J, Cappell J, Fletcher JS, Schwamborn K. 2018 Mass Spectrometry Imaging and Integration with Other Imaging Modalities for Greater Molecular Understanding of Biological Tissues. *Molecular Imaging and Biology* 20: 888–901 [PubMed: 30167993]
166. Kriegsmann K, Longuespée R, Hundemer M, Zgorzelski C, Casadonte R, Schwamborn K, Weichert W, Schirmacher P, Harms A, Kazdal D, Leichsenring J, Stenzinger A, Warth A, Fresnais M, Kriegsmann J, Kriegsmann M. in press. Combined Immunohistochemistry after Mass Spectrometry Imaging for superior spatial information. *PROTEOMICS – Clinical Applications* 0: 1800035

167. Amoscato AA, Sparvero LJ, He RR, Watkins S, Bayir H, Kagan VE. 2014 Imaging Mass Spectrometry of Diversified Cardiolipin Molecular Species in the Brain. *Analytical Chemistry* 86: 6587–95 [PubMed: 24949523]
168. Sparvero LJ, Amoscato AA, Fink AB, Anthonymuthu T, New LA, Kochanek PM, Watkins S, Kagan VE, Bayir H. 2016 Imaging mass spectrometry reveals loss of polyunsaturated cardiolipins in the cortical contusion, hippocampus, and thalamus after traumatic brain injury. *J Neurochem* 139: 659–75 [PubMed: 27591733]
169. Tian H, Sparvero LJ, Amoscato AA, Bloom A, Bayir H, Kagan VE, Winograd N. 2017 Gas Cluster Ion Beam Time-of-Flight Secondary Ion Mass Spectrometry High-Resolution Imaging of Cardiolipin Speciation in the Brain: Identification of Molecular Losses after Traumatic Injury. *Analytical Chemistry* 89: 4611–9 [PubMed: 28306235]
170. Mohammadi AS, Phan NTN, Fletcher JS, Ewing AG. 2016 Intact lipid imaging of mouse brain samples: MALDI, nanoparticle-laser desorption ionization, and 40 keV argon cluster secondary ion mass spectrometry. *Analytical and Bioanalytical Chemistry* 408: 6857–68 [PubMed: 27549796]
171. Barré FPY, Claes BSR, Dewez F, Peutz-Kootstra CJ, Munch-Petersen HF, Grønbæk K, Lund A, Heeren RMA, Côme CRM, Cillero-Pastor B. 2018 Specific lipid and metabolic profiles of R-CHOP-resistant diffuse large B-cell lymphoma elucidated by matrix-assisted laser desorption ionization mass spectrometry imaging and in vivo imaging. *Analytical Chemistry*
172. Bakker B, Eijkel GB, Heeren RMA, Karperien M, Post JN, Cillero-Pastor B. 2017 Oxygen-Dependent Lipid Profiles of Three-Dimensional Cultured Human Chondrocytes Revealed by MALDI-MSI. *Analytical Chemistry* 89: 9438–44 [PubMed: 28727417]
173. Zavalin A, Todd EM, Rawhouser PD, Yang J, Norris JL, Caprioli RM. 2012 Direct imaging of single cells and tissue at sub-cellular spatial resolution using transmission geometry MALDI MS. *Journal of Mass Spectrometry* 47: i–i
174. Lanni EJ, Dunham SJ, Nemes P, Rubakhin SS, Sweedler JV. 2014 Biomolecular imaging with a C60-SIMS/MALDI dual ion source hybrid mass spectrometer: instrumentation, matrix enhancement, and single cell analysis. *J Am Soc Mass Spectrom* 25: 1897–907 [PubMed: 25183225]
175. Winograd N 2015 Imaging Mass Spectrometry on the Nanoscale with Cluster Ion Beams. *Analytical Chemistry* 87: 328–33 [PubMed: 25458665]
176. Bich C, Touboul D, Brunelle A. 2014 Cluster TOF-SIMS imaging as a tool for micrometric histology of lipids in tissue. *Mass Spectrometry Reviews* 33: 442–51 [PubMed: 24265115]
177. Mahoney CM. 2013 Cluster secondary ion mass spectrometry : principles and applications. Hoboken, New Jersey: Wiley
178. Winograd N 2018 Gas Cluster Ion Beams for Secondary Ion Mass Spectrometry. *Annual Review of Analytical Chemistry* 11: 29–48
179. Tian H, Maci ek D, Postawa Z, Garrison BJ, Winograd N. 2016 CO₂ Cluster Ion Beam, an Alternative Projectile for Secondary Ion Mass Spectrometry. *Journal of The American Society for Mass Spectrometry* 27: 1476–82 [PubMed: 27324648]
180. Angerer TB, Blenkinsopp P, Fletcher JS. 2015 High energy gas cluster ions for organic and biological analysis by time-of-flight secondary ion mass spectrometry. *International Journal of Mass Spectrometry* 377: 591–8
181. Sämfors S, Ståhlman M, Klevstig M, Borén J, Fletcher JS. 2017 Localised lipid accumulation detected in infarcted mouse heart tissue using ToF-SIMS. *International Journal of Mass Spectrometry* In press
182. Sparvero LJ, Amoscato AA, Kochanek PM, Pitt BR, Kagan VE, Bayir H. 2010 Mass-spectrometry based oxidative lipidomics and lipid imaging: applications in traumatic brain injury. *Journal of Neurochemistry* 115: 1322–36 [PubMed: 20950335]
183. Sparvero LJ, Amoscato AA, Dixon CE, Long JB, Kochanek PM, Pitt BR, Bayir H, Kagan VE. 2012 Mapping of phospholipids by MALDI imaging (MALDI-MSI): realities and expectations. *Chemistry and Physics of Lipids* 165: 545–62 [PubMed: 22692104]
184. Ji J, Kline AE, Amoscato A, Samhan-Arias AK, Sparvero LJ, Tyurin VA, Tyurina YY, Fink B, Manole MD, Puccio AM, Okonkwo DO, Cheng JP, Alexander H, Clark RS, Kochanek PM, Wipf

