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Chemical tools to study lipid signaling

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Chapter 1

General introduction

Lipids and their diversity

Lipids are defined as hydrophobic biomolecules that dissolve in organic solvents, but not in water. They have a wide variety of functions inside the cell, such as structural building block of membranes, energy storage and cell signaling. The discovery that aspirin affects prostaglandin synthesis demonstrated that lipids can modulate the immune system and that enzymes involved in their metabolism constitute interesting drug targets.¹ Many different signaling activities have subsequently been discovered for multiple lipid classes, such as endocannabinoids, resolvins, steroid hormones and vitamins A and D.²⁻⁵ However, many signaling lipids have a low abundance, high lipophilicity and are short-lived, and as a consequence the mode of action and protein interaction partners of many lipids have remained elusive.

In an effort to systematically classify the rapidly expanding collection of identified lipids, the Lipid Metabolites and Pathways Strategy (LIPID MAPS) consortium has come up with a more concise definition of a lipid. In this definition, a lipid comprises a small hydrophobic or amphipathic molecule that is formed at least partially by the condensation of ketoacyl thioesters and/or isoprene units.⁶ Based on these two building blocks, eight major lipid classes are defined: fatty acyls, glycerolipids, prenol lipids, sphingolipids, glycerophospholipids, sterol lipids, saccharolipids and polyketides (Figure 1).⁷

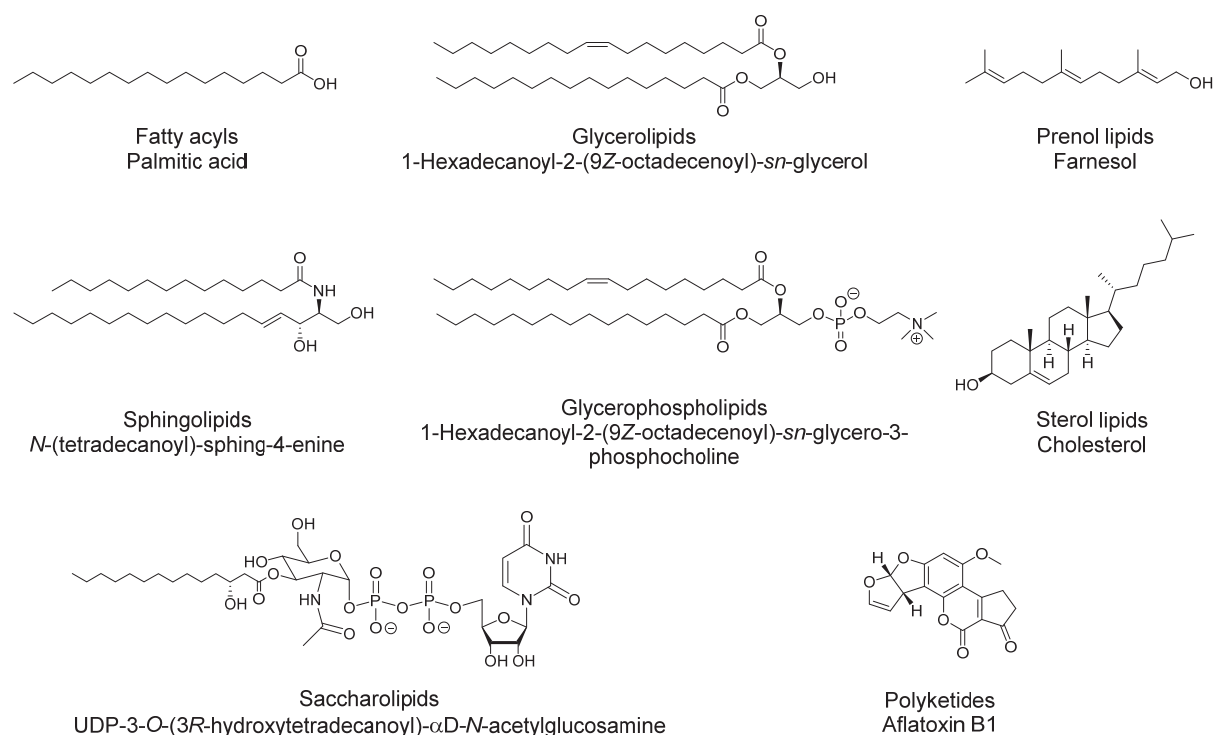


Figure 1 | Representative structures of the eight lipid categories as defined by the LIPID MAPS consortium.^{6,7}

