Electron Activated Dissociation for near complete characterization of lipids from single MS/MS spectrum using ZenoTOF 7600

Tomáš Korba, AMEDIS

11. September, 2023

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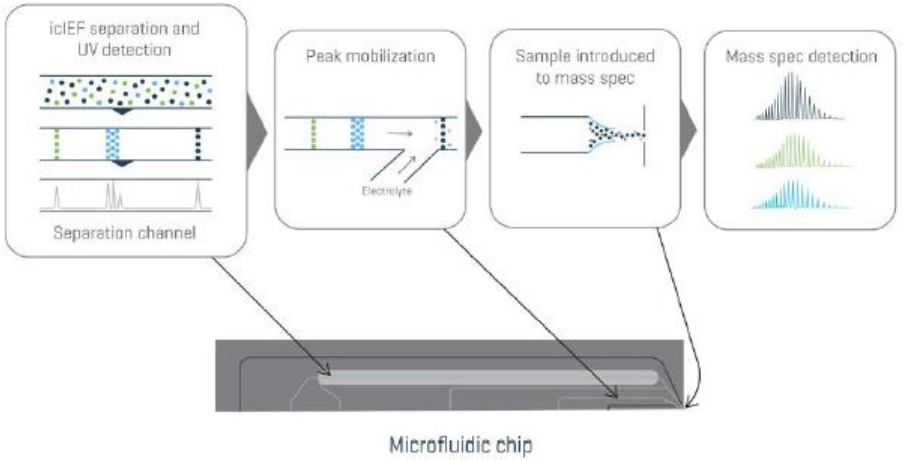
Intabio ZT

IMAGED CAPILLARY ISOELECTRIC FOCUSING (ICIEF)-UV/MS



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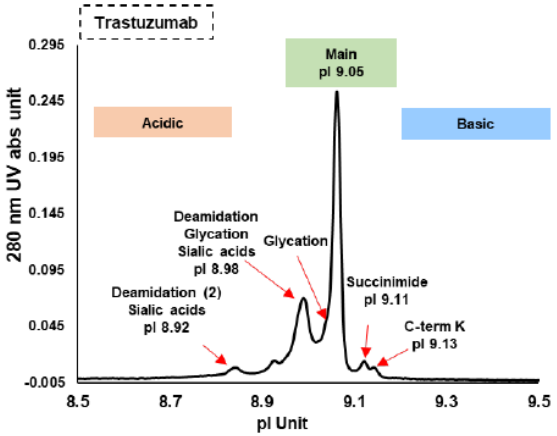
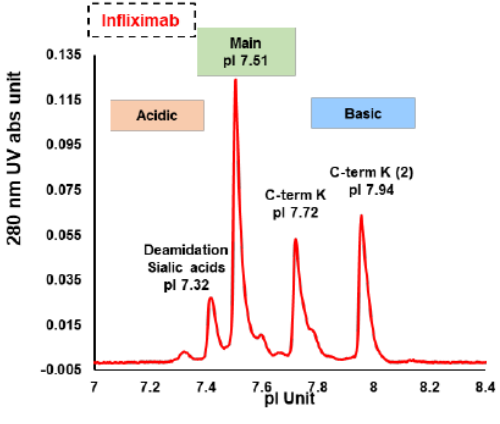
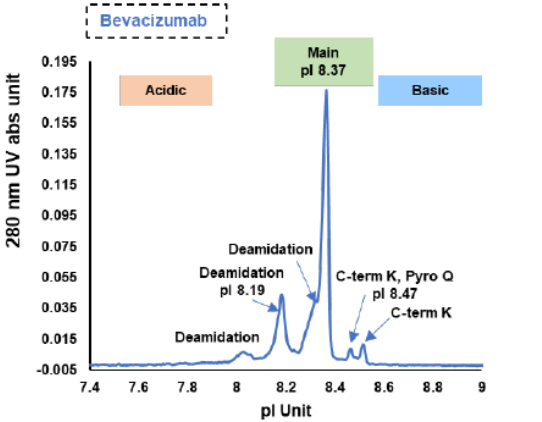
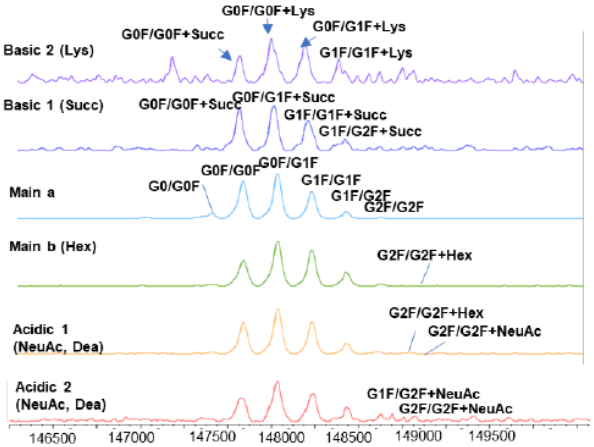
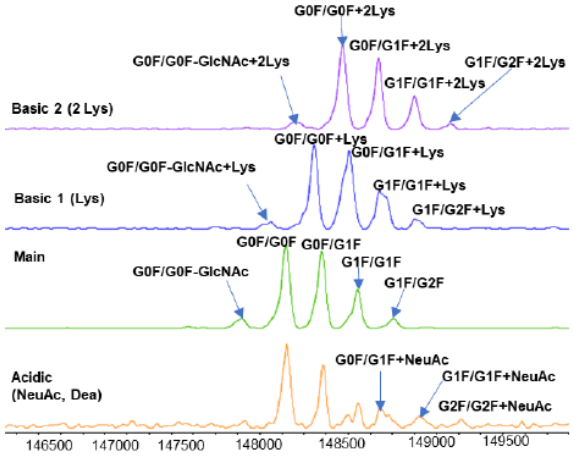
Intabio ZT

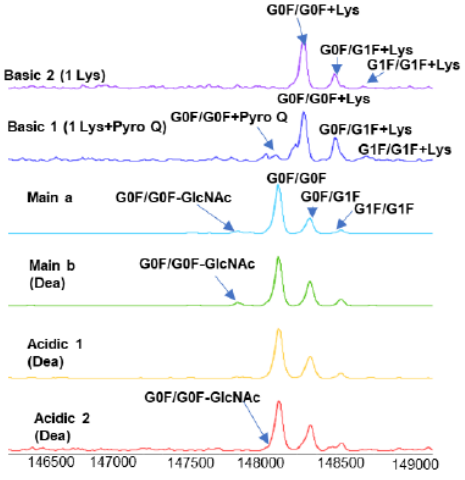

Trastuzumab
Main
pI 9.05
Acidic
Basic
280 nm UV abs unit
0.295
0.245
0.195
0.145
0.095
0.045
-0.005
Deamidation
Glycation
Sialic acids
pI 8.98
Glycation
Deamidation (2)
Sialic acids
pI 8.92
Succinimide
pI 9.11
C-term K
pI 9.13
8.5
8.7
8.9
9.1
9.3
9.5
pI Unit


Infliximab
Main
pI 7.51
Acidic
Basic
280 nm UV abs unit
0.135
0.115
0.095
0.075
0.055
0.035
0.015
-0.005
Deamidation
Sialic acids
pI 7.32
C-term K
pI 7.72
C-term K (2)
pI 7.94
7
7.2
7.4
7.6
7.8
8
8.2
8.4
pI Unit


