

# The use of AP-MALDI for structural insight into viral envelope lipids and other biomedical applications

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# Zoonotic Enveloped Viruses are a Significant Current and Future Public Health Threat

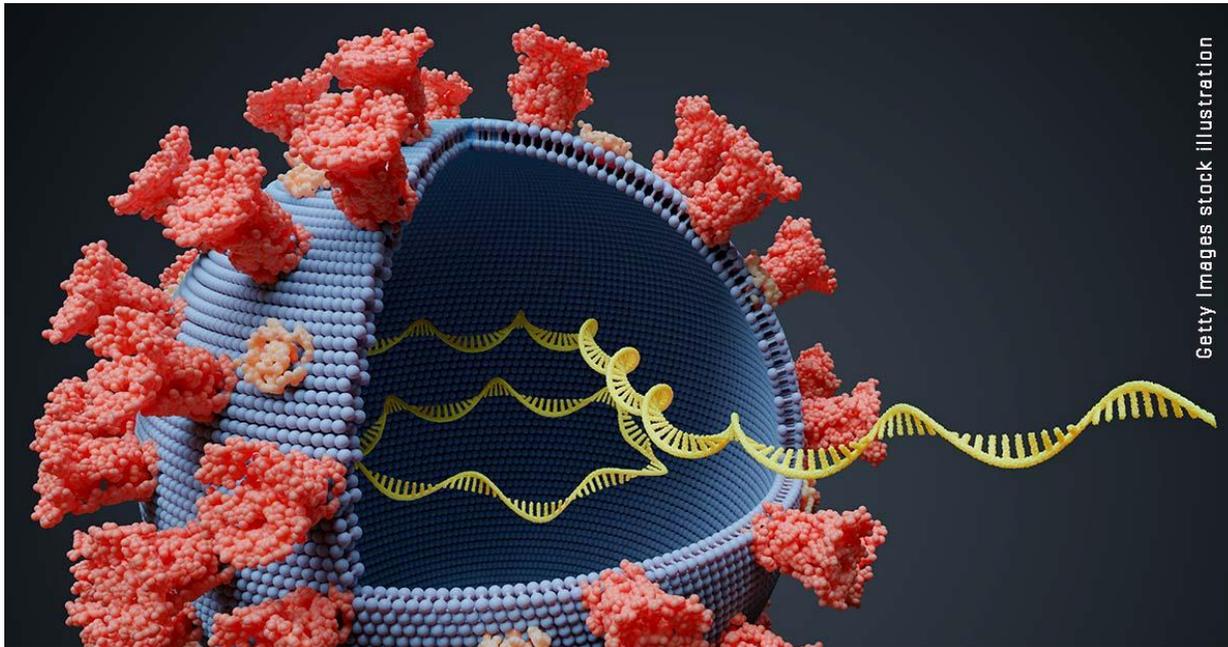


Image Credit: vchal / Shutterstock

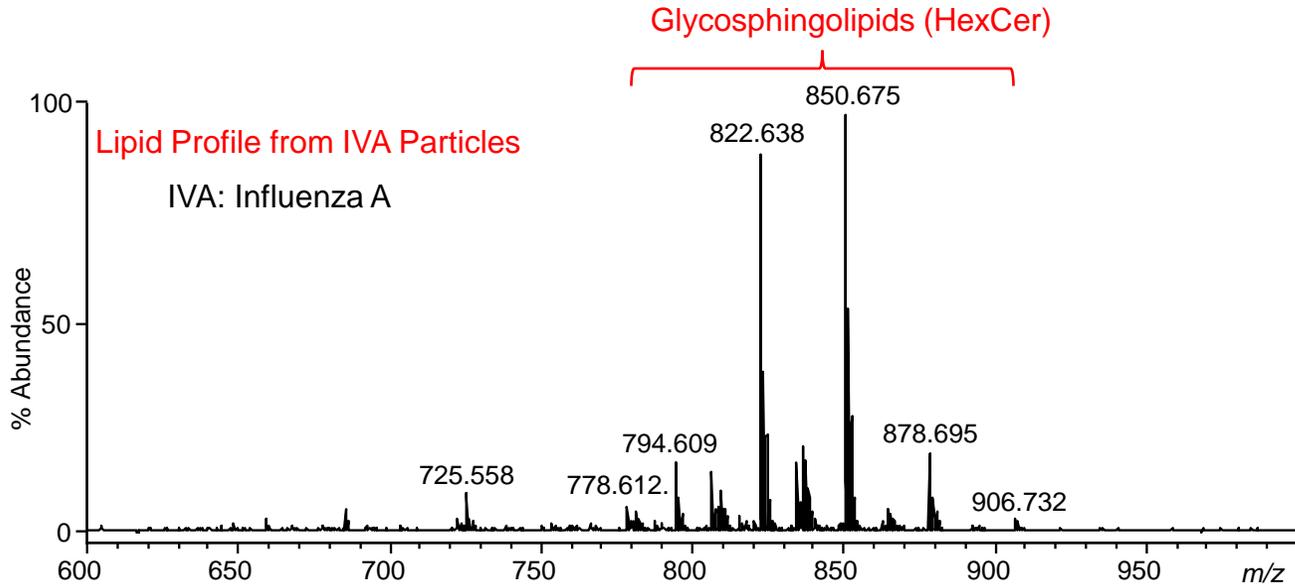
Viral Envelope: intimately tied to the virus's ability to successfully replicate

