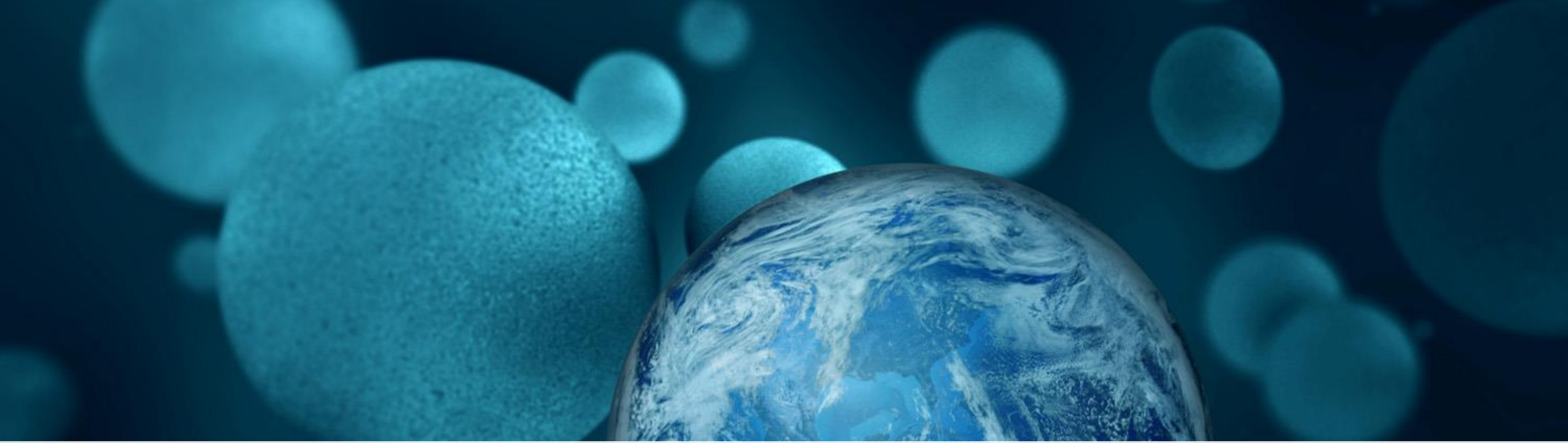


ThermoFisher SCIENTIFIC

Let's Chew the Fat with Orbitrap Mass Spectrometry

Outline

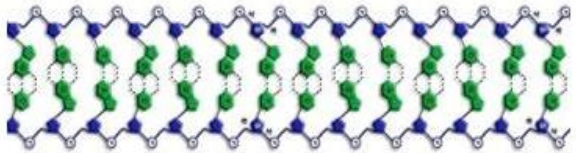
- Introduction – Lipidomics the fastest growing omics
- Challenges – Lipid complexity and solutions
- LC/MS workflow – Q Exactive HF-X and LipidSearch software
- Example – Analysis of human plasma lipid profiling
- Advanced Challenges – Orbitrap Fusion Lumos
- Summary

Omics - Insights to Biology

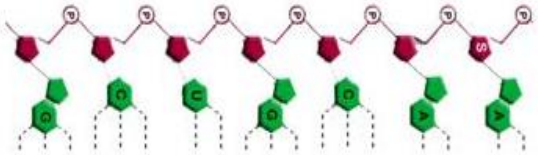
Environment

Microbiome

DNA

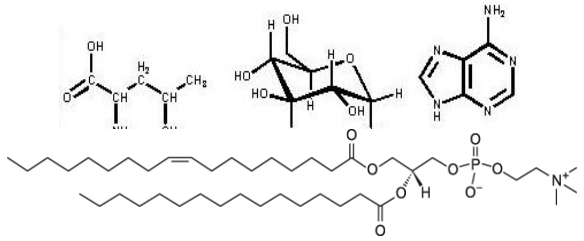


RNA



Proteins

Metabolites + Lipids



Genomics – 22,000 genes

Biological potential

Transcriptomics – 100,000 transcripts

Response to conditions

Proteomics – 1,000,000 proteoforms

Biological Function

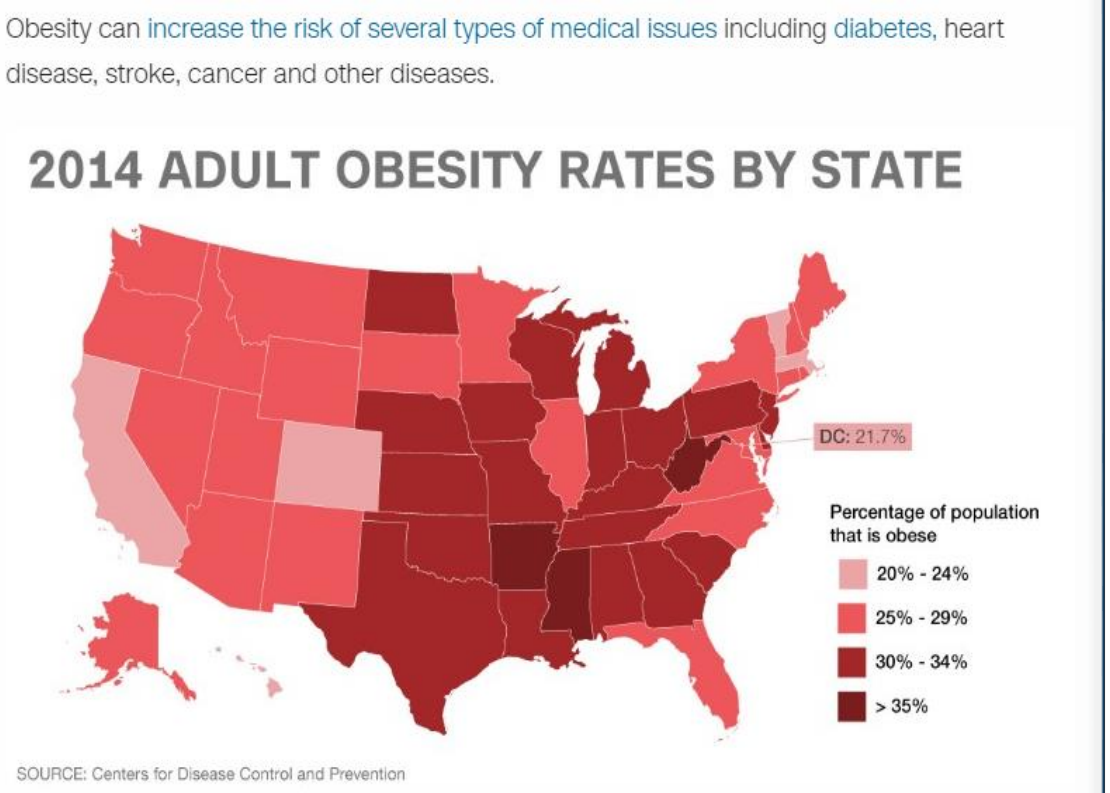
Metabolomics >5,000 compounds

Lipidomics >30,000 species

Physiological state/phenotype

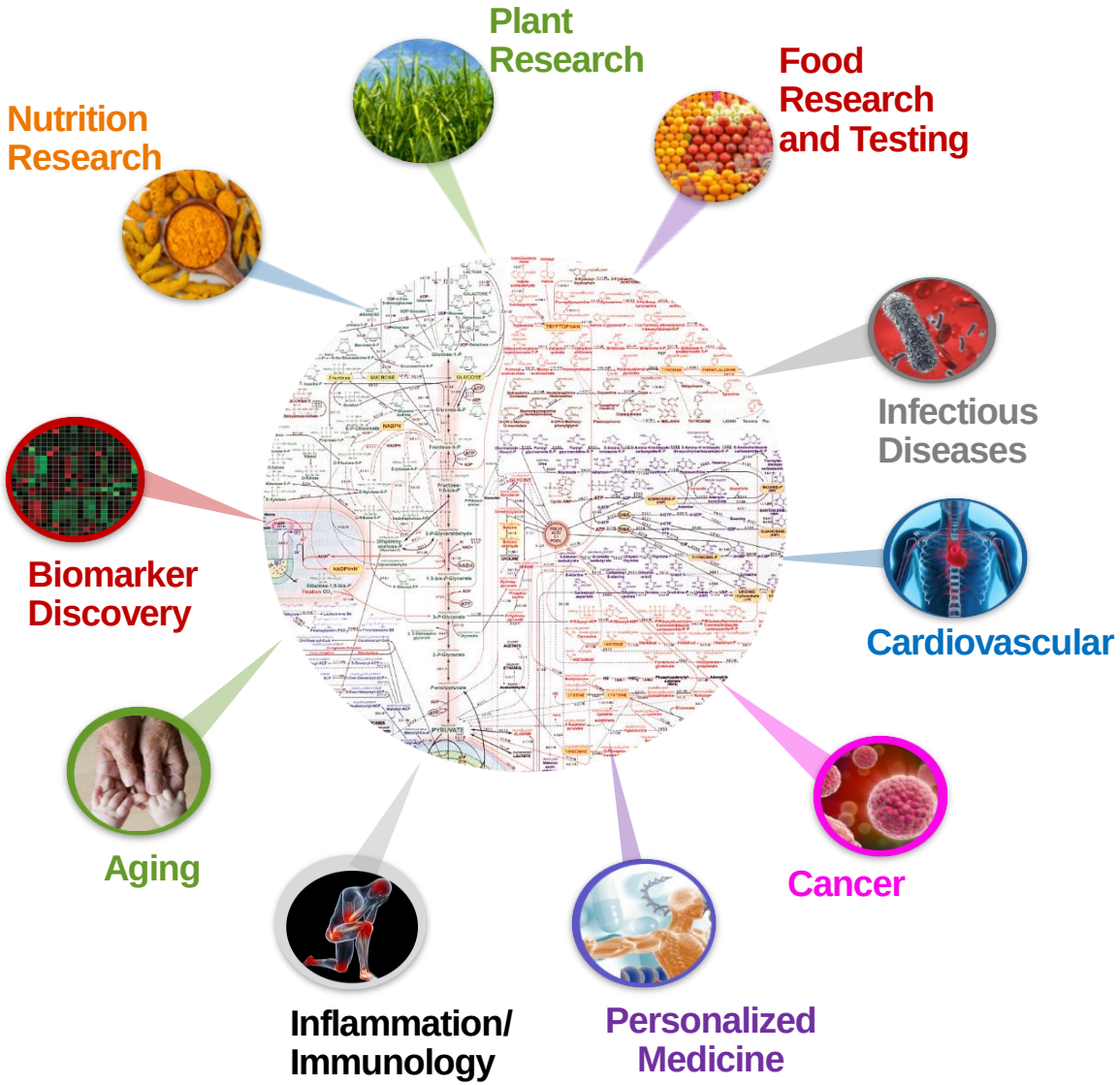
Lipids Play Important Roles in Development of Obesity and Life-Threatening Disease

Obesity is associated with a cluster of lipid abnormalities, such as high fasting triglyceride levels and low HDL cholesterol,

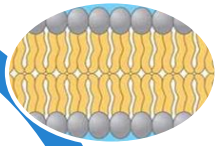


- Obesity is an epidemic, not only in the US, but is a growing health concern worldwide
- Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in children and adolescents
- NAFLD often progresses to non-alcoholic steatohepatitis (NASH)
- NASH may lead to fibrosis and liver cirrhosis, ultimately leading to liver failure or carcinoma

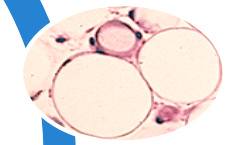
Lipid Biology—Plays an Important Role in Disease and Nutrition



Biological functions of lipids



Cell Membranes
Cell membranes are made from a double layer of phospholipids



Energy Storage
Lipid play an important rule in storing energy

Insulation
Fats are important for heat insulation

Cell-cell communication
Steroids are a group of lipids involved in cellular communication

Lipidomics Reveals Lipid Species That May Be Predictive of Disease Progression

JOURNAL OF LIPID RESEARCH ASBMB

Biomarkers of NAFLD progression: a lipidomics approach to an epidemic[1]

D. Lee Gorden,*,† David S. Myers,‡ Pavlina T. Ivanova,‡ Eoin Fahy,§ Mano R. Maurya,§ Shakti Gupta,§ Jun Min,§ Nathanael J. Spann,† Jeffrey G. McDonald,# Samuel L. Kelly,** Jingjing Duan,** M. Cameron Sullards,** Thomas J. Leiker,†† Robert M. Barkley,†† Oswald Quehenberger,‡‡,§§ Aaron M. Armando,§§ Stephen B. Milne,‡ Thomas P. Mathews,‡ Michelle D. Armstrong,‡ Chijun Li,## Willie V. Melvin,* Ronald H. Clements,* M. Kay Washington,## Alisha M. Mendonsa,† Joseph L. Witztum,‡‡ Ziqiang Guan,## Christopher K. Glass,† Robert C. Murphy,†† Edward A. Dennis,§§,*** Alfred H. Merrill, Jr.,** David W. Russell,# Shankar Subramaniam,2,§§,*** and H. Alex Brown2,‡,†††

Departments of Surgery,* Cancer Biology,† Pharmacology,‡ Pathology, Microbiology, and Immunology,## Biochemistry, and the Vanderbilt Institute of Chemical Biology,††† Vanderbilt University Medical Center, Nashville, TN; Department of Bioengineering, School of Engineering,§ and Departments of Cellular and Molecular Medicine and Medicine,† University of California, San Diego, La Jolla, CA; Department of Molecular Genetics,# University of Texas Southwestern Medical Center, Dallas, TX; Schools of Biology, Chemistry, and Biochemistry, and the Parker H. Petit Institute for Bioengineering and Bioscience,** Georgia Institute of Technology, Atlanta, GA; Department of Pharmacology,†† University of Colorado at Denver, Aurora, CO; Departments of Medicine,‡‡ Pharmacology,§§ and Chemistry and Biochemistry,*** School of Medicine, University of California, San Diego, La Jolla, CA; and Department of Biochemistry,# Duke University Medical Center, Durham, NC