Bevacizumab
Main
pI 8.37
Acidic
Basic
280 nm UV abs unit
0.195
0.175
0.155
0.135
0.115
0.095
0.075
0.055
0.035
0.015
-0.005
Deamidation
Deamidation
pI 8.19
Deamidation
C-term K, Pyro Q
pI 8.47
C-term K
7.4
7.6
7.8
8
8.2
8.4
8.6
8.8
9
pI Unit


Basic 2 (Lys)
G0F/G0F+Lys
G0F/G0F+Succ
G0F/G1F+Lys
G1F/G1F+Lys
Basic 1 (Succ)
G0F/G0F+Succ
G0F/G1F+Succ
G1F/G1F+Succ
G1F/G2F+Succ
Main a
G0/G0F
G0F/G0F
G0F/G1F
G1F/G1F
G1F/G2F
G2F/G2F
Main b (Hex)
G2F/G2F+Hex
Acidic 1
(NeuAc, Dea)
G2F/G2F+Hex
G2F/G2F+NeuAc
Acidic 2
(NeuAc, Dea)
G1F/G2F+NeuAc
G2F/G2F+NeuAc
146500
147000
147500
148000
148500
149000
149500


Basic 2 (2 Lys)
G0F/G0F+2Lys
G0F/G0F-GlcNAc+2Lys
G0F/G1F+2Lys
G1F/G1F+2Lys
G1F/G2F+2Lys
Basic 1 (Lys)
G0F/G0F+Lys
G0F/G0F-GlcNAc+Lys
G0F/G1F+Lys
G1F/G1F+Lys
G1F/G2F+Lys
Main
G0F/G0F
G0F/G1F
G0F/G0F-GlcNAc
G1F/G1F
G1F/G2F
Acidic
(NeuAc, Dea)
G0F/G1F+NeuAc
G1F/G1F+NeuAc
G2F/G2F+NeuAc
146500
147000
147500
148000
148500
149000
149500


Basic 2 (1 Lys)
G0F/G0F+Lys
G0F/G1F+Lys
G1F/G1F+Lys
Basic 1 (1 Lys+Pyro Q)
G0F/G0F+Lys
G0F/G0F+Pyro Q
G0F/G1F+Lys
G1F/G1F+Lys
Main a
G0F/G0F
G0F/G0F-GlcNAc
G0F/G1F
G1F/G1F
Main b
(Dea)
G0F/G0F-GlcNAc
Acidic 1
(Dea)
Acidic 2
(Dea)
G0F/G0F-GlcNAc
146500
147000
147500
148000
148500
149000

Electron Activated Dissociation

WITH ZENO TOF

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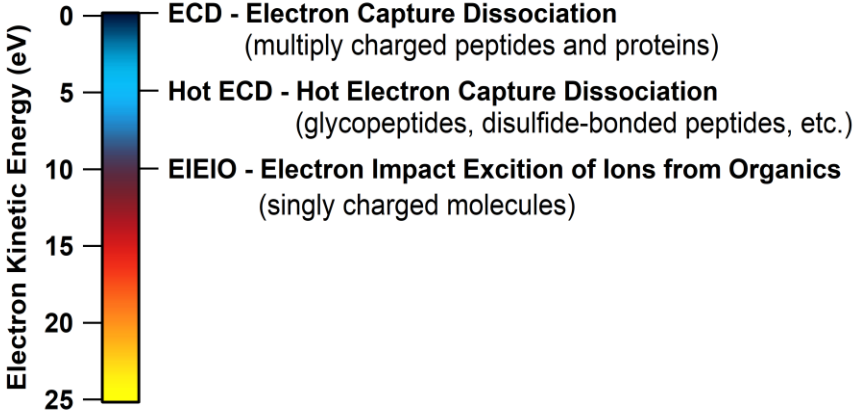
Is there a need for alternative fragmentation ?

COMPLEMENTARY AND INFORMATION RICH FOR STRUCTURE ELUCIDATION WITH EAD

- Collision induced dissociation (CID) is a soft, thermal fragmentation technique
  - Often leads to cleavage of most labile sites
  - Results in few diagnostic fragments
  - Insufficient cleavage without protonation sites
- Electron activated dissociation (EAD cell) offers complementary fragmentation information
  - Radical dissociation mechanism
  - Can maintain labile modifications
  - Potential to result in many diagnostic fragments

Electron activated dissociation (EAD)

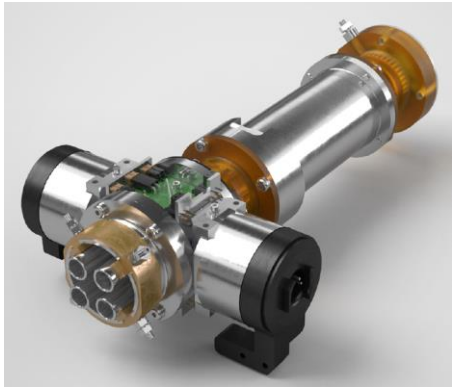
- Free electrons are captured by ions and form a radical state which then fragments
  - Electrons introduced with different energies will induce fragmentation in different molecule types

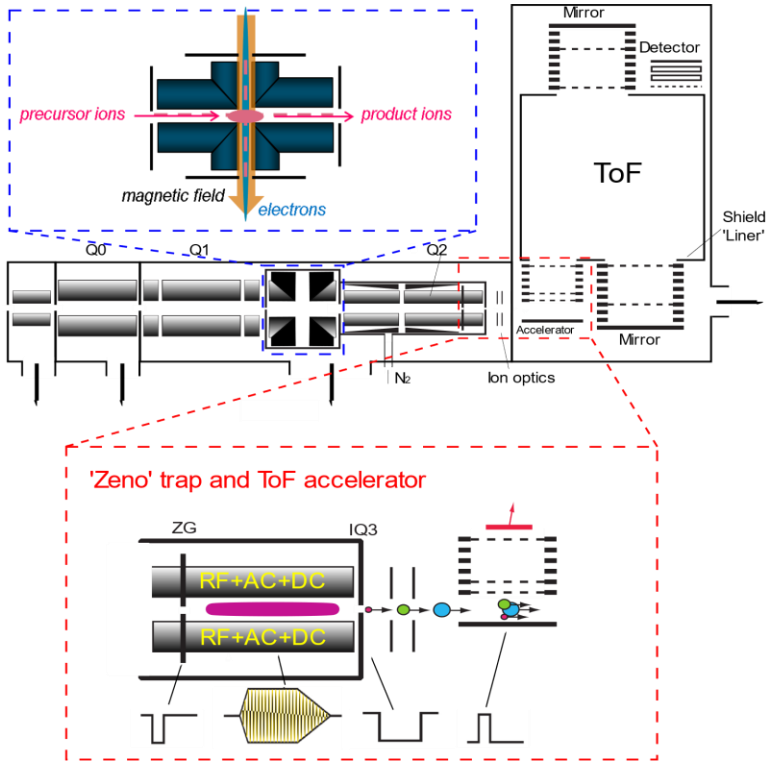


T. Baba *et al. Anal.Chem*. 2014

How SCIEX solves the problem?