The variation in chemical characteristics between lipid families makes it a challenge to analyze multiple lipid species in one experiment. Different methods of sample preparation and analytical techniques are required to detect and quantify different classes of lipids.⁸ Adding to the challenge is the wide range of metabolic modifications lipids can undergo, thereby yielding potential new bioactive lipid species. Since its inception, the field of lipidomics, which is defined as the analysis of lipids and their interacting partners within a biological system, has made great strides forward.⁹ Standardization of protocols, increased availability of isotopically labelled lipids and the high mass accuracy and resolution of modern mass spectrometers have enabled the measurement of many different lipid species in complex biological samples.¹⁰ However, bulk analysis of lipids after sample homogenization and lipid extraction discards the information on spatial distribution, therefore other methods are required to investigate lipid localization and protein interaction partners. Spatial information is important to study the role of lipids as signaling molecules, as signaling events occur through localized alterations in membrane lipid composition and activation of specific receptors such as peroxisome proliferator-activated receptors (PPARs)¹¹ and the G-protein coupled receptors GPR40¹² and GPR120.¹³ In recent years, several technical advances in mass spectrometry and innovative chemical biology strategies have shed light on the lipid-protein interaction landscape and thereby facilitate studies of their biological function.

Polyunsaturated fatty acids

Fatty acids are an important component of cell membranes and are integral to a wide variety of lipid classes. In vertebrates, fatty acid production is initiated in the cytosol by a system collectively referred to as fatty acid synthase.¹⁴ When this system completes the biosynthesis of an acyl chain 16 carbon atoms in length, it releases the formed palmitate for further processing by enzymes located at the endoplasmic reticulum. One of these processes is the introduction of one or more *cis* double bonds, which is performed in eukaryotes by desaturases. This results in the formation of mono- or polyunsaturated fatty acids (PUFAs), where the double bonds in the latter are separated by methylene groups. When incorporated into membrane lipids, *cis* double bonds introduce disorder and increase fluidity, which has an impact on membrane protein function and on the assembly of lipid rafts.¹⁵

Fatty acids have common and systematic names, but are also referred to by a nomenclature that denotes the number of carbon atoms of the acyl chain, the number of double bonds, and the position of the first double bond relative to the terminal methyl, termed the omega (ω , or *n*) carbon. The major PUFAs found in mammals are omega-3 and omega-6 fatty acids. As mammals lack the enzymes required for certain biosynthetic steps, these fatty acids are conditionally essential nutrients. In principle, only α -linolenic acid (ALA, 18:3 *n*-3) and linoleic acid (LA, 18:2 *n*-6) are essential for the biosynthesis of other PUFAs. However, the multiple chain elongation and desaturation steps to form higher order PUFAs like eicosapentaenoic acid (EPA, 20:5 *n*-3) and docosahexaenoic acid (DHA, 22:6 *n*-3) are highly inefficient, and the bulk of these fatty acids are derived from the diet (Figure 2).¹⁶ Diets rich in omega-3 fatty acids, along with omega-3 supplements, are of great interest as they are linked to a number of health benefits, such as reduced risk for ischemic stroke,¹⁷ neuroprotective and antidepressant effects,^{18,19} and improvements in chronic inflammatory conditions.^{20–22} Moreover, they are essential for neuronal development²³ and inadequate intake is associated with many neurological diseases, including depression,²⁴ anxiety disorders,²⁵ and neuroinflammation.^{26,27} However, as often as these benefits are reported, they are also the subject of discussion. For example, omega-3 fatty acids are known to prevent a number of risk factors for cardiovascular disease, including high blood pressure,²⁸ reduced arterial compliance,²⁹ cardiac arrhythmias³⁰ and abnormal platelet reactivity and thrombosis.³¹ However, this prevention of cardiovascular risk factors does not translate to benefit to human health. Recent meta-analyses^{32,33} of clinical trials investigating omega-3 supplementation found no clinical benefit for fatal or nonfatal coronary heart disease or any major vascular events. This discrepancy highlights the limited knowledge on the molecular and cellular pathways by which omega-3 fatty acids confer their benefits. It has been suggested that many of the beneficial effects on human health derive from modulation of the immune system,^{21,34} which has been supported by the discovery of anti-inflammatory oxidized metabolites.³⁵

