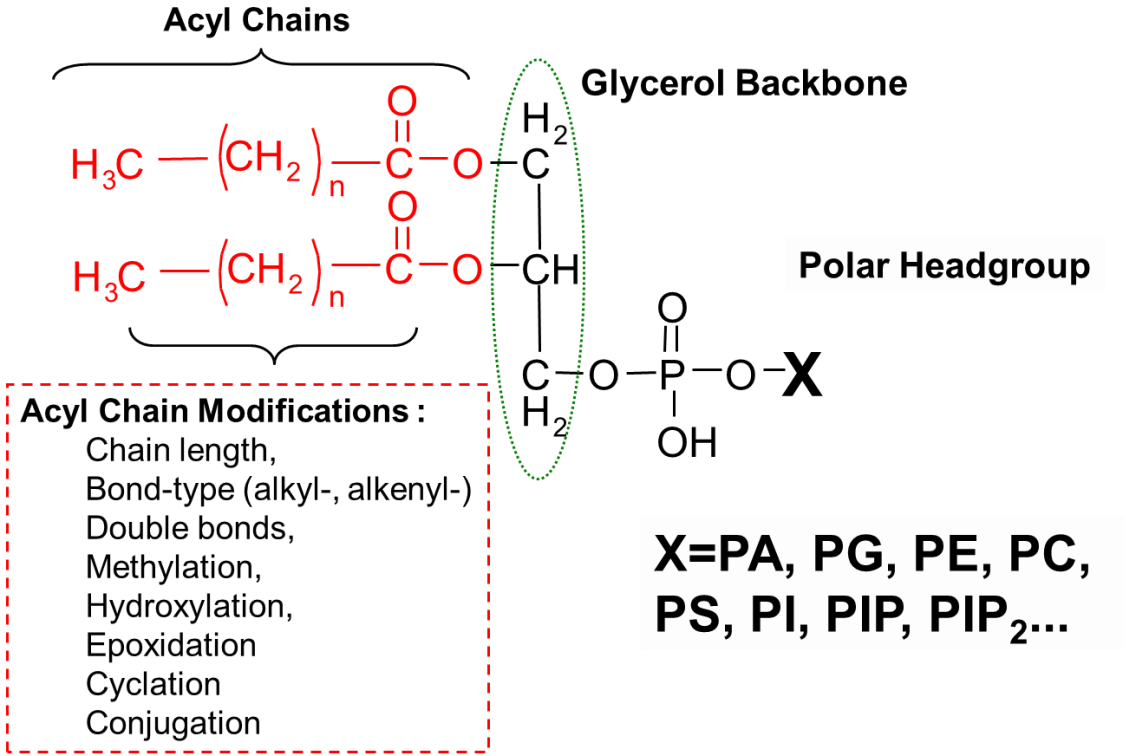
Electron activated dissociation (EAD) for lipidomics

Using the ZenoTOF 7600 system

SCIEX
The Power of Precision

The challenge of structural diversity

STRUCTURAL DETAILS OF GLYCEROPHOSPHOLIPIDS



- The potential number of unique glycerophospholipids is > 12,000
- Fatty acid modifications increases this number

SCIEX
The Power of Precision

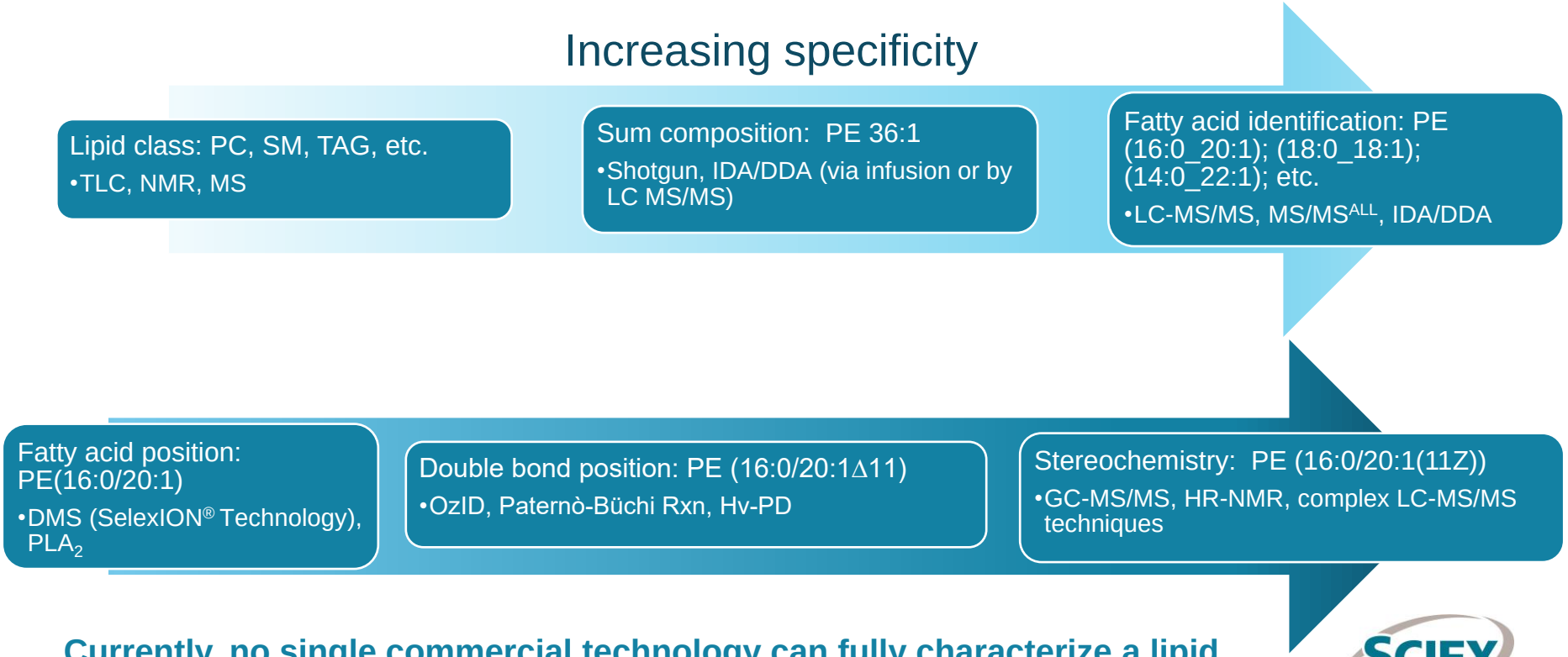
Complexity as a function of specificity: PE 36:1

TRANSLATION OF SUM COMPOSITION TO SPECIFIC LIPID MOLECULAR SPECIES

Class Level	Sum-Composition Level	Fatty Acid Level	Positional Isomer Level	Double Bond Position(s)	Cis/Trans
PE	PE 36:1	PE(14:0_22:1)	PE(14:0/22:1)	**1**	**2**
		PE(14:1_22:0)	PE(22:1/14:0)	**1**	**2**
		PE(16:0_20:1)	PE(14:1/22:0)	**3** - (Δ8, Δ9, Δ11)	**6**
		PE(16:1_20:0)	PE(22:0/14:1)	**3** - (Δ8, Δ9, Δ11)	**6**
		PE(18:0_18:1)	PE(16:0/20:1)	**4** - (Δ13, Δ11, Δ9, Δ8)	**8**
			PE(20:1/16:0)	**4** - (Δ13, Δ11, Δ9, Δ8)	**8**
			PE(16:1/20:0)	**2** - (Δ9, Δ6)	**4**
			PE(20:0/16:1)	**2** - (Δ9, Δ6)	**4**
			PE(18:0/18:1)	**4** - (Δ12, Δ11, Δ9, Δ7)	**8**
			PE(18:1/18:0)	**4** - (Δ12, Δ11, Δ9, Δ7)	**8**
Possibilities:	1	5	10	28	**56**