- P, Kagan VE, Bayir H. 2012 Lipidomics identifies cardiolipin oxidation as a mitochondrial target for redox therapy of brain injury. *Nat Neurosci* 15: 1407–13 [PubMed: 22922784]
185. Hankin JA, Farias SE, Barkley RM, Heidenreich K, Frey LC, Hamazaki K, Kim H-Y, Murphy RC. 2011 MALDI Mass Spectrometric Imaging of Lipids in Rat Brain Injury Models. *Journal of the American Society for Mass Spectrometry* 22: 1014–21 [PubMed: 21953042]
186. Woods AS, Colsch B, Jackson SN, Post J, Baldwin K, Roux A, Hoffer B, Cox BM, Hoffer M, Rubovitch V, Pick CG, Schultz JA, Balaban C. 2013 Gangliosides and Ceramides Change in a Mouse Model of Blast Induced Traumatic Brain Injury. *Acs Chemical Neuroscience* 4: 594–600 [PubMed: 23590251]
187. Roux A, Muller L, Jackson SN, Post J, Baldwin K, Hoffer B, Balaban CD, Barbacci D, Schultz JA, Gouty S, Cox BM, Woods AS. 2016 Mass spectrometry imaging of rat brain lipid profile changes over time following traumatic brain injury. *Journal of Neuroscience Methods* 272: 19–32 [PubMed: 26872743]
188. Barbacci DC, Roux A, Muller L, Jackson SN, Post J, Baldwin K, Hoffer B, Balaban CD, Schultz JA, Gouty S, Cox BM, Woods AS. 2017 Mass Spectrometric Imaging of Ceramide Biomarkers Tracks Therapeutic Response in Traumatic Brain Injury. *ACS Chemical Neuroscience* 8: 2266–74 [PubMed: 28745861]
189. Stübiger G, Wuczkowski M, Bicker W, Belgacem O. 2014 Nanoparticle-Based Detection of Oxidized Phospholipids by MALDI Mass Spectrometry: Nano-MALDI Approach. *Analytical Chemistry* 86: 6401–9 [PubMed: 24914456]
190. Tian H, Sparvero LJ, Blenkinsopp P, Amoscato AA, Watkins SC, Bayir H, Kagan VE, Winograd N. 2019 Secondary ion mass spectrometry images cardiolipins and phosphatidylethanolamines at the subcellular level. *Angewandte Chemie International Edition* in press
191. Reuter L, Tukey P, Maloney L, Pani J, Smith S. 1990 Human perception and visualization. Presented at Proceedings of the 1st conference on Visualization
192. Brazil E, Fernstrom M. 2011 Navigation of Data In The Sonification Handbook. Berlin, Germany: Logos Publishing House
193. Dubus G, Bresin R. 2013 A Systematic Review of Mapping Strategies for the Sonification of Physical Quantities. *PLOS ONE* 8: e82491
194. Bywater RP, Middleton JN. 2016 Melody discrimination and protein fold classification. *Heliyon* 2: e00175
195. Hegg JC, Middleton J, Robertson BL, Kennedy BP. 2018 The sound of migration: exploring data sonification as a means of interpreting multivariate salmon movement datasets. *Heliyon* 4: e00532
196. Clendinning JP, Marvin EW. 2016 The Musicians Guide to Theory and Analysis New York: W.W. Norton and Co.
197. Tomlinson L, Fuller C, Pierce SS. 2012 Making musical serialism using mass spectrometry and nuclear magnetic resonance. In Abstracts, 64th Southeast Regional Meeting of the American Chemical Society Raleigh, NC, United States
198. Middleton JN, Dowd D. 2008 Web-Based Algorithmic Composition from Extramusical Resources. *Leonardo* 41: 128–35
199. Koelmel JP, Kroeger NM, Ulmer CZ, Bowden JA, Patterson RE, Cochran JA, Beecher CWW, Garrett TJ, Yost RA. 2017 LipidMatch: an automated workflow for rule-based lipid identification using untargeted high-resolution tandem mass spectrometry data. *BMC bioinformatics* 18: 331– [PubMed: 28693421]
200. Ulmer CZ, Koelmel JP, Ragland JM, Garrett TJ, Bowden JA. 2017 LipidPioneer : A Comprehensive User-Generated Exact Mass Template for Lipidomics. *J Am Soc Mass Spectrom* 28: 562–5 [PubMed: 28074328]
201. O'Connor A, Brasher CJ, Slatter DA, Meckelmann SW, Hawksworth JI, Allen SM, O'Donnell VB. 2017 LipidFinder: A computational workflow for discovery of lipids identifies eicosanoid-phosphoinositides in platelets. *JCI insight* 2
202. Fahy E, Alvarez-Jarreta J, Brasher CJ, Nguyen A, Hawksworth JI, Rodrigues P, Meckelmann S, Allen SM, O'Donnell VB. 2018 LipidFinder on LIPID MAPS: peak filtering, MS searching and statistical analysis for lipidomics. *Bioinformatics*: bty679-bty

