Improving Metabolomics and Lipidomics Analyses with Ozone-Based Approaches

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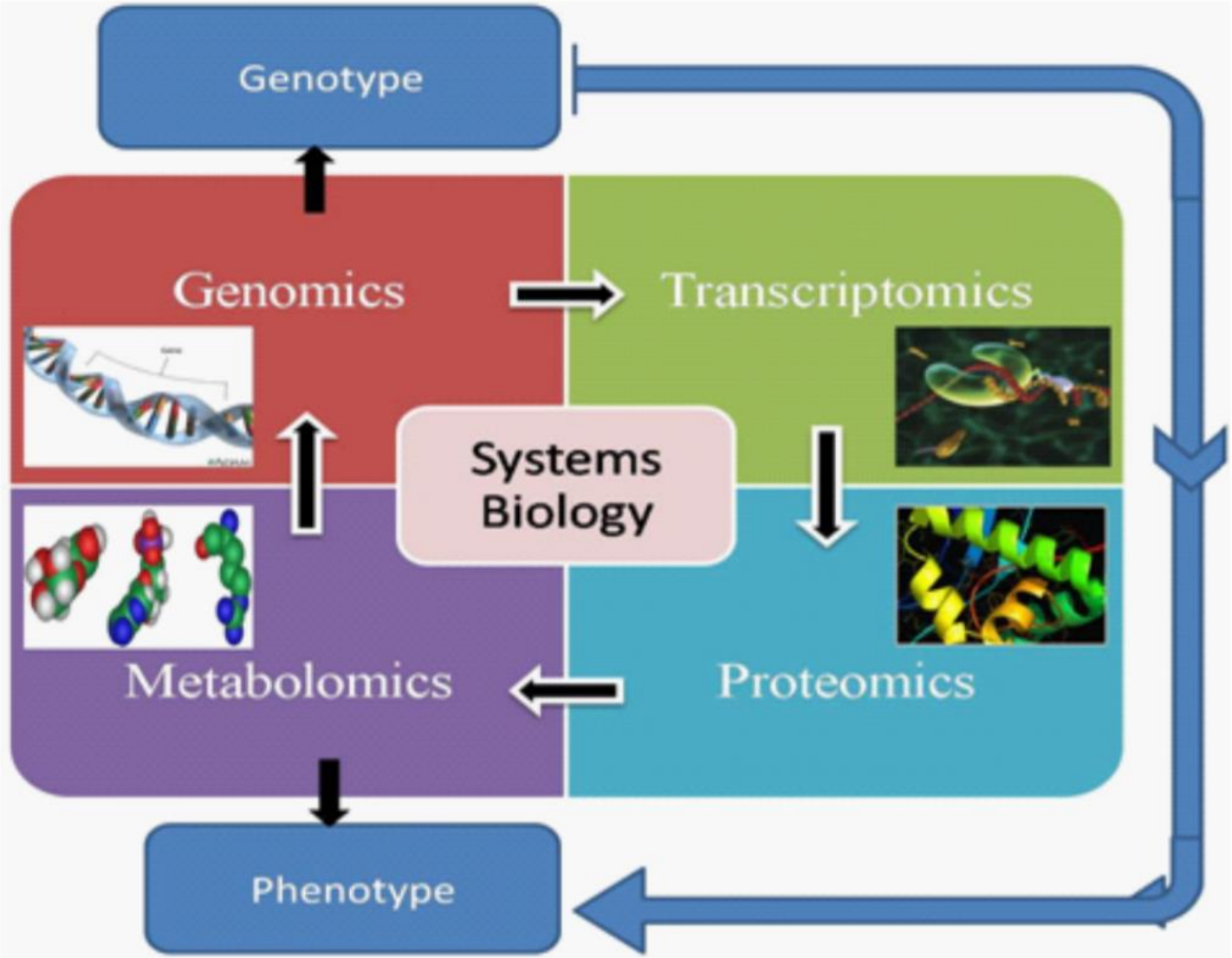
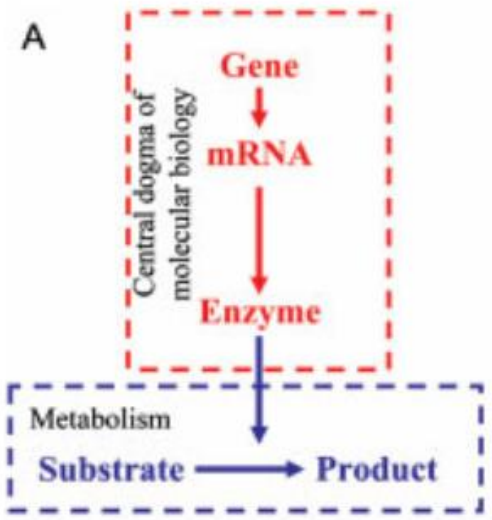
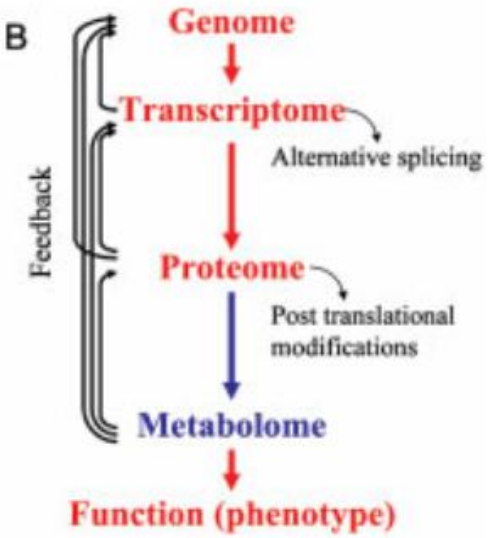
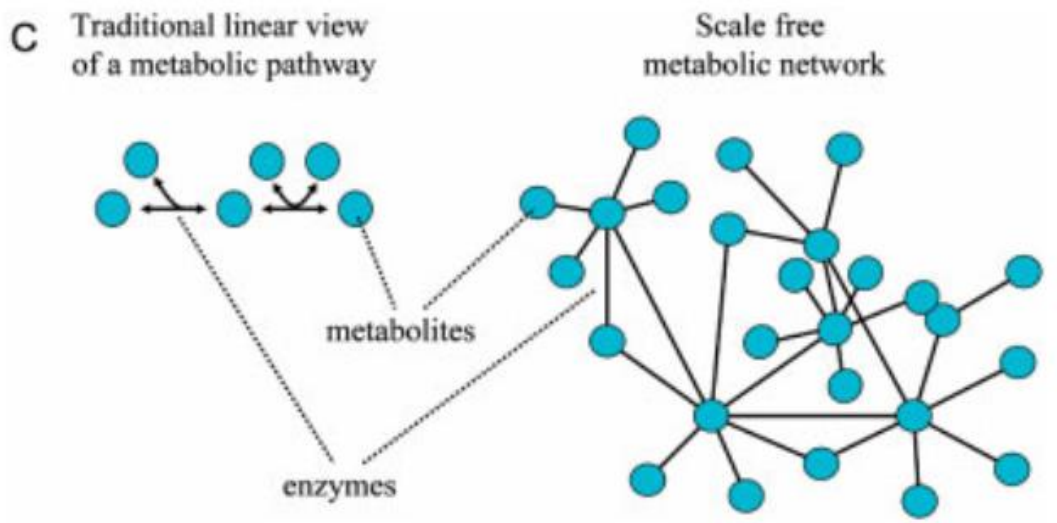
Tuesday, June 4th, 2019

Conventional –Omics Analysis







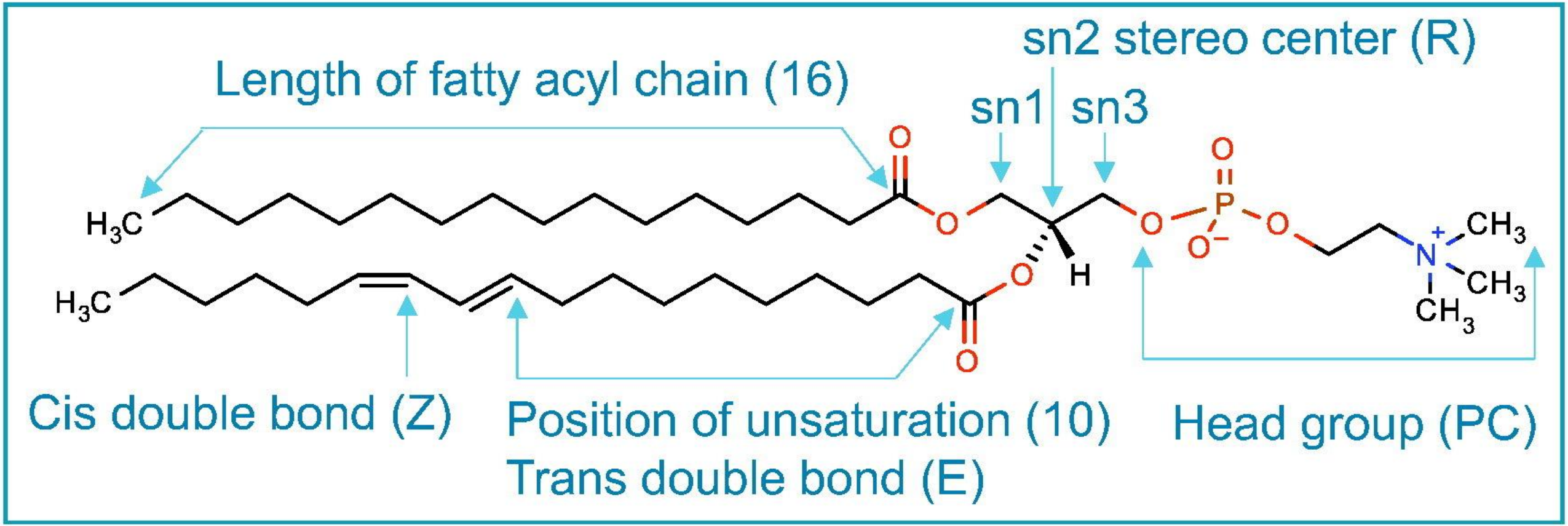


Nanda, T. et al. *Journal of Computer Science & Systems Biology.* **2011**, *S13*, doi:10.4172/jcsb.S13-003.
Hollywood, K. et al. *Proteomics*. **2006**, *6*, 4716-4723.

Conventional –Omics Analysis

Technique	Advantages	Disadvantages
Chromatography (LC, GC, SFC, etc.)	• Pre-MS separation • Reduced suppression • Capable of isomer separations	• Time of analysis (mins/hours) • Non-specific separations • Extensive method development for challenging separations

Challenging Separations



"UHPLC-HRMS/MS often does not provide sufficient information to characterize all structural details of lipid molecules"

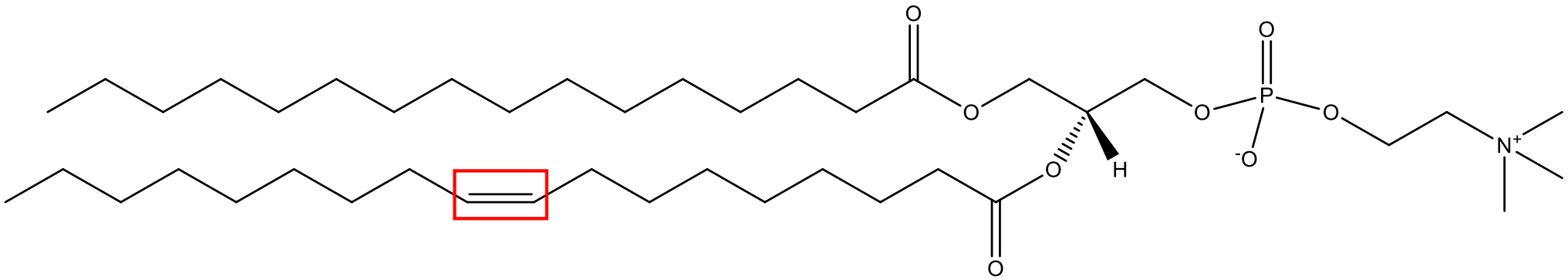
Koelmel, J.P., et al. *Biochimica et Biophysica Acta.* **2017**, *1862*, 766-770.

Conventional –Omics Analysis

Technique	Advantages	Disadvantages
Chromatography (LC, GC, SFC, etc.)	• Pre-MS separation • Reduced suppression • Capable of isomer separations	• Time of analysis (mins/hours) • Non-specific separations • Extensive method development for challenging separations
High Resolution Accurate Mass HRAM Mass Spectrometry	• Resolution of isobars • More accurate identification	• **Cannot differentiate isomers**
Tandem Mass Spectrometry (MS/MS)	• Improved S:N for targeted • Structural Information	• **Cannot always differentiate isomers (i.e., stereoisomers)** • **Not universal for functional groups**

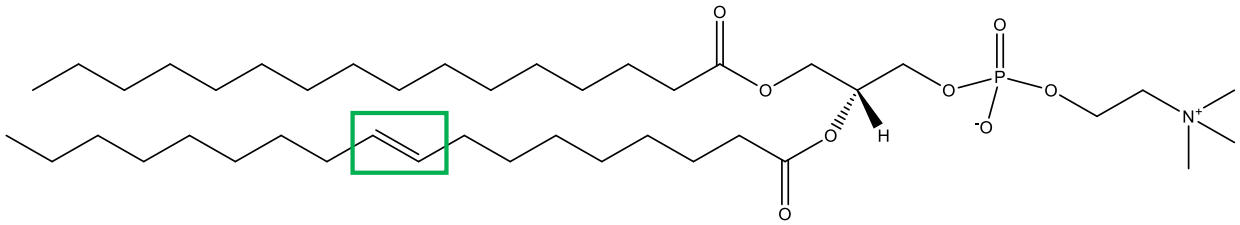
What other strategies can we incorporate?