INTEGRATED MS/MS ASSEMBLY WITH ELECTRON ACTIVATED DISSOCIATION (EAD)





- EAD cell for electron based fragmentation
- Zeno trap for enhancement of low abundant fragment ions

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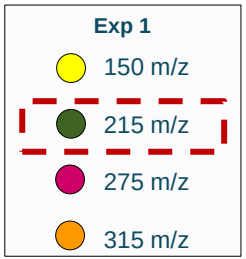
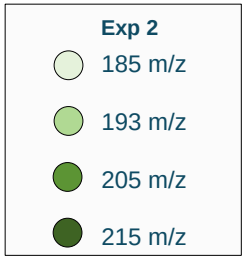
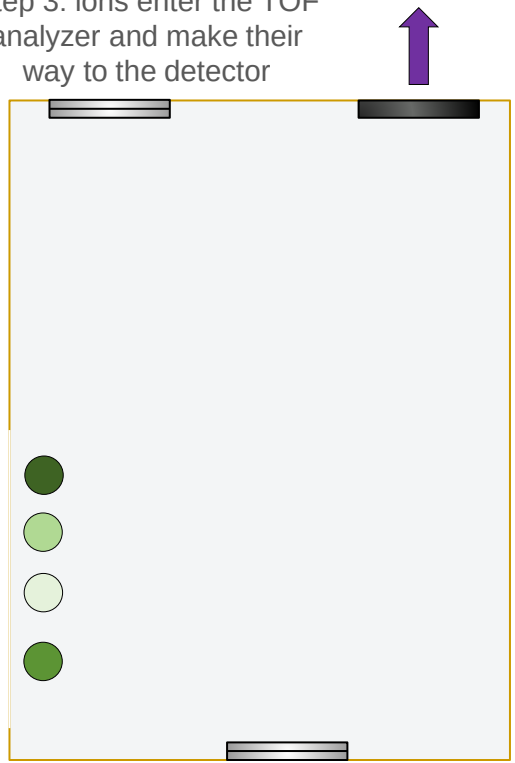
Electron activated dissociation (EAD)



Step 2: fragmentation in EAD cell with optimized KE



Step 3: ions enter the TOF analyzer and make their way to the detector

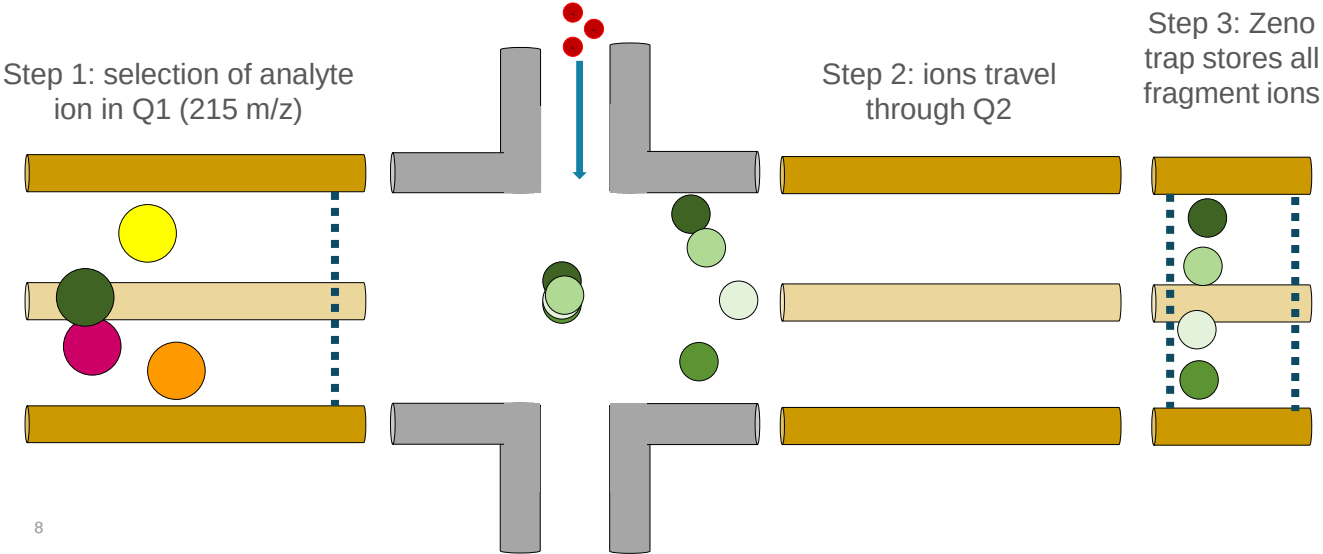


Electrons

Step 1: selection of analyte ion in Q1 (215 m/z)

Step 2: ions travel through Q2

Step 3: Zeno trap stores all fragment ions



EAD for small molecules

ELECTRON ACTIVATED DISSOCIATION (EAD)

- Distinguishing of isomers
- Drug metabolite identification, position of modification
- Position of glucuronation (N / O)
- Endogenous metabolites forms
- Position of polar groups on saccharides
- Additional fragments for compound confirmation

EAD for biomolecules

ELECTRON ACTIVATED DISSOCIATION (EAD)

- Improved bottom-up characterization performance to meet the challenges of complex next gen therapeutics
  - Confirmation of PTMs (glycosylation, disulfide-bonds, phosphorylation, sulfation, …)
  - Detailed determination of aa isomers (isoAsp / Asp, Ile / Leu)
  - Fragmentation of singularly, doubly and multiply charged ions
  - Comprehensive sequence coverage
- Allows for sequence information directly from the intact molecule (top/middle down)
  - Sequence coverage from single experiment
- Wide range of electron energy adjustments (up to 25 eV) allows for high degree of selectivity for backbone fragmentation and maintenance of side chain

EAD general facts

ELECTRON ACTIVATED DISSOCIATION (EAD)

- Routine and reliable similar to CID MS/MS: set and forget
- Potential to support quantification
- Only alternative fragmentation technique with sensitivity improvements using the **Zeno trap**

Lipid characterization

WITH ZENO EAD MS/MS

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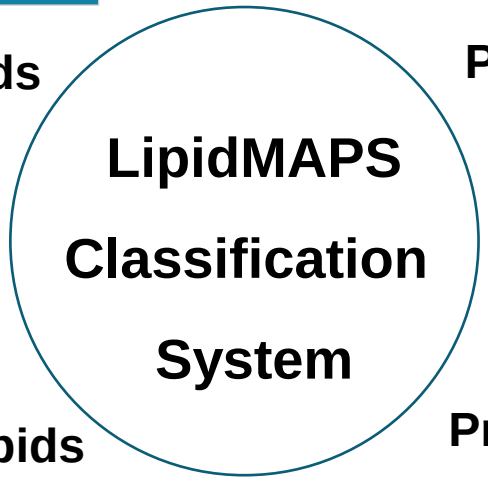
The Lipidome

There are >150,000 distinct lipid molecular species in the lipidome



Fatty acyls

- Eicosanoids
- Docosanoids
- Fatty Alcohols
- Fatty Aldehydes
- Nitrated Fatty Acids
- Etc…