203. Collins JR, Edwards BR, Fredricks HF, Van Mooy BA. 2016 LOBSTAHS: An Adduct-Based Lipidomics Strategy for Discovery and Identification of Oxidative Stress Biomarkers. *Anal Chem* 88: 7154–62 [PubMed: 27322848]
204. Ni Z, Angelidou G, Hoffmann R, Fedorova M. 2017 LPPtiger software for lipidome-specific prediction and identification of oxidized phospholipids from LC-MS datasets. *Sci Rep* 7: 15138 [PubMed: 29123162]
205. O'Donnell VB, Murphy RC, Watson SP. 2014 Platelet lipidomics: modern day perspective on lipid discovery and characterization in platelets. *Circulation research* 114: 1185–203 [PubMed: 24677238]
206. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. 2011 KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic acids research* 40: D109–D14 [PubMed: 22080510]

Figure 1.

Structural formulae of major glycerophospholipids and their oxidation products.

Glycerophospholipids have two fatty acid residues that are attached at the *sn*-1, *sn*-2 positions of the glycerol backbone and polar groups that occupy the *sn*-3 position. PUFA are usually present at the *sn*-2 position. OxPLs include a group of oxygenated PUFA-lipids where oxygen-containing functional groups such as hydroperoxy-, hydroxy-, epoxy- and keto- are positioned on the fatty acid in the *sn*-2 position.

Figure 2.

Schema showing truncation of oxPE and its reaction with nucleophilic amino acids.

(a) structure of 1-octadecanoyl-2-(15-hydroperoxy-5E,8E,11E,13E-eicosatetraenoyl)-sn-glycero-3-phosphoethanolamine and possible leaving groups [b; (i)-malondialdehyde, (ii) acrolein, (iii) 4-hydroxyhexenal, (iv) 4-hydroxynonenal, (v) 4-oxononenal] and remaining groups [c; (i) 1-octadecanoyl-2-(15-oxo-5Z,8Z,13E-pentadecatrienoyl)-sn-glycero-3-phosphoethanolamine, (ii) 1-octadecanoyl-2-(15,12-di-oxo-5Z,8Z,13E-pentadecatrienoyl)-sn-glycero-3-phosphoethanolamine, (iii) 1-octadecanoyl-2-(15-oxo-12-hydroxy-5Z,8Z,13E-

pentadecatrienoyl)-*sn*-glycero-3-phospho-ethanolamine] electrophiles. (**d**) reaction schema showing the reaction of oxidized-PE derived electrophiles with nucleophilic amino acid side chains (i) histidine, (ii) cysteine and (iii) lysine

Figure 3.