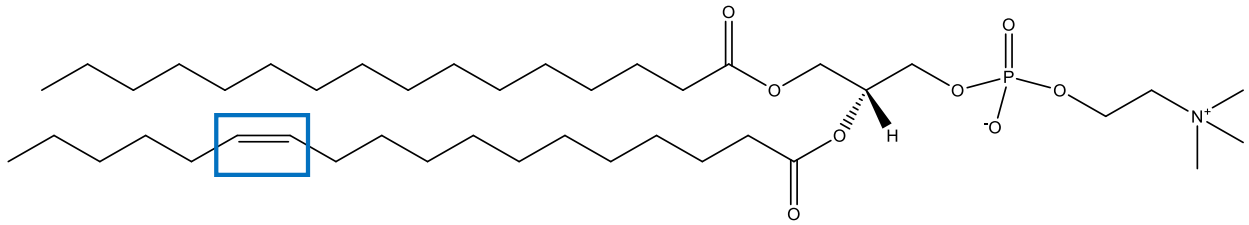
Challenging Separations



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

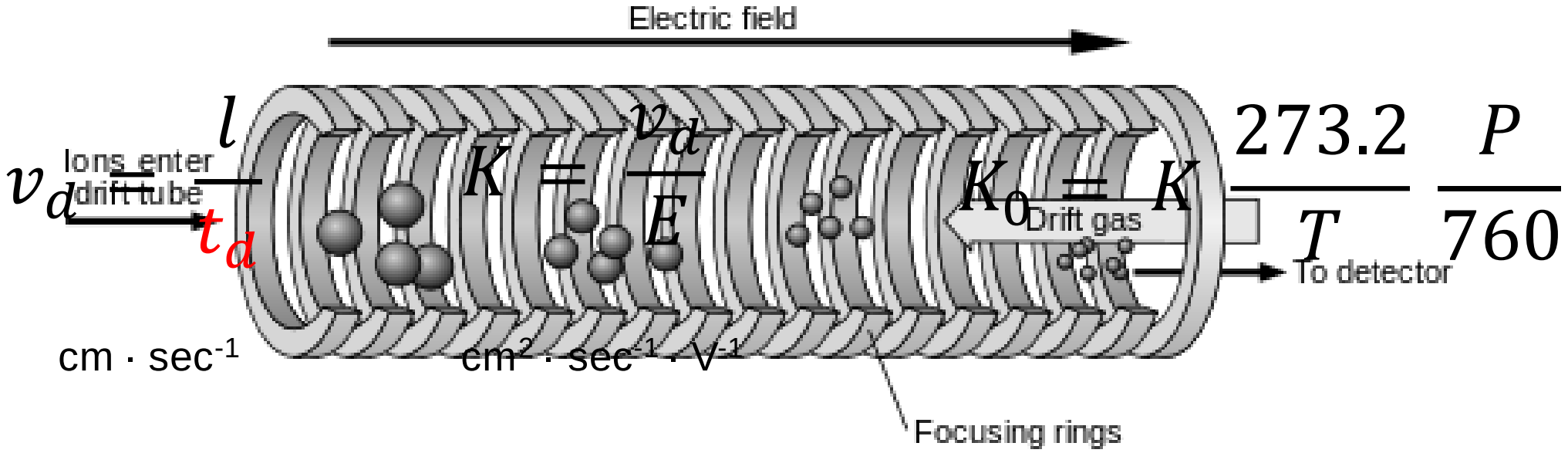
UHPLC-HRMS/MS → PC 16:0_18:1



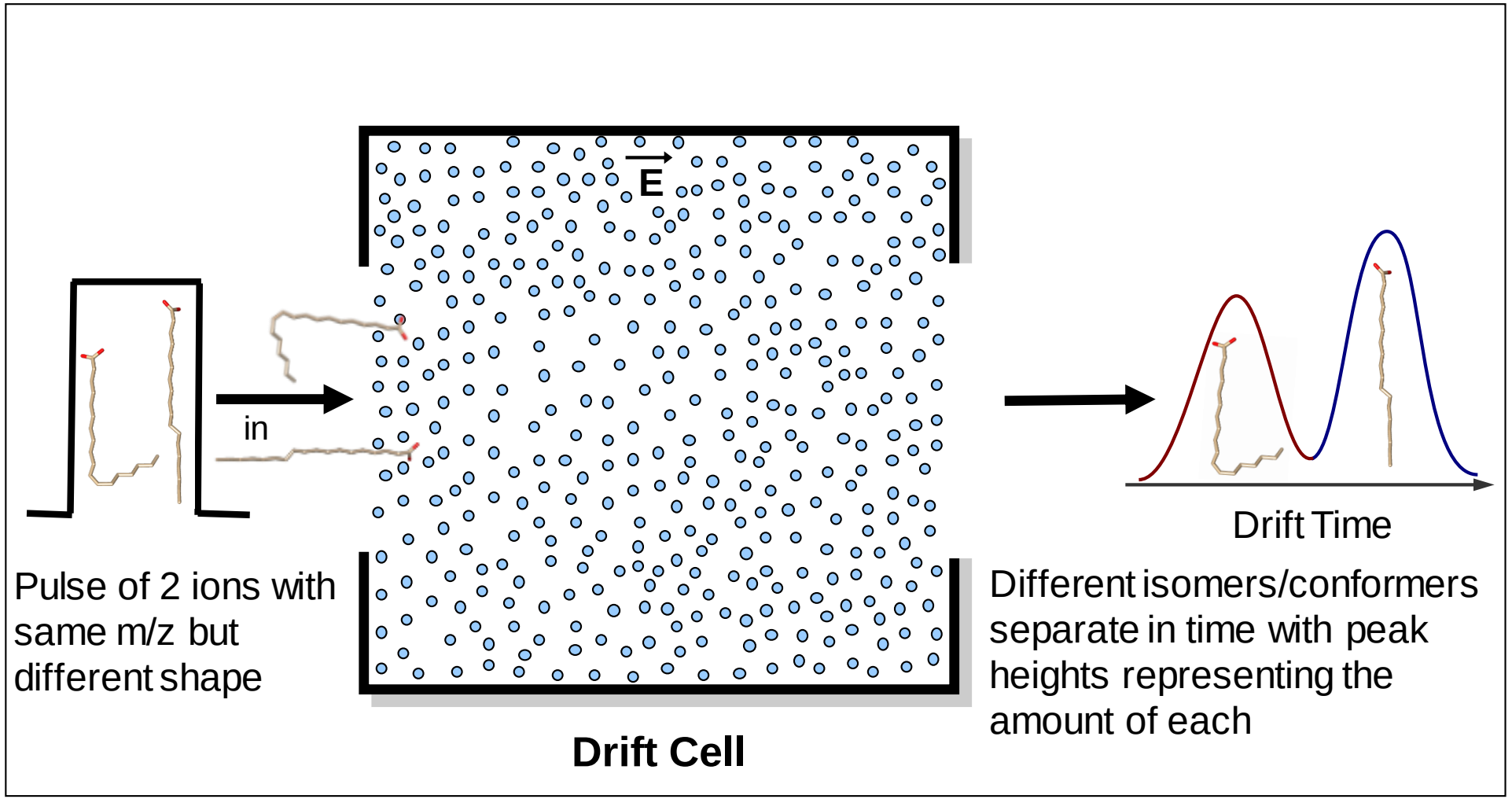


Drift Tube Ion Mobility (DT-IMS)

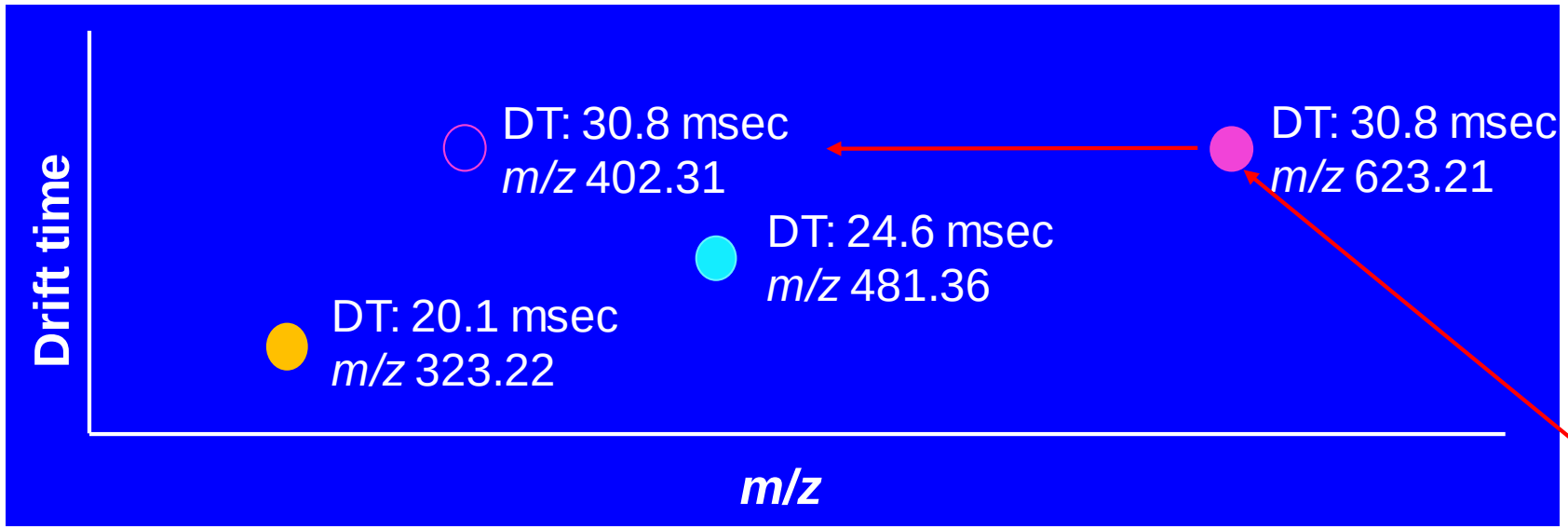
- Ions pulsed into drift tube with **constant electric field**
- Ions experience collisions with drift gas molecules and are separated based on **size, shape, and charge**
  - Smaller ions experience fewer collisions, travel at higher velocity
- Drift time (t_d) measured with detector (i.e., mass spectrometry)
- Ions have characteristic **mobility factor K**

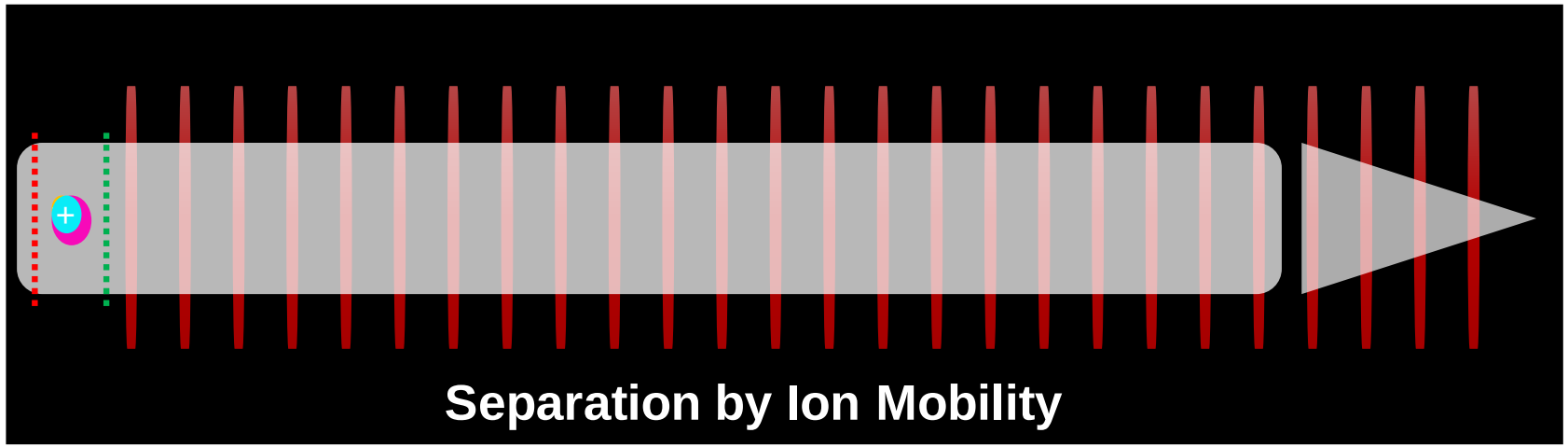


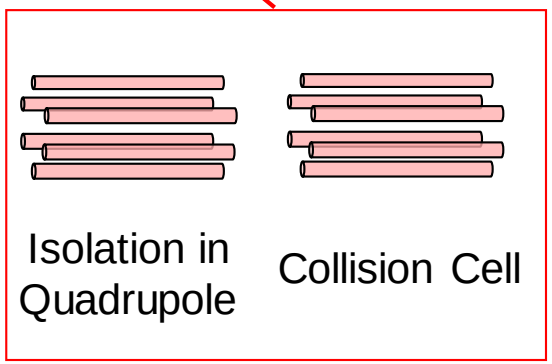
Ion Mobility Concept

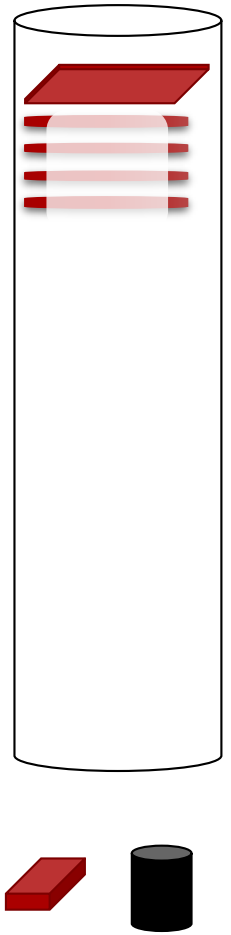


Introduction to IM-MS



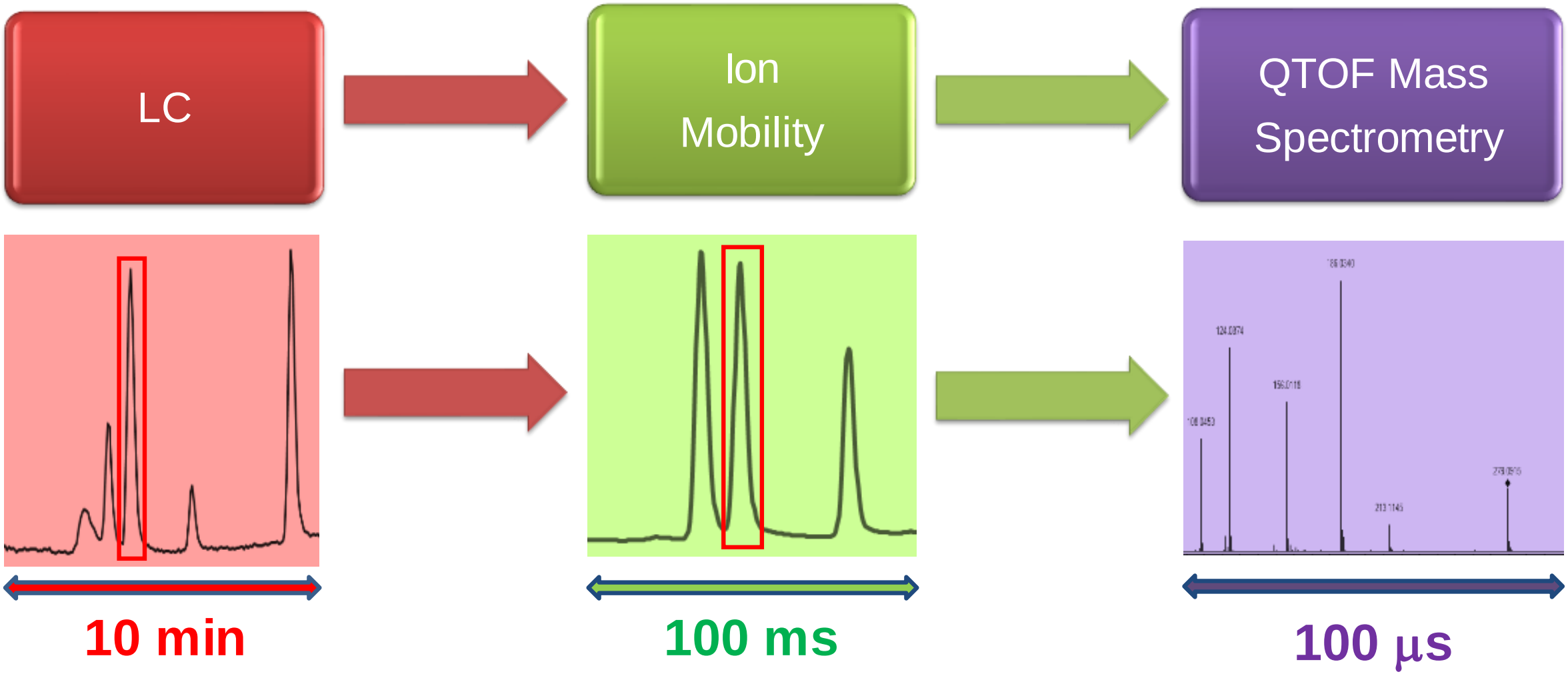






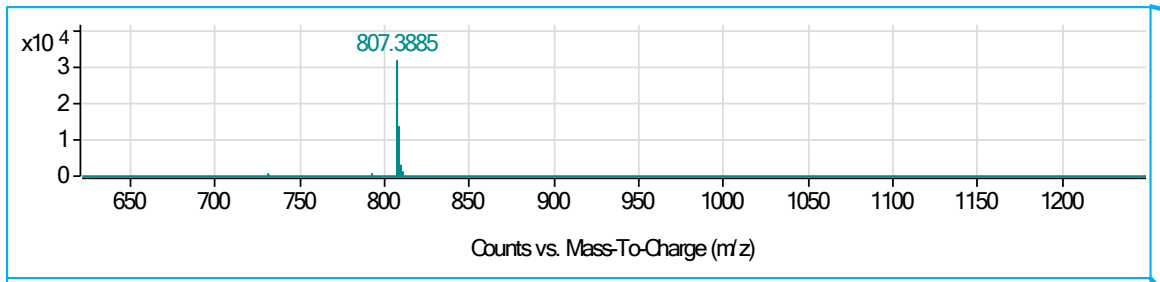
MS/MS Mode

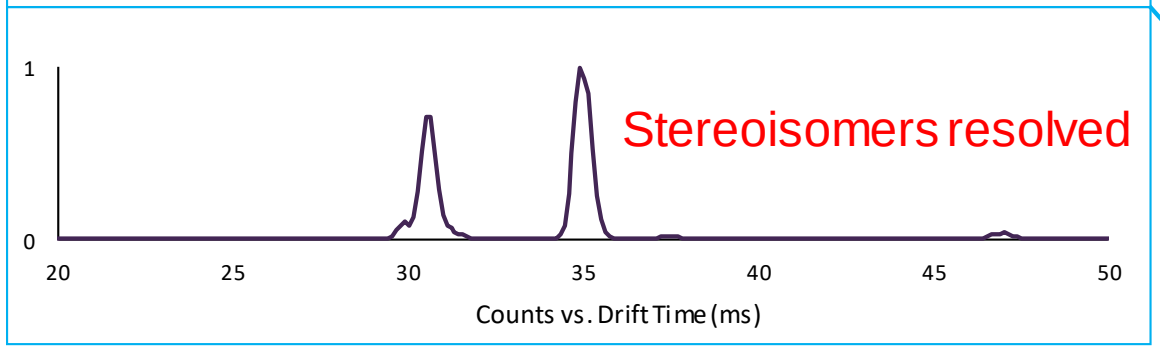
LC-IM-MS: Ideal Time-Scale Marriage



Ion Mobility Advantages

- Additional separation of components in complex mixtures
  - Chromatographic coeluting compounds; Isobars and isomers