Abstract The spectrum of nonalcoholic fatty liver disease (NAFLD) includes steatosis, nonalcoholic steatohepatitis (NASH), and cirrhosis. Recognition and timely diagnosis of these different stages, particularly NASH, is important for both potential reversibility and limitation of complications. Liver biopsy remains the clinical standard for definitive diagnosis. Diagnostic tools minimizing the need for invasive procedures or that add information to histologic data are important in novel management strategies for the growing epidemic of NAFLD. We describe an "omics" approach to detecting a reproducible signature of lipid metabolites, aqueous intracellular metabolites, SNPs, and mRNA transcripts in a double-blinded study of patients with different stages of NAFLD that involves profiling liver biopsies, plasma, and urine samples. Using linear discriminant analysis, a panel of 20 plasma metabolites that includes glycerophospholipids, sphingolipids, sterols, and various aqueous small molecular weight components involved in cellular metabolic pathways, can be used to differentiate between NASH and steatosis. This identification of differential biomolecular signatures has the potential to improve clinical diagnosis and facilitate therapeutic intervention of NAFLD.—Gorden, D. L., D. S. Myers, P. T. Ivanova, E. Fahy, M. R. Maurya, S. Gupta, J. Min, N. J. Spann, J. G. McDonald, S. L. Kelly, J. Duan, M. C. Sullards, T. J. Leiker, R. M. Barkley, O. Quehenberger, A. M. Armando, S. B. Milne, T. P. Mathews, M. D. Armstrong, C. Li, W. V. Melvin, R. H. Clements, M. K. Washington, A. M. Mendonsa, J. L. Witztum, Z. Guan, C. K. Glass, R. C. Murphy, E. A. Dennis, A. H. Merrill, Jr., D. W. Russell, S. Subramaniam, and H. A. Brown. **Biomarkers of NAFLD progression: a lipidomics approach to an epidemic.** *J. Lipid Res.* **2015.** 56: **722–736.**

Supplementary key words diagnostic tools • mass spectrometry • phospholipids • sphingolipids • nonalcoholic fatty liver disease • nonalcoholic steatohepatitis

Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming one of the most common forms of liver disease in

Abbreviations: CE, cholesteryl ester; DAG, diacylglycerol; ECM, extracellular matrix; FDR, false discovery rate; F16BP, fructose-1,6-bisphosphate; GO, gene ontology; GPL, glycerophospholipid; HCC, hepatocellular carcinoma; KEGG, Kyoto Encyclopedia of Genes and Genomes; LDA, linear discriminant analysis; LPE, lysophosphatidylethanolamine; MMP, matrix metalloproteinase; MRM, multiple reaction monitoring; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PC, phosphatidylcholine; PE, phosphatidylethanolamine; RNA-Seq, RNA sequence; S/N, steatosis/normal; SOP, standard operating procedure; TAG, triacylglycerol; TCA, the citric acid or tricarboxylic acid cycle.

This work was primarily supported by National Institutes of Health Grant GM U54069338 to the LIPID MAPS Consortium. The authors declare no competing interest.

Manuscript received 11 November 2014 and in revised form 17 January 2015.

Published, JLR Papers in Press, January 17, 2015

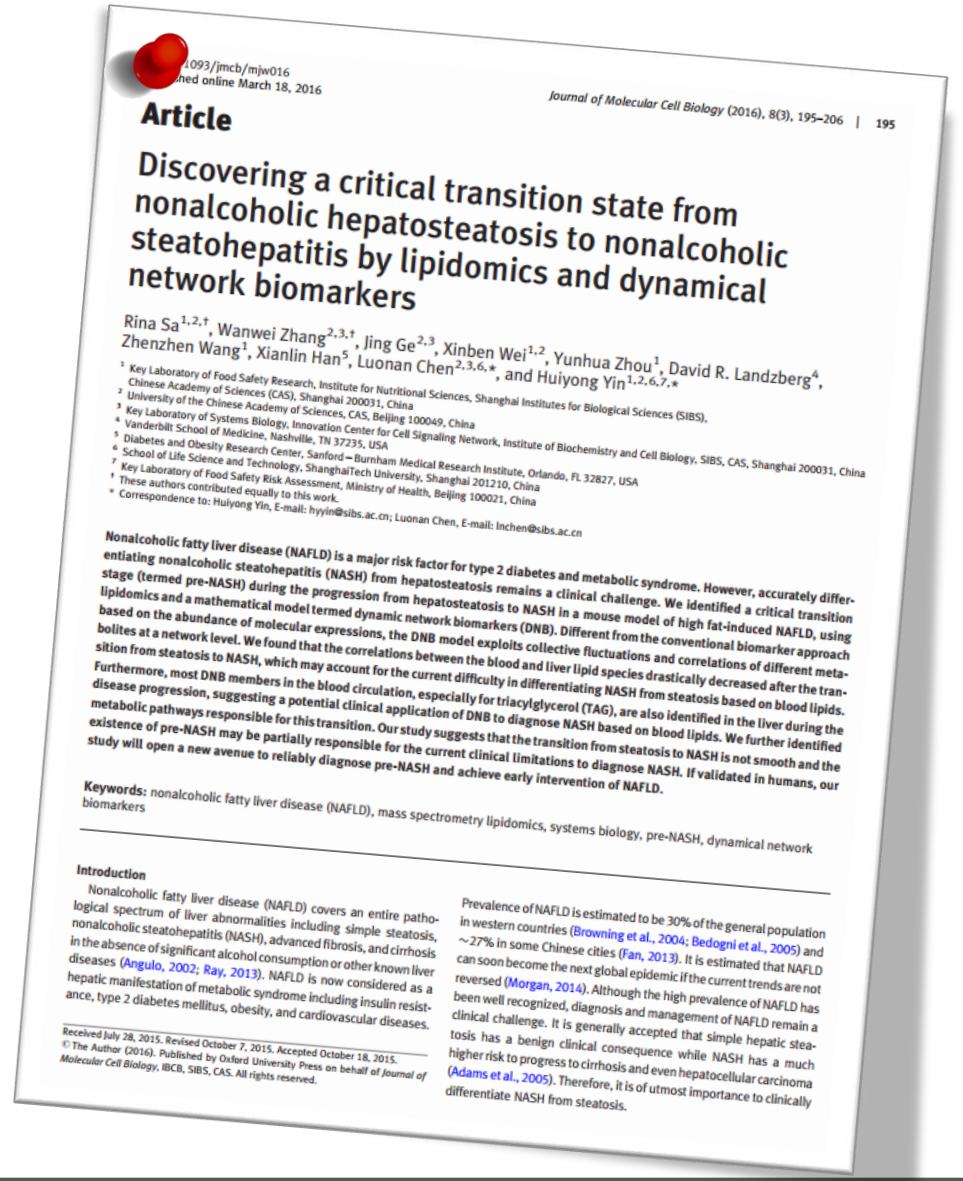
DOI 10.1194/jlr.P056002

[1] Guest editor for this article was Arthur A. Spector, University of Iowa (Emeritus).

[2] To whom correspondence should be addressed.

e-mail: alex.brown@vanderbilt.edu (H.A.B.); shankar@ucsd.edu (S.S.)

[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of seven figures, four tables, and five data files.



4093/jmcb/mjw016
hed online March 18, 2016

Article

Discovering a critical transition state from nonalcoholic hepatosteatosis to nonalcoholic steatohepatitis by lipidomics and dynamical network biomarkers

Rina Sa[1,2,†], Wanwei Zhang[2,3,†], Jing Ge[2,3], Xinben Wei[1,2], Yunhua Zhou[1], David R. Landzberg[4], Zhenzhen Wang[1], Xianlin Han[5], Luonan Chen[2,3,6,*], and Huiyong Yin[1,2,6,7,*]

[1] Key Laboratory of Food Safety Research, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS), Shanghai 200031, China
[2] University of the Chinese Academy of Sciences, CAS, Beijing 100049, China
[3] Key Laboratory of Systems Biology, Innovation Center for Cell Signaling Network, Institute of Biochemistry and Cell Biology, SIBS, CAS, Shanghai 200031, China
[4] Vanderbilt School of Medicine, Nashville, TN 37235, USA
[5] Diabetes and Obesity Research Center, Sanford–Burnham Medical Research Institute, Orlando, FL 32827, USA
[6] School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China
[7] Key Laboratory of Food Safety Risk Assessment, Ministry of Health, Beijing 100021, China
† These authors contributed equally to this work.
* Correspondence to: Huiyong Yin, E-mail: hyyin@sibs.ac.cn; Luonan Chen, E-mail: lnchen@sibs.ac.cn

Nonalcoholic fatty liver disease (NAFLD) is a major risk factor for type 2 diabetes and metabolic syndrome. However, accurately differentiating nonalcoholic steatohepatitis (NASH) from hepatosteatosis remains a clinical challenge. We identified a critical transition stage (termed pre-NASH) during the progression from hepatosteatosis to NASH in a mouse model of high fat-induced NAFLD, using lipidomics and a mathematical model termed dynamic network biomarkers (DNB). Different from the conventional biomarker approach based on the abundance of molecular expressions, the DNB model exploits collective fluctuations and correlations of different metabolites at a network level. We found that the correlations between the blood and liver lipid species drastically decreased after the transition from steatosis to NASH, which may account for the current difficulty in differentiating NASH from steatosis based on blood lipids. Furthermore, most DNB members in the blood circulation, especially for triacylglycerol (TAG), are also identified in the liver during the disease progression, suggesting a potential clinical application of DNB to diagnose NASH based on blood lipids. We further identified metabolic pathways responsible for this transition. Our study suggests that the transition from steatosis to NASH is not smooth and the existence of pre-NASH may be partially responsible for the current clinical limitations to diagnose NASH. If validated in humans, our study will open a new avenue to reliably diagnose pre-NASH and achieve early intervention of NAFLD.

Keywords: nonalcoholic fatty liver disease (NAFLD), mass spectrometry lipidomics, systems biology, pre-NASH, dynamical network biomarkers

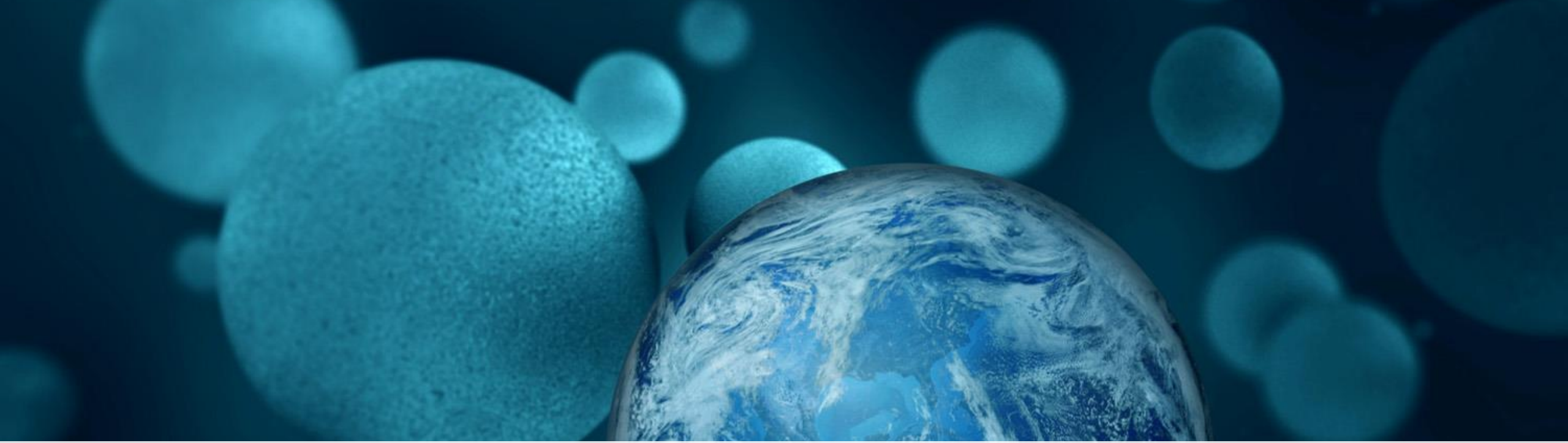
Introduction

Nonalcoholic fatty liver disease (NAFLD) covers an entire pathological spectrum of liver abnormalities including simple steatosis, nonalcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis in the absence of significant alcohol consumption or other known liver diseases (Angulo, 2002; Ray, 2013). NAFLD is now considered as a hepatic manifestation of metabolic syndrome including insulin resistance, type 2 diabetes mellitus, obesity, and cardiovascular diseases.

Prevalence of NAFLD is estimated to be 30% of the general population in western countries (Browning et al., 2004; Bedogni et al., 2005) and ~27% in some Chinese cities (Fan, 2013). It is estimated that NAFLD can soon become the next global epidemic if the current trends are not reversed (Morgan, 2014). Although the high prevalence of NAFLD has been well recognized, diagnosis and management of NAFLD remain a clinical challenge. It is generally accepted that simple hepatic steatosis has a benign clinical consequence while NASH has a much higher risk to progress to cirrhosis and even hepatocellular carcinoma (Adams et al., 2005). Therefore, it is of utmost importance to clinically differentiate NASH from steatosis.

Received July 28, 2015. Revised October 7, 2015. Accepted October 18, 2015.