Glycerolipids

- Monoglycerides
- Diglycerides
- Triglycerides
- Glcosylmonoglycerides
- Glcosyldiglycerides
- Etc…

Glycero-phospholipids

- PC, PE, PS, PI
- PG, PA, PIP, PIP_2, PIP_3, CL, Etc…

Sphingolipids

- Sphingoid Bases
- Ceramides
- Phosphosphingolipids
- Neutral Glycosphingolipids
- Acidic Glycosphingolipids
- Etc…

Sterol lipids

- Cholesteryl Esters
- Steryl Esters
- Stigmasterols
- Etc…

Prenol lipids

- Isoprenoids
- Quinones
- Polyprenols
- Etc…

Saccharolipids

- Acylaminosugars
- Acylaminosugar glycans
- Etc…

Polyketides

- Flavanoids
- Linear polyketides
- Polyenes
- Etc…

The challenge of specificity

THE MULTIPLE LEVELS OF LIPID STRUCTURAL SPECIFICITY

Lipid class: PE, SM, TAG, etc…
- TLC, NMR, MS

Sum composition: PE 36:1
- Shotgun, IDA/DDA (via infusion or by LC MS/MS)

Fatty acid identification: PE (16:0_20:1); (18:0_18:1); (14:0_22:1); etc…
- LC-MS/MS, MS/MS^{ALL}, IDA/DDA

Fatty acid position: PE(16:0/20:1)
- DMS (SelexION® Technology), PLA_2

Double bond position: PE (16:0/20:1Δ11)
- OzID, Paternò-Büchi Rxn, Hv-PD

Stereochemistry: PE (16:0/20:1(11Z))
- GC-MS/MS, HR-NMR, Complex LC-MS/MS techniques

EAD-based fragmentation can be used to fully characterize the structures of lipid molecular species

Photo by K8 on Unsplash

Complexity as a function of specificity: PE 36:1

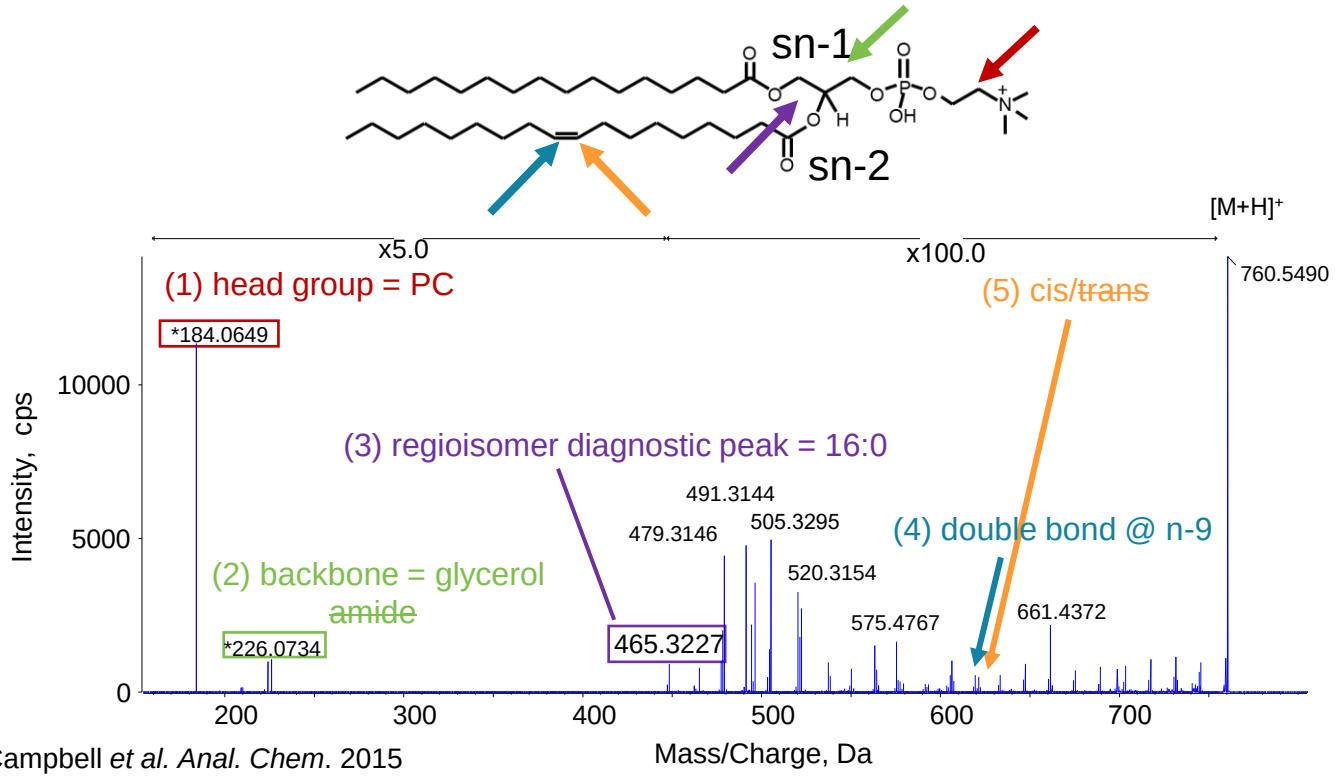
TRANSLATION OF SUM COMPOSITION TO SPECIFIC LIPID MOLECULAR SPECIES

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**

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

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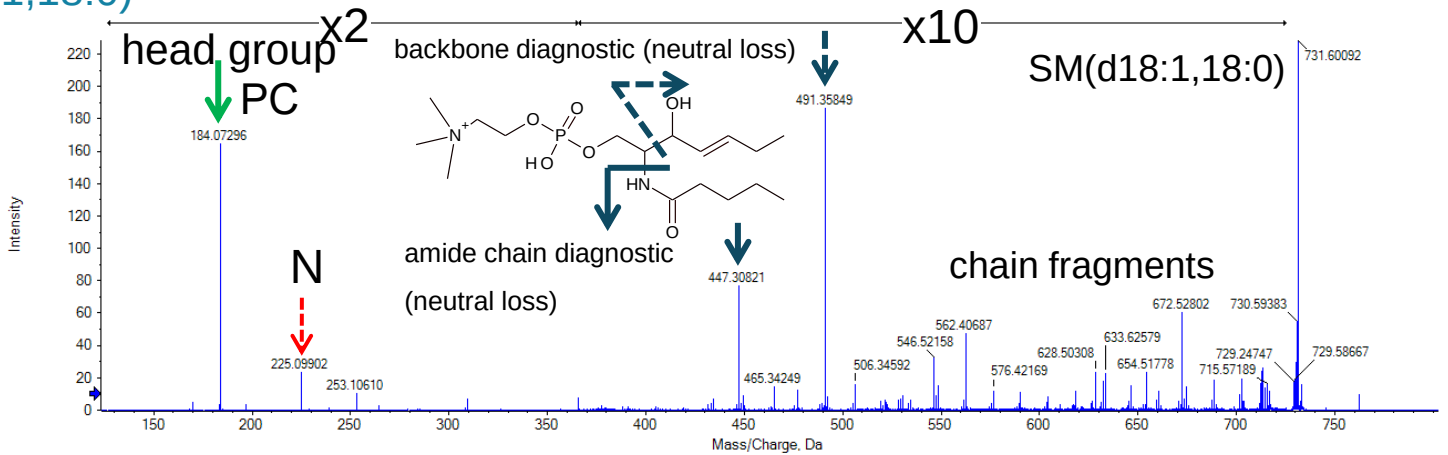
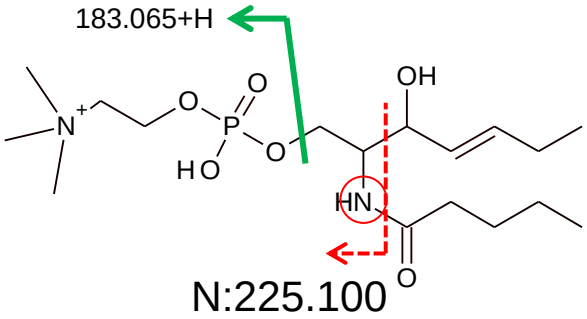
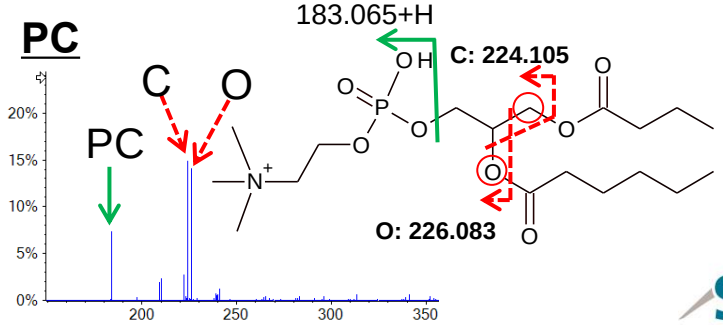
EIEIO of sphingomyelins (SMs)