Ion Mobility Advantages

- Additional separation of components in complex mixtures
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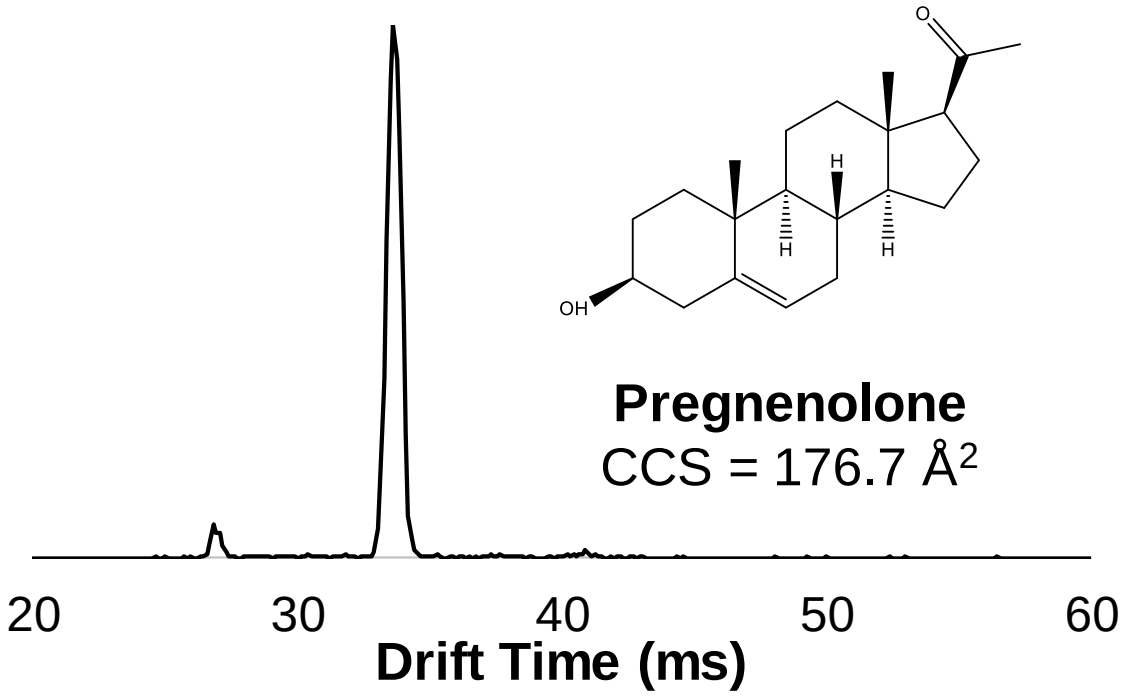
- Calculation of collision cross section (CCS)
  - Useful for identification – Can be coupled with RT and *m/z* databases



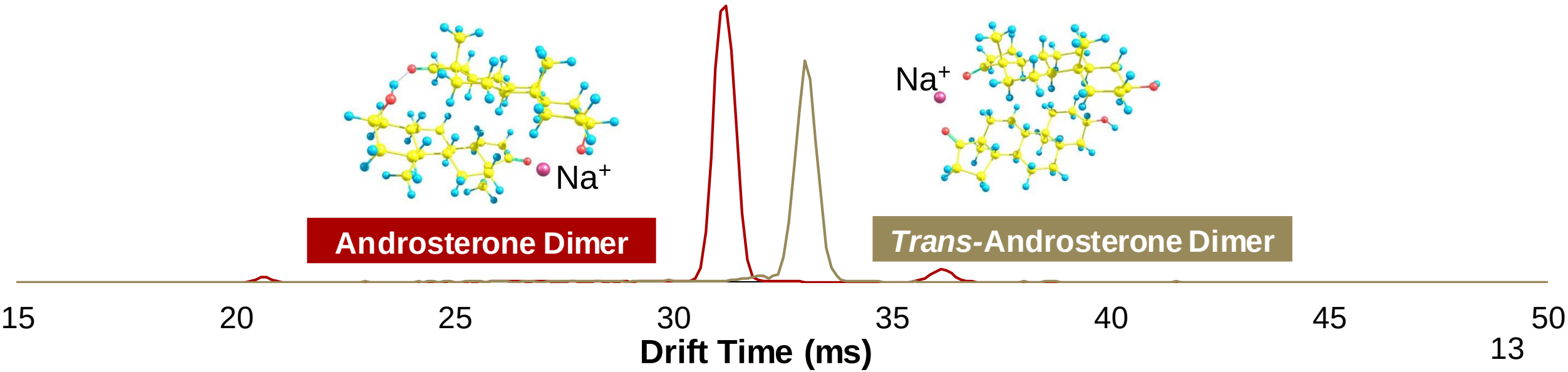
Collision Cross Section (Ω):

$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_B T)^{1/2}} \left[\frac{1}{m_I} + \frac{1}{m_B}\right]^{1/2} \frac{t_d E}{L} \frac{760}{P} \frac{T}{273.2} \frac{1}{N}$$

ze	Analyte Charge State	t_d	Corrected Drift Time
k_B	Boltzman Constant	E	Electric Field
T	Drift Tube Temperature	L	Drift Tube Length
m_I	Mass, Ion	P	Drift Tube Pressure
m_B	Mass, Buffer Gas	N	Drift Tube Number Density

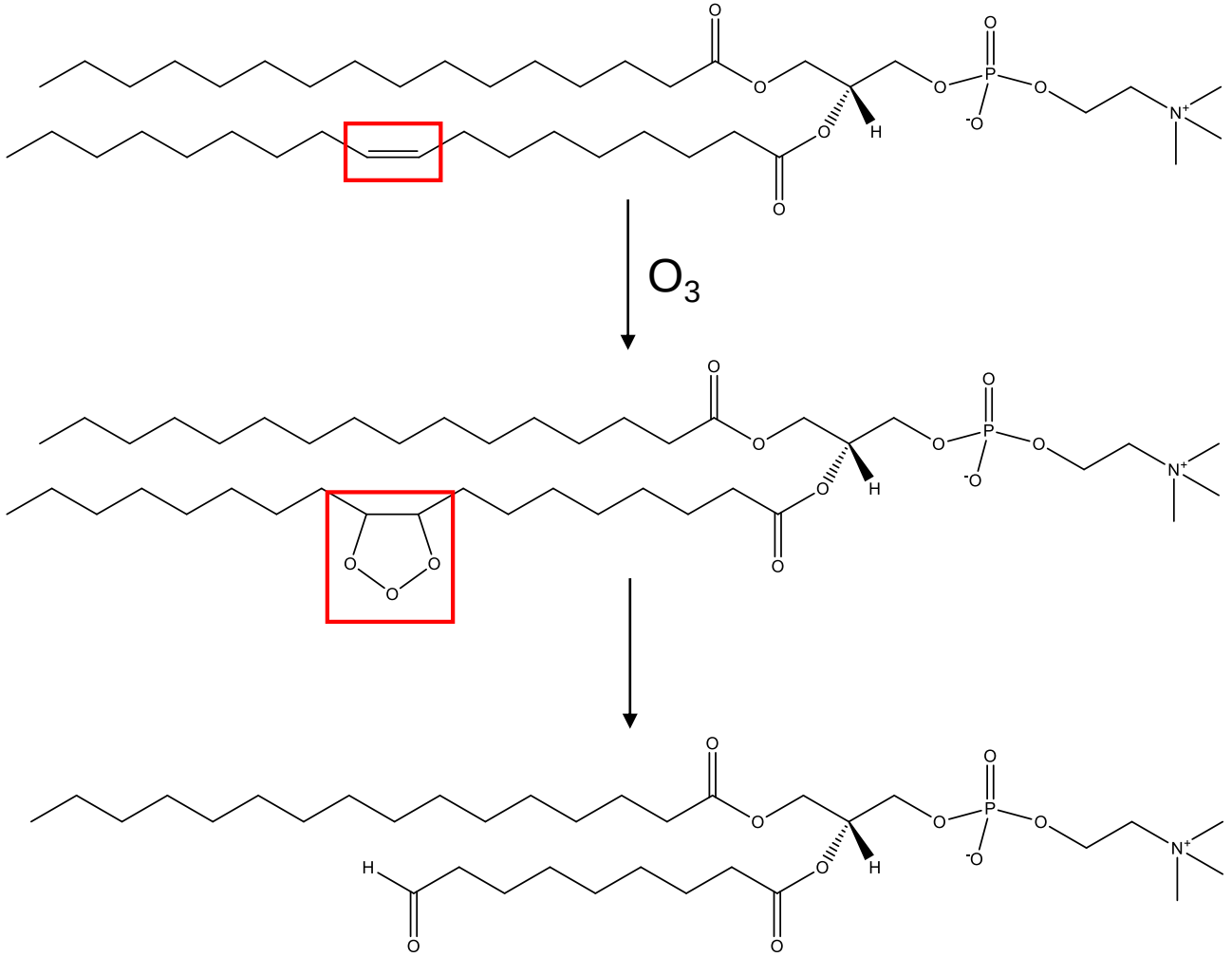
Ion Mobility Advantages

- Additional separation of components in complex mixtures
  - Chromatographic coeluting compounds; Isobars and isomers
- Calculation of collision cross section (CCS)
  - Useful for identification – Can be coupled with RT and *m/z* databases
- Structural information for gas-phase complexes
  - Binding strength; Complex shape and size



Mechanism of Ozonolysis

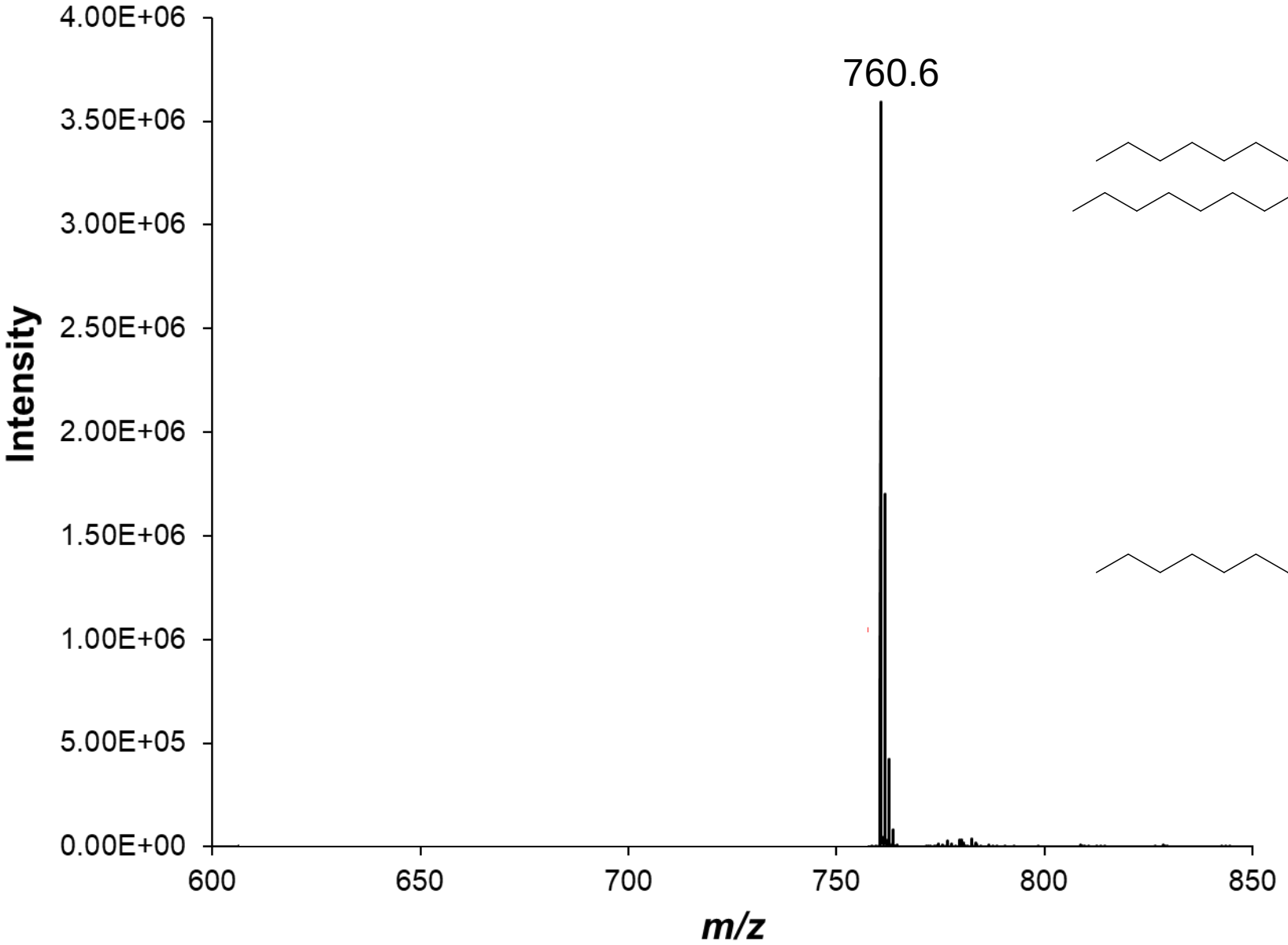
1-Palmitoyl-2-oleoyl-glycerol-3-phosphocholine
PC 16:0/18:1(9Z) (POPC)