The Challenge of specificity

THE MANY LAYERS OF LIPID STRUCTURAL SPECIFICITY

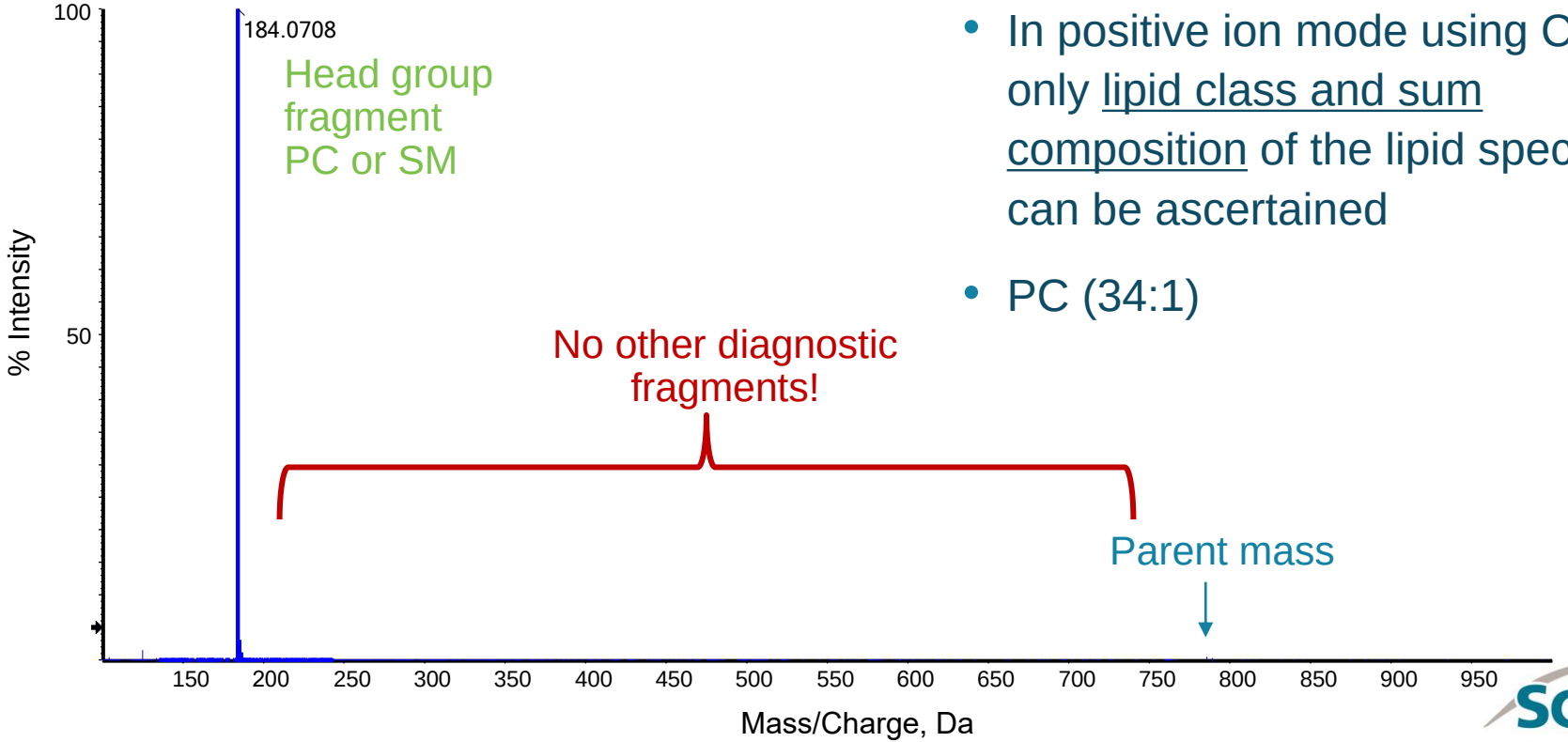


Currently, no single commercial technology can fully characterize a lipid

SCIEX
The Power of Precision

Collision induced dissociation for lipidomics

IDA SPECTRUM OF 788.6185

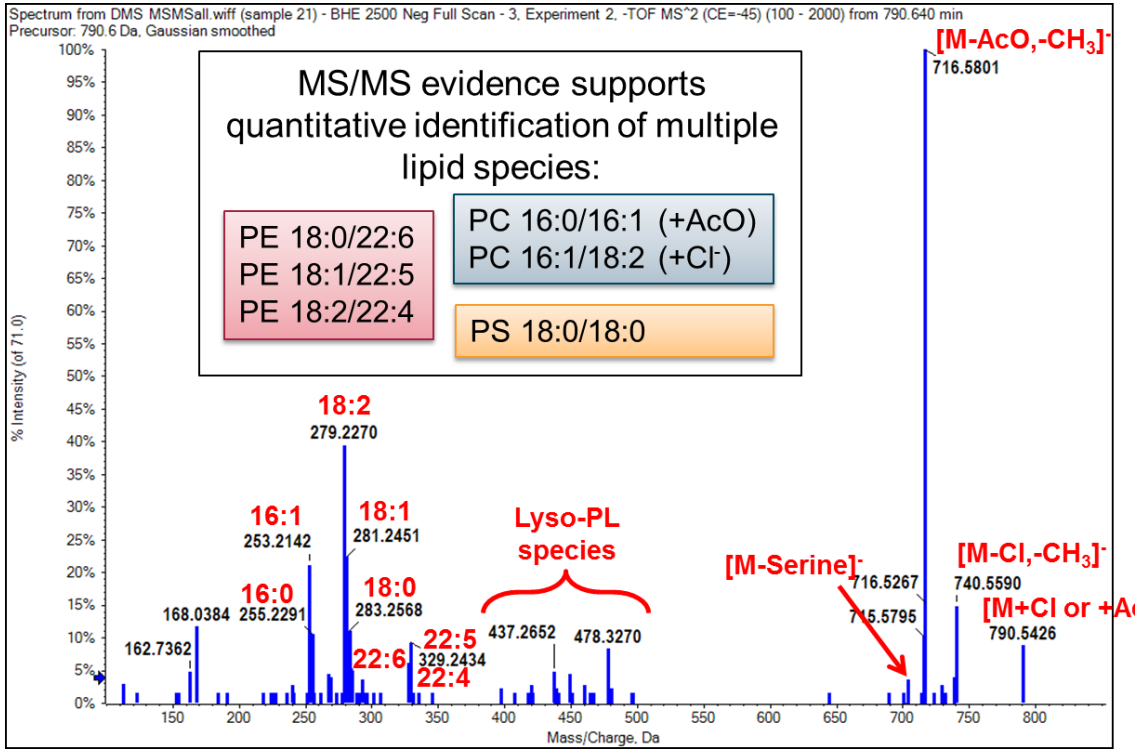


- In positive ion mode using CID, only lipid class and sum composition of the lipid species can be ascertained
- PC (34:1)

SCIEX
The Power of Precision

Example of isobaric interference in infusion-based lipidomics

COMPLICATED INTERPRETATION



- Many lipids share the same precursor mass
- Many of those same precursors, share the same fragments
- Careful data analysis is needed to successfully interpret shotgun data
- Quantification strategies must accommodate shared fragment ions