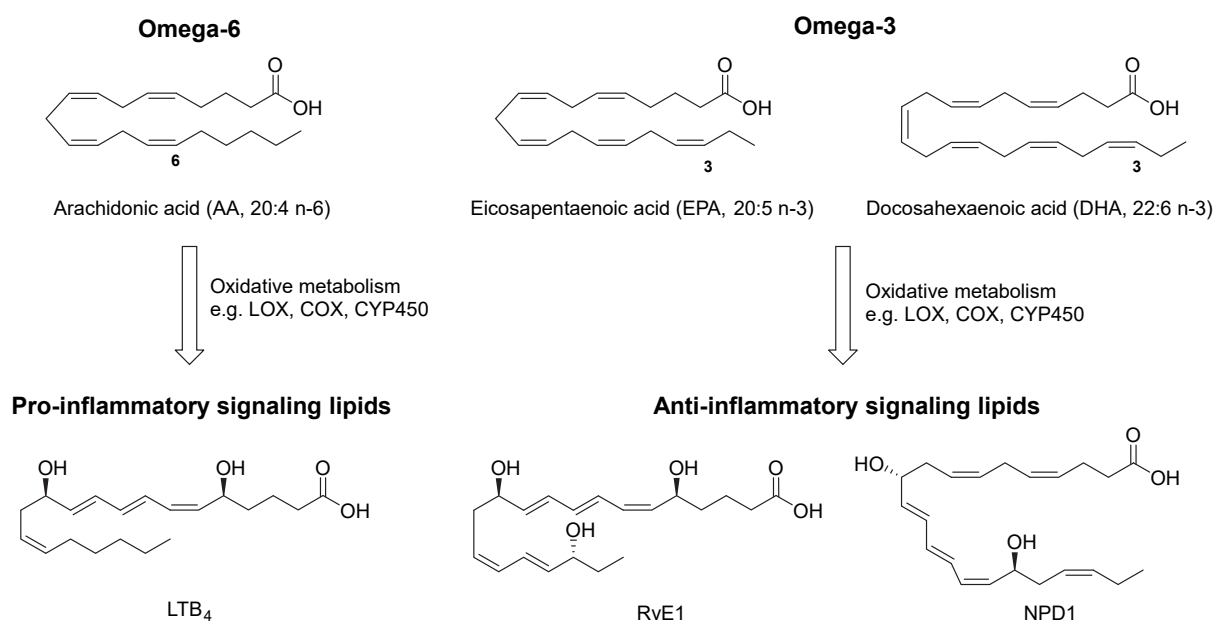


Figure 2 | Omega-3 and omega-6 fatty acids and examples of their oxidative metabolites. COX, cyclooxygenase; LOX, lipoxygenase; CYP450, cytochrome P450; NPD1, neuroprotectin D1; RvE1, resolvin E1; LTB₄, leukotriene B₄.

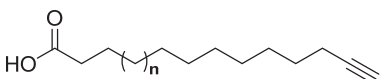
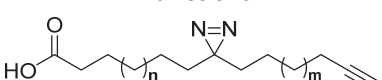
Oxidized derivatives of PUFAs have long been established as immunomodulatory signaling lipids. Arachidonic acid (AA, 20:4 n-6) can be oxidized by cyclooxygenases (COX) and lipoxygenases (LOX) into bioactive eicosanoids such as prostaglandins and leukotrienes, respectively (Figure 2). Most of these metabolites are pro-inflammatory and inhibition of their biosynthesis by non-steroidal anti-inflammatory drugs (NSAIDs) results in alleviation of pain, swelling and fever. In contrast, oxidized metabolites of omega-3 fatty acids act as anti-inflammatory lipids involved in the resolution of inflammation.³ Although many of these metabolites and their anti-inflammatory effects have been reported, their mechanisms and biosynthetic pathways remain to be characterized. Consequently, the proteins involved in their biosynthesis, degradation and signaling are of interest for investigation as potential drug targets for inflammatory diseases.²²

Tools to study lipid signaling molecules

The field of chemical biology has developed two main approaches to study lipids: design and synthesis of chemically modified lipids to track their metabolism and localization, and chemical tools to identify the protein interaction partners involved in their signaling functions.