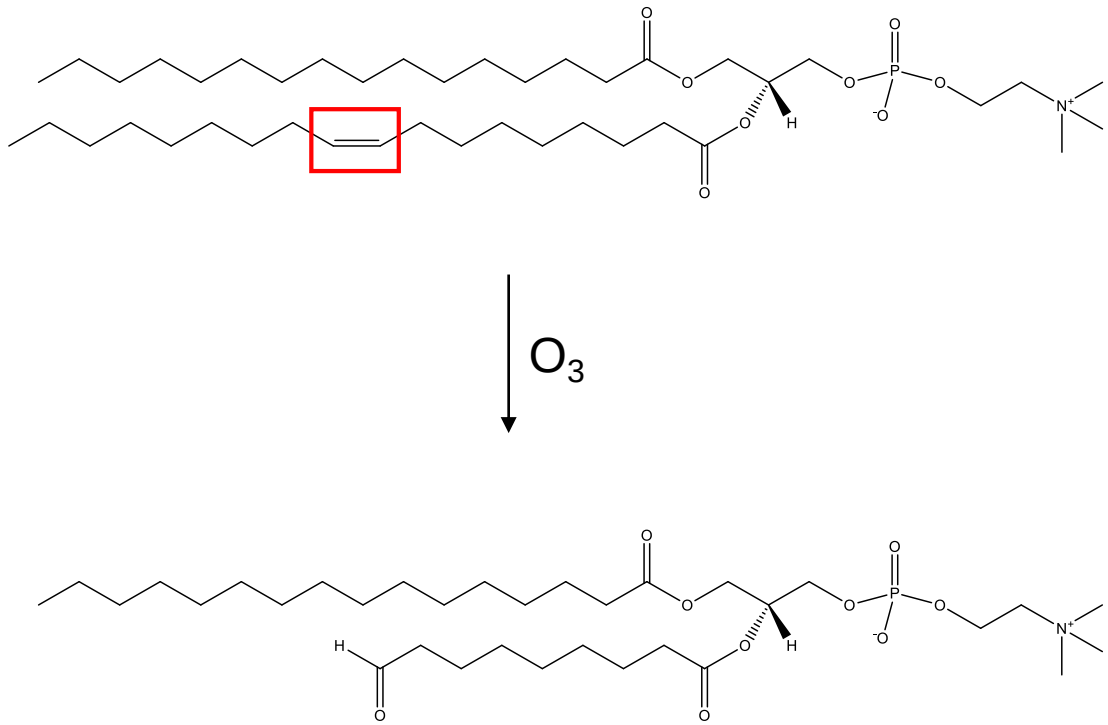


Addition of Ozone Across Double Bond
→ Molozonide

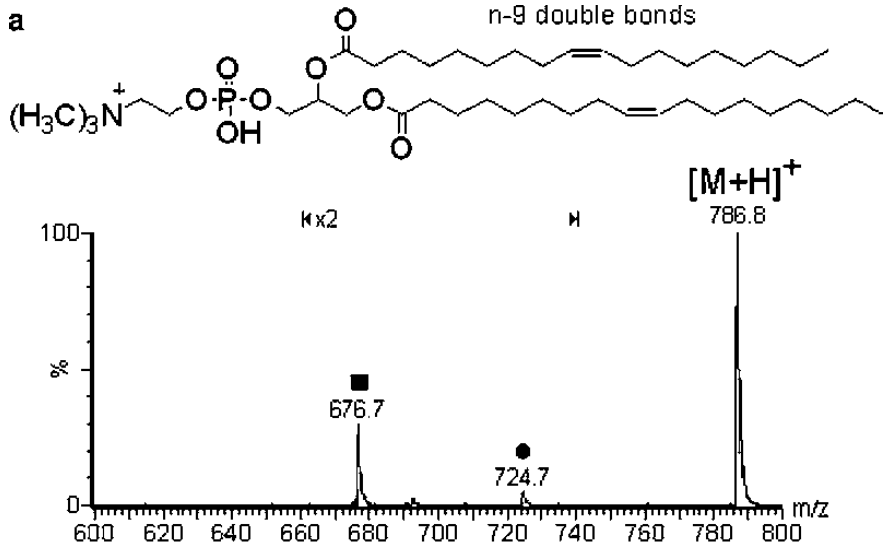
Aldehyde Product
-C_9H_{18} + O

Ozonolysis with Mass Spectrometry

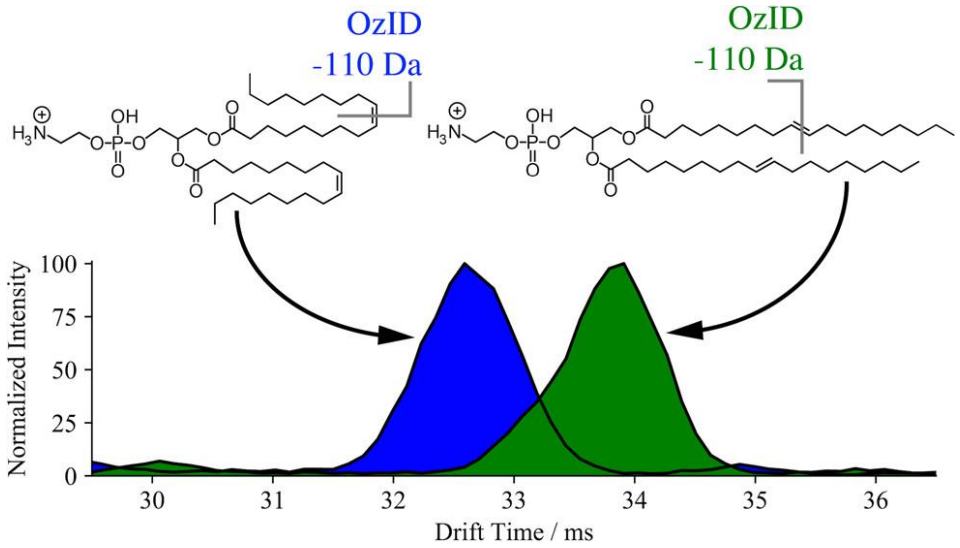




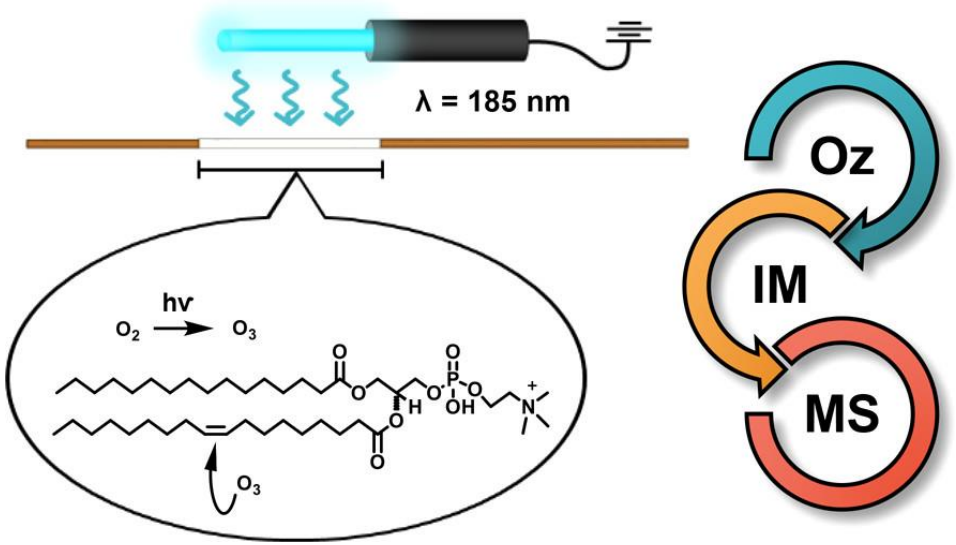
Ozonolysis for Lipidomics



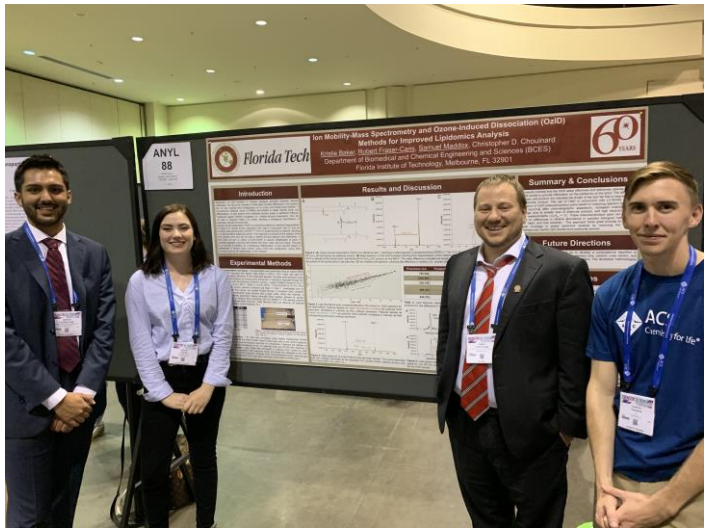
Thomas, M. et al. *Methods in Molecular Biology.* **2009**, *Lipidomics,* 413-441.



Poad, B.L.J. et al. *Analytical Chemistry.* **2018**, *90 (2),* 1292-1300.

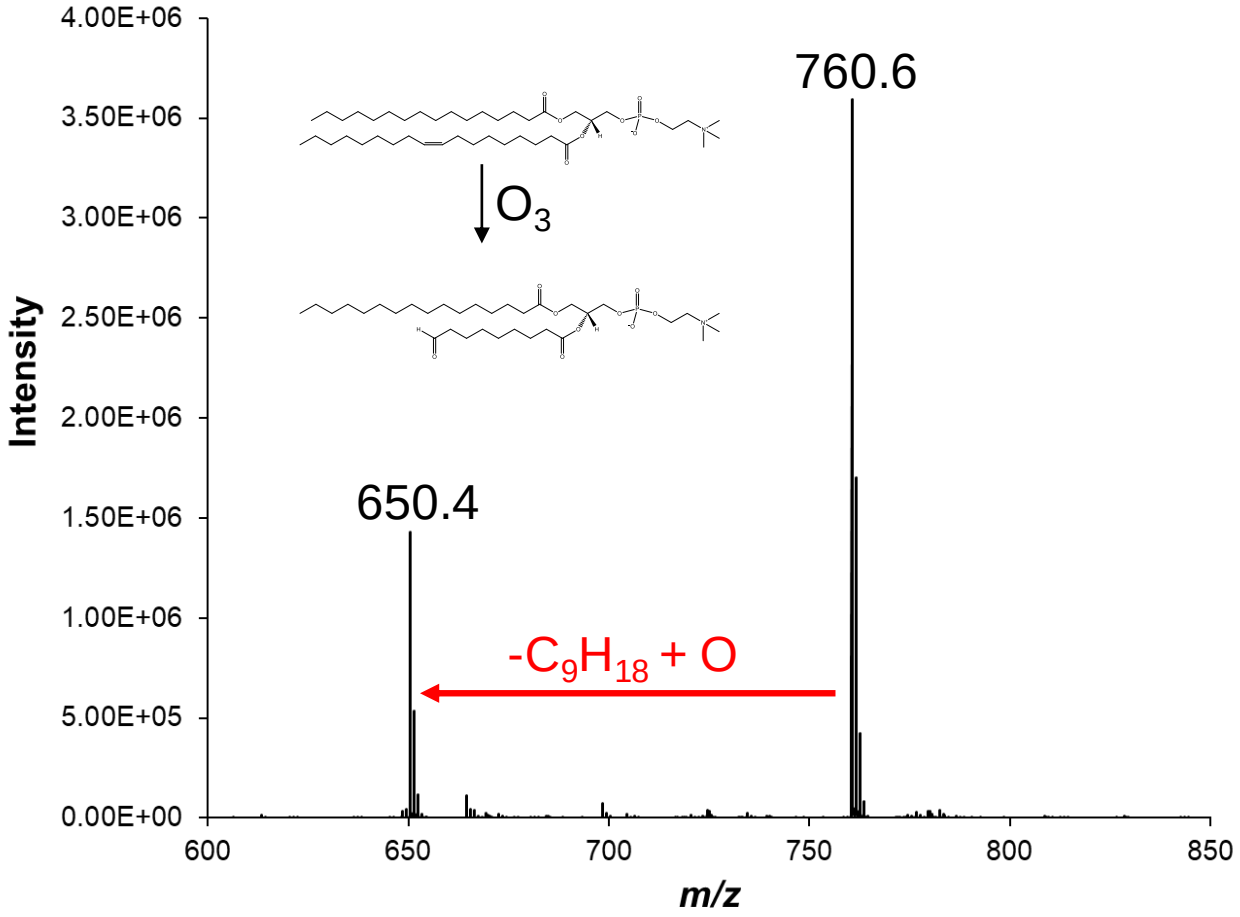


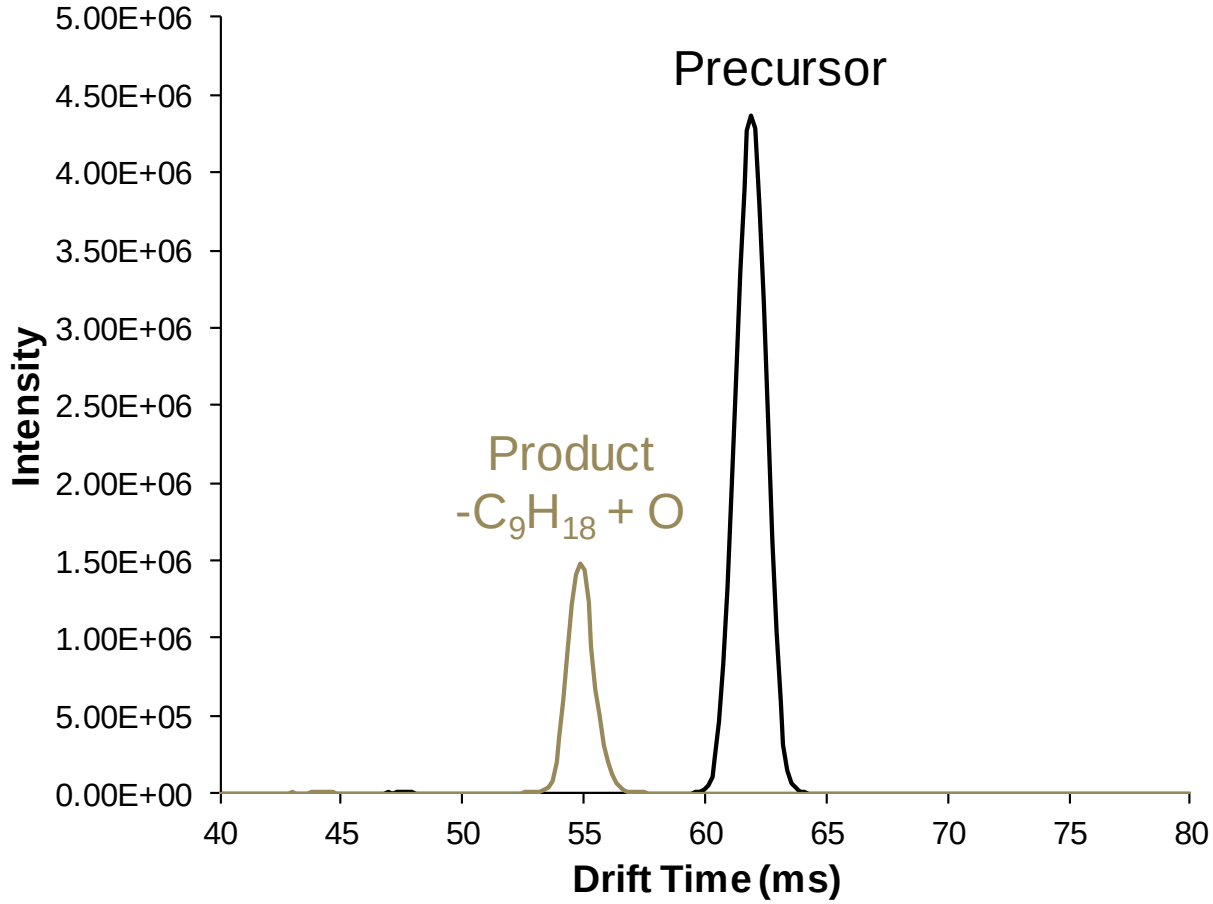
Harris, R.A. et al. *Analytical Chemistry.* **2018**, *90 (3),* 1915-1924.



Maddox, S. et al. *ACS National Meeting,* **2019**, *ANYL 88. Manuscript in Preparation (2019).*

Ozonolysis with Mass Spectrometry

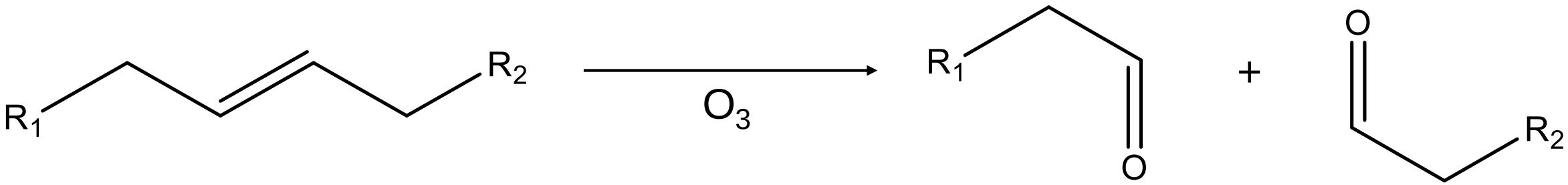




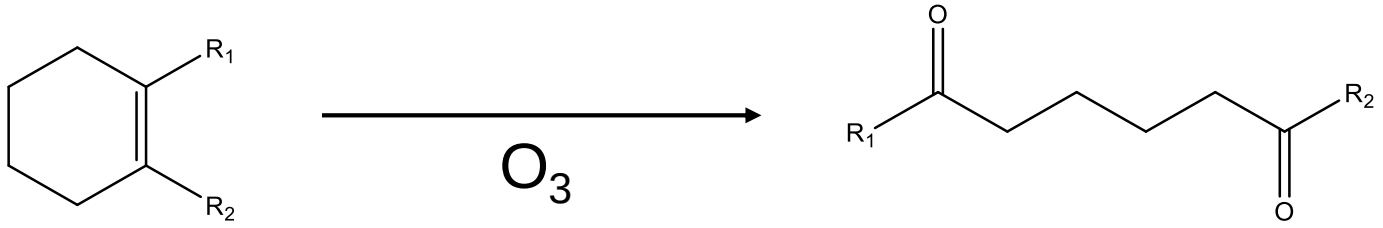
Can we use ozonolysis to augment structural differences for improving IMS separations?