- Entry (fusion)
- Assembly
- Exit (budding)
- Protection

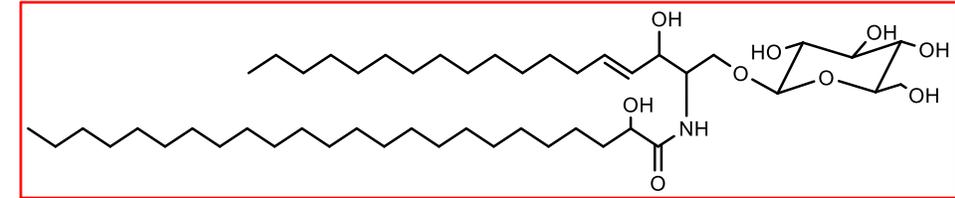
- Zoonotic viral diseases represent a serious and imminent threat to public health
- Top list of emerging pathogens are zoonotic enveloped viruses (e.g., Ebola, Nipah, Lassa fever, MERS, and SARS)<sup>1</sup>.

<sup>1</sup>Sweileh WM. Global Health. 2017;13(1):9 DOI: [10.1186/s12992-017-0233-9](https://doi.org/10.1186/s12992-017-0233-9).

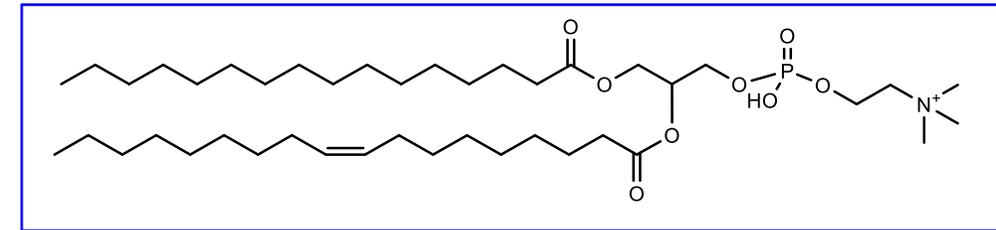
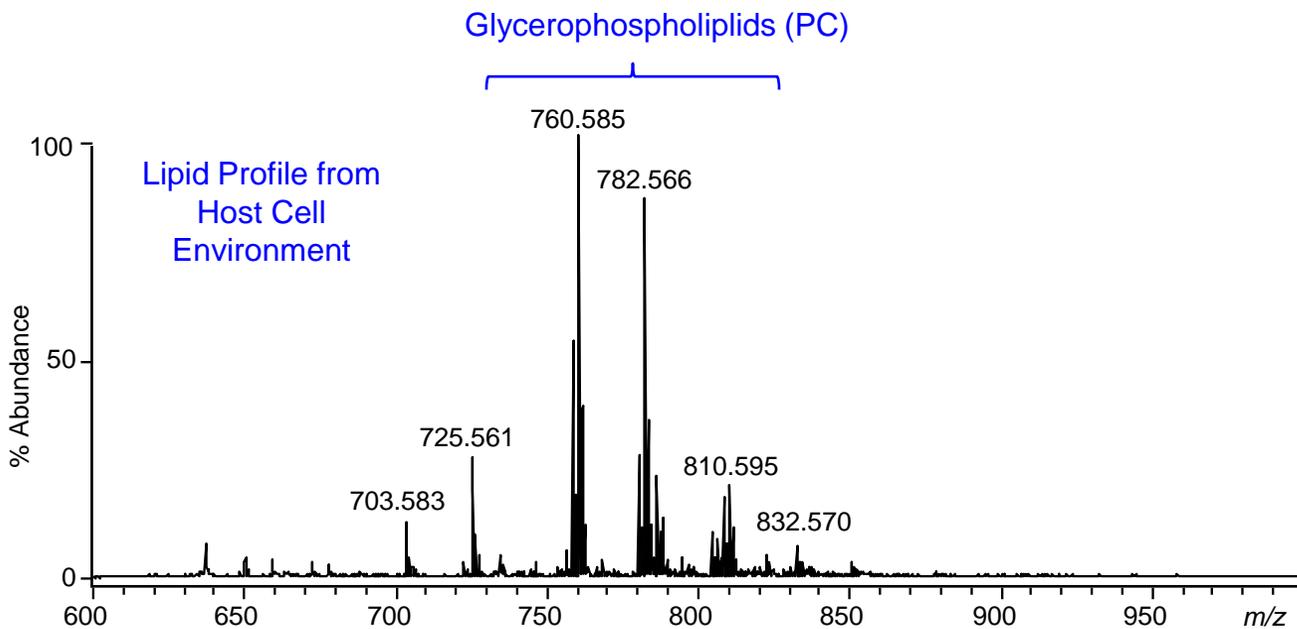
# Rapid Lipid Detection: MALDI MS



- Total lipid extract (MTBE)
- Matrix: DHB (1:1, MeOH:IPA)
- Positive ion mode
- MALDI TOF