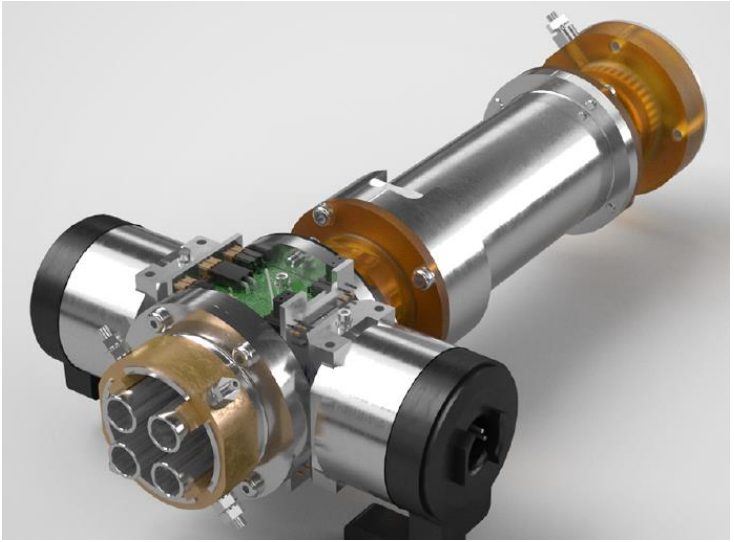
SCIEX
The Power of Precision

Characterization of lipid species

SCIEX
The Power of Precision

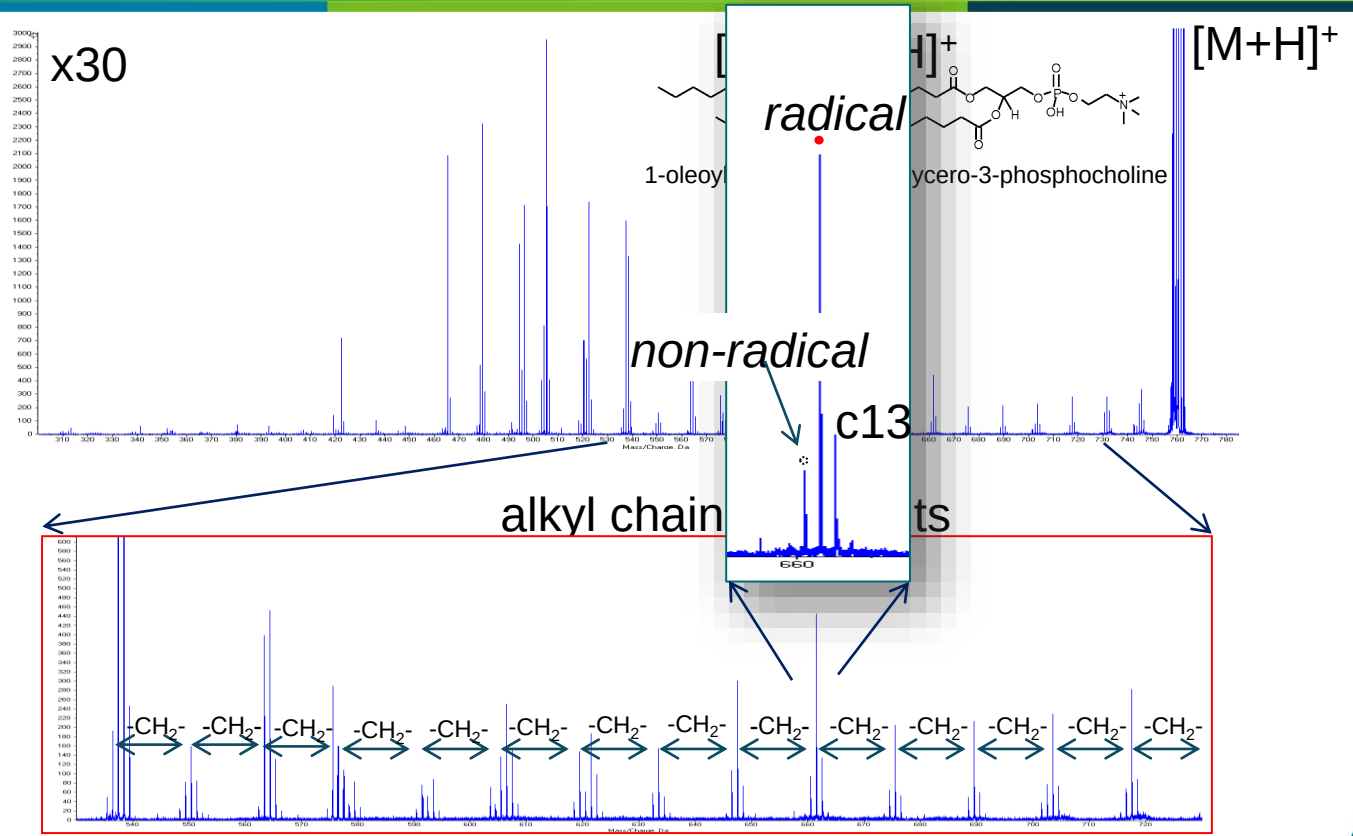
Benefits of EAD technology

- EAD kinetic energy can be tuned depending on analyte type
- EAD fragmentation is reproducible at a quantitative level
- Fast fragmentation and acquisition
  - Electron irradiation times ~ 10-30 msec
  - System can do DDA on LC time scale up to 20 Hz
  - Maintaining spectral resolution of 30,000
- EAD is optimized to limit secondary fragmentation (unless desired)
- EAD does not require further activation of charged reduced species by CID, but workflow is possible



EIEIO (5eV): Alkyl chain dissociation

ZenoTOF 7600 System

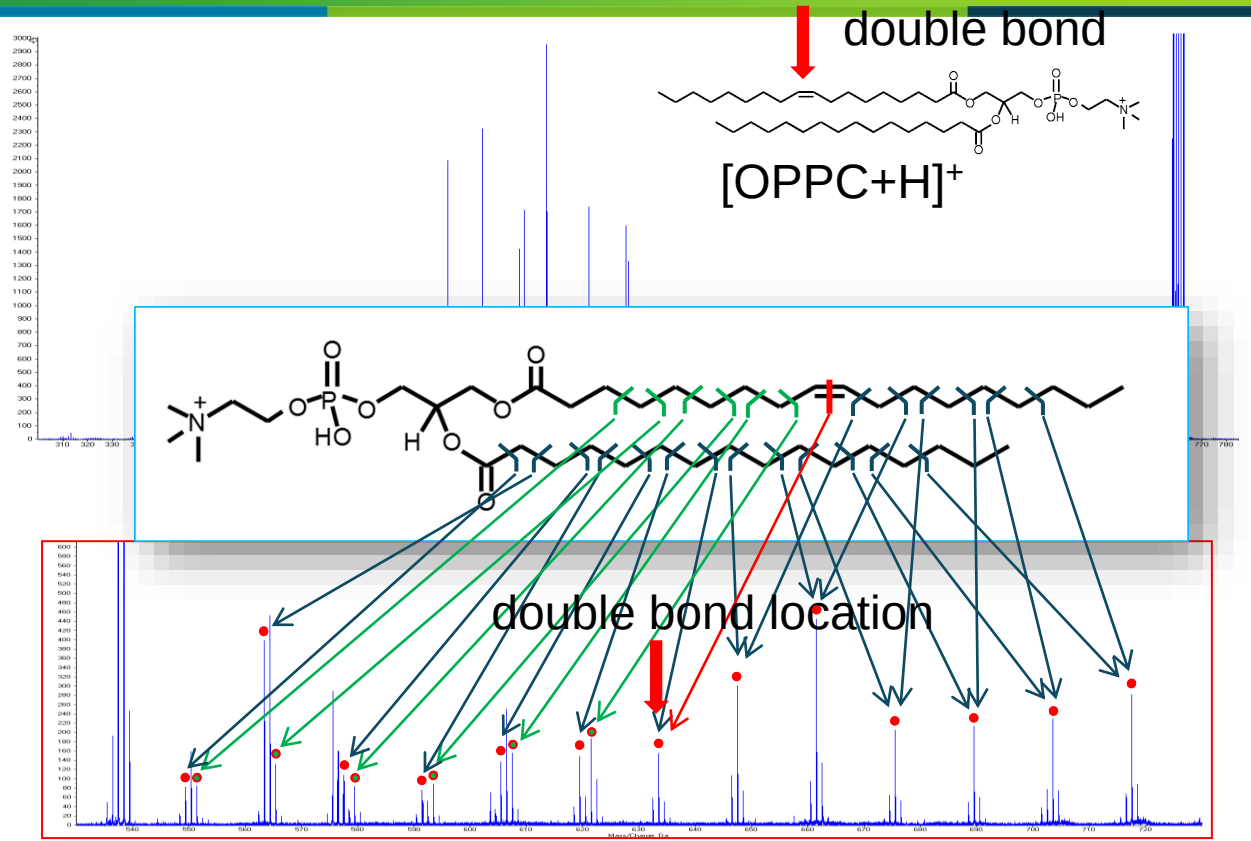


Campbell and Baba, (2015) *Anal. Chem.* **87(11)**, 5837–5845

SCIEX The Power of Precision

EIEIO (5eV): double bond location

ZenoTOF 7600 System



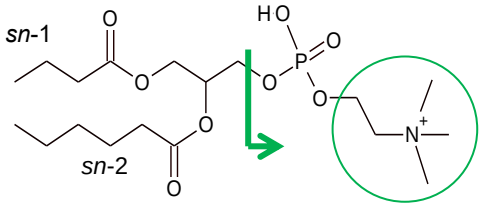
Campbell and Baba, (2015) *Anal. Chem.* **87(11)**, 5837–5845

SCIEX
The Power of Precision

What can EAD do for lipids?