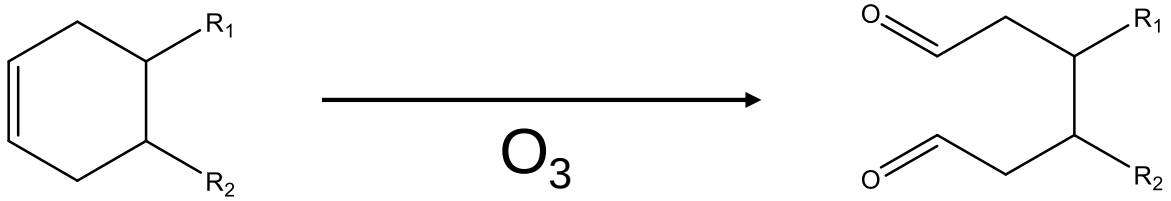
The Ozonolysis Mechanism

Ozone-Induced Dissociation (OzID)



Ozone-Induced Rearrangement (OzIR)



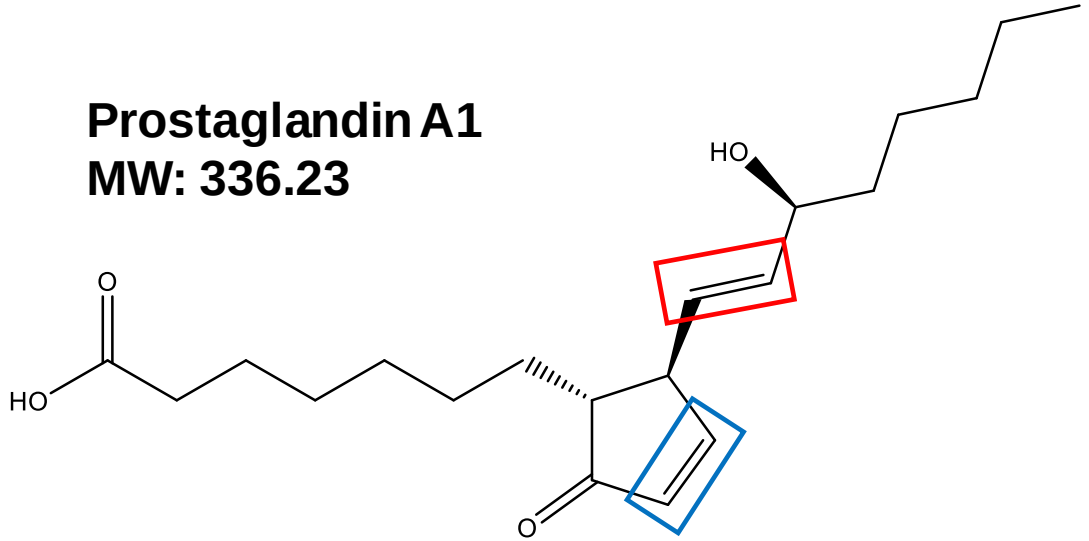


Prostaglandins

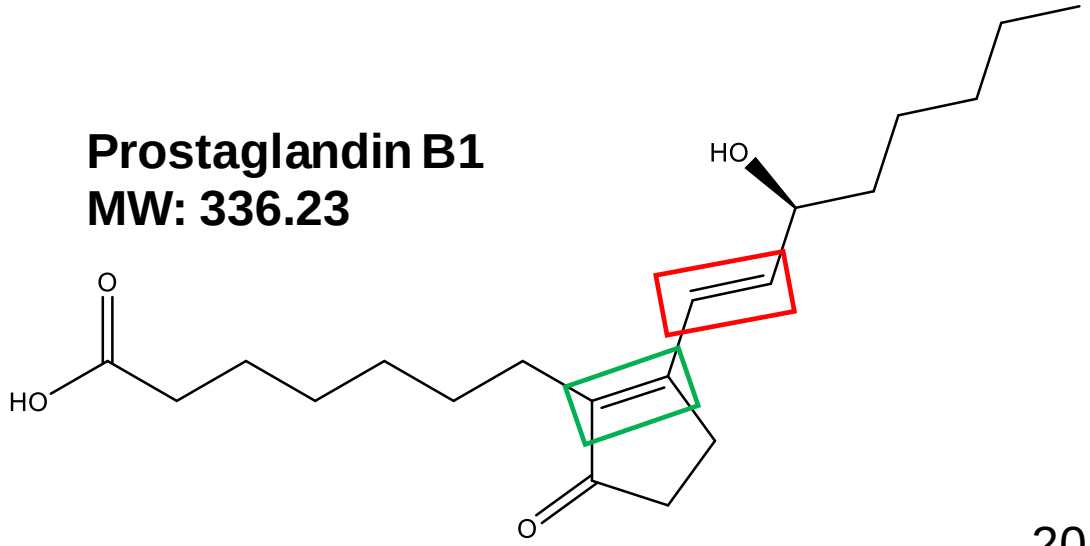
- Lipid autacoids derived from arachidonic acid

- Sustain homeostatic functions
- Mediate pathogenic mechanisms
- Promote/resolve inflammatory response

Prostaglandin A1
MW: 336.23



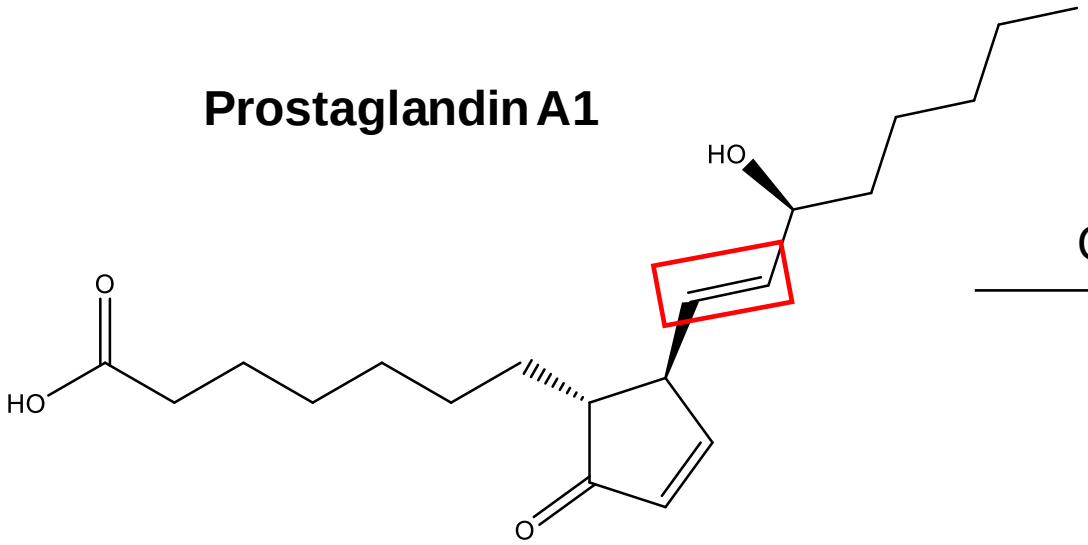
Prostaglandin B1
MW: 336.23



Ricciotti, E. et al. *Arteriosclerosis, Thrombosis, and Vascular Biology.* **2011**, *31,* 986-1000.

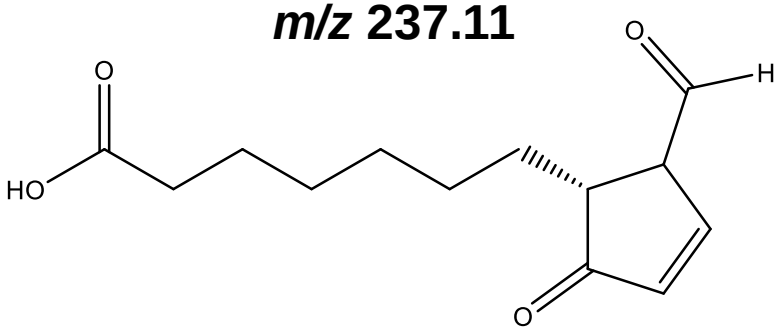
Prostaglandins with Ozonolysis

Prostaglandin A1

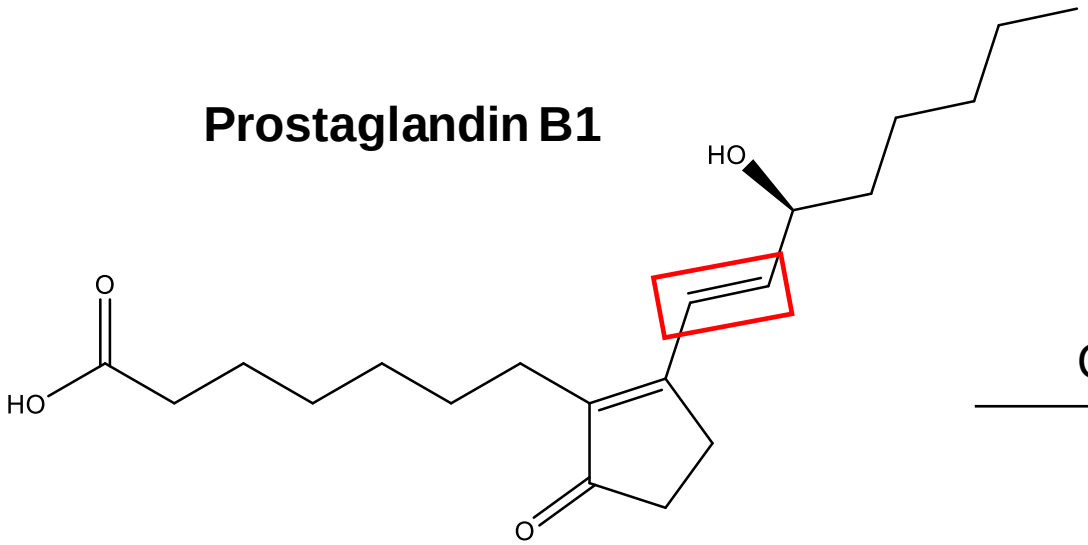


O_3

[M-H]$^-$
***m/z* 237.11**



Prostaglandin B1



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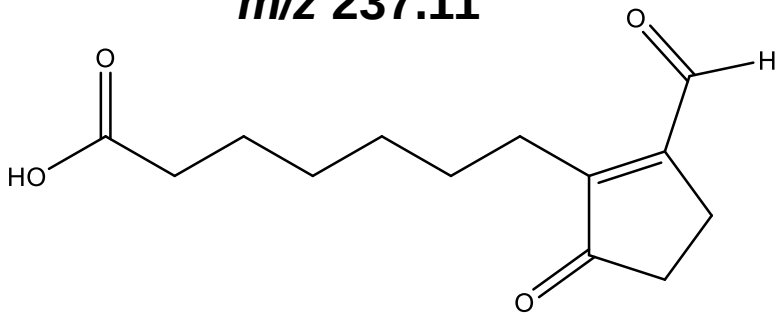
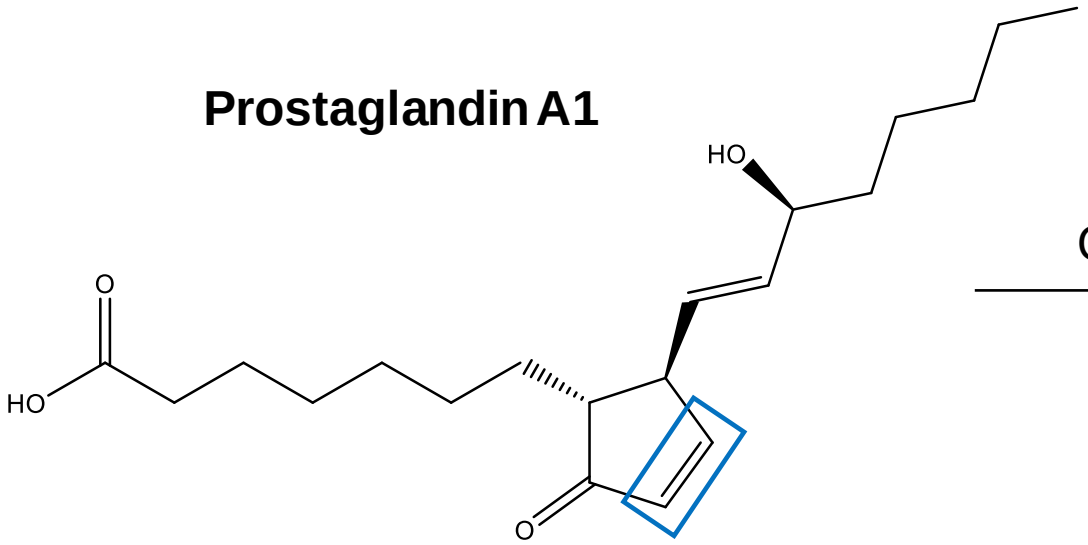


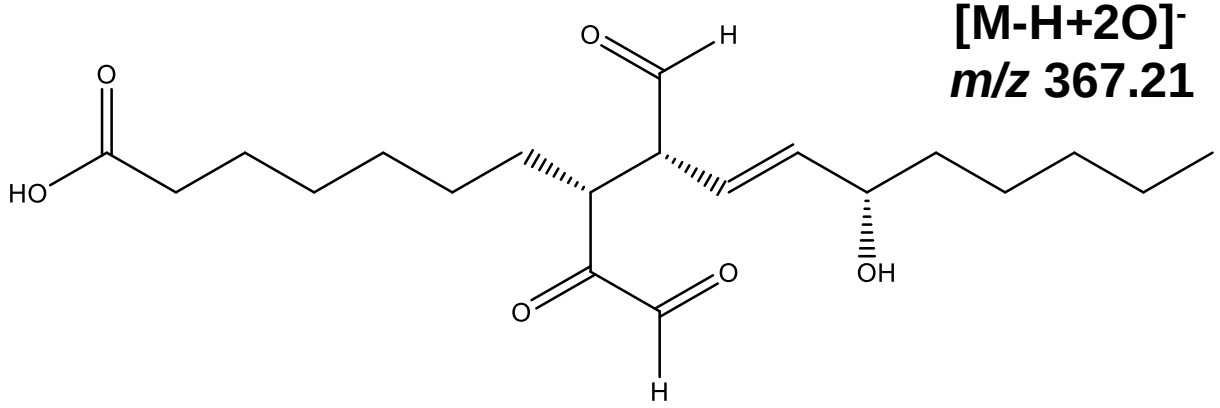
Maddox, S. et al. *Manuscript in Preparation.*

Prostaglandins with Ozonolysis

Prostaglandin A1



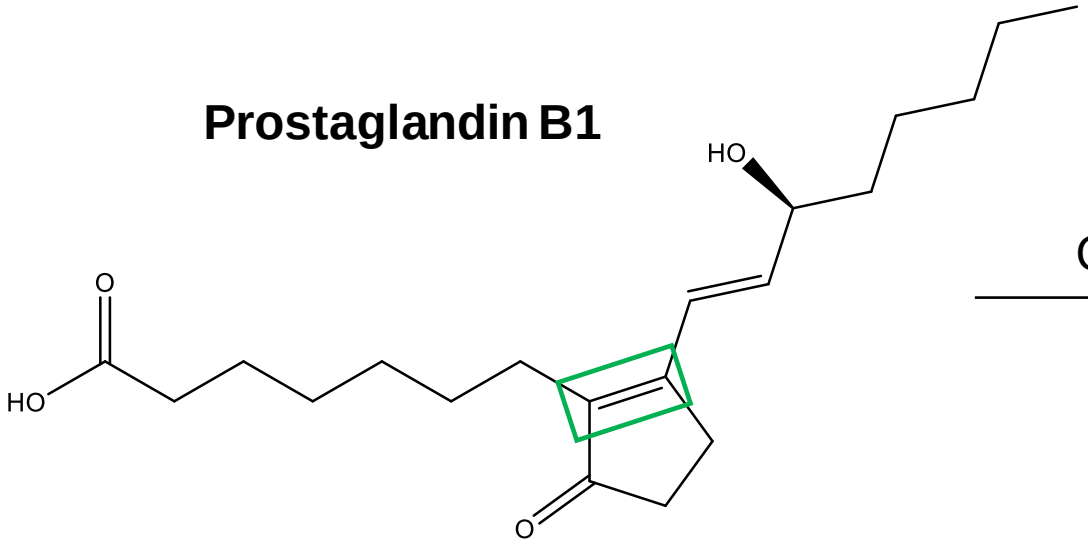
O_3



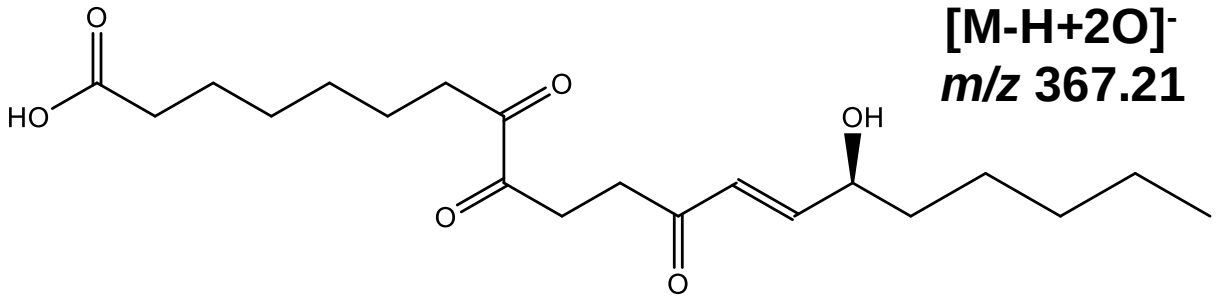
[M-H+2O]$^-$
***m/z* 367.21**

Ozone-Induced Rearrangement (OzIR)