To track lipids in a biological system, they can be functionalized with a reporter tag. These tags traditionally consist of radioactive isotopes³⁶ and fluorophores.^{37,38} However, radioactive isotopes require special equipment and procedures, while fluorophores are relatively large and rigid structures, affecting metabolism, distribution and trafficking.^{39,40} A more versatile and less intrusive modification is the introduction of an alkyne (Table 1).^{41,42} Using bioorthogonal ligation chemistry, such as 'click' chemistry, alkyne-functionalized lipids can be ligated to fluorescent groups for detection, or to affinity handles for isolation.⁴³ These functionalized lipids have been used for the visualization of lipids in membranes, their metabolism and modification of proteins by lipids, for example myristoylation⁴⁴ or prenylation⁴⁵ of proteins.^{46–49} However, these tools will only reveal covalent lipid-protein interactions. To study noncovalent lipid-protein interactions, the chemical tool requires additional functionality.

Table 1 | General structures and exemplary applications of fatty-acid based chemical tools.

General structure	Functionality
<p>Monofunctional</p> 	<p>Tracking metabolism^{49–51} Incorporation as post-translational modification⁴⁷ Localization by Raman spectroscopy or fluorescence microscopy^{46,52}</p>
<p>Bifunctional</p> 	<p>All of the above, as well as: Mapping protein-lipid interactions^{53,54} Mapping protein-protein interactions⁵⁵</p>

To detect lipid-interacting proteins, a photoactivatable group, such as a diazirine, benzophenone or aryl azide, can be incorporated into an alkyne-tagged lipid, providing a bifunctionalized lipid. Irradiation with light results in the formation of a reactive intermediate that can form a stable, covalent bond with an interacting protein.⁵⁶ The bioorthogonal handle can subsequently be ligated to a fluorophore to visualize the interaction partner. Alternatively, ligation to an affinity handle such as biotin allows for enrichment and identification of the tagged protein by mass spectrometry. Bifunctional lipids have been successfully used to map lipid-protein interaction networks, lipid signaling functions, lipid metabolism, lipid localization and protein-protein interactions.^{53–55,57} However, currently reported lipid probes do not cover all lipid classes. In part, this is due to the challenges of chemically synthesizing these functionalized lipids.

Aim and outline

The overarching aim of the research presented in this thesis was to develop and apply chemical tools to study lipid metabolism, transport and signaling.

Chapter 2 reviews lipid-based photoaffinity probes published in the last decade and their applications. Additionally, a list of promiscuous lipid binding proteins is presented and the challenges inherent to lipid probes are discussed. **Chapter 3** describes the synthesis of photoaffinity probes based on the omega-3 fatty acid DHA and an oxidized derivative, 17-hydroxy-DHA (17-HDHA), using a combination of chemical and chemoenzymatic transformations. The probes are used in comparative photoaffinity-based protein profiling, which reveals that PTGR1 is capable of converting 17-HDHA to 17-oxo-DHA in human macrophages. **Chapter 4** investigates the design and application of a photoaffinity probe based on the neuroprotective ethanolamide derivative of DHA, DHEA. This probe is used to study the molecular mechanism of action of this signaling lipid in a microglial cell line. **Chapter 5** reports on an improved method for the synthesis of DHA derivatives. Regioselective hydrobromination of DHA enables the synthesis of DHA-alkyne, which is used to study DHA metabolism and intracellular exchange by flow cytometry. **Chapter 6** describes the investigation of WOBE437, a reported inhibitor of anandamide uptake with no known protein target. The effects of WOBE437 on anandamide uptake and metabolism are investigated, and a photoaffinity probe is used to identify the protein targets of this inhibitor. **Chapter 7** demonstrates the use of cyclopropene lipids to perform live-cell fluorescence microscopy using quenched fluorophores. A cyclopropene-modified version of arachidonic acid is synthesized for the purpose of studying anandamide transport and localization in real-time. **Chapter 8** summarizes the work described in this thesis and provides future directions.