FRAGMENTATION PATTERNS

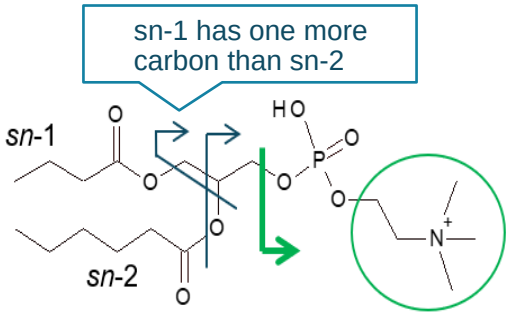
- EAD produces diagnostic fragments of:
  - Head group
    - PC (SM), PE, PS, PI, PA, PG



Head group variations

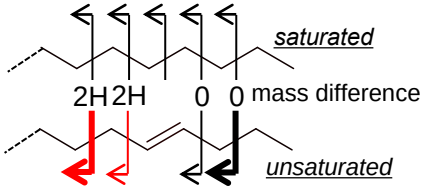
Backbone (glycerol/sphingoid)

- Regioisomerism (sn-1, sn-2, etc.)



Chain structure

- Length and double bond positions
- Double bond stereochemistry



SCIEX
The Power of Precision

Lipid species characterization

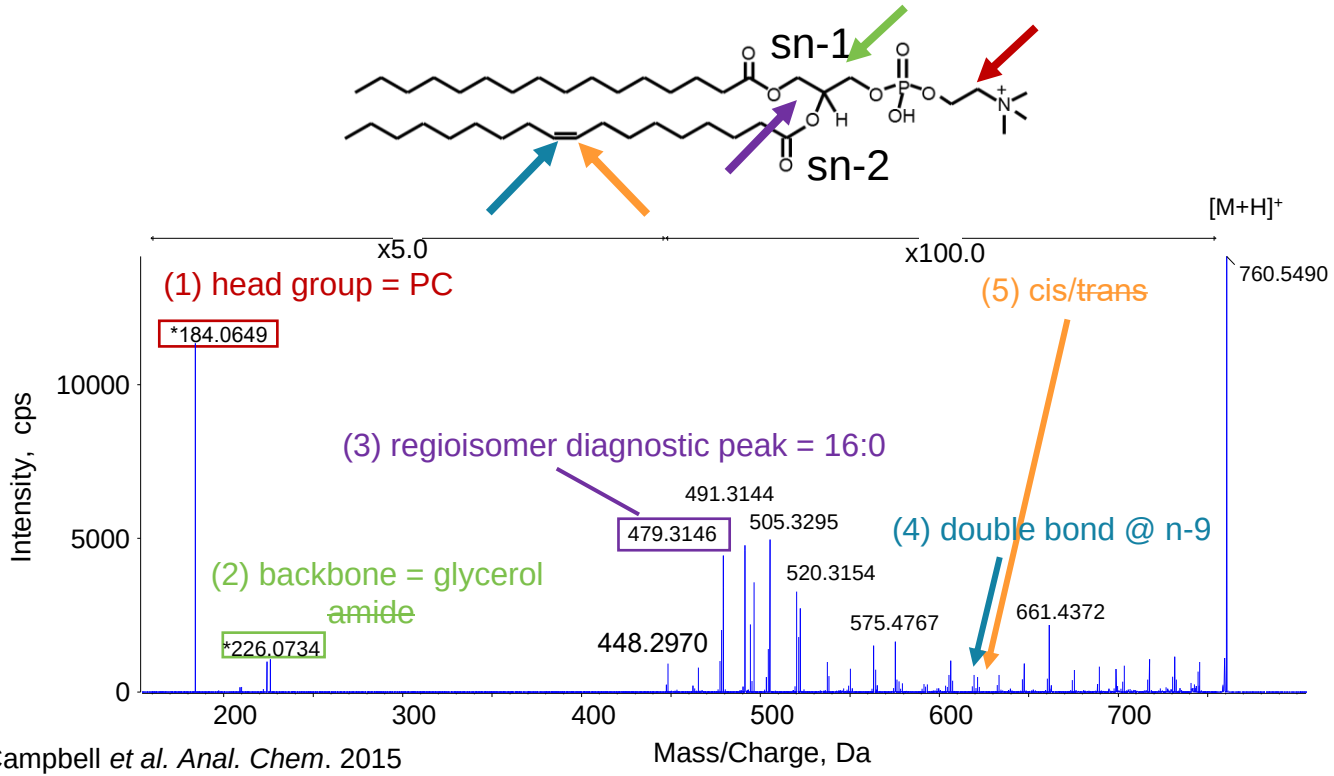
WITH ZenoTOF 7600 SYSTEM

- Electron activated dissociation (EAD) can provide:
  - Structural elucidation of lipid species within one LC-MS/MS analysis
    - Lipid class
    - Fatty acid identification
    - Fatty acid position
    - Double bond position
    - Double bond stereochemistry

EAD for lipid characterization

ZenoTOF 7600 System

single experiment → *de novo* analysis → PC 16:0 / 18:1(n-9:cis)