ThermoFisher SCIENTIFIC



ThermoFisher SCIENTIFIC

Addressing the Challenges in Untargeted Lipidomics

Challenges of Lipid Identification and Quantification

Lipidomics Challenges	Requirements of LC-MS^n Platform
Diversity in structures and physical chemical properties	Higher resolving power for both the HPLC separation and for the MS analysis
Thousands of isomeric and isobaric species	Faster effective MS/MS scan speed, excellent mass accuracy and multiple dissociation techniques
Very low to very high concentrations	High sensitivity and wide dynamic range for both MS and MS^n spectra and for quantitation
Time consuming lipid ID	Dedicated software for automated lipid molecular identification and quantification

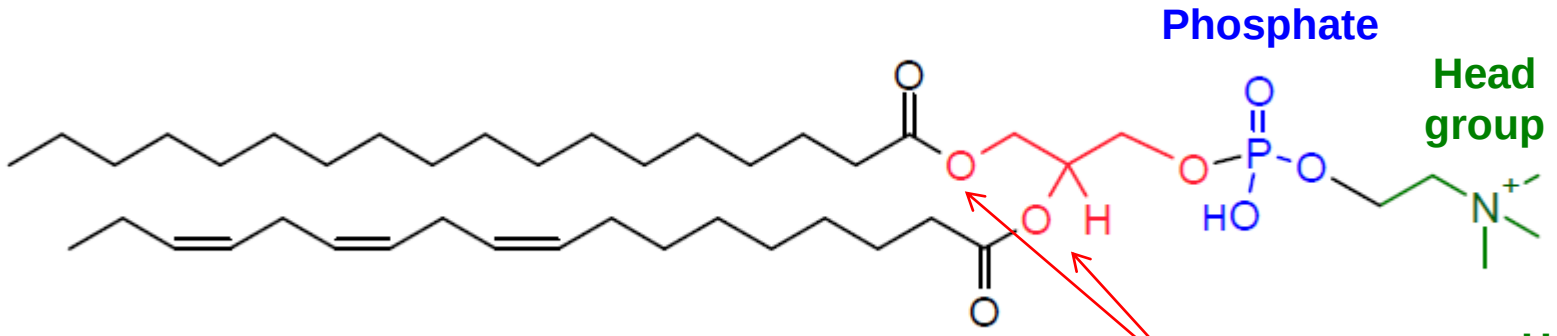
LipidMaps – Lipid Classification

Category	Abbrev.	Example
Fatty acyls	FA	oleic acid (1-octadecenoic acid)
Glycerolipids	GL	1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycerol
Glycero-phospholipids	GP	1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine
Sphingolipids	SP	N-(tetradecanoyl)-sphing-4-enine
Sterol lipids	ST	cholest-5-en-3β-ol
Prenol lipids	PR	2E,6E-farnesol
Saccharolipids	SL	UDP-3-O-(3R-hydroxy-tetradecanoyl)-αd-N-acetylglucosamine
Polyketides	PK	aflatoxin B_1

References:

Fahy E, et al., **A comprehensive classification system for lipids.** *J. Lipid Res.* (2005) 46: 839-861.
Fahy E, et al., **Update of the LIPID MAPS comprehensive classification system for lipids.** *J. Lipid Res.* (2009) 50:S9-S14

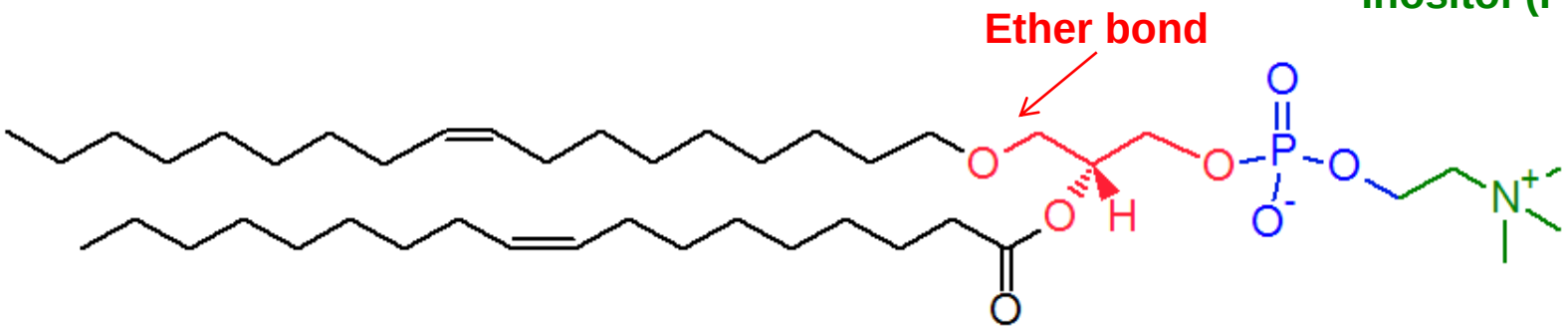
Lipid Nomenclature: Glycerophospholipids



Molecular species composition:
PC 18:0/18:3

Sum composition:
PC 36:3

Hydrogen (PA)
Choline (PC)
Ethanolamine (PE)
Serine (PS)
Glycerol (PG)
Inositol (PI)



Molecular species composition:
PC 18:1p/18:1e

Sum composition:
PC 36:2e

Challenges of Lipid Identification and Quantification

Lipidomics Challenges	Requirements of LC-MS^n Platform
Diversity in structures and physical chemical properties	Higher resolving power for both the HPLC separation and for the MS analysis
Thousands of isomeric and isobaric species	Faster effective MS/MS scan speed, excellent mass accuracy and multiple dissociation techniques
Very low to very high concentrations	High sensitivity and wide dynamic range for both MS and MS^n spectra and for quantitation
Time consuming lipid ID	Dedicated software for automated lipid molecular identification and quantification

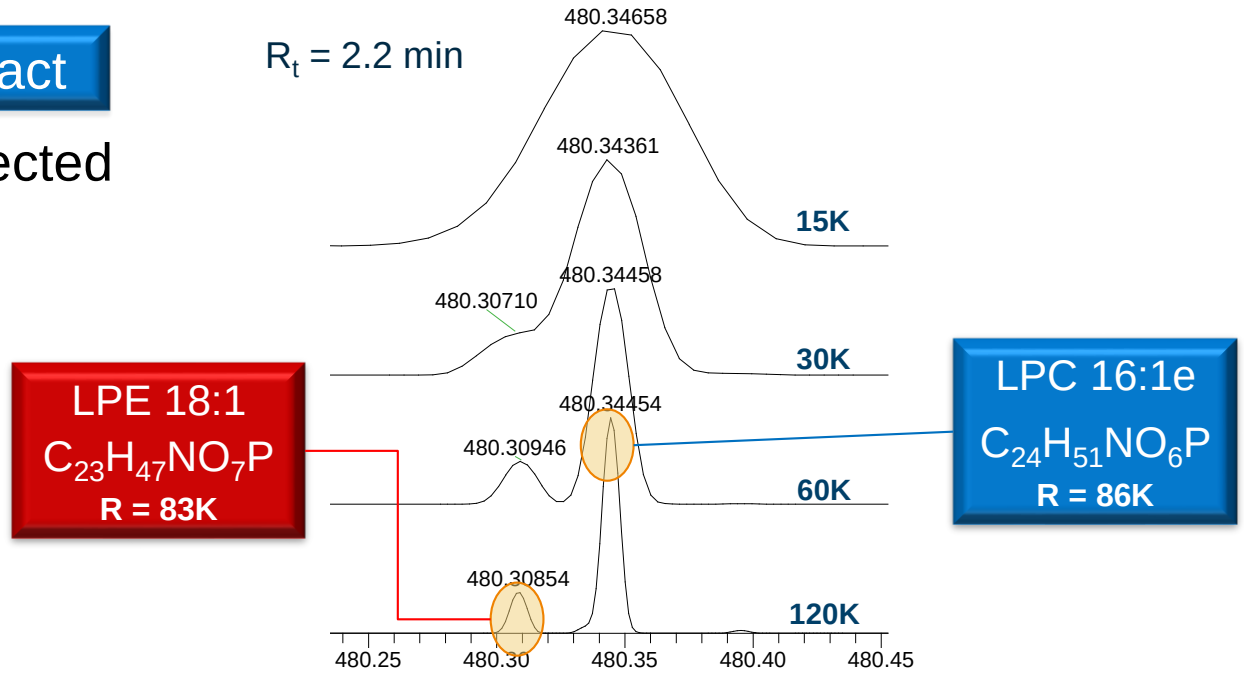
Resolving Isobaric Species Improves ID and Quan

Bovine Heart Extract

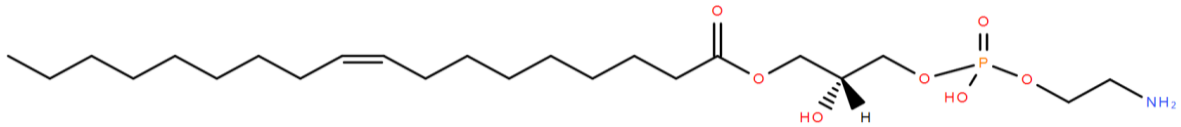
500ng/µL x 2µL injected

30min LC-MS run

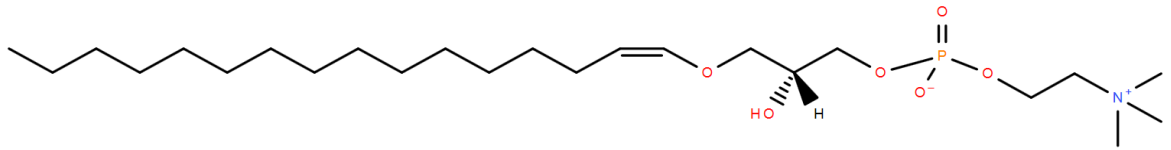
Q Exactive HF



Lyso PE 18:1



LPC 16:1e/16:0p

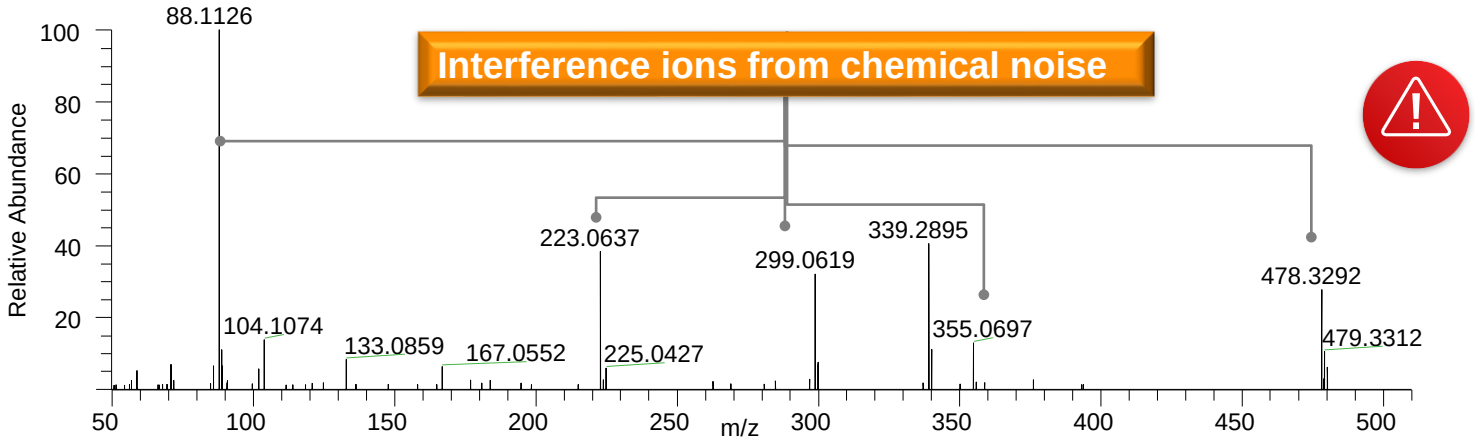


ThermoFisher SCIENTIFIC

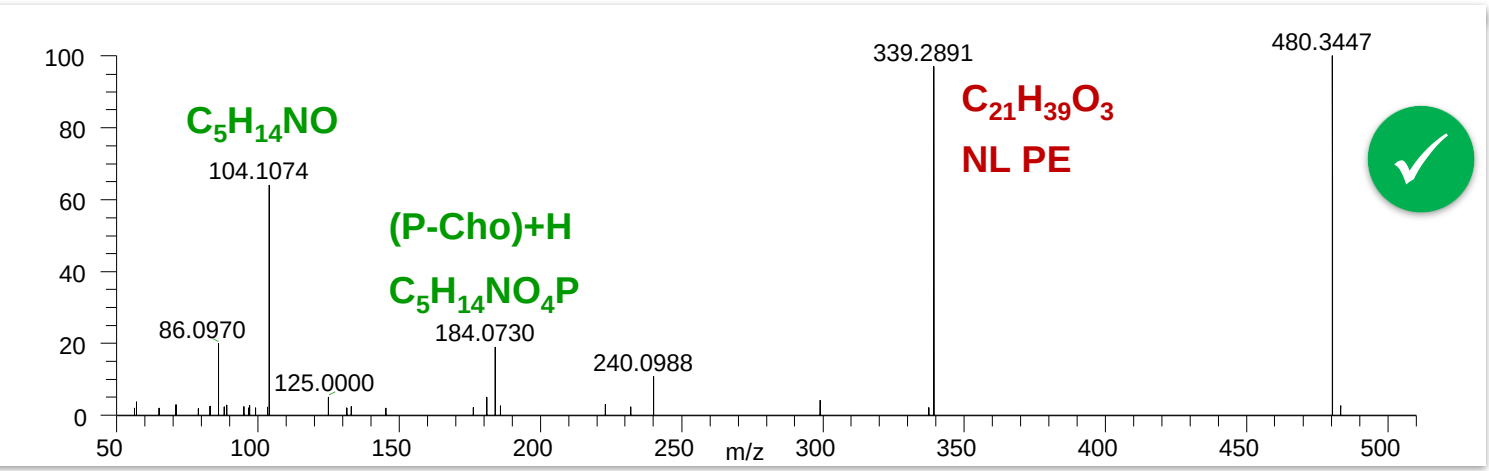
Higher Specificity with 1-amu Isolation Width MS^2

LPC(16:0p)+H and **LPE(18:1)+H** from Bovine heart extract, 0.5µg/µL x 2µL injected

IW: 4.0 amu



IW: 1.0 amu



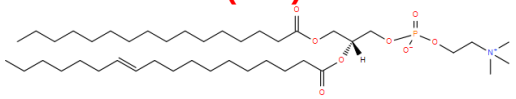
ThermoFisher SCIENTIFIC

LC/MS and MS/MS are Required to Identify Lipid Molecular Species

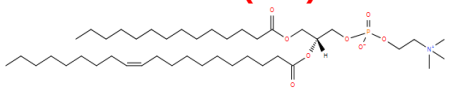
25 PC Isomers sharing same formula

LipidIon	m/z	Formula
PC(10:0/24:1)+H	760.5851	C42 H83 O8 N1 P1
PC(11:0/23:1)+H	760.5851	C42 H83 O8 N1 P1
PC(12:0/22:1)+H	760.5851	C42 H83 O8 N1 P1
PC(13:0/21:1)+H	760.5851	C42 H83 O8 N1 P1
PC(14:0/20:1)+H	760.5851	C42 H83 O8 N1 P1
PC(14:1/20:0)+H	760.5851	C42 H83 O8 N1 P1
PC(15:0/19:1)+H	760.5851	C42 H83 O8 N1 P1
PC(16:0/18:1)+H	760.5851	C42 H83 O8 N1 P1
PC(16:1/18:0)+H	760.5851	C42 H83 O8 N1 P1
PC(17:0/17:1)+H	760.5851	C42 H83 O8 N1 P1
PC(17:1/17:0)+H	760.5851	C42 H83 O8 N1 P1
PC(18:0/16:1)+H	760.5851	C42 H83 O8 N1 P1
PC(18:1/16:0)+H	760.5851	C42 H83 O8 N1 P1
PC(19:1/15:0)+H	760.5851	C42 H83 O8 N1 P1
PC(20:0/14:1)+H	760.5851	C42 H83 O8 N1 P1
PC(20:1/14:0)+H	760.5851	C42 H83 O8 N1 P1
PC(21:1/13:0)+H	760.5851	C42 H83 O8 N1 P1
PC(22:0/12:1)+H	760.5851	C42 H83 O8 N1 P1
PC(22:1/12:0)+H	760.5851	C42 H83 O8 N1 P1
PC(23:0/11:1)+H	760.5851	C42 H83 O8 N1 P1
PC(23:1/11:0)+H	760.5851	C42 H83 O8 N1 P1
PC(24:0/10:1)+H	760.5851	C42 H83 O8 N1 P1
PC(24:1/10:0)+H	760.5851	C42 H83 O8 N1 P1
PC(25:1/9:0)+H	760.5851	C42 H83 O8 N1 P1
PC(26:1/8:0)+H	760.5851	C42 H83 O8 N1 P1