References

- (1) Vane, J. R. Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-like Drugs. *Nature. New Biol.* **1971**, 231 (25), 232–235.
- (2) Pandey, R.; Mousawy, K.; Nagarkatti, M.; Nagarkatti, P. Endocannabinoids and Immune Regulation. *Pharmacol. Res.* **2009**, 60 (2), 85–92.
- (3) Serhan, C. N.; Petasis, N. A. Resolvins and Protectins in Inflammation Resolution. *Chem. Rev.* **2011**, 111 (10), 5922–5943.
- (4) Marshall-Gradisnik, S.; Green, R.; Brenu, E.; Weatherby, R. Anabolic Androgenic Steroids Effects on the Immune System: A Review. *Open Life Sci.* **2009**, 4 (1), 19–33.
- (5) Mora, J. R.; Iwata, M.; Andrian, U. H. V. Vitamin Effects on the Immune System. *Nat. Rev. Immunol.* **2008**, 8 (9), 685–698.
- (6) Fahy, E.; Cotter, D.; Sud, M.; Subramaniam, S. Lipid Classification, Structures and Tools. *Biochim. Biophys. Acta* **2011**, 1811 (11), 637–647.
- (7) Fahy, E.; Subramaniam, S.; Murphy, R. C.; Nishijima, M.; Raetz, C. R. H.; Shimizu, T.; Spener, F.; Meer, G. van; Wakelam, M. J. O.; Dennis, E. A. Update of the LIPID MAPS Comprehensive Classification System for Lipids. *J. Lipid Res.* **2009**, 50, S9–S14.
- (8) Yang, K.; Han, X. Lipidomics: Techniques, Applications, and Outcomes Related to Biomedical Sciences. *Trends Biochem. Sci.* **2016**, 41 (11), 954–969.
- (9) Wenk, M. R. The Emerging Field of Lipidomics. *Nat. Rev. Drug Discov.* **2005**, 4 (7), 594–610.
- (10) Wenk, M. R. Lipidomics: New Tools and Applications. *Cell* **2010**, 143, 888–895.
- (11) Berger, J.; Moller, D. E. The Mechanisms of Action of PPARs. *Annu. Rev. Med.* **2002**, 53 (1), 409–435.
- (12) Tan, J. K.; McKenzie, C.; Mariño, E.; Macia, L.; Mackay, C. R. Metabolite-Sensing G Protein-Coupled Receptors—Facilitators of Diet-Related Immune Regulation. *Annu. Rev. Immunol.* **2017**, 35 (1), 371–402.
- (13) Oh, D. Y.; Talukdar, S.; Bae, E. J.; Imamura, T.; Morinaga, H.; Fan, W.; Li, P.; Lu, W. J.; Watkins, S. M.; Olefsky, J. M. GPR120 Is an Omega-3 Fatty Acid Receptor Mediating Potent Anti-Inflammatory and Insulin-Sensitizing Effects. *Cell* **2010**, 142 (5), 687–698.
- (14) Nelson, D. L.; Lehninger, A. L.; Cox, M. M. *Lehninger Principles of Biochemistry*; Macmillan, 2008.
- (15) Calder, P. C. Docosahexaenoic Acid. *Ann. Nutr. Metab.* **2016**, 69 (Suppl. 1), 8–21.
- (16) Spector, A. A. Essentiality of Fatty Acids. *Lipids* **1999**, 34 (1), S1–S3.
- (17) Hengeveld, L. M.; Praagman, J.; Beulens, J. W. J.; Brouwer, I. A.; van der Schouw, Y. T.; Sluijs, I. Fish Consumption and Risk of Stroke, Coronary Heart Disease, and Cardiovascular Mortality in a Dutch Population with Low Fish Intake. *Eur. J. Clin. Nutr.* **2018**, 72 (7), 942–950.
- (18) Larrieu, T.; Layé, S. Food for Mood: Relevance of Nutritional Omega-3 Fatty Acids for Depression and Anxiety. *Front. Physiol.* **2018**, 9, 1047.
- (19) Bazinet, R. P.; Layé, S. Polyunsaturated Fatty Acids and Their Metabolites in Brain Function and Disease. *Nat. Rev. Neurosci.* **2014**, 15 (12), 771–785.
- (20) Calder, P. C. Marine Omega-3 Fatty Acids and Inflammatory Processes: Effects, Mechanisms and Clinical Relevance. *Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids* **2015**, 1851 (4), 469–484.
- (21) Calder, P. C. Omega-3 Fatty Acids and Inflammatory Processes: From Molecules to Man. *Biochem. Soc. Trans.* **2017**, 45 (5), 1105–1115.
- (22) Serhan, C. N.; Levy, B. D. Resolvins in Inflammation: Emergence of the pro-Resolving Superfamily of Mediators. *J. Clin. Invest.* **2018**, 128 (7), 2657–2669.
- (23) Salem, N.; Litman, B.; Kim, H.-Y.; Gawrisch, K. Mechanisms of Action of Docosahexaenoic Acid in the Nervous System. *Lipids* **2001**, 36 (9), 945–959.
- (24) Liu, J. J.; Green, P.; John Mann, J.; Rapoport, S. I.; Sublette, M. E. Pathways of Polyunsaturated Fatty Acid Utilization: Implications for Brain Function in Neuropsychiatric Health and Disease. *Brain Res.* **2015**, 1597, 220–246.
- (25) Müller, C. P.; Reichel, M.; Mühle, C.; Rhein, C.; Gulbins, E.; Kornhuber, J. Brain Membrane Lipids in Major Depression and Anxiety Disorders. *Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids* **2015**, 1851 (8), 1052–1065.
- (26) Kiecolt-Glaser, J. K.; Belury, M. A.; Porter, K.; Beversdorf, D. Q.; Lemeshow, S.; Glaser, R. Depressive Symptoms, Omega-6:Omega-3 Fatty Acids, and Inflammation in Older Adults. *Psychosom. Med.* **2007**, 69 (3), 217–224.
- (27) Calder, P. C. N-3 Polyunsaturated Fatty Acids, Inflammation, and Inflammatory Diseases. *Am. J. Clin. Nutr.* **2006**, 83 (6), 1505S–1519S.
- (28) Geleijnse, J. M.; Giltay, E. J.; Grobbee, D. E.; Donders, A. R. T.; Kok, F. J. Blood Pressure Response to Fish Oil Supplementation: Metaregression Analysis of Randomized Trials. *J. Hypertens.* **2002**, 20 (8), 1493–1499.
- (29) Nestel, P.; Shige, H.; Pomeroy, S.; Cehun, M.; Abbey, M.; Raederstorff, D. The N-3 Fatty Acids Eicosapentaenoic Acid and Docosahexaenoic Acid Increase Systemic Arterial Compliance in Humans. *Am. J. Clin. Nutr.* **2002**, 76 (2), 326–330.