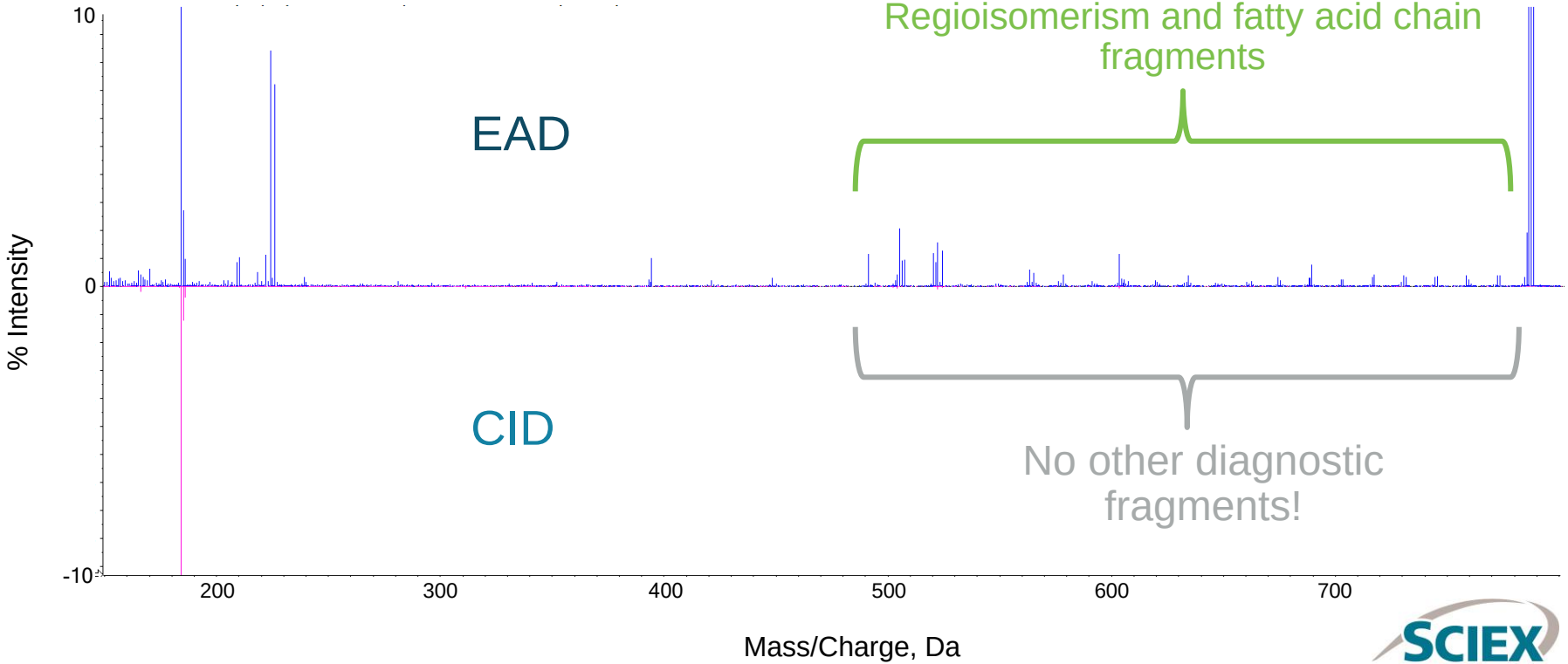


Campbell *et al. Anal. Chem*. 2015

SCIEX The Power of Precision

Inverted overlay

TIC OF 788.6185

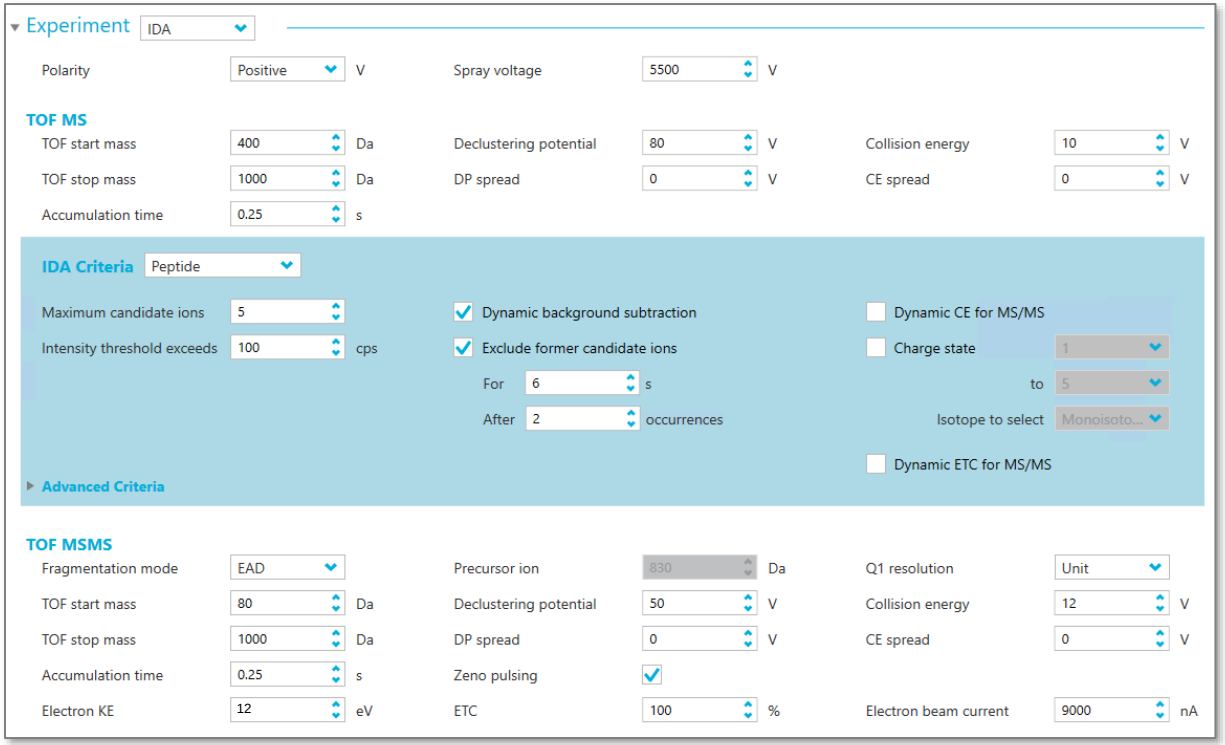


SCIEX
The Power of Precision

Method setup

SCIEX OS Software

SCIEX OS SOFTWARE 2.0



- IDA acquisition
- EAD fragmentation with high kinetic energy – 12 eV
- Pulsing with Zeno trap ON
- Current of 9000 nA

SCIEX The Power of Precision

Experimental design

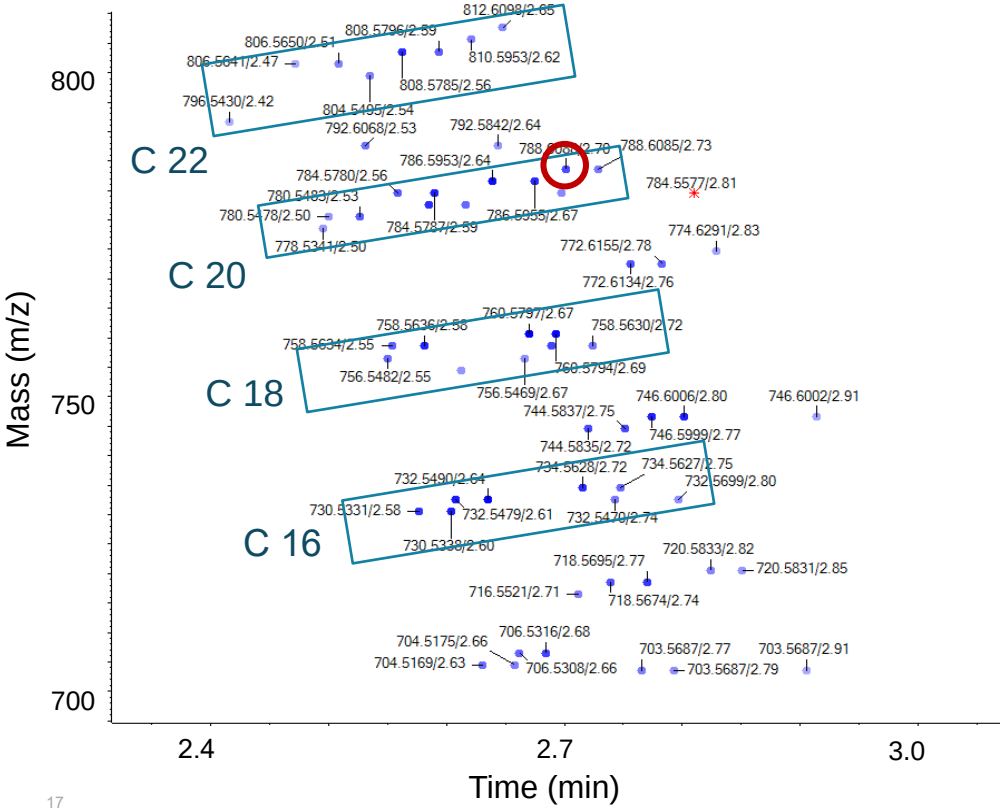
C2C12 MYOTUBES

- Collaboration with Dr. Katsu Fenai's group at U. Utah
- Separation achieved using Phenomenex Cyano column (P/N:00D-4254-Y0)
  - Normal phase chromatography for class-based separation
- Cells extracted using modified LLE: 3-phase extraction
- Data collected using LC-based data dependent acquisition