Prostaglandin B1



O_3



[M-H+2O]$^-$
***m/z* 367.21**

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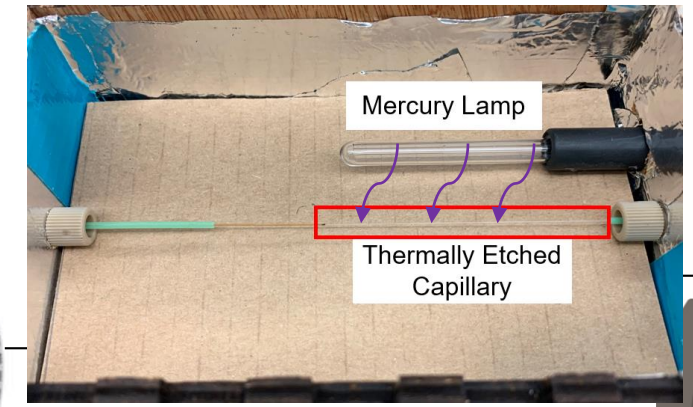
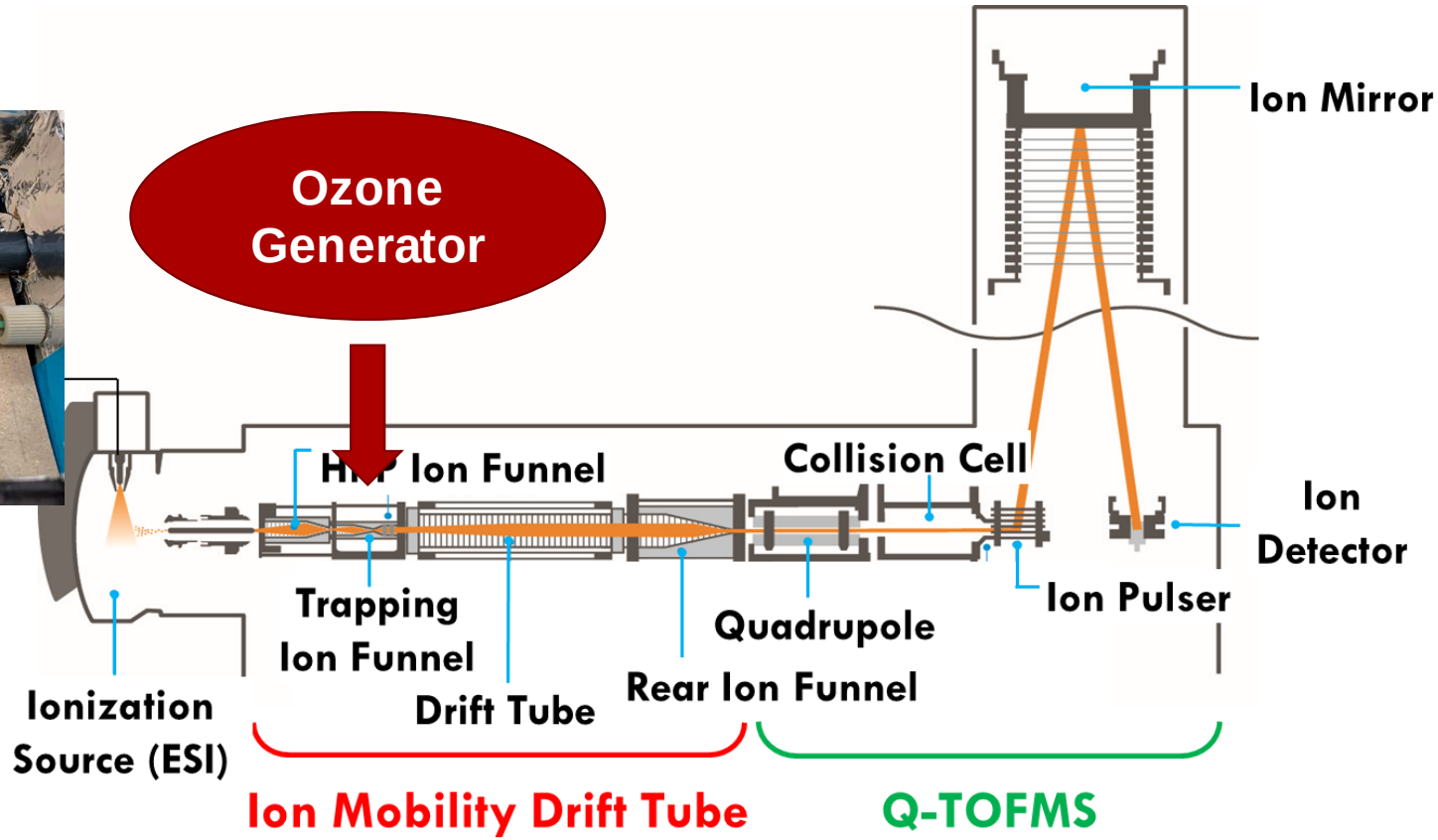
Experimental Setup

Agilent 1290 Infinity II UHPLC



- Mercury lamp produces 185 nm radiation
- Thermally etched silica capillary creates "window" for exposure
- Inexpensive option

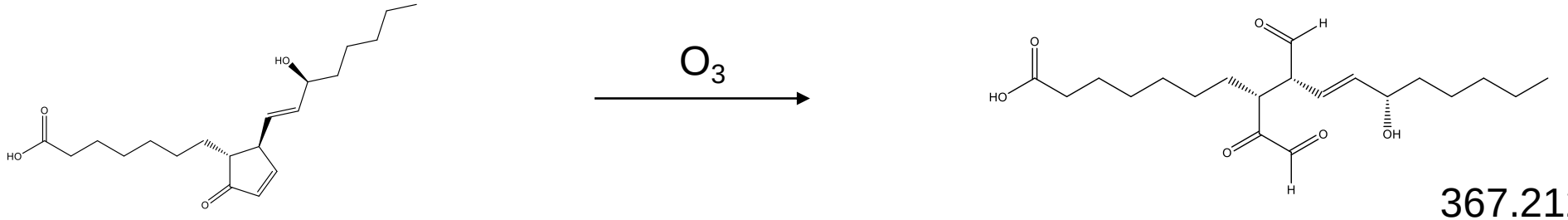
Agilent 6560 IM-QTOF

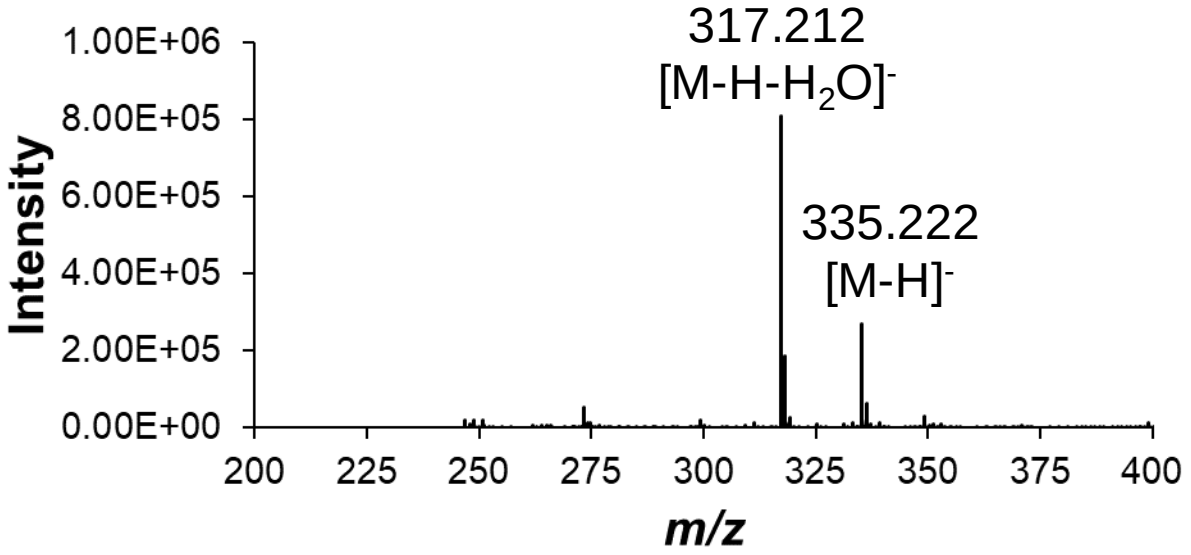


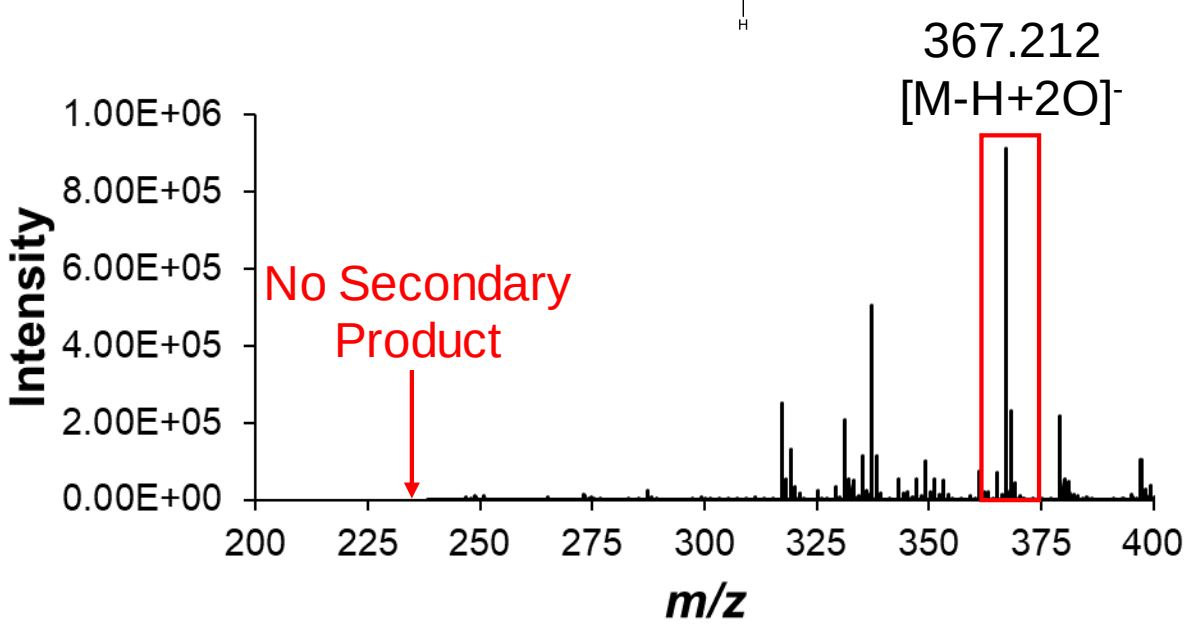
- 78 cm Drift Tube
- 4.00 Torr Nitrogen Drift Gas
- 25 °C

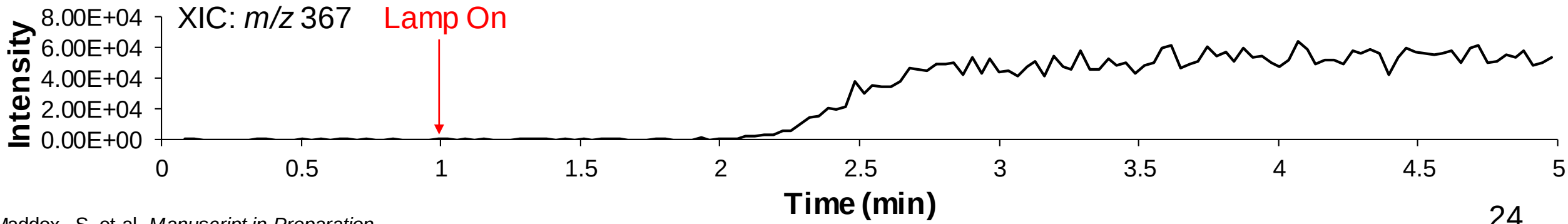
Harris, R.A. et al. *Analytical Chemistry*, **2018**, *90 (3)*, 1915-1924.
Stinson, C.A. et al. *Analyst*, **2016**, *141*, 3696-3704.
Maddox, S. et al. *Manuscript in Preparation.*

OzIR of Prostaglandins









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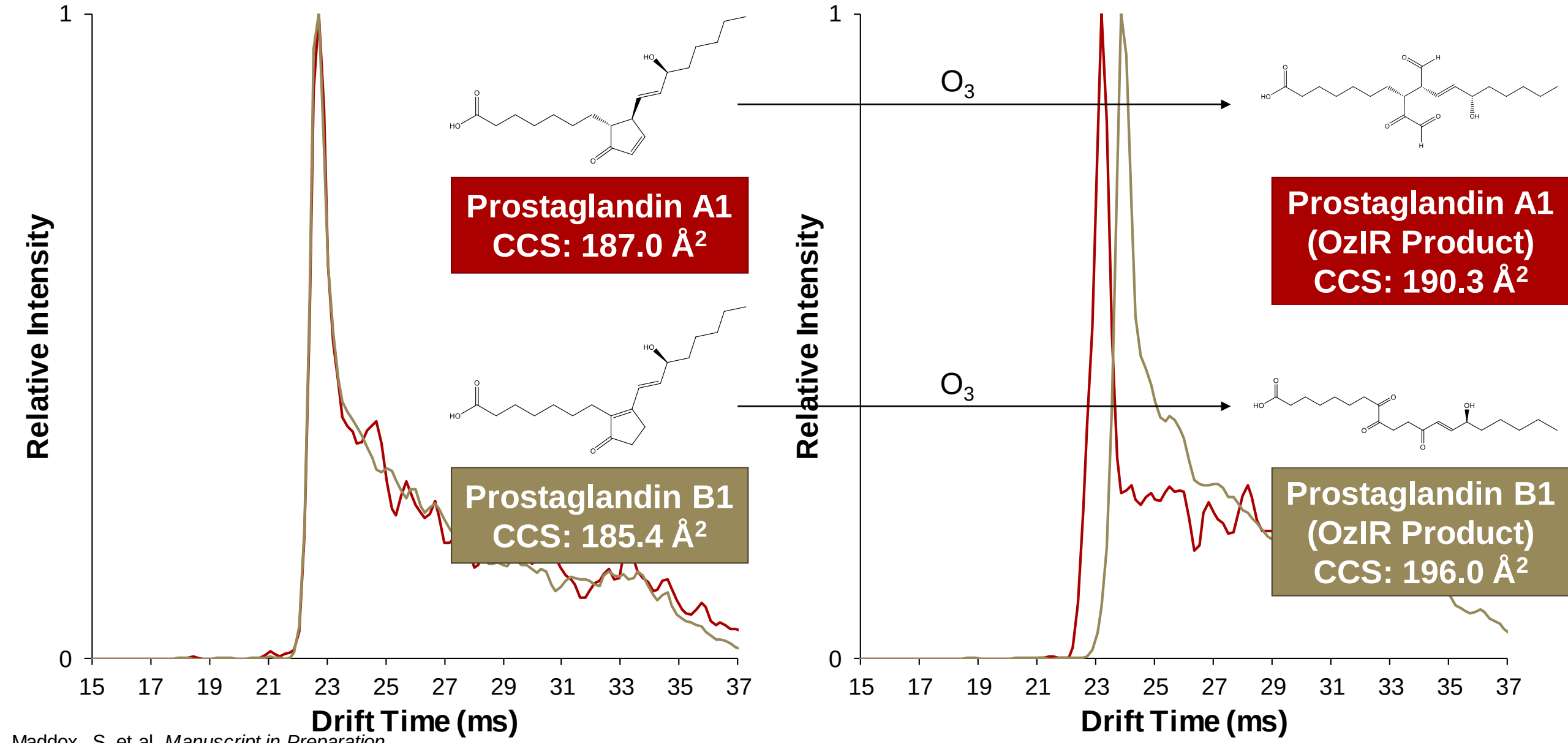