SM(d18:1,18:0)



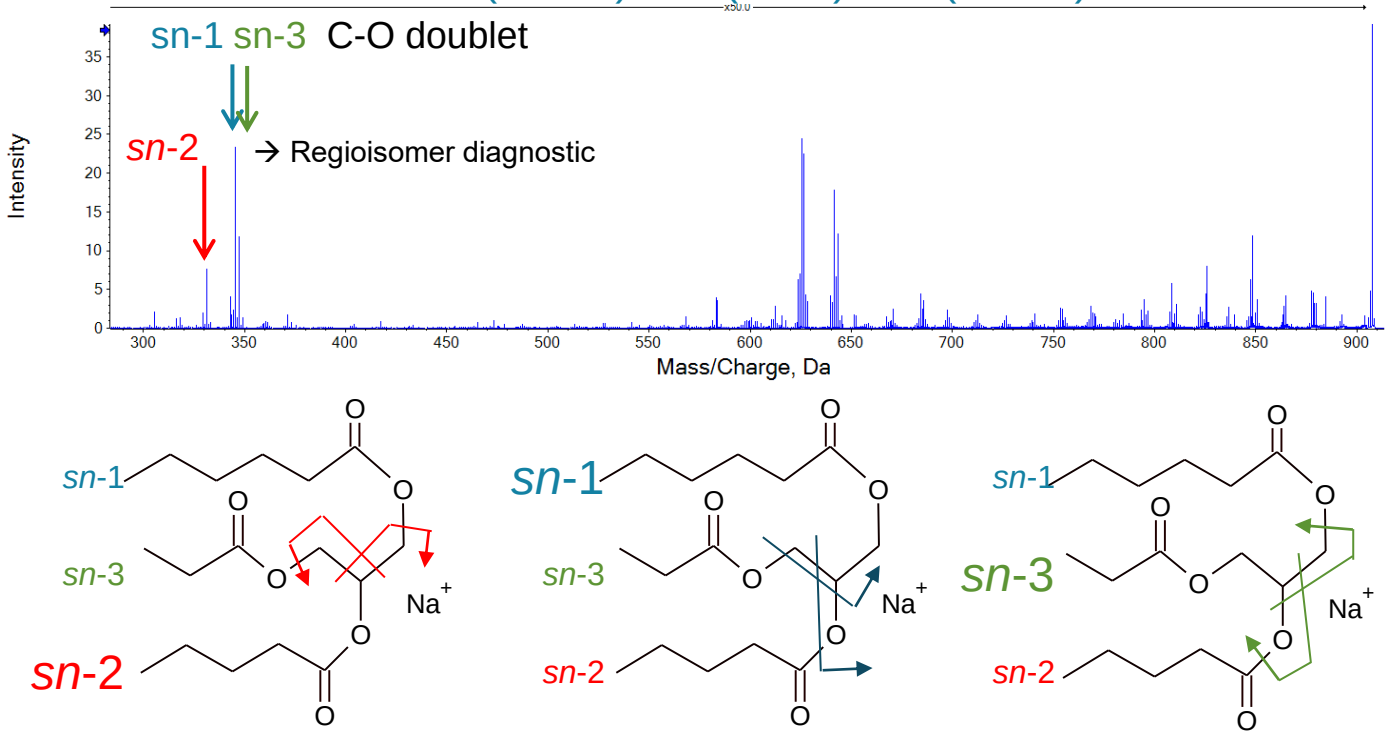




Baba et al. (2016) *J Lipid Res*. **57(11)**:858-867.

Differentiation of regioisomers

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



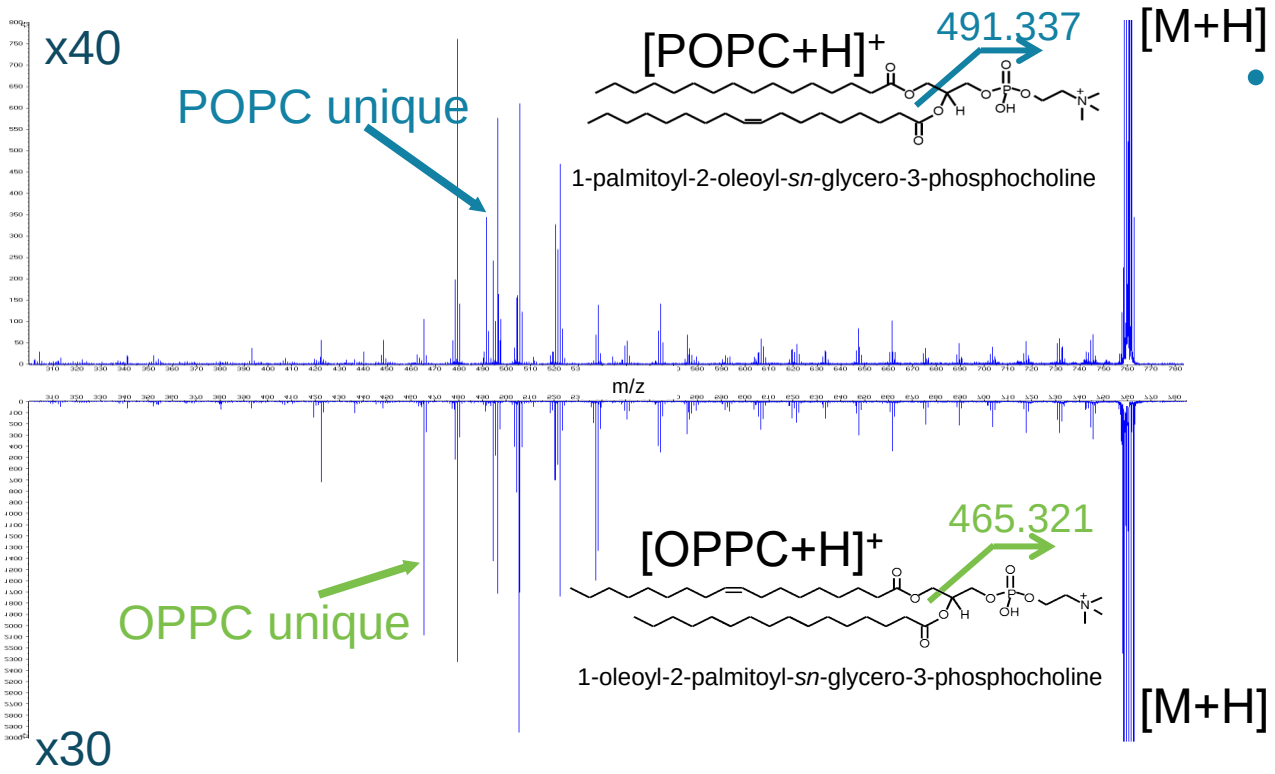
- sn-2 attachment point can be differentiated from the sn-1 position **or** sn-3 position through examination of the dual chain loss fragment ions