Zeno trap EAD IDA of lipids

ZenoTOF 7600 System

IDA EXPLORER - FATTY ACID CHARACTERIZATION ON LC TIME FRAME



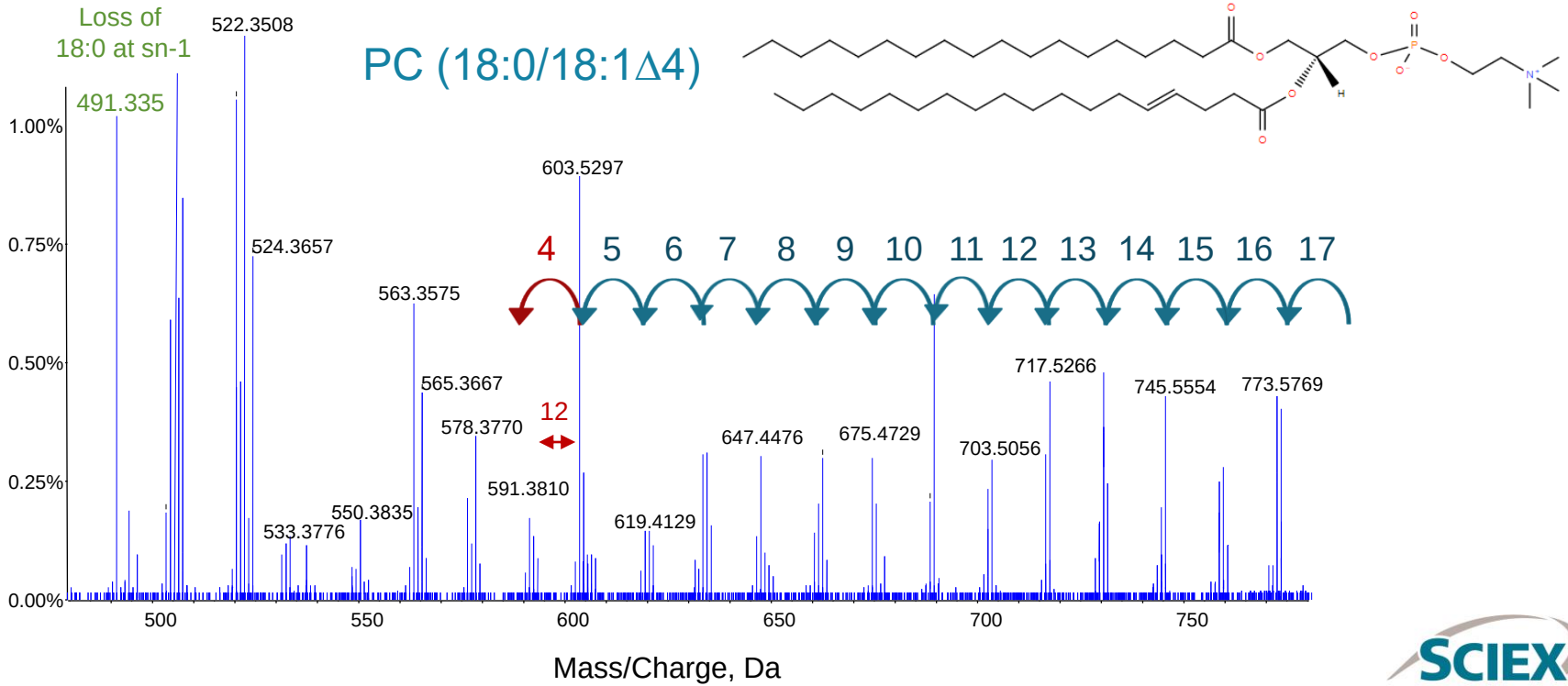
- Lipids extracted from C2C12 myotubes
- Lipids with different chain lengths appear as ladders increasing in mass (blue boxes)
- Unsaturated lipids for each chain length elute over time on normal phase chromatography
- Red circle indicates the lipid shown on next slide

SCIEX The Power of Precision

Double bond position by LC-MS

ZenoTOF 7600 System

EIEIO SPECTRUM OF 788.6185 (PC) – COLLECTED BY DATA DEPENDENT LC-MS

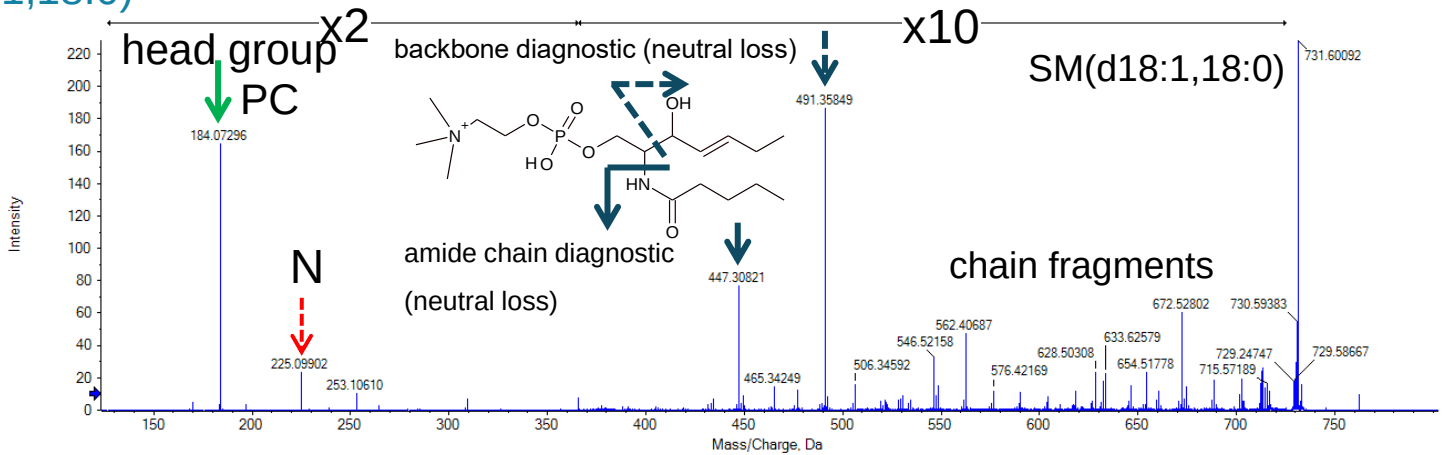


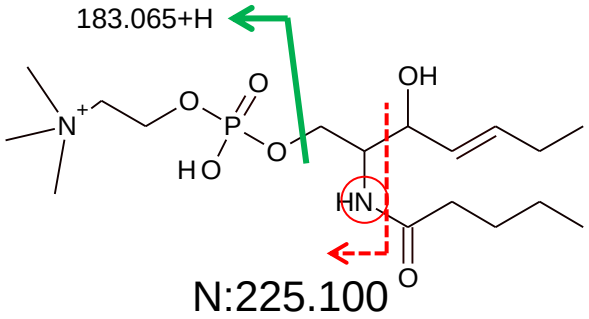
SCIEX
The Power of Precision

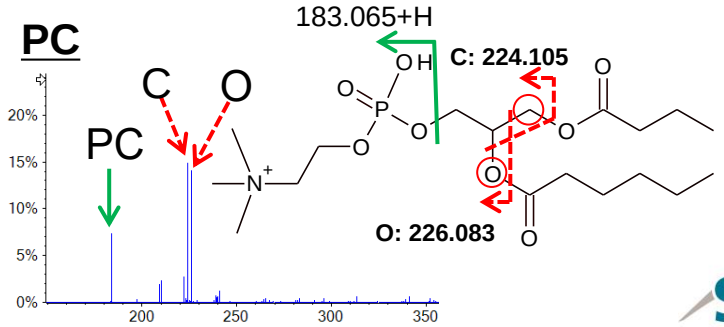
EIEIO of sphingomyelins (SMs)

ZenoTOF 7600 System

SM(d18:1,18:0)







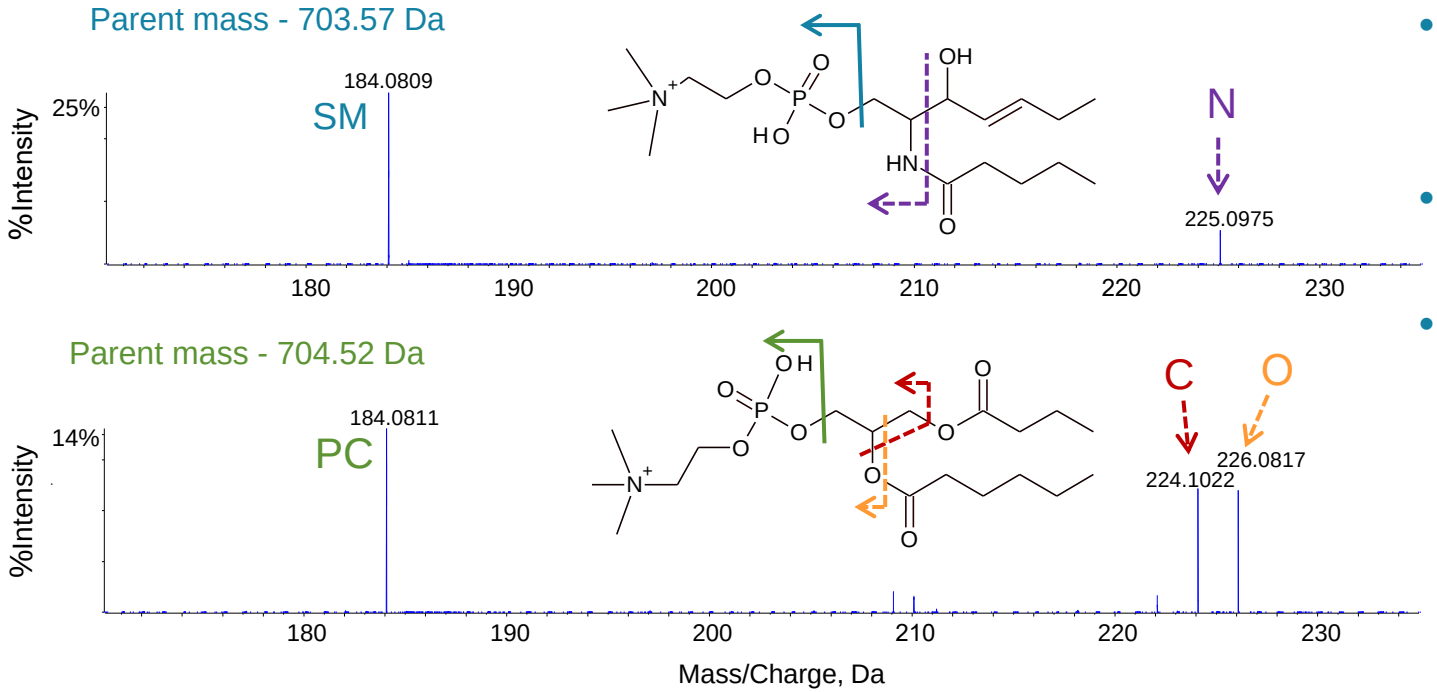
Baba et al. (2016) *J Lipid Res*. **57(11)**:858-867.