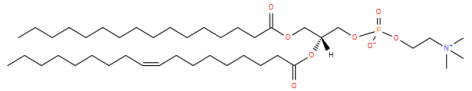
PC 16:0/18:1(11E)



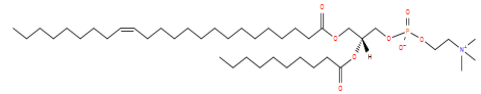
PC 14:0/20:1(11Z)



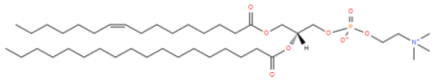
PC 16:0/18:1(9Z)



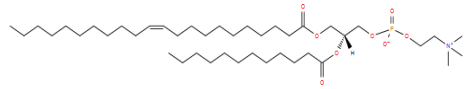
PC 24:1(15Z)/10:0

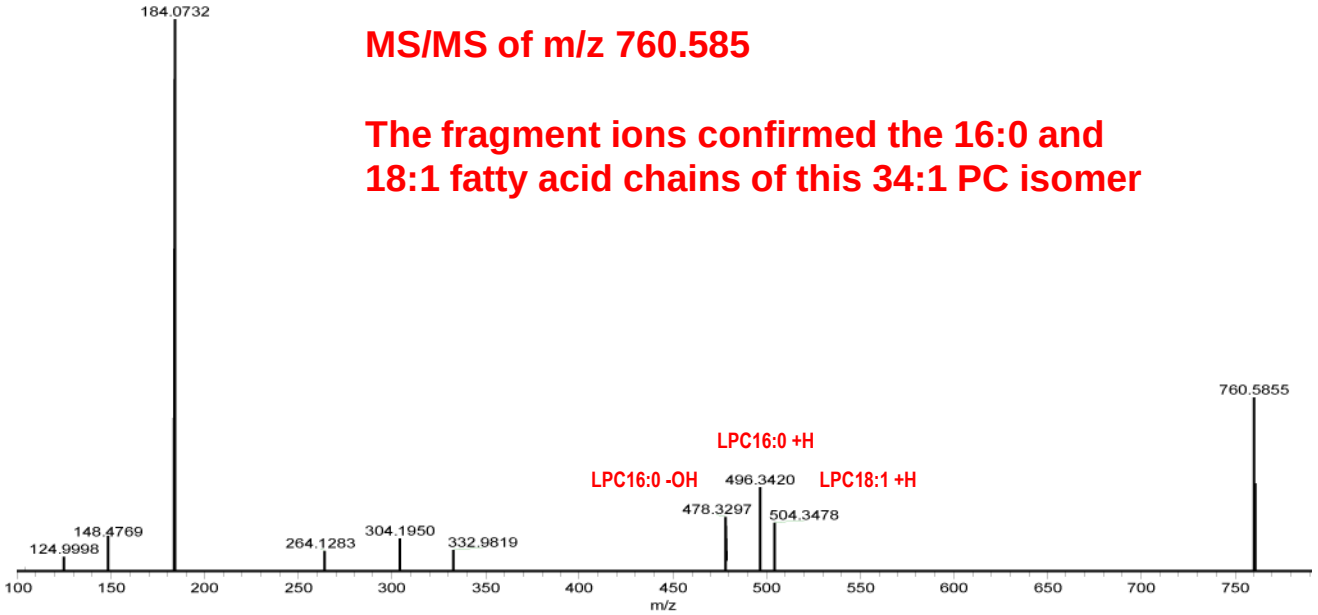


PC 16:1/18:0(9Z)/18:0



PC 22:1(11Z)/12:0





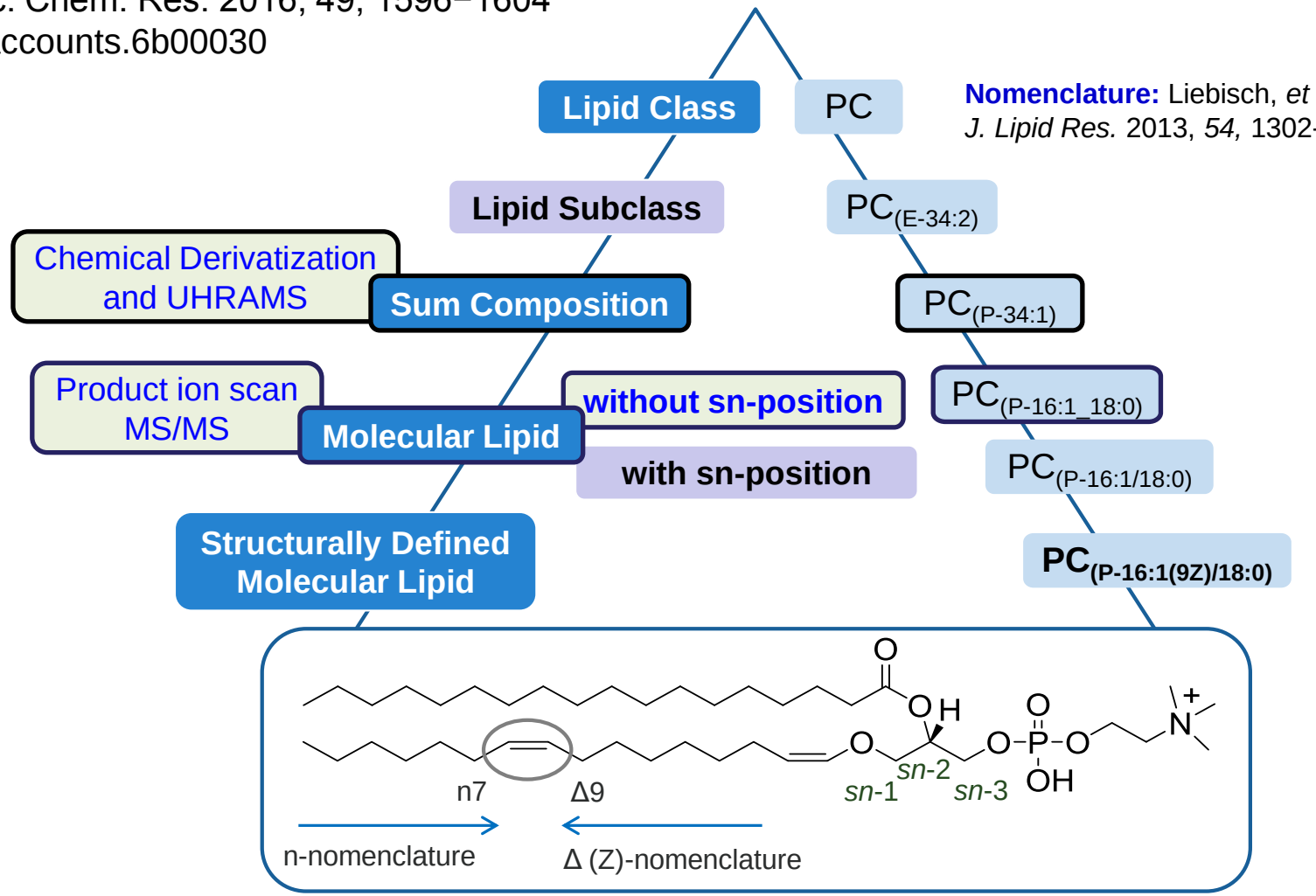
MS/MS of m/z 760.585

The fragment ions confirmed the 16:0 and 18:1 fatty acid chains of this 34:1 PC isomer

ThermoFisher SCIENTIFIC

Hierarchal Scheme of Lipid Classification

Ryan and Reid. Acc. Chem. Res. 2016, 49, 1596−1604
DOI: 10.1021/acs.accounts.6b00030



Nomenclature: Liebisch, *et al.* *J. Lipid Res.* 2013, *54,* 1302-1309.

Adapted from: Ekroos, K. In Lipidomics, Ch 1. 2012 Wiley-VCH Verlag GmbH & Co

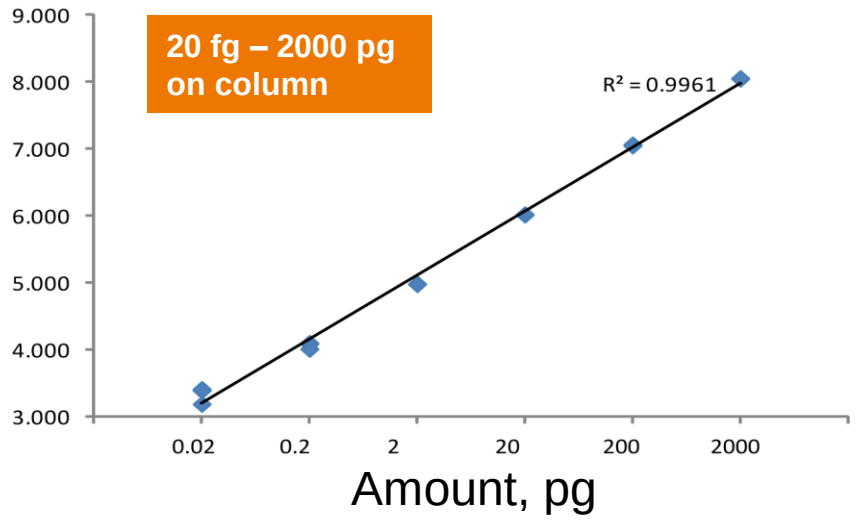
Challenges of Lipid Identification and Quantification

Lipidomics Challenges	Requirements of LC-MS^n Platform
Diversity in structures and physical chemical properties	Higher resolving power for both the HPLC separation and for the MS analysis
Thousands of isomeric and isobaric species	Faster effective MS/MS scan speed, excellent mass accuracy and multiple dissociation techniques
Very low to very high concentrations	High sensitivity and wide dynamic range for both MS and MS^n spectra and for quantitation
Time consuming lipid ID	Dedicated software for automated lipid molecular identification and quantification

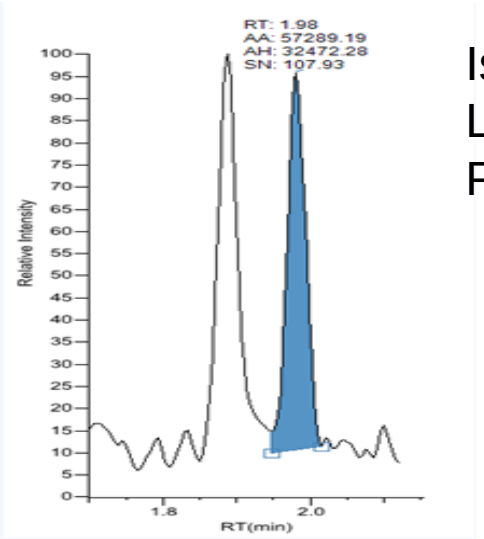
Orbitrap Based Mass Spectrometers Offer High Sensitivity and Wide Dynamic Range

QE HF MS

17:0-14:1 PC spiked in bovine liver lipid extract



LOQ 1.6 fmol, LOD 46 amol



Isotopic labeled Leucine spiked in Plasma

Orbitrap Fusion Lumos MS

ThermoFisher SCIENTIFIC

Challenges of Lipid Identification and Quantification

Lipidomics Challenges	Requirements of LC-MS^n Platform
Diversity in structures and physical chemical properties	Higher resolving power for both the HPLC separation and for the MS analysis
Thousands of isomeric and isobaric species	Faster effective MS/MS scan speed, excellent mass accuracy and multiple dissociation techniques
Very low to very high concentrations	High sensitivity and wide dynamic range for both MS and MS^n spectra and for quantitation
Time consuming lipid ID	Dedicated software for automated lipid molecular identification and quantification

High Throughput Lipid Identification and Quantitation

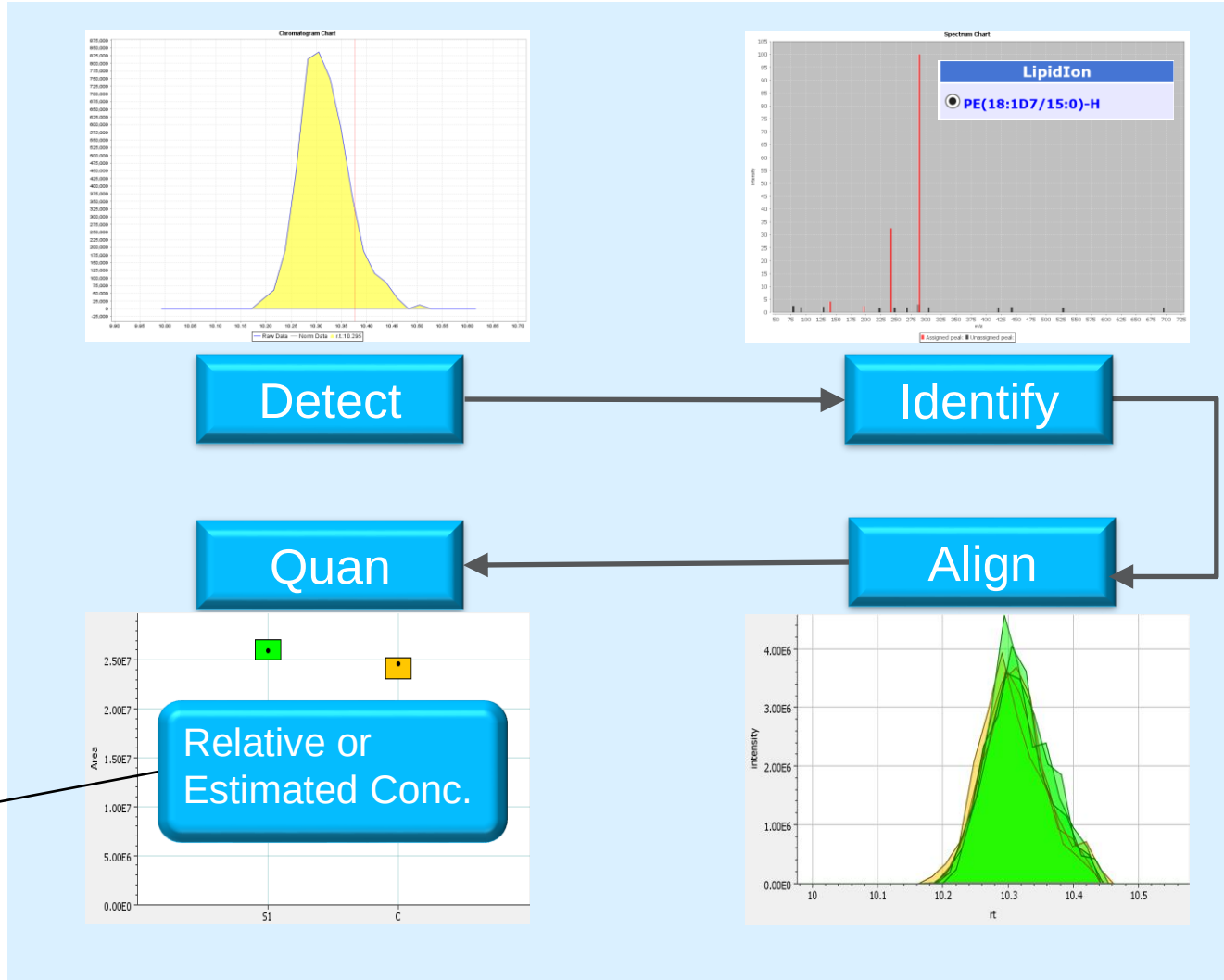
LipidSearch software addresses the time-consuming ID bottleneck in lipidomics