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

Differentiation of acyl chain position

ZenoTOF 7600 System

EAD MS/MS - POPC / OPPC IDENTIFICATION



- POPC vs OPPC
  - Using EAD, the position of the fatty acyl chains can be determined
  - Diagnostic fragments are shown for each lipid species

L Campbell *et al.* (2015) *Anal. Chem.* **87,11**, 5837–5845

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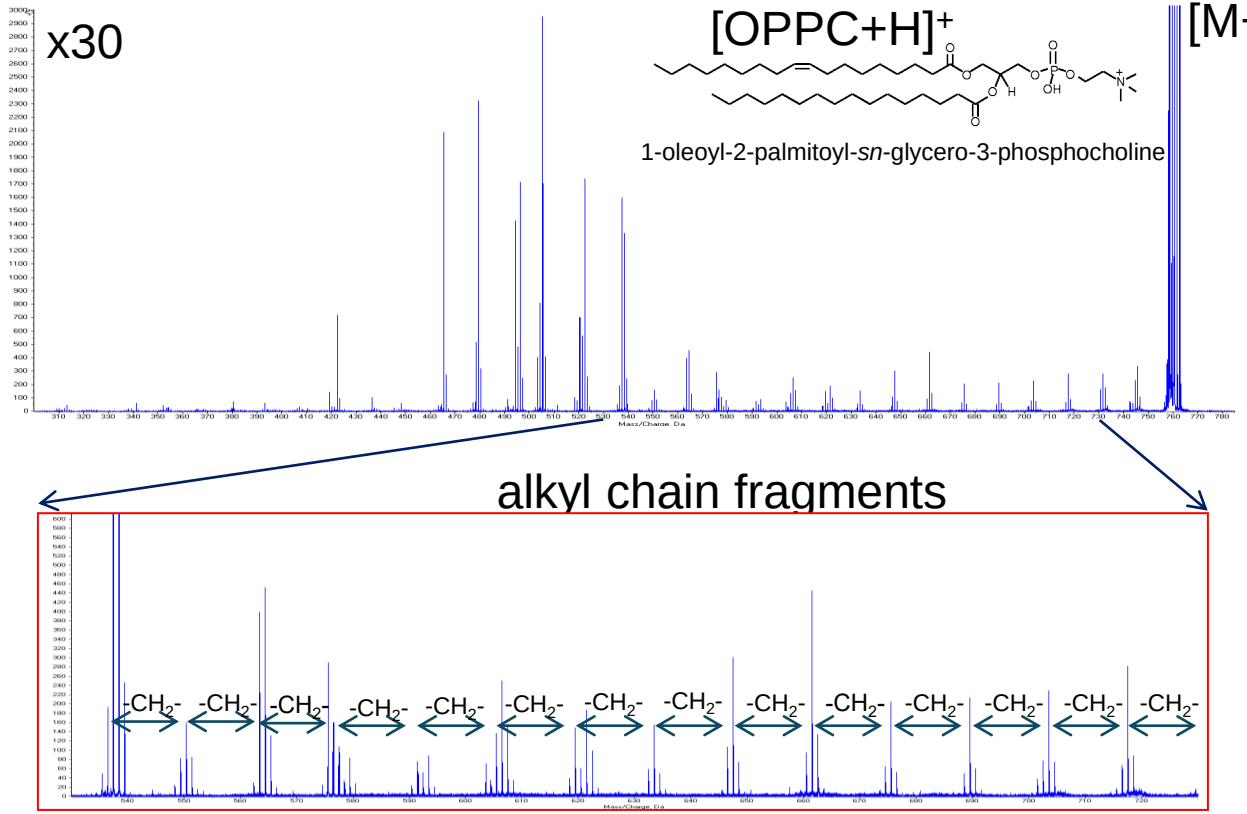
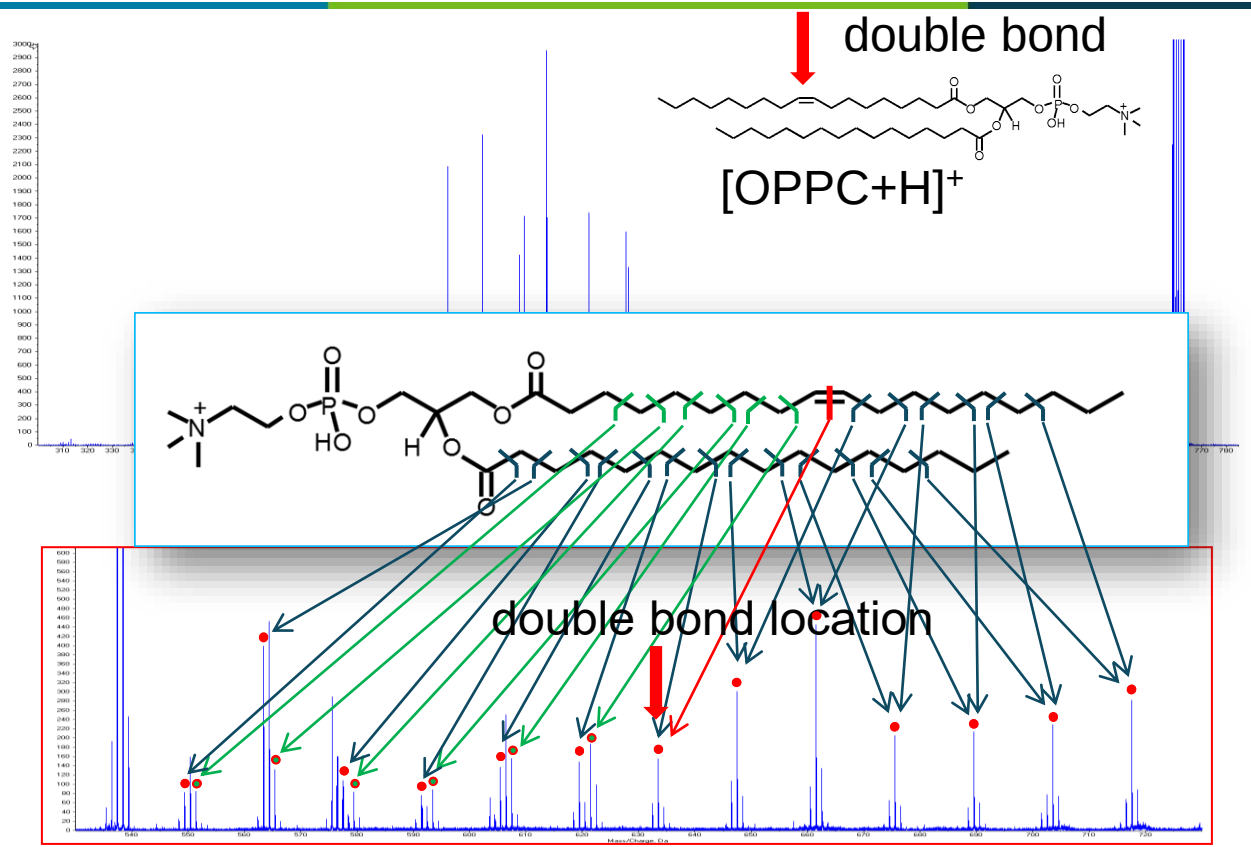
EAD-based fragmentation of OPPC: alkyl chain dissociation