- (30) von Schacky, C. Omega-3 Fatty Acids: Antiarrhythmic, Proarrhythmic or Both? *Curr. Opin. Clin. Nutr. Metab. Care* **2008**, *11* (2), 94–99.
- (31) Foundation, B. N. *Unsaturated Fatty Acids: Nutritional and Physiological Significance: The Report of the British Nutrition Foundation's Task Force*, Chapman & Hall, 1992.
- (32) Aung, T.; Halsey, J.; Kromhout, D.; Gerstein, H. C.; Marchioli, R.; Tavazzi, L.; Geleijnse, J. M.; Rauch, B.; Ness, A.; Galan, P.; Chew, E. Y.; Bosch, J.; Collins, R.; Lewington, S.; Armitage, J.; Clarke, R. Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease Risks: Meta-Analysis of 10 Trials Involving 77 917 Individuals. *JAMA Cardiol.* **2018**, *3* (3), 225–233.
- (33) Abdelhamid, A. S.; Brown, T. J.; Brainard, J. S.; Biswas, P.; Thorpe, G. C.; Moore, H. J.; Deane, K. H.; AlAbdulghafoor, F. K.; Summerbell, C. D.; Worthington, H. V.; Song, F.; Hooper, L. Omega-3 Fatty Acids for the Primary and Secondary Prevention of Cardiovascular Disease. *Cochrane Database Syst. Rev.* **2018**, No. 11.
- (34) de Bus, I.; Witkamp, R.; Zuilhof, H.; Albada, B.; Balvers, M. The Role of N-3 PUFA-Derived Fatty Acid Derivatives and Their Oxygenated Metabolites in the Modulation of Inflammation. *Prostaglandins Other Lipid Mediat.* **2019**, *144*, 106351.
- (35) Bannenberg, G. L.; Chiang, N.; Ariel, A.; Arita, M.; Tjonahen, E.; Gotlinger, K. H.; Hong, S.; Serhan, C. N. Molecular Circuits of Resolution: Formation and Actions of Resolvins and Protectins. *J. Immunol.* **2005**, *174* (7), 4345–4355.
- (36) Brown, M. S.; Goldstein, J. L. Cholesterol Feedback: From Schoenheimer's Bottle to Scap's MELADL. *J. Lipid Res.* **2009**, *50* (Supplement), S15–S27.
- (37) Wüstner, D. Fluorescent Sterols as Tools in Membrane Biophysics and Cell Biology. *Chem. Phys. Lipids* **2007**, *146* (1), 1–25.
- (38) Gimpl, G.; Gehrig-Burger, K. Probes for Studying Cholesterol Binding and Cell Biology. *Steroids* **2011**, *76* (3), 216–231.
- (39) Laguerre, A.; Schultz, C. Novel Lipid Tools and Probes for Biological Investigations. *Curr. Opin. Cell Biol.* **2018**, *53*, 97–104.
- (40) Sezgin, E.; Can, F. B.; Schneider, F.; Clausen, M. P.; Galiani, S.; Stanly, T. A.; Waithe, D.; Colaco, A.; Honigsmann, A.; Wüstner, D.; Platt, F.; Eggeling, C. A Comparative Study on Fluorescent Cholesterol Analogs as Versatile Cellular Reporters. *J. Lipid Res.* **2016**, *57* (2), 299–309.
- (41) Gaebler, A.; Milan, R.; Straub, L.; Hoelper, D.; Kuerschner, L.; Thiele, C. Alkyne Lipids as Substrates for Click Chemistry-Based in Vitro Enzymatic Assays. *J. Lipid Res.* **2013**, *54* (8), 2282–2290.
- (42) Robichaud, P. P.; Poirier, S. J.; Boudreau, L. H.; Doiron, J. A.; Barnett, D. A.; Boilard, E.; Surette, M. E. On the Cellular Metabolism of the Click Chemistry Probe 19-Alkyne Arachidonic Acid. *J. Lipid Res.* **2016**, *57* (10), 1821–1830.