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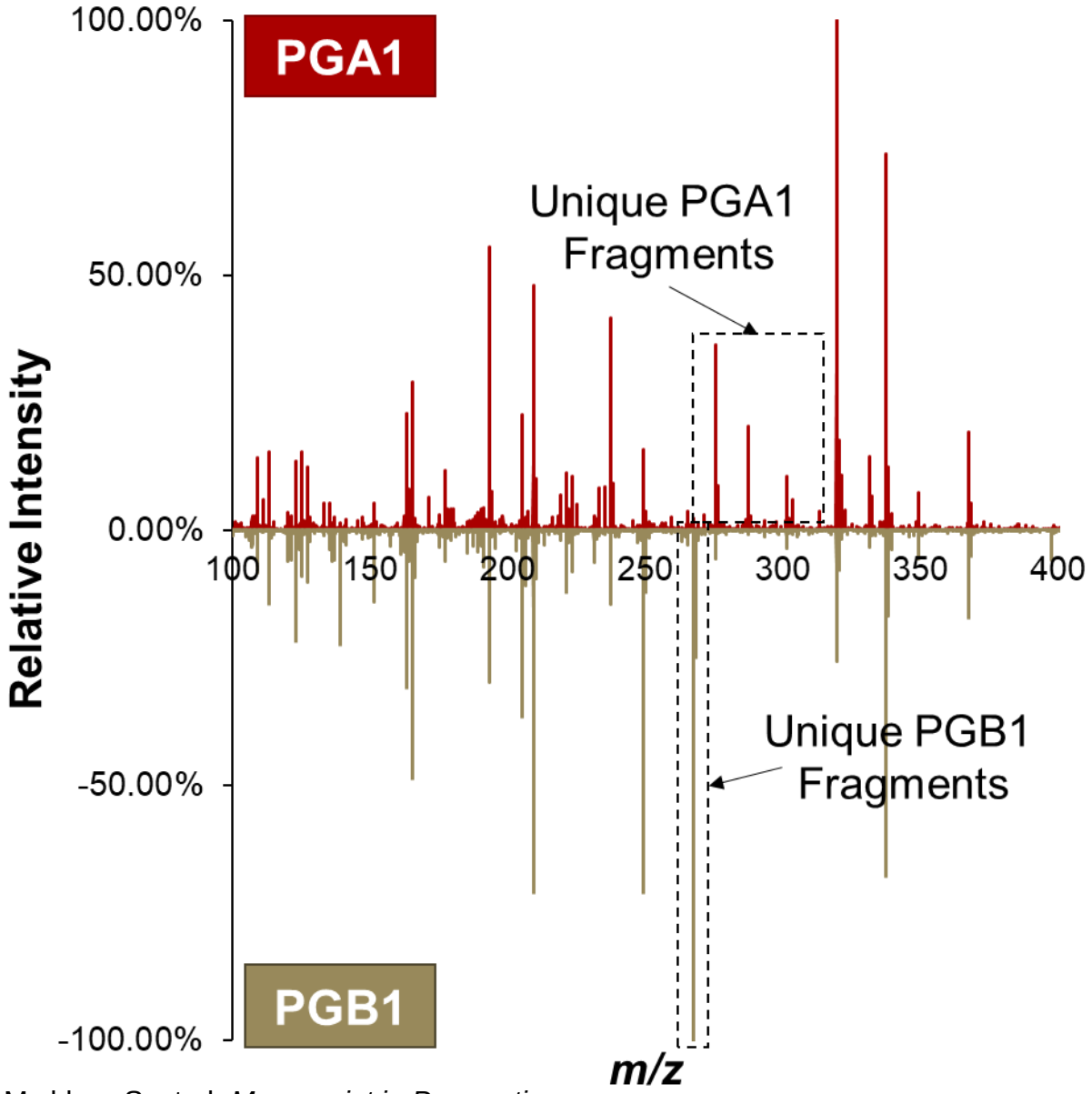
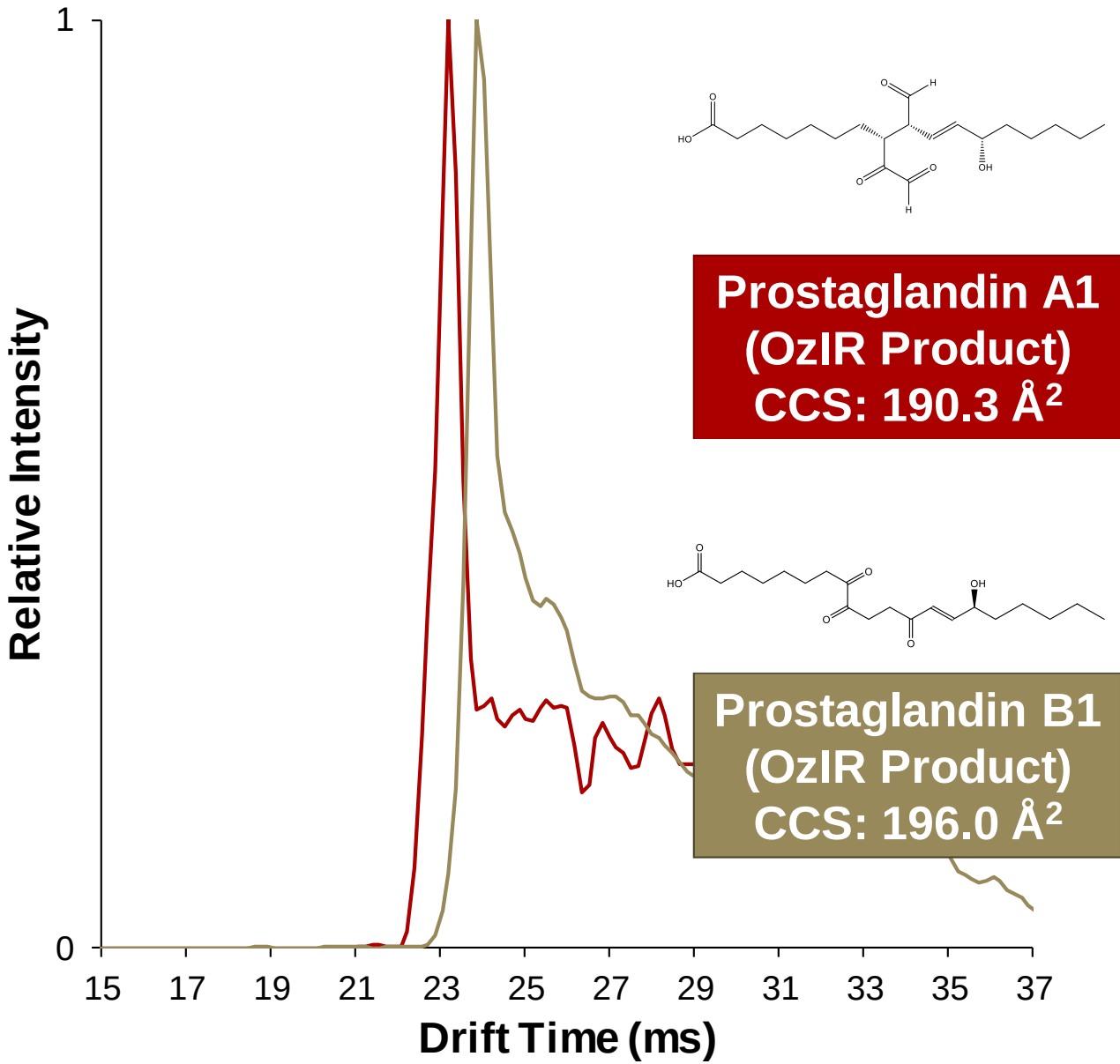
OzIR of Prostaglandins





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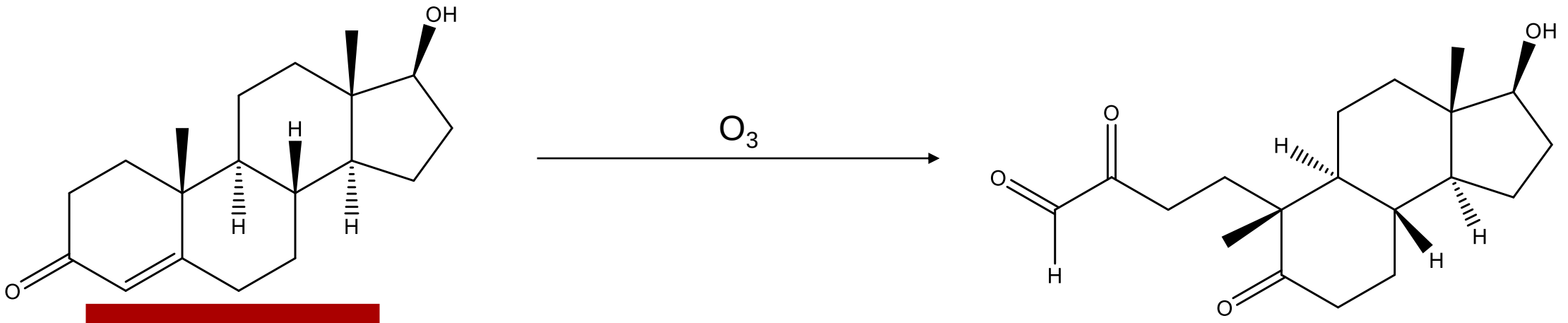
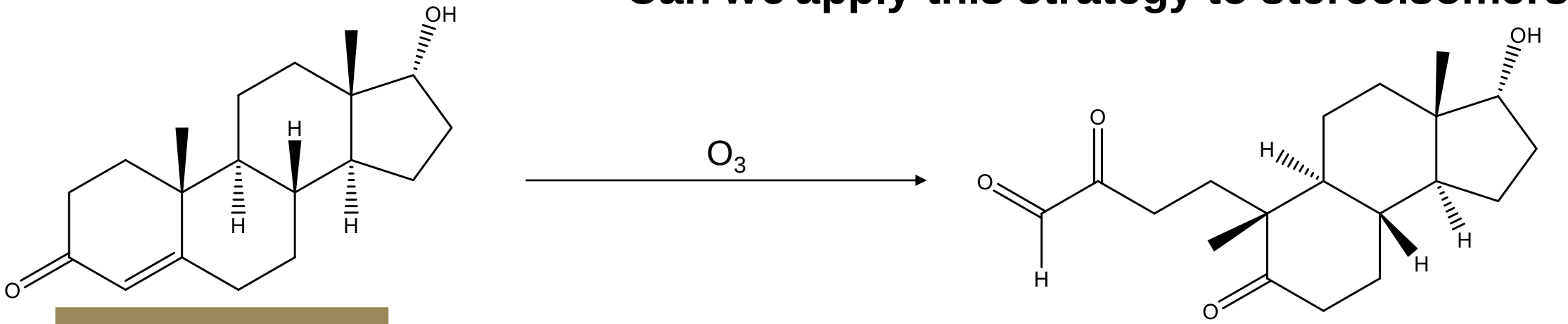
OzIR of Steroids



Testosterone

Can we apply this strategy to stereoisomers?



Epitestosterone

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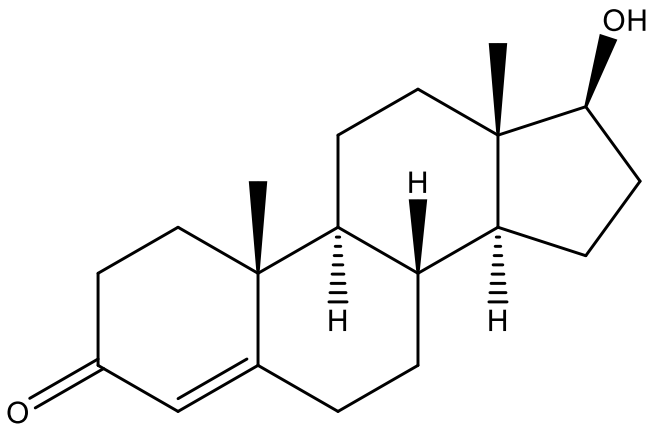
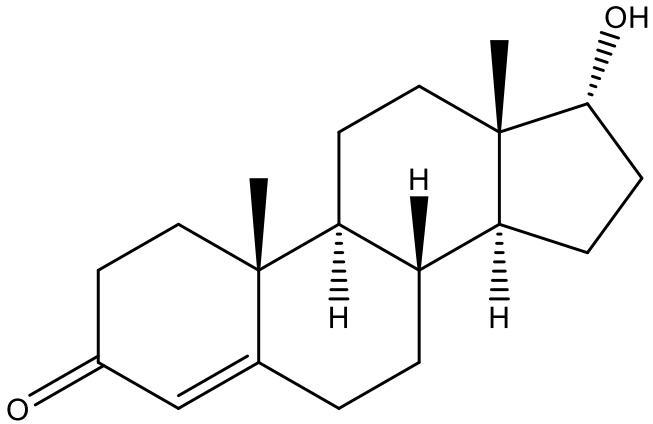
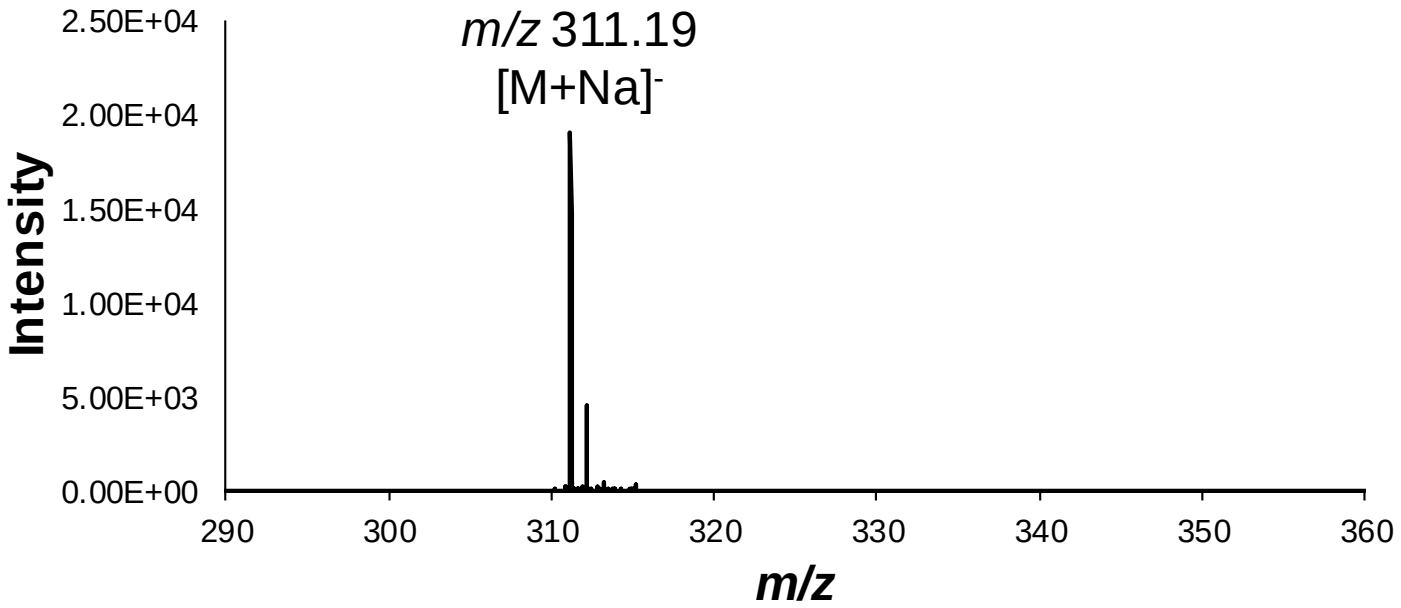
OzIR of Steroids