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

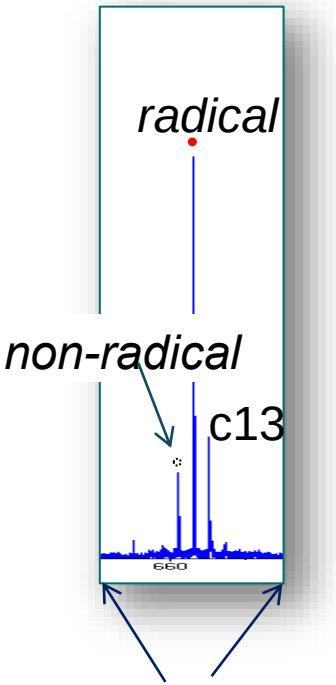
EAD-based fragmentation of OPPC: alkyl chain dissociation

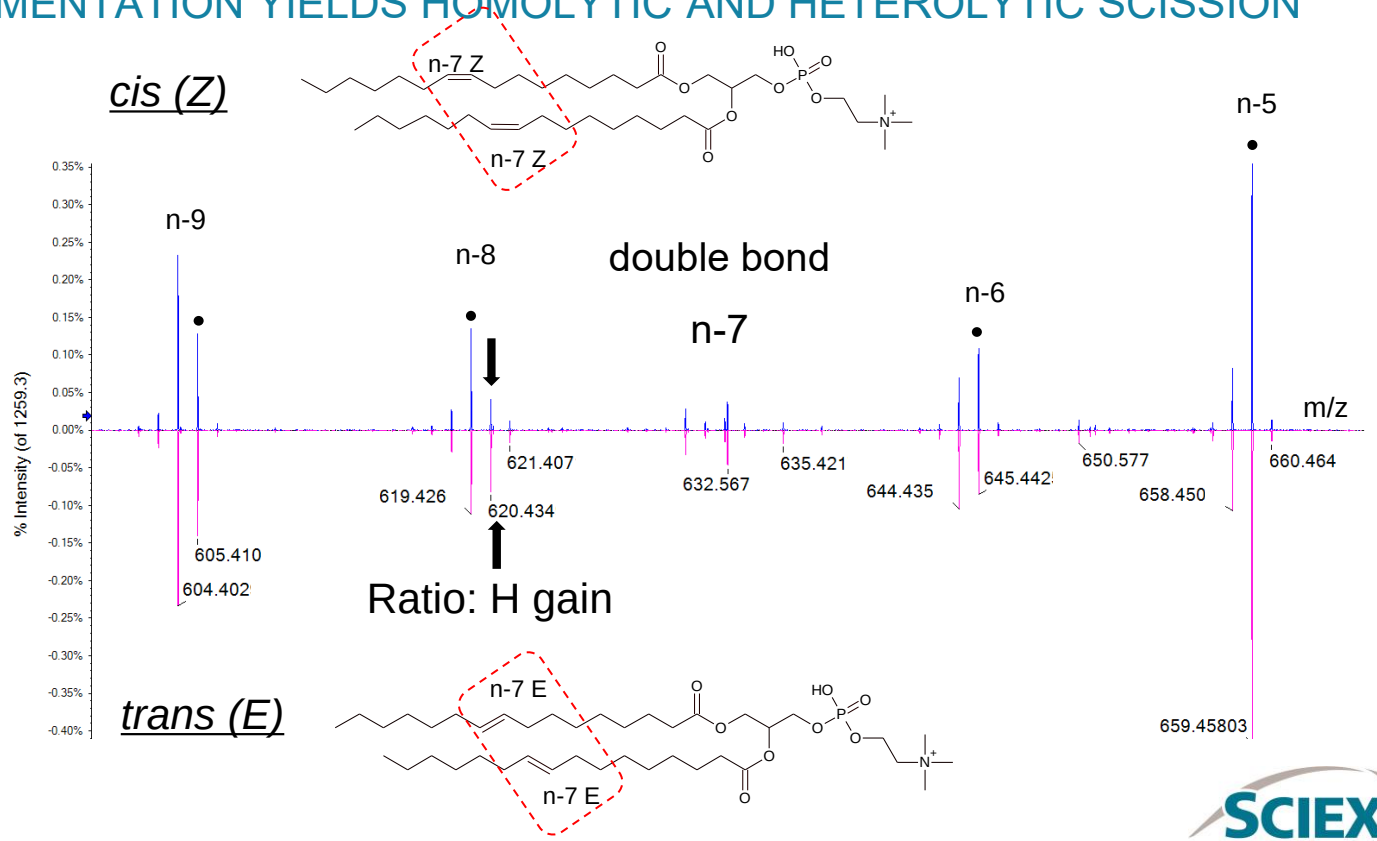


Campbell and Baba, (2015) *Anal. Chem.* **87(11)**, 5837–5845

Distinguishing between cis and trans double bonds

EAD-BASED FRAGMENTATION YIELDS HOMOLYTIC AND HETEROLYTIC SCISSION PRODUCTS





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