Structural characterization of oxCL molecular species obtained from ileum of mice exposed to total body irradiation (9.5Gy) using LC-MS/MS Fusion Lumos spectrometer (Thermo Fisher, San Jose, CA).

A. Typical base peak profile of CL molecular species with m/z 1465.9755 in the range of retention time (RT) from 15 to 30 min. **B.** Full MS^2 spectra of CL molecular species with m/z 1465.9755 at RT 21.8 min (a) and 23.6 min (b). Inserts; MS^2 spectra in the m/z range from 690 to 720. **C.** Structural formulas and MS^2 fragmentation pattern of 13-HODE-CL (a) and 9-HODE-CL (b). **D.** Full MS^3 spectra of CL molecular species with m/z 1465.9755 $\rightarrow$ 295 at RT 21.8 min (a) and 23.6 min (b). Structural formulae and fragmentation patterns of 13-HODE (insert – a) and 9-HODE (insert – b). Mice were exposed to total body irradiation at dose of 9.5Gy. Two days after exposure mice were sacrificed and lipids were extracted by SPE. CLs molecular species were separated by reverse phase chromatography (C18 column was used) and analyzed by ESI-MS/MS using Fusion Lumos (ThermoFisher, San Jose, CA). Two distinct peaks at retention time 21.8 and 23.6 min with m/z 1465.9755 were identified as 13-HODE-CL (left panels) and 9-HODE-CL (right panels).

Figure 4.

A schema illustrating hydrolysis and re-esterification of phospholipids and their oxidation products by PLA₂.

Several phospholipases A₂ such as LpPLA₂, calcium independent iPLA₂γ and iPLA₂β release oxidatively modified fatty acids. This approach can significantly reduce the diversification and simplify the initial analysis of oxPL species. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; PA, phosphatidic acid; CL, cardiolipin; LPC, lyso-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; LPI, lyso-phosphatidylinositol; LPS, lyso-phosphatidylserine; LPG, lyso-phosphatidylglycerol; LPA, lyso-phosphatidic acid; mCL, mono-lyso-cardiolipin.

Figure 5.

A schema illustrating oxidation and externalization of PS on the surface of apoptotic cells and their engulfment by macrophages. In normal cells, non-oxidized PS is localized exclusively in the inner leaflet of plasma membrane. APT, aminophospholipid translocase.

Figure 6.

Detection and identification of triglycerides and their oxidatively truncated species.

A. LC-MS profiles and LC-MS/MS spectra of TAG individual molecular species C16:0/C18:2/C18:1 (a) and their oxidatively truncated derivatives C16:0/C9-ONA/C18:1(b).

B. Typical LC-MS profiles and LC-MS/MS spectrum of truncated TAG (C16:0/C9-ONA/C18:1) species before and after incubation with 2,4-dinitrophenylhydrazine, DNPH (a).

After derivatization with DNPH the peak at m/z 766 almost disappears (see lower left panel, a) and a new product at m/z 946 is recorded (right upper panel, a). Before TAG derivatization (at the same retention time) the peak at m/z 946 was not observed (right lower panel, a). Structural LC-MS/MS analysis of truncated TAG (C16:0/C9-ONA/C18:1) is shown in panels b and c. Possible structure of the DNPH modified product is inserted. LC-ESI-MS/MS analysis of TAGs/CEs was performed on a Dionex LC system (UltiMate 3000 auto sampler) that was coupled to a Q-Exactive hybrid-quadrupole-orbitrap mass spectrometer (ThermoFisher, Inc. San Jose, CA). TAGs/CEs were separated on a reverse phase column (Luna 3 μm C18 (2) 100A, 150 × 1.0 mm, (Phenomenex)) at a flow rate of 0.065 mL/min. The analysis was performed using gradient solvents (methanol and propanol) containing 0.1% NH₄OH.

Figure 7.

MALDI detection and analysis of molecular species of CL in freshly frozen tissue sections from brains of control rats and after traumatic brain injury (TBI).

A. Optical image of an H&E stained coronal rat brain section 3 h after TBI. Point of impact is marked with an arrow. **B.** MALDI imaging of a serial section from the same brain showing the loss of CL(74:7) from the contusional and peri-contusional areas.