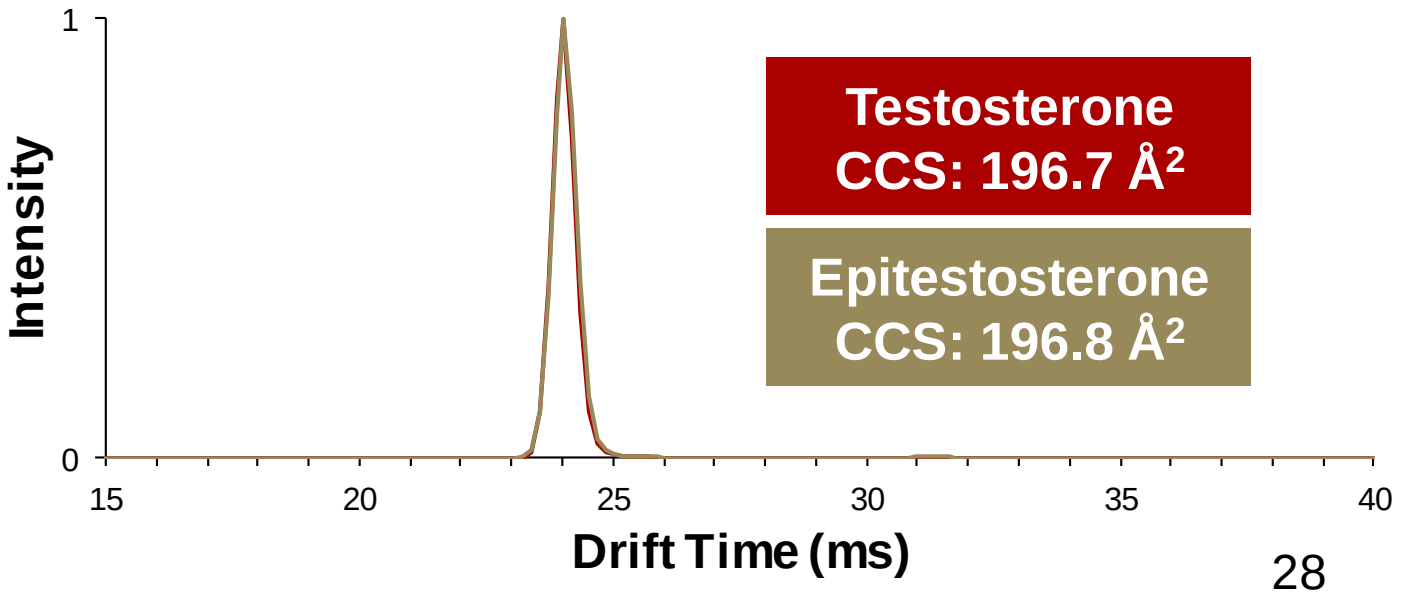


Testosterone



Epitestosterone





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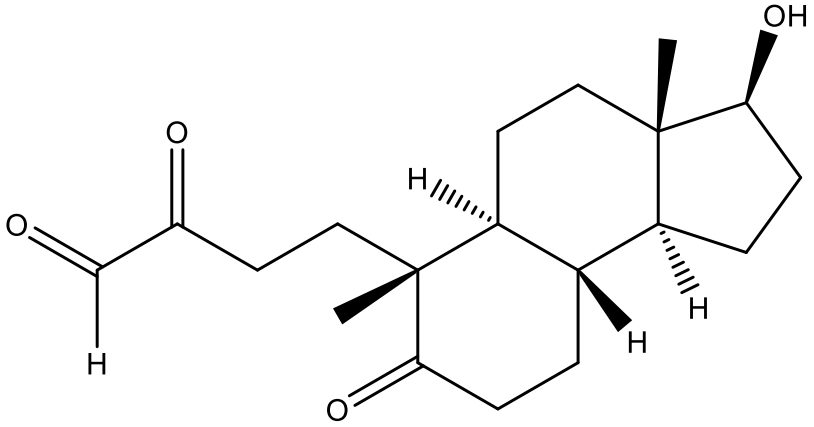
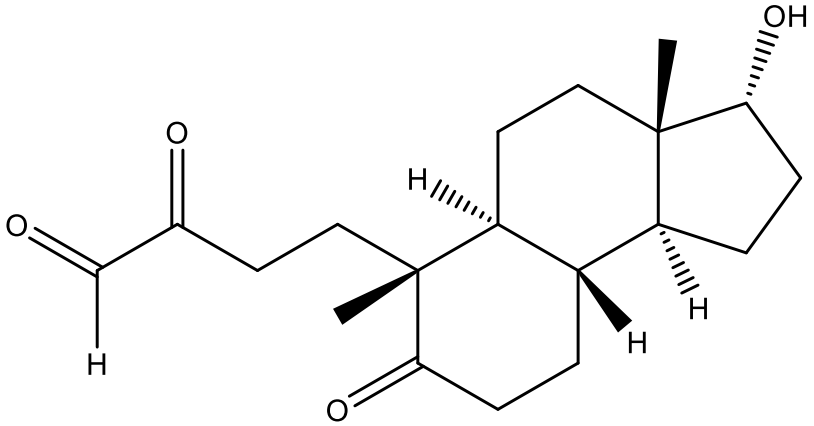
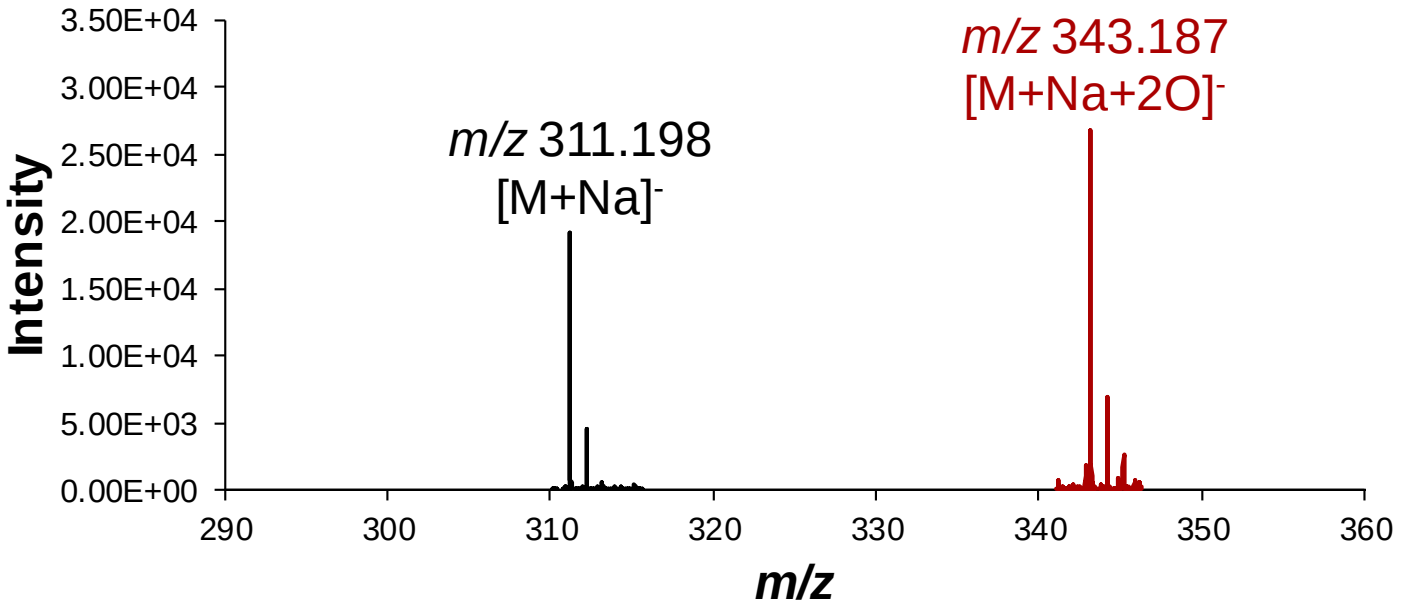
OzIR of Steroids

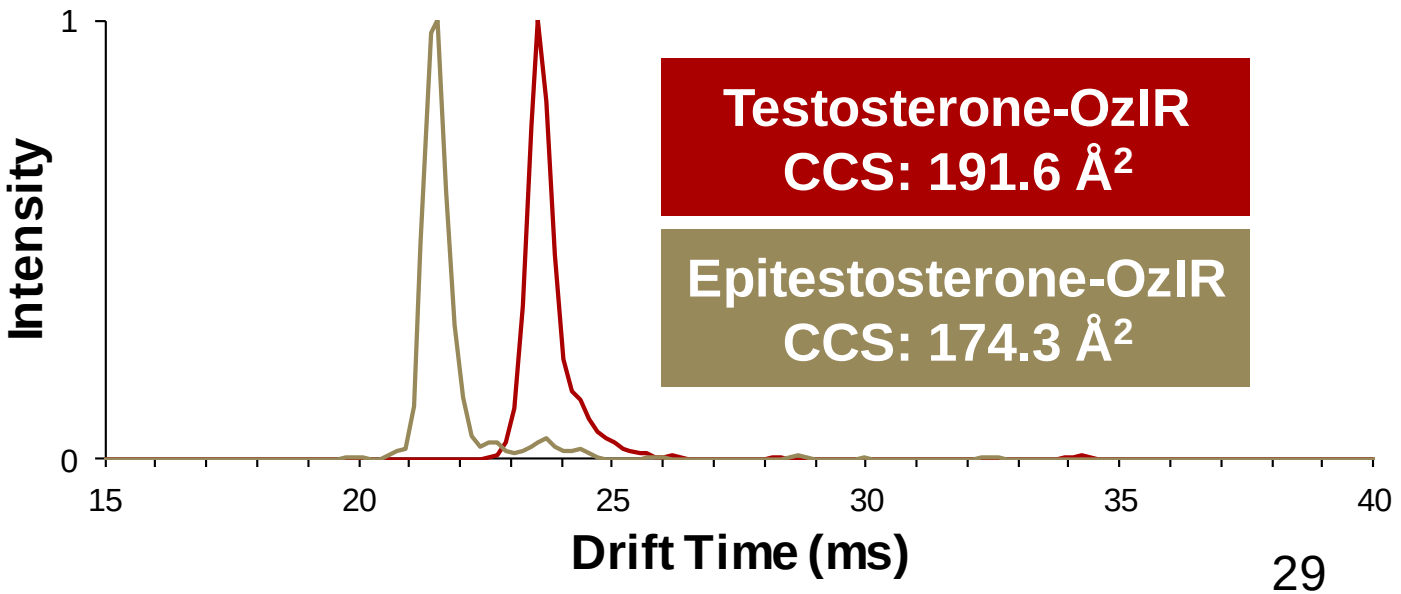


Testosterone-OzIR



Epitestosterone-OzIR





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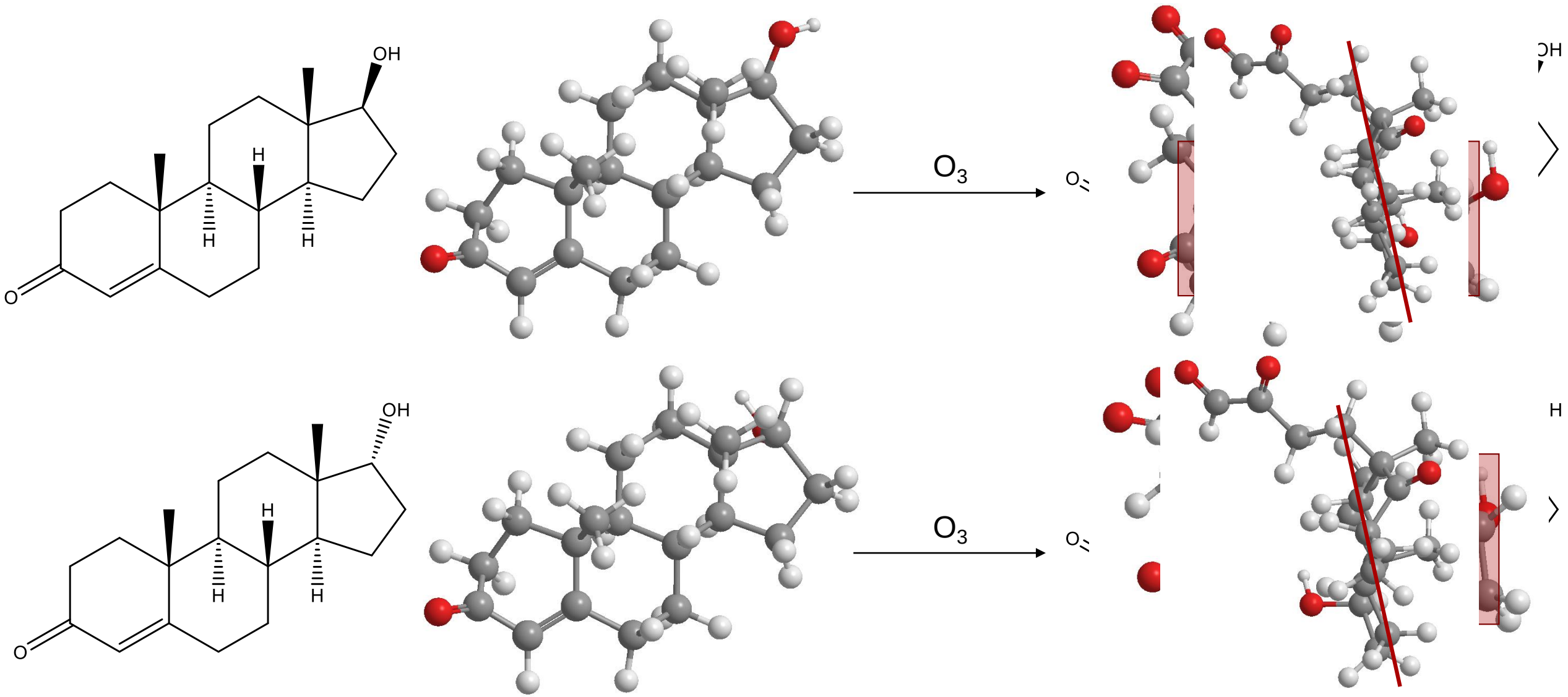
OzIR of Steroids



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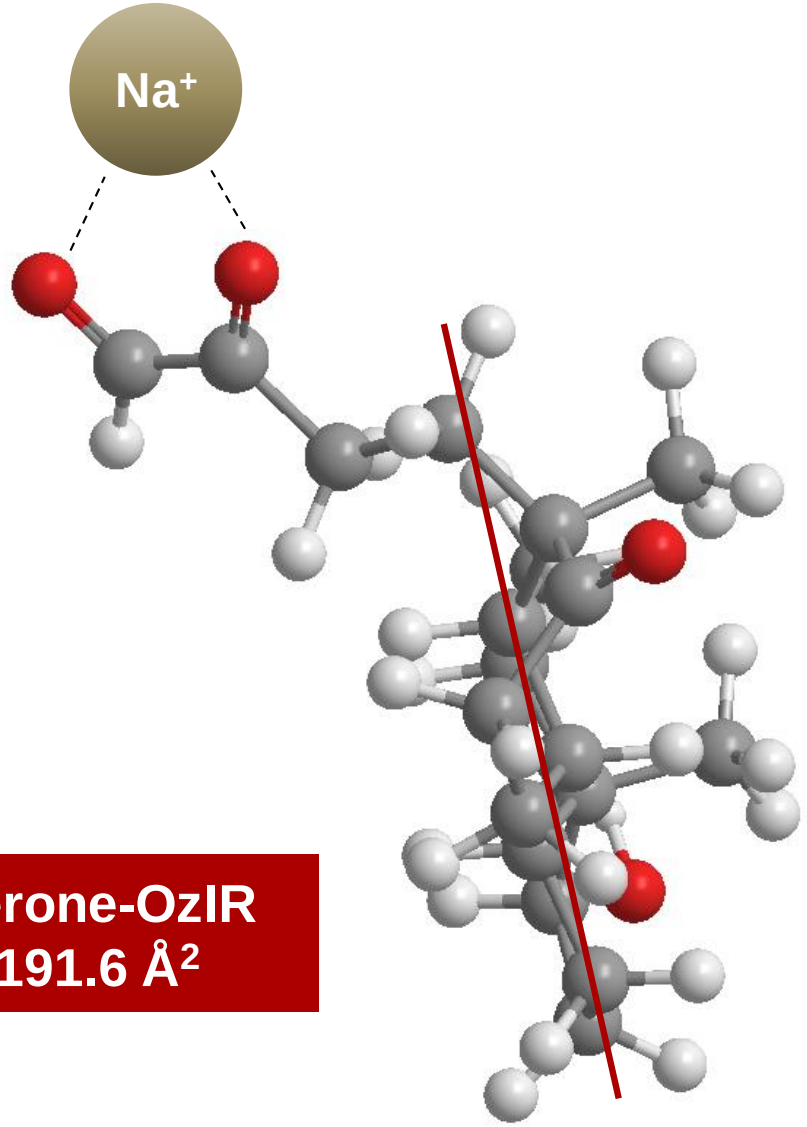
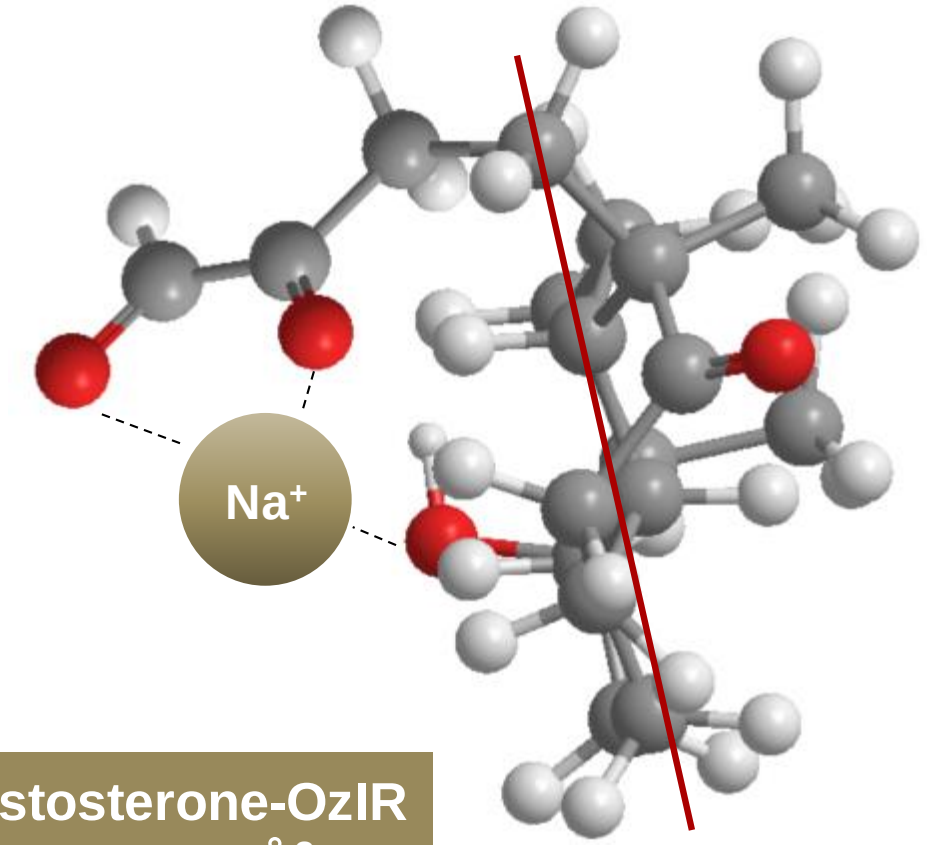
OzIR of Steroids



Testosterone-OzIR
CCS: 191.6 Å²



Epitestosterone-OzIR
CCS: 174.3 Å²

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Summary & Conclusions

- Ozone-induced rearrangement (OzIR) is a promising tool for differentiation of double bond-containing compounds in the –omics
  - Improved IM resolution based on the structural changes induced with OzIR
  - Different fragmentation patterns observed for some small molecule isomers
- OzID/OzIR a simple, low-cost tool to be implemented with IM-MS, HRMS, and MS/MS platforms with little to no reduction in throughput
- Application to other biomolecular classes in the –omics??

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60 YEARS

Florida Tech

Questions?