SCIEX The Power of Precision

Ease of distinguishing common isobars

ZenoTOF 7600 System

DIAGNOSTIC BACKBONES BY EAD MS/MS

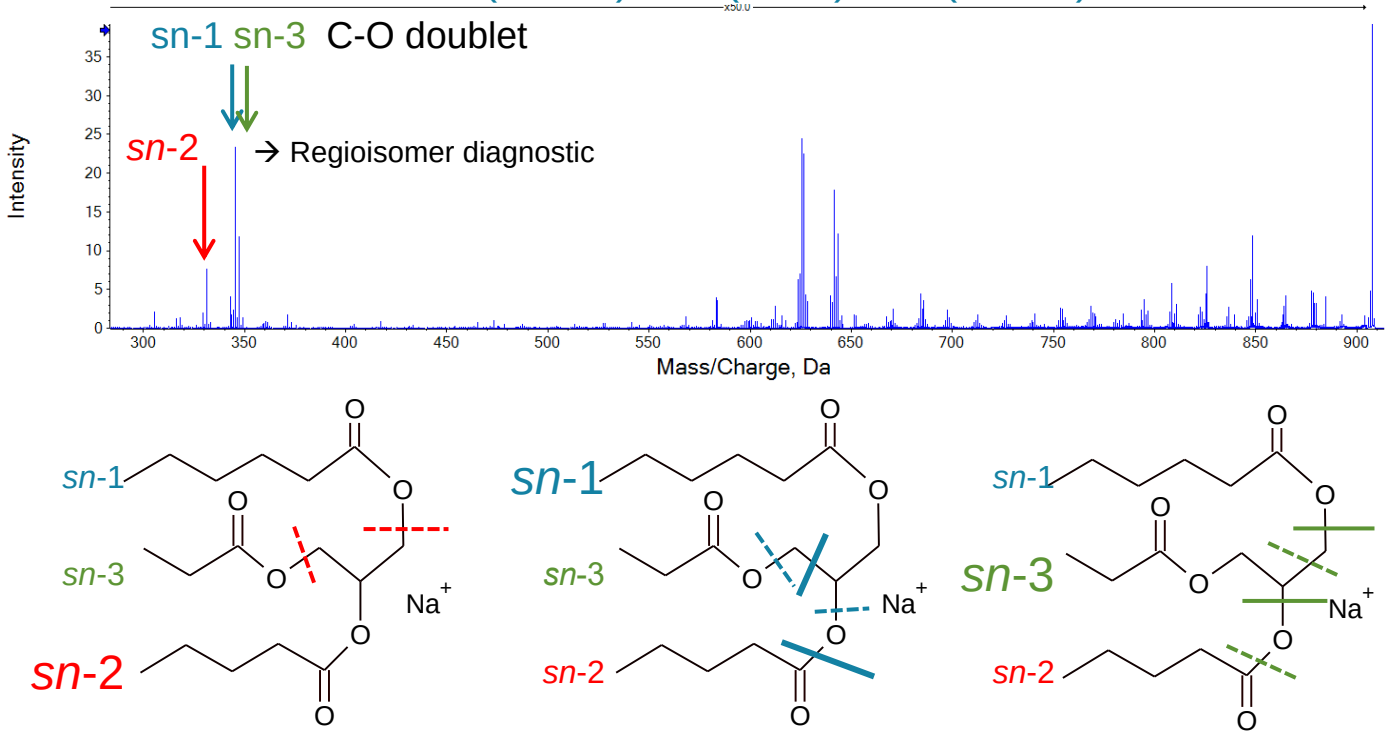


- Sphingomyelins vs. phosphatidylcholines
- PC head group at m/z 184
- Fragment ions containing either nitrogen (m/z 225) **or** carbon and oxygen (m/z 224 and 226) to distinguish SM from PC

SCIEX The Power of Precision

Differentiation of regioisomers

EAD MS/MS - TAG 18:1(N-12Z)/18:1(N-9Z)/18:1(N-12Z)



- sn-2 attachment point can be differentiated from the sn-1 position **or** sn-3 position through examination of the dual chain loss fragment ions

Baba et al. (2016) *J Lipid Res*. **57(11)**:2015-2027.

Summary of applicability

ZenoTOF 7600 System

Phospholipids

	class	double bond locations	
		protonated	sodiated
PC	✓	✓✓✓	✓
PE	✓	✓	✓
PS	✓	✓	✓
PA	✓	x	✓
PG	✓	x	✓
PI	✓	x	✓

including plasmalogens and ethers

Other adducts can be formed and detected using EIEIO

Sphingolipids

	class	double bond locations	
		protonated	sodiated
Cer	✓	✓	✓
HexCer	✓	✓	✓✓
LacCer	✓	✓	✓
SM	✓	✓✓	✓