LipidSearch

Lipid ID & Quan

- HRAM (all file types)
- LC/dd MS-MS/MS^3 data
- ID based on MS^2 & MS^3
- Integrate multiple groups and rel. quan
- Calculate estimated concentration

Lipid Molec	Calc Mass	Formula	Rt min	Main Area [c*]	Main Area [s1**]	CV [c]	CV [s1]	Est. ng/mL [c]	Est. ng/mL [s1]	Fold Change (s1/c)
PE(d7-18:1/15:0)	710.5591	C38 H67 O8 N1 P1 D7	10.3	2.52E+07	2.70E+07	4.6	3.9	830	830	1.00
PE(18:1/20:4)	765.5309	C43 H76 O8 N1 P1	9.8	2.62E+07	7.44E+07	5.4	6.0	863	2,284	2.65
PE(16:0/18:1)	717.5309	C39 H76 O8 N1 P1	11.5	2.37E+07	6.65E+07	4.3	3.7	780	2,042	2.62
PE(18:0/20:3)	769.5622	C43 H80 O8 N1 P1	9.9	2.39E+07	6.20E+07	6.4	3.6	789	1,904	2.41
PE(18:0/18:2)	743.5465	C41 H78 O8 N1 P1	12.1	2.91E+07	7.25E+07	2.2	2.2	961	2,227	2.32
PE(16:0/20:4)	739.5152	C41 H74 O8 N1 P1	9.6	8.64E+07	2.06E+08	4.6	8.6	2,850	6,325	2.22
PE(18:0/18:1)	745.5622	C41 H80 O8 N1 P1	10.9	8.63E+07	1.69E+08	0.2	1.1	2,847	5,176	1.82
PE(18:1/18:1)	743.5465	C41 H78 O8 N1 P1	9.3	1.11E+08	1.95E+08	4.4	4.6	3,648	5,992	1.64
PE(16:0/22:6)	763.5152	C43 H74 O8 N1 P1	9.2	1.85E+08	2.90E+08	12.6	5.7	6,100	8,903	1.46



ThermoFisher SCIENTIFIC

Untargeted Lipidomics using Orbitrap Analyzer and Lipid Search 4.1 Software

- Robust and reproducible lipidome profiling
- Automatic data processing using Lipid Search 4.1 software
- Orbitrap high resolving power differenciates overlap isobaric species and backgrounds
- Confident lipid identification and precise quantitation
- Highly efficient analysis with scan-to-scan polarity switching

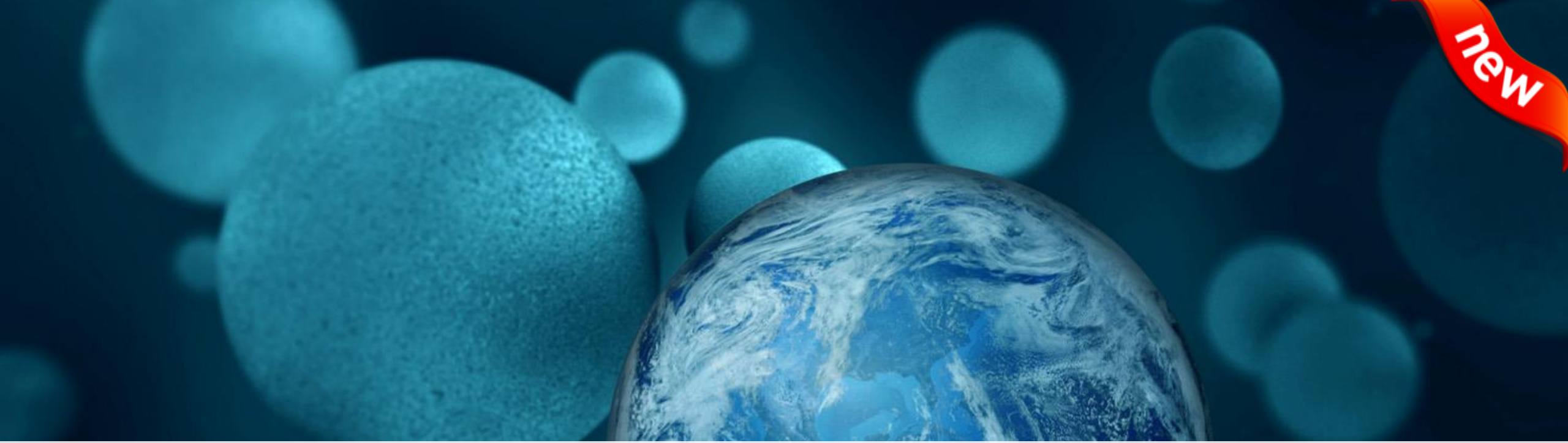
Cells, Tissue, Plasma samples

Crude Lipid Extract

Total lipid extracts are analyzed by LC-dd-MS/MS

Lipid molecular species are identified, quantified and biological insights derived from powerful software

- Thermo Scientific™ Accucore™ C30 LC column
- Thermo Scientific™ Vanquish™ UHPLC System
- Thermo Scientific™ Quadrupole-Orbitrap™ MS
- Thermo Scientific™ LipidSearch™ Software



ThermoFisher SCIENTIFIC

Novel High-Throughput Lipid Profiling and Quantitation

Untargeted Lipidomics using Orbitrap Analyzer and Lipid Search 4.1 Software

new

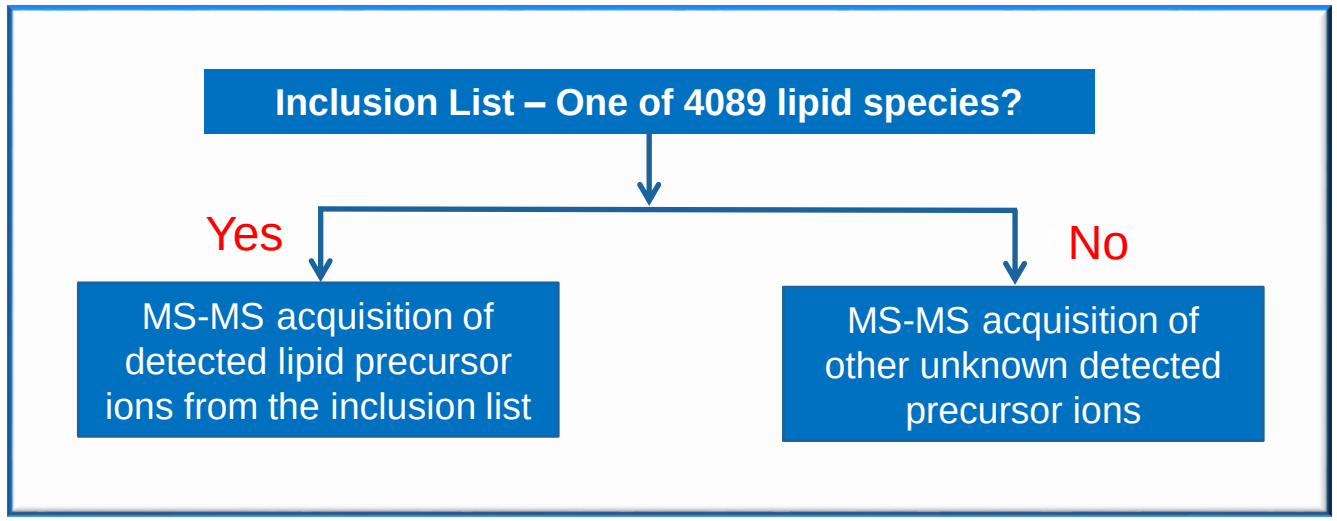
- Human Plasma Samples
  - Control: healthy patient
  - Diabetic: diabetic patient
- **Lipid Extraction**
  - 60 µL Plasma
  - 10 µL of SPLASH
  - Final volume 100 µL
- **LC/MS Analysis**
  - Triplicate inj., 2 µL
  - Pos. & Neg ion separate
  - Inclusion list >4000 lipids

Cells, Tissue, Plasma samples

Crude Lipid Extract

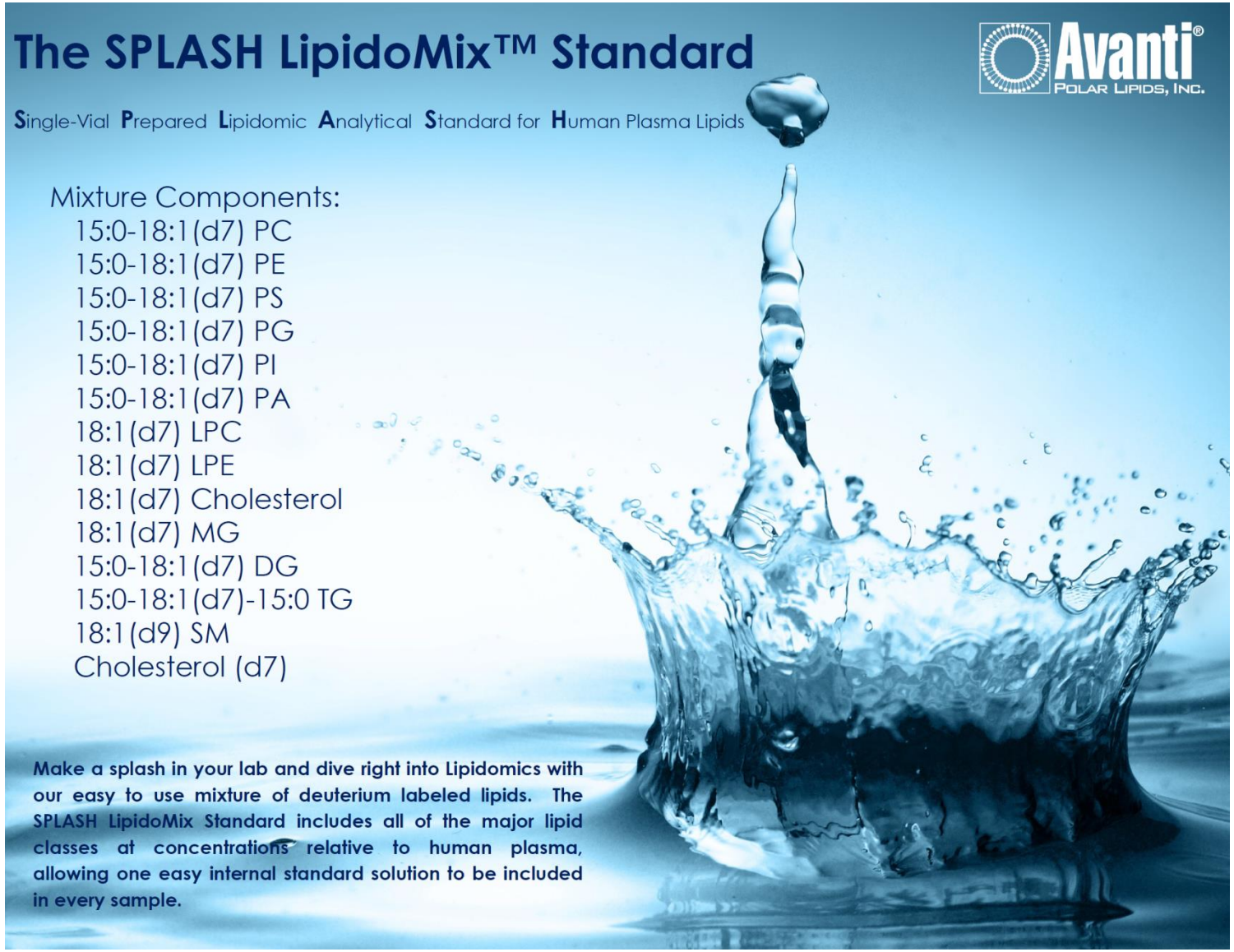
Total lipid extracts are analyzed by LC-dd-MS/MS

Lipid molecular species are identified, quantified and biological insights derived from powerful software