- (43) Hein, J. E.; Fokin, V. V. Copper-Catalyzed Azide–Alkyne Cycloaddition (CuAAC) and beyond: New Reactivity of Copper(I) Acetylides. *Chem. Soc. Rev.* **2010**, *39* (4), 1302–1315.
- (44) Wright, M. H.; Paape, D.; Storck, E. M.; Serwa, R. A.; Smith, D. F.; Tate, E. W. Global Analysis of Protein N-Myristoylation and Exploration of N-Myristoyltransferase as a Drug Target in the Neglected Human Pathogen *Leishmania Donovanii*. *Chem. Biol.* **2015**, *22* (3), 342–354.
- (45) Storck, E. M.; Morales-Sanfrutos, J.; Serwa, R. A.; Panyain, N.; Lanyon-Hogg, T.; Tolmachova, T.; Ventimiglia, L. N.; Martin-Serrano, J.; Seabra, M. C.; Wojciak-Stothard, B.; Tate, E. W. Dual Chemical Probes Enable Quantitative System-Wide Analysis of Protein Prenylation and Prenylation Dynamics. *Nat. Chem.* **2019**, *11* (6), 552–561.
- (46) Gaebler, A.; Penno, A.; Kuerschner, L.; Thiele, C. A Highly Sensitive Protocol for Microscopy of Alkyne Lipids and Fluorescently Tagged or Immunostained Proteins. *J. Lipid Res.* **2016**, *57* (10), 1934–1947.
- (47) Tate, E. W.; Kalesh, K. A.; Lanyon-Hogg, T.; Storck, E. M.; Thinon, E. Global Profiling of Protein Lipidation Using Chemical Proteomic Technologies. *Curr. Opin. Chem. Biol.* **2015**, *24*, 48–57.
- (48) Hofmann, K.; Thiele, C.; Schött, H.-F.; Gaebler, A.; Schoene, M.; Kiver, Y.; Friedrichs, S.; Lütjohann, D.; Kuerschner, L. A Novel Alkyne Cholesterol to Trace Cellular Cholesterol Metabolism and Localization. *J. Lipid Res.* **2014**, *55* (3), 583–591.
- (49) Thiele, C.; Papan, C.; Hoelper, D.; Kusserow, K.; Gaebler, A.; Schoene, M.; Piotrowitz, K.; Lohmann, D.; Spandl, J.; Stevanovic, A.; Shevchenko, A.; Kuerschner, L. Tracing Fatty Acid Metabolism by Click Chemistry. *ACS Chem. Biol.* **2012**, *7* (12), 2004–2011.
- (50) Gaebler, A.; Milan, R.; Straub, L.; Hoelper, D.; Kuerschner, L.; Thiele, C. Alkyne Lipids as Substrates for Click Chemistry-Based in Vitro Enzymatic Assays. *J. Lipid Res.* **2013**, *54* (8), 2282–2290.
- (51) Thiele, C.; Wunderling, K.; Leyendecker, P. Multiplexed and Single Cell Tracing of Lipid Metabolism. *Nat. Methods* **2019**, *16* (11), 1123–1130.
- (52) Jamieson, L. E.; Greaves, J.; McLellan, J. A.; Munro, K. R.; Tomkinson, N. C. O.; Chamberlain, L. H.; Faulds, K.; Graham, D. Tracking Intracellular Uptake and Localisation of Alkyne Tagged Fatty Acids Using Raman Spectroscopy. *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* **2018**, *197*, 30–36.
- (53) Niphakis, M. J.; Lum, K. M.; Cognetta III, A. B.; Correia, B. E.; Ichu, T.-A.; Olucha, J.; Brown, S. J.; Kundu, S.; Piscitelli, F.; Rosen, H.; Cravatt, B. F. A Global Map of Lipid-Binding Proteins and Their Ligandability in Cells. *Cell* **2015**, *161* (7), 1668–1680.