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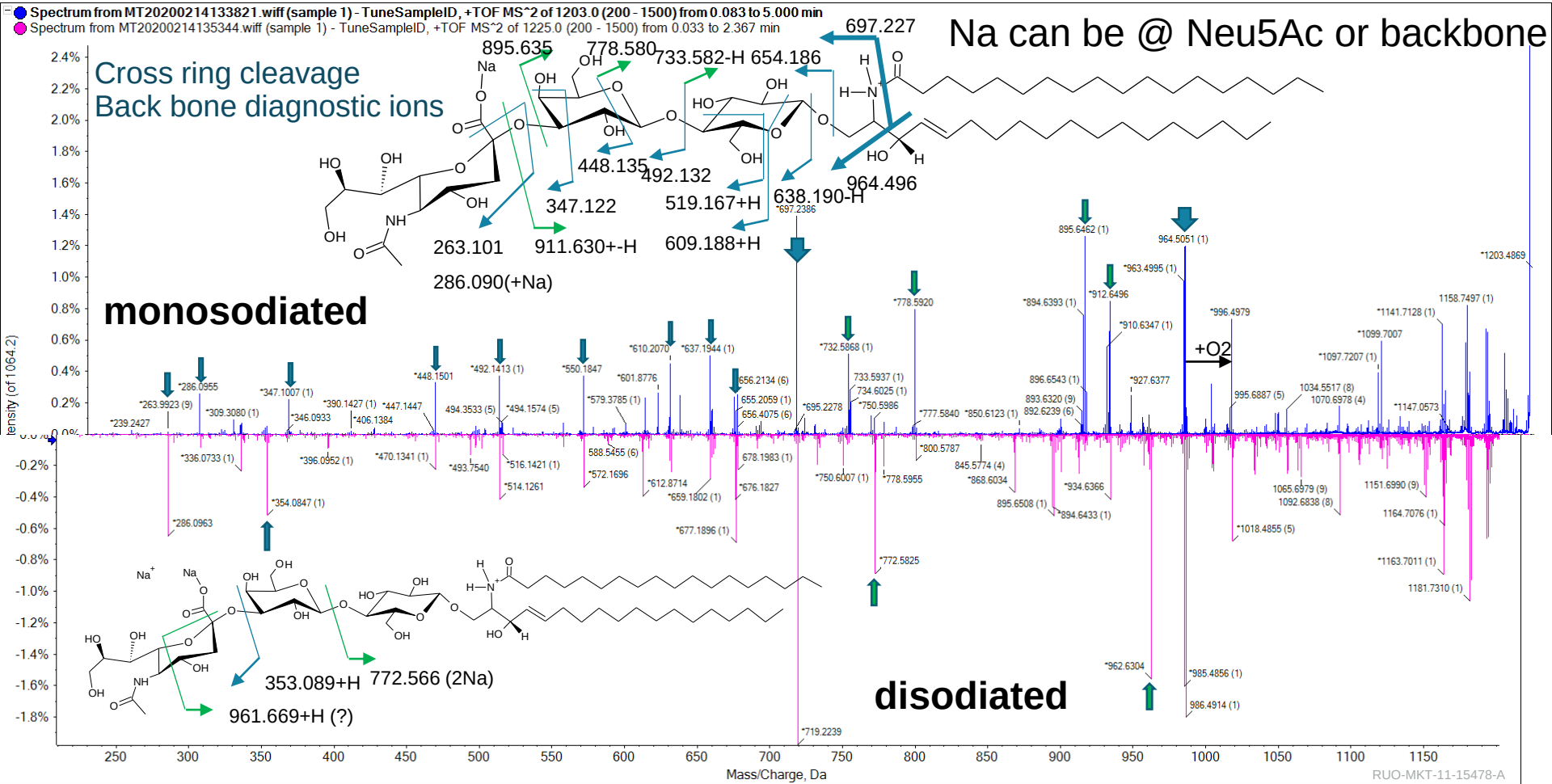
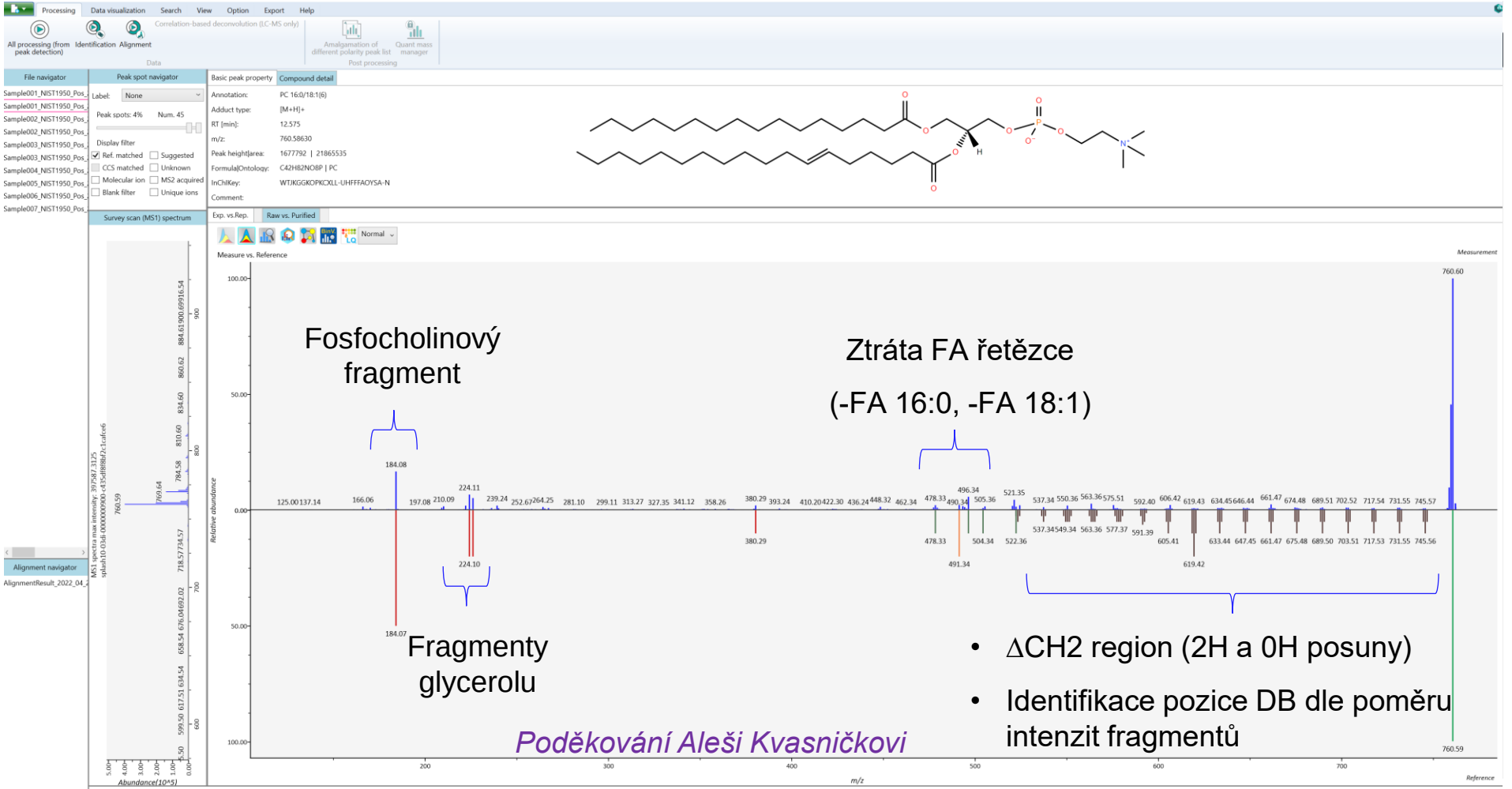
GM3-sodiated 1+: EIEIO



Sneak peek: MS-DIAL 5 alpha -> LC-MS data s EAD fragmentací (ZenoTOF 7600)



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

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Thank you

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