ThermoFisher SCIENTIFIC

The SPLASH™ Lipidomix® Mass Spec Standard

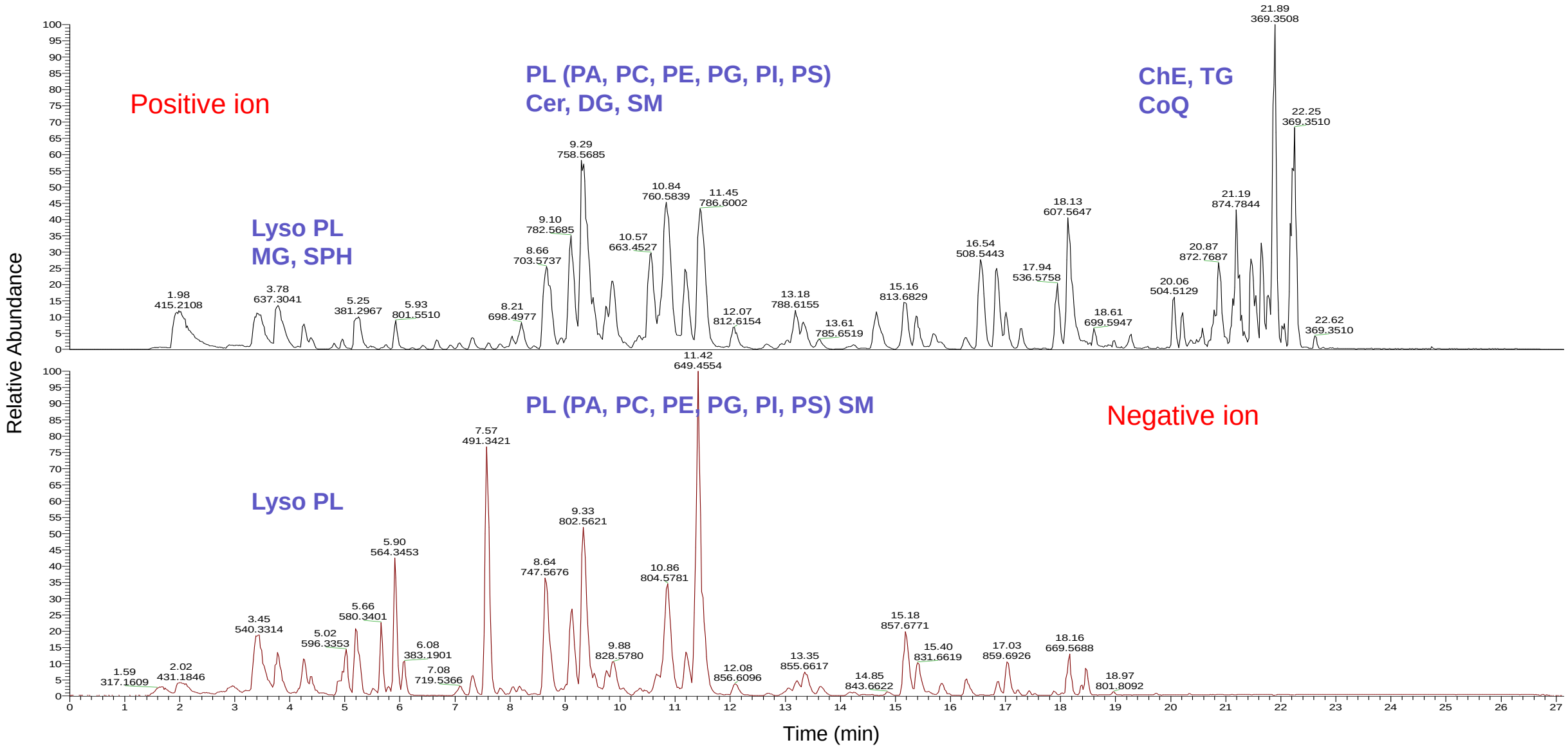


Number of Lipid Species Covered by the Inclusion List

Components	Lipid Class	# Lipid Species	FA Sum Comp.
15:0-18:1(d7) PC	PC	492	17:0 - 51:1
15:0-18:1(d7) PE	PE	511	16:0 - 48:2
15:0-18:1(d7) PS	PS	398	27:1 - 48:6
15:0-18:1(d7) PG	PG	332	32:1 - 49:6
15:0-18:1(d7) PI	PI	188	32:1 - 42:6
15:0-18:1(d7) PA	PA	302	33:1 - 49:8
18:1(d7) LPC	LPC	129	14:0 - 33:3
18:1(d7) LPE	LPE	54	14:0 - 22:0
18:1(d7) Chol Ester	Chol Ester	57	16:2 - 26:5
18:1(d7) MG	MG	164	15:2 - 38:0
15:0-18:1(d7) DG	DG	509	28:4 - 57:4
15:0-18:1(d7)-15:0 TG	TG	759	30:0 - 72:5
18:1(d9) SM	SM	178	30:2 - 53:6
Cholesterol (d7)	Cholesterol	1	
Total		**4074**	

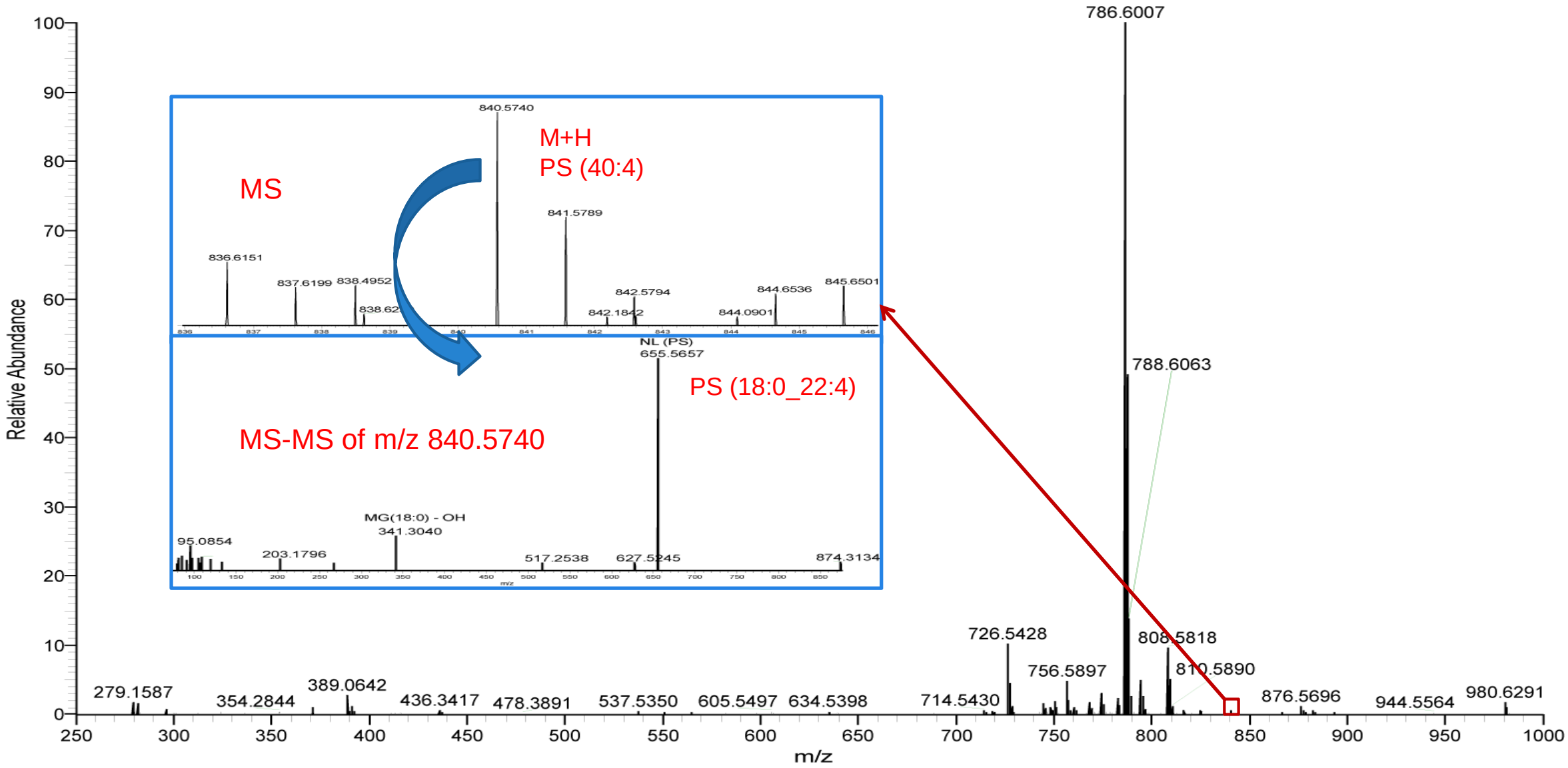
- The precursor ions of targeted lipid species are generated *in-silico* using the lipid database included in the Lipid Search software.
- The lipid class selection corresponds to the isotopic labeled lipid counterparts of Splash standard mixture.
- The range of fatty acyl sum composition per lipid class was selected based on previous discovery experimental data.

Total Ion Chromatograms: Positive and Negative Ion MS Scans



ThermoFisher SCIENTIFIC

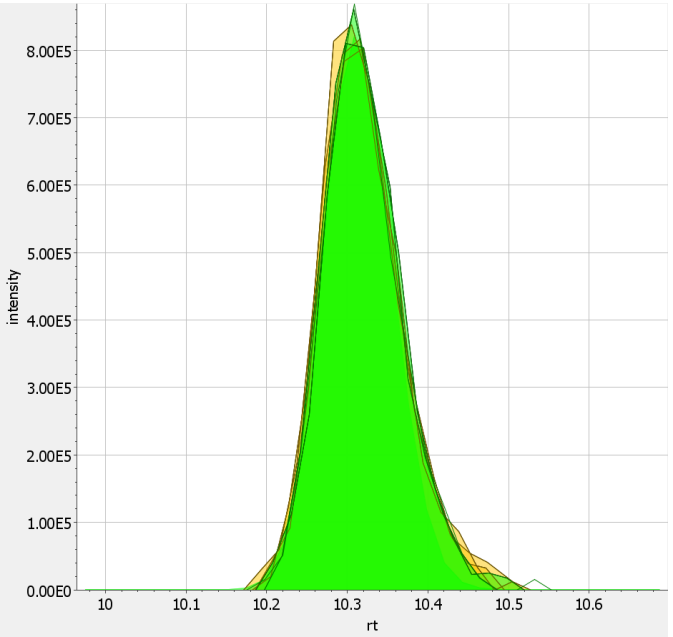
Confident Identification of Low Abundant PS Species



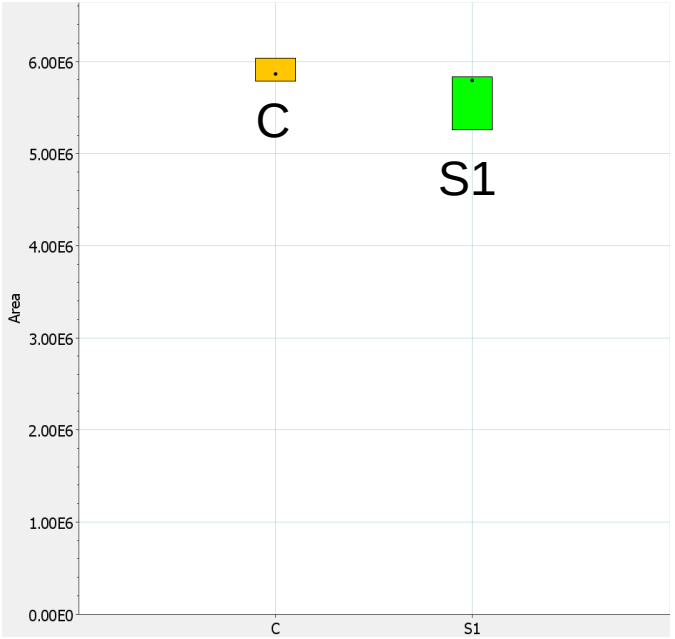
ThermoFisher SCIENTIFIC

LipidSearch Batch Data Processing and Quantitation

d_7-18:1/15:0 PE, 830 ng/mL



Control vs. Diabetic



Lipid Molec key	Ion key	Grade	Polarity	BaseRt	Obs Mz	Delta(m/z)	Delta(ppm)	Ion Formula	Area	Area RSD
PE(18:1D7/15:0)	-H	A	N	10.3064	709.5532	0.0013	1.8211	C38 H66 O8 N1 P1 D7	5.629E06	5.753E0
PE(18:1D7/15:0)	-H	A	N	10.3064	709.5537	0.0018	2.5224	C38 H66 O8 N1 P1 D7	5.894E06	2.177E0
PE(18:1D7/15:0)	**+H**	**B**	**P**	**10.3064**	**711.5661**	**-0.0003**	**-0.4384**	**C38 H68 O8 N1 P1 D7**	**2.599E07**	**3.960E0**
PE(18:1D7/15:0)	**+H**	**C**	**P**	**10.3064**	**711.5655**	**-0.0010**	**-1.3456**	**C38 H68 O8 N1 P1 D7**	**2.429E07**	**4.482E0**

Processing Steps:

1. Search each data file
2. Merge the search results
   Pos. and Neg. ion
3. Report includes ID's
   a) Estimated Quan (IS) or
   b) Rel. Amounts (no IS)

ThermoFisher SCIENTIFIC

Identification and Quantification for Partial List of PE Lipids in Human Plasma

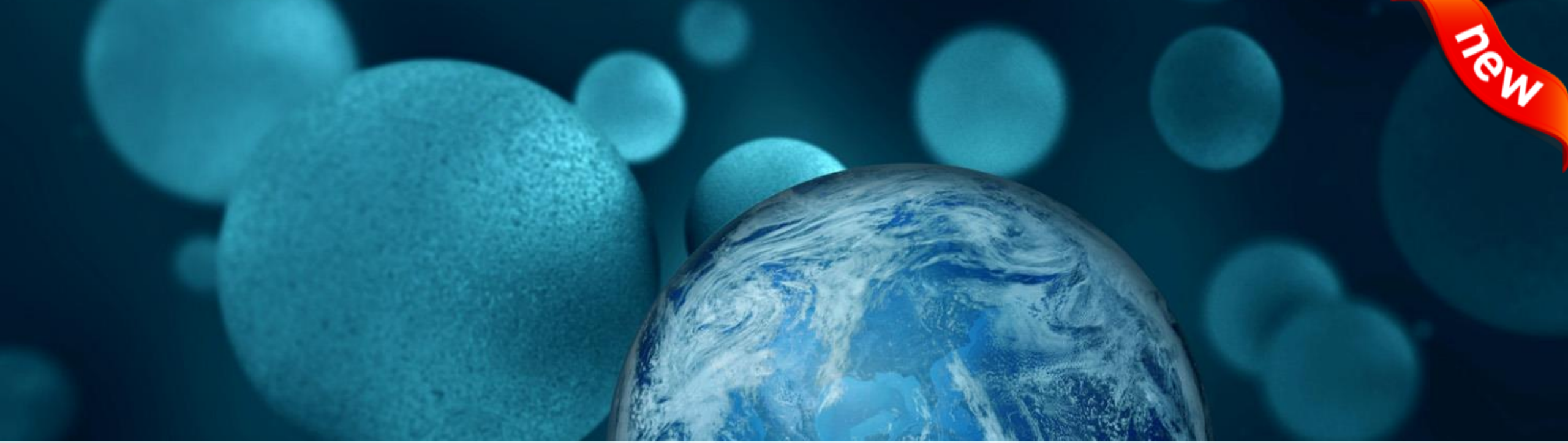
Lipid Molec	Calc Mass	Formula	RT min	Area control	Area diabetic	CV control	CV diabetic	Conc. ng/ml, control	Conc. ng/ml, diabetic	Fold Change
PE(18:1/20:4)	765.5309	C43 H76 O8 N1 P1	9.8	2.62E+07	7.44E+07	5.4	6.0	863	2284	2.65
PE(18:0/18:2)	743.5465	C41 H78 O8 N1 P1	12.1	2.91E+07	7.25E+07	2.2	2.2	961	2227	2.32
PE(16:0/20:4)	739.5152	C41 H74 O8 N1 P1	9.6	8.64E+07	2.06E+08	4.6	8.6	2850	6325	2.22
PE(18:0/18:1)	745.5622	C41 H80 O8 N1 P1	10.9	8.63E+07	1.69E+08	0.2	1.1	2847	5176	1.82
PE(18:1/18:1)	743.5465	C41 H78 O8 N1 P1	9.3	1.11E+08	1.95E+08	4.4	4.6	3648	5992	1.64
PE(16:0/22:6)	763.5152	C43 H74 O8 N1 P1	9.2	1.85E+08	2.90E+08	12.6	5.7	6100	8903	1.46
PE(20:0/18:2)	771.5778	C43 H82 O8 N1 P1	11.5	7.71E+07	1.18E+08	4.9	3.8	2542	3613	1.42
PE(18:0/20:4)	767.5465	C43 H78 O8 N1 P1	9.1	5.29E+07	7.66E+07	3.2	2.3	1746	2354	1.35
PE(18:0/20:4)	767.5465	C43 H78 O8 N1 P1	11.8	1.62E+08	2.23E+08	2.5	3.0	5347	6846	1.28
PE(18:0/22:6)	791.5465	C45 H78 O8 N1 P1	11.3	9.61E+07	1.23E+08	0.5	2.1	3169	3783	1.19
PE(18:0p/18:2)	727.5516	C41 H78 O7 N1 P1	13.3	5.28E+07	6.68E+07	8.0	6.8	1741	2053	1.18
PE(18:1p/20:4)	749.5359	C43 H76 O7 N1 P1	10.8	4.03E+07	5.09E+07	5.5	13.7	1328	1564	1.18
PE(16:0p/22:6)	747.5203	C43 H74 O7 N1 P1	10.1	5.56E+07	6.68E+07	3.0	4.4	1832	2052	1.12
PE(18:0/22:6)	791.5465	C45 H78 O8 N1 P1	8.7	3.40E+07	4.06E+07	1.4	7.2	1122	1246	1.11
PE(20:0/20:4)	795.5778	C45 H82 O8 N1 P1	11.2	2.63E+07	2.84E+07	4.2	3.8	867	872	1.01
PE(18:1D7/15:0), IS	710.5591	C38 H67 O8 N1 P1 D7	10.3	2.52E+07	2.70E+07	4.6	3.9	830	830	1.00
PE(18:0p/20:4)	751.5516	C43 H78 O7 N1 P1	13.0	9.90E+07	1.05E+08	1.5	2.6	3265	3220	0.99
PE(18:0p/22:6)	775.5516	C45 H78 O7 N1 P1	12.4	4.68E+07	4.02E+07	1.4	4.0	1544	1233	0.80