including hydroxy

Acylglycerols

	class	double bond locations	
		protonated	sodiated
DG	x	x	✓
MGDG	✓	x	✓
TG	✓	x	✓✓

SCIEX
The Power of Precision

Double bond stereochemistry

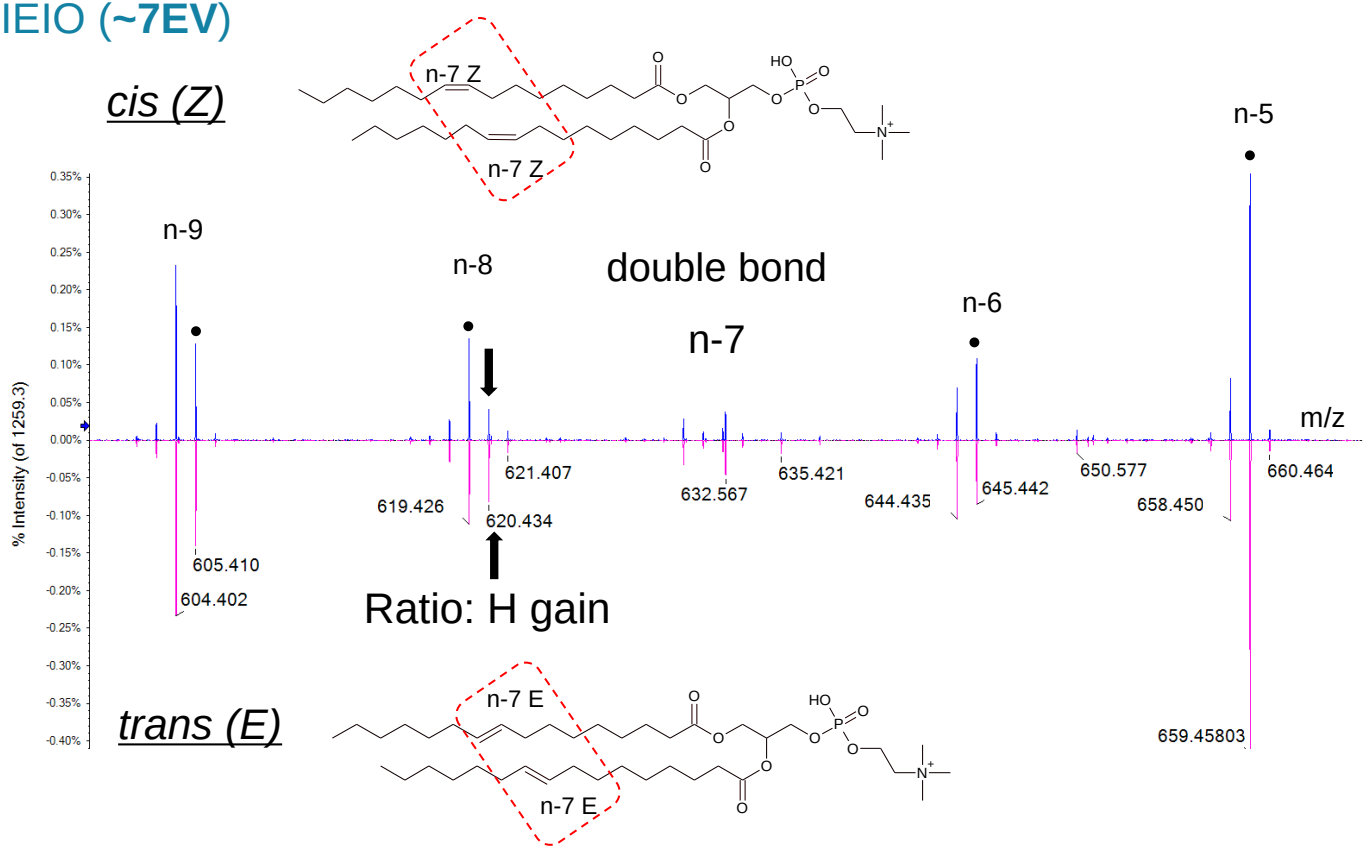
CIS/TRANS

SCIEX
The Power of Precision

Finding cis/trans difference

ZenoTOF 7600 System

EIEIO (~7EV)



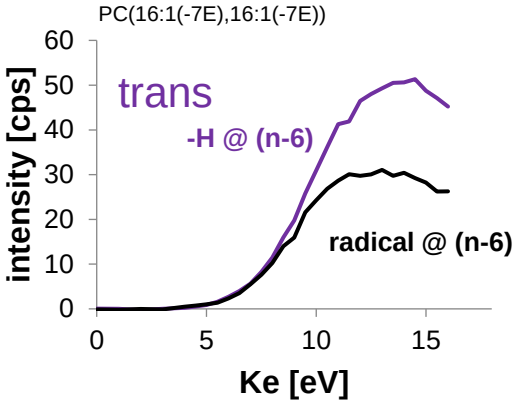
Biological significance:

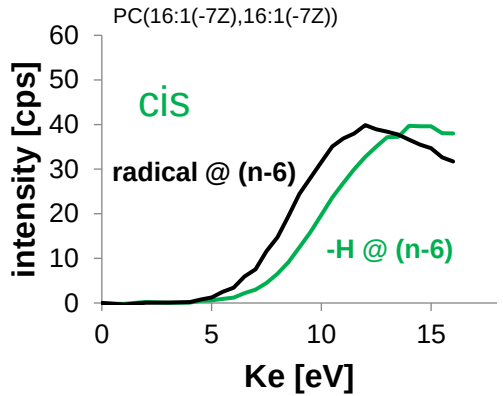
- Nutrition
- Food adulteration
- Biomarker research
- Membrane fluidity
- Lipid biological function

Baba, et al. (2017) *Anal. Chem.* **89(14),** 7307–7315

SCIEX The Power of Precision

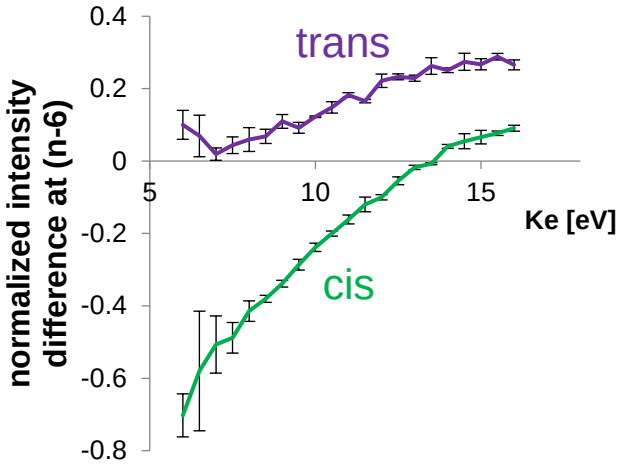
Energy dependence





normalized intensity difference

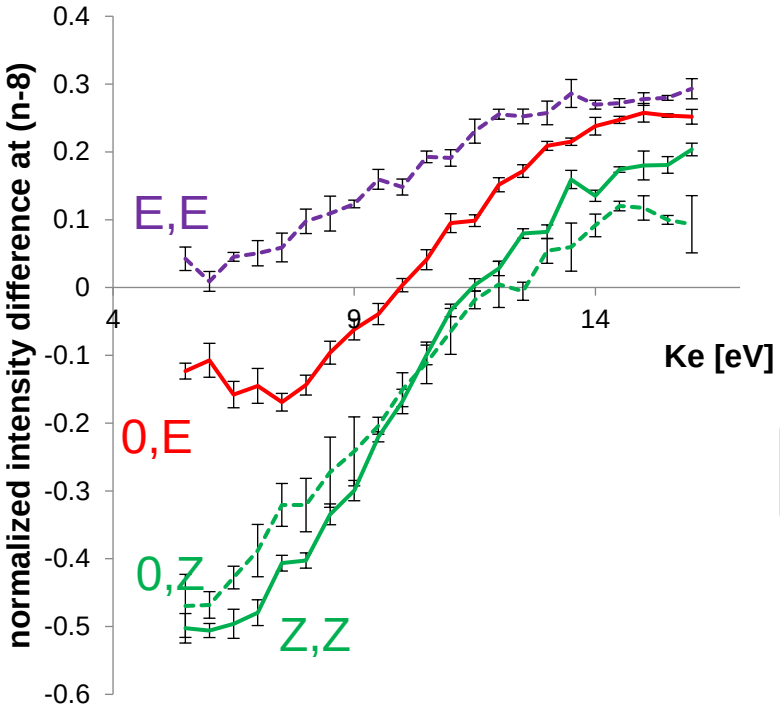
$$\overline{D} = \frac{\text{[H loss] - [radical]}}{\text{[H loss] + [radical]}}$$



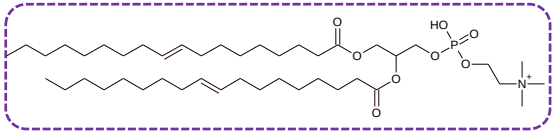
$\overline{D}$ indicates trans/cis difference significantly

Baba, et al. (2017) *Anal. Chem.* **89(14),** 7307–7315

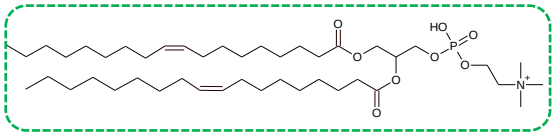
Mixed acyls: cis-POPC / trans-POPC



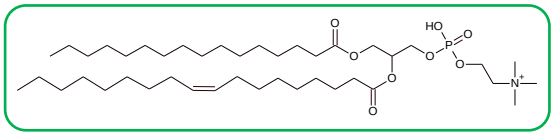
PC(18:1(-9E),18:1(-9E))



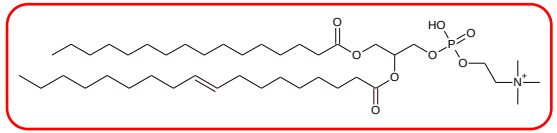
PC(18:1(-9Z),18:1(-9Z))



cis-POPC: PC(16:0,18:1(-9Z))



trans-POPC: PC(16:0,18:1(-9E))



Baba, et al. (2017) *Anal. Chem.* **89(14),** 7307–7315

Summary

NEAR COMPLETE LIPID CHARACTERIZATION IN ONE EXPERIMENT

- EIEIO for lipids is a powerful tool for characterization
  - In one LC-MS data dependent acquisition:
    - Lipid class
    - Fatty acid identification
    - Fatty acid position
    - Double bond position
    - Double bond stereochemistry

SCIEX

The Power of Precision

Trademarks / Licensing

The SCIEX clinical diagnostic portfolio is For In Vitro Diagnostic Use. Rx Only. Product(s) not available in all countries. For information on availability, please contact your local sales representative or refer to https://sciex.com/diagnostics. All other products are For Research Use Only. Not for use in Diagnostic Procedures.

SCIEX
The Power of Precision