- (54) Haberkant, P.; Raijmakers, R.; Wildwater, M.; Sachsenheimer, T.; Brügger, B.; Maeda, K.; Houweling, M.; Gavin, A.-C.; Schultz, C.; van Meer, G.; Heck, A. J. R.; Holthuis, J. C. M. In Vivo Profiling and Visualization of Cellular Protein–Lipid Interactions Using Bifunctional Fatty Acids. *Angew. Chem. Int. Ed.* **2013**, *52* (14), 4033–4038.
- (55) Peng, T.; Hang, H. C. Bifunctional Fatty Acid Chemical Reporter for Analyzing S-Palmitoylated Membrane Protein–Protein Interactions in Mammalian Cells. *J. Am. Chem. Soc.* **2015**, *137* (2), 556–559.
- (56) Haberkant, P.; Holthuis, J. C. M. Fat & Fabulous: Bifunctional Lipids in the Spotlight. *Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids* **2014**, *1841* (8), 1022–1030.
- (57) Haberkant, P.; Stein, F.; Höglinger, D.; Gerl, M. J.; Brügger, B.; Van Veldhoven, P. P.; Krijgsveld, J.; Gavin, A.-C.; Schultz, C. Bifunctional Sphingosine for Cell-Based Analysis of Protein-Sphingolipid Interactions. *ACS Chem. Biol.* **2016**, *11* (1), 222–230.