Identification and Quantitation Summary for Control and Diabetic Human Plasma

Lipid Sub-class	# Filtered Species	Estimated Concentration
AcCa	3	
Cer	26	
CerG1-G3	5	
ChE	17	☑
DG	25	☑
LPC	36	☑
LPE	2	☑
MG	8	☑
PA	2	☑
PC	427	☑
PE	41	☑
PG	1	☑
PI	14	☑
PS	9	☑
SM	124	☑
TG	340	☑
Total Species	1078	1044

Criteria for Data Filtering:

1. Specified main adduct ion
2. Quality of Identification
3. Peak Height
4. CV < 30%



ThermoFisher SCIENTIFIC

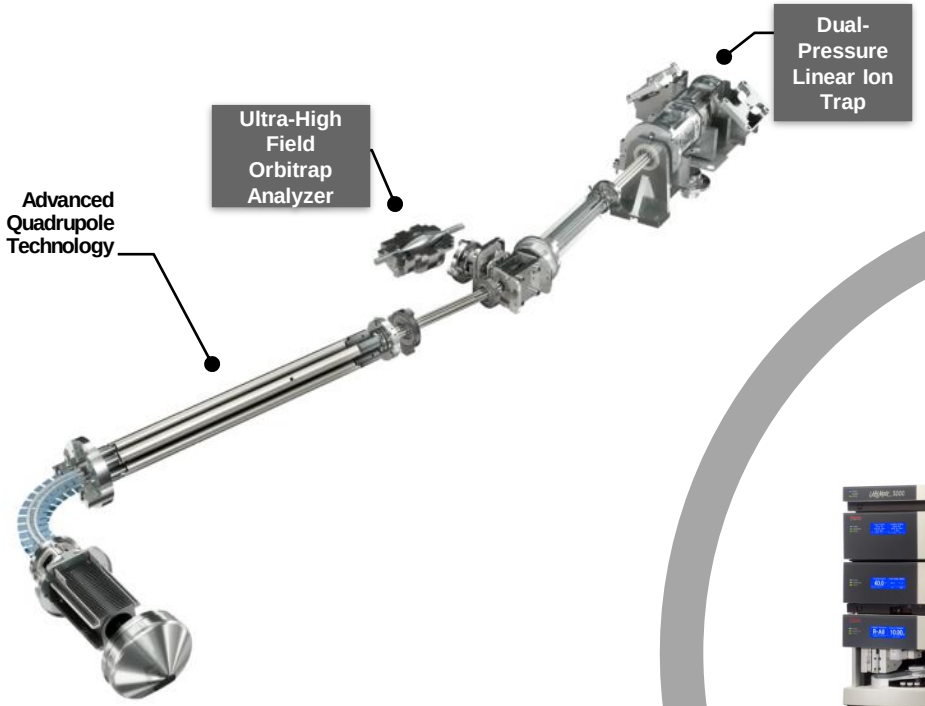
Next Generation Tools for Deeper Lipid Characterization

Advanced Challenges of Lipid Structure Characterization and Isotopic Analysis

Advanced Lipidomics Challenges	Requirements of LC-MS^n Platform
Separate and perform accurate quantification of isotopomers to elucidate biological mechanisms	Ultra-high resolution up to 1M to resolve isotopomers and perform quantitation
Isomeric lipid species with differences in carbon-carbon double bond position(s)	Fragmentation that provides unique structural information for carbon-carbon double bond location

Unique Features of Orbitrap Fusion Lumos MS – A Tribrid Orbitrap Mass Spectrometer

new



HCD MS/MS

CID MS^n

- LIPID IDENTIFICATION REQUIRES MORE THAN ONE TYPE OF DISSOCIATION TECHNIQUE
- ION TRAP MS^N EXPERIMENTS PROVIDE MORE DEFINITIVE STRUCTURAL INFORMATION

UVPD

- UNIQUE FRAGMENTATION FOR LIPIDOMICS CHARACTERIZATION
- OPTIONAL ON NEW AND EXISTING SYSTEMS

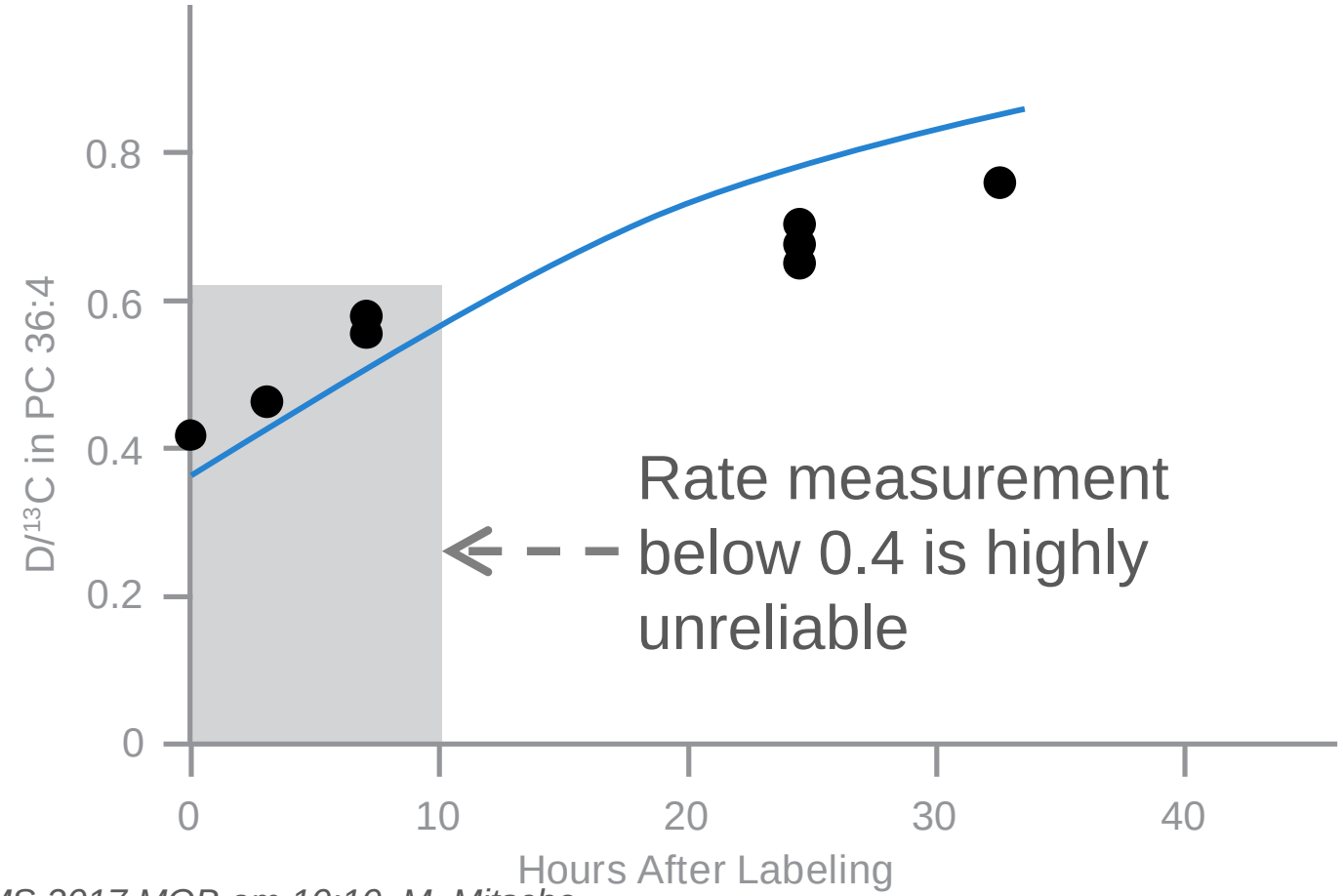
1M

- ULTRA-HIGH RESOLUTION FOR FINE ISOTOPE STRUCTURE
- OPTIONAL ON NEW AND EXISTING SYSTEMS

ThermoFisher SCIENTIFIC

Low-Resolution Measurements for Lipid Flux Analysis

^{13}C and D are unresolved in M+1, QQQ MS

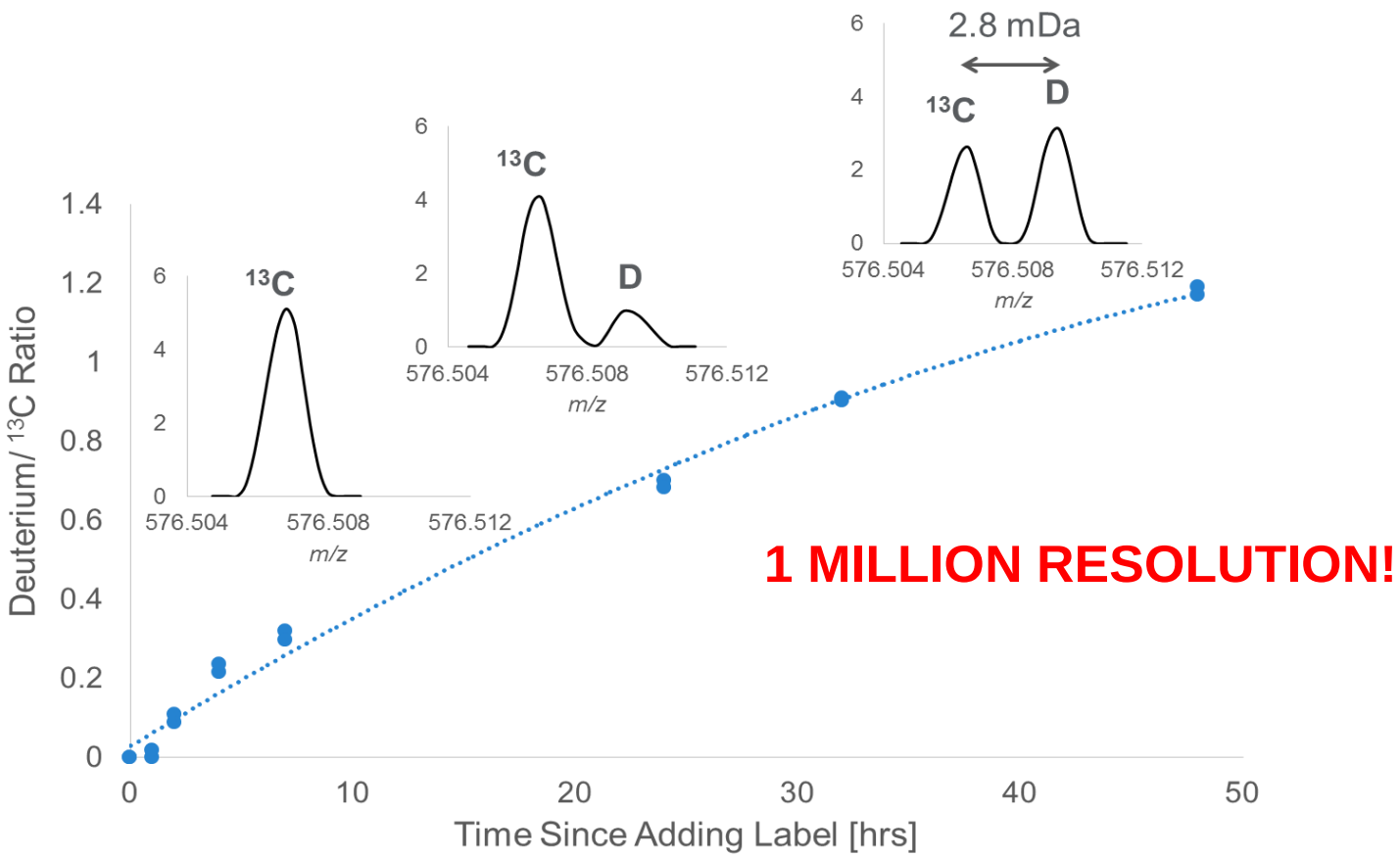


ASMS 2017 MOB am 10:10, M. Mitsche

Isotope Dilution Quantitation Using a Low Resolution Instrument

- ^{13}C background worsens detection limits translating into lack of accuracy of D incorporation rate measurements
- General lack of accuracy and precision
- Removing background noise requires separating ^{13}C and D, 2.8 mDa apart
- Only ultra-high resolution instruments can perform this

New 1M Ultra-High Resolution For Lipid Flux Analysis



Rate of deuterium incorporation for TAG 52:3 in human hepatoma cells (HuH7) labeled with 5% D_2O

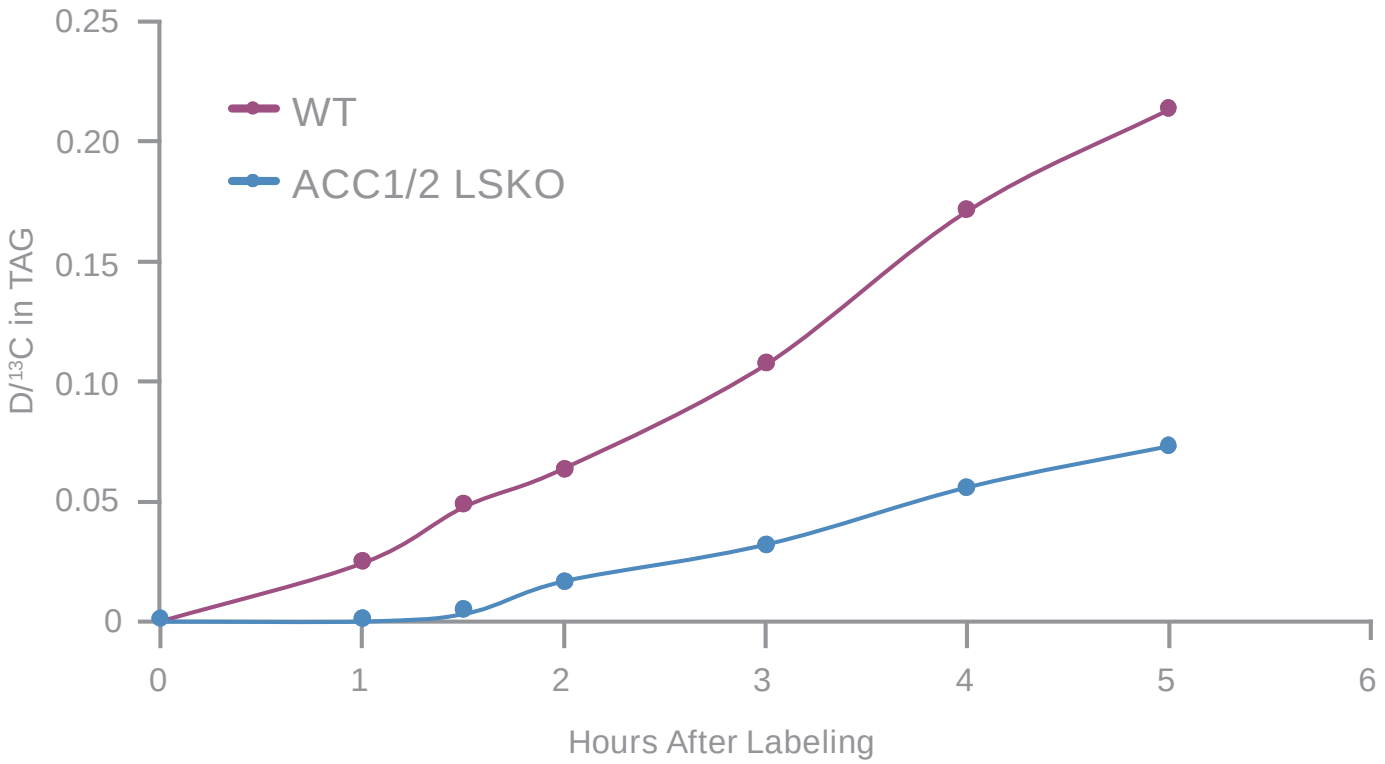
ASMS 2017 MOB am 10:10, M. Mitsche

Resolving Natural ^{13}C From Labeled Deuterium Isotope

- The Triacylglyceride precursor ion was fragmented using high energy collisional activation
- Deuterium incorporation levels were plotted for the fragment ion at *m/z* 576.5

New 1M Ultra-High Resolution for Lipid Flux Analysis

1 MILLION RESOLUTION!



Rate of deuterium incorporation for TAG 52:3 in ACC1/2 liver-specific knockout and wild type mouse derived primary hepatocyte cells labeled with 5% D_2O

ASMS 2017, MOB am 10:10, M. Mitsche

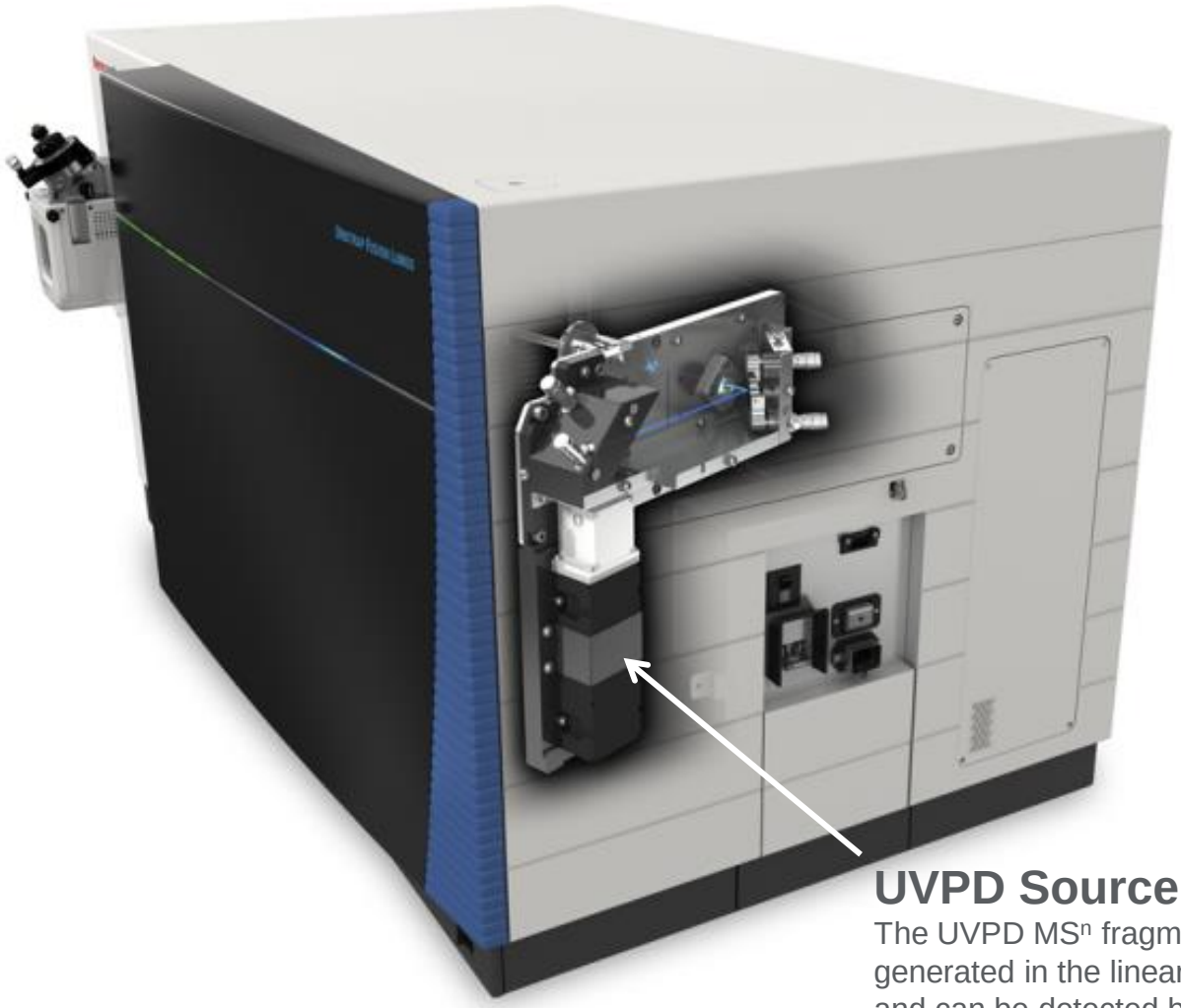
Resolving Natural ^{13}C From Labeled Deuterium Isotope

- ACC1 and ACC2 are rate limiting enzymes in fatty acid biosynthesis
- Removing enzymes from liver reduces fatty acid biosynthesis based on radio-labeling studies
- Deuterium uptake levels were measured for the TAG52:3 fragment ion at *m/z* 576.5

Advanced Challenges of Lipid Structure Characterization and Isotopic Analysis

Advanced Lipidomics Challenges	Requirements of LC-MS^n Platform
Separate and perform accurate quantification of isotopomers to elucidate biological mechanisms	Ultra-high resolution up to 1M to resolve isotopomers and perform quantitation
Isomeric lipid species with differences in carbon-carbon double bond position(s)	Fragmentation that provides unique structural information for carbon-carbon double bond location

UVPD Implementation (Class 1 Laser System)



UVPD Source

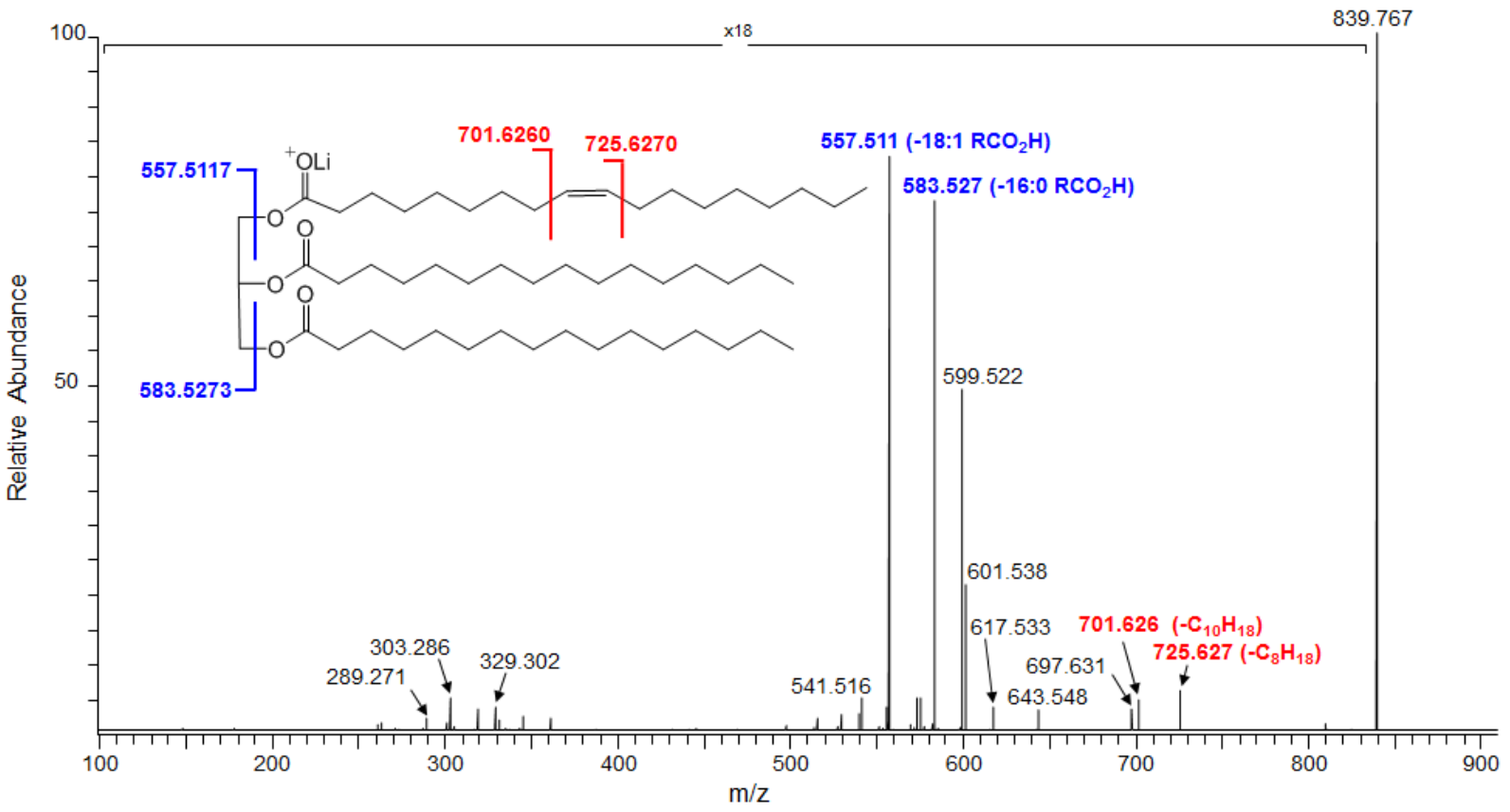
The UVPD MS^n fragments are generated in the linear ion trap and can be detected by either the ion trap or Orbitrap

new

Compact Footprint

- UVPD source is embedded inside the instrument, directly connected to the dual-pressure linear ion trap
- UVPD source employs a 213 nm laser with 2.5 kHz repetition rate delivering >1.2 µJ/pulse
- UVPD is a field upgradable option

UVPD For Comprehensive Lipid Characterization



Locating Double Bonds

- HRAM UVPD MS^2 spectrum of $[M+Li]^+$ precursor ions of TG 16:0/16:0/18:1
- Fragments identify acyl chains
- UVPD unique fragments identify location of double bonds within the acyl chains

ASMS 2017, WOD 03:10 pm : Reid G.et al.

Summary

- Developed an accessible untargeted lipidomics workflow
- Thousands of major lipid species were identified and quantified in a single run
- This LC MS-MS workflow can be applied to any complex biological samples including plasma, serum, tissues, cells and food
- The Fusion Lumos 1M option enables lipid fluxomics LC/MS experiments that are not possible using lower resolution instruments
- UVPD enables location of lipid double bonds and is only available on Orbitrap Fusion Lumos MS

Acknowledgment

- **Thermo Fisher Scientific**
  - Reiko Kiyonami
  - David Peake
  - Elena Sokol
  - Andreas Huhmer
  - Tabiwang Arrey
  - Alexander Harder
  - Romain Huguet
  - Anastasia Kalli
  - Julian Saba
  - Seema Sharma
  - Vlad Zabrouskov
- **MITSUI KNOWLEDGE INDUSTRY**
  - Yasuto Yokoi
  - Yukihiro Fukamachi
- **The University Of Melbourne**
  - Gavin Reid
- **The University Of Texas Southwestern Medical Center**
  - Matthew Mitsche
  - Jeff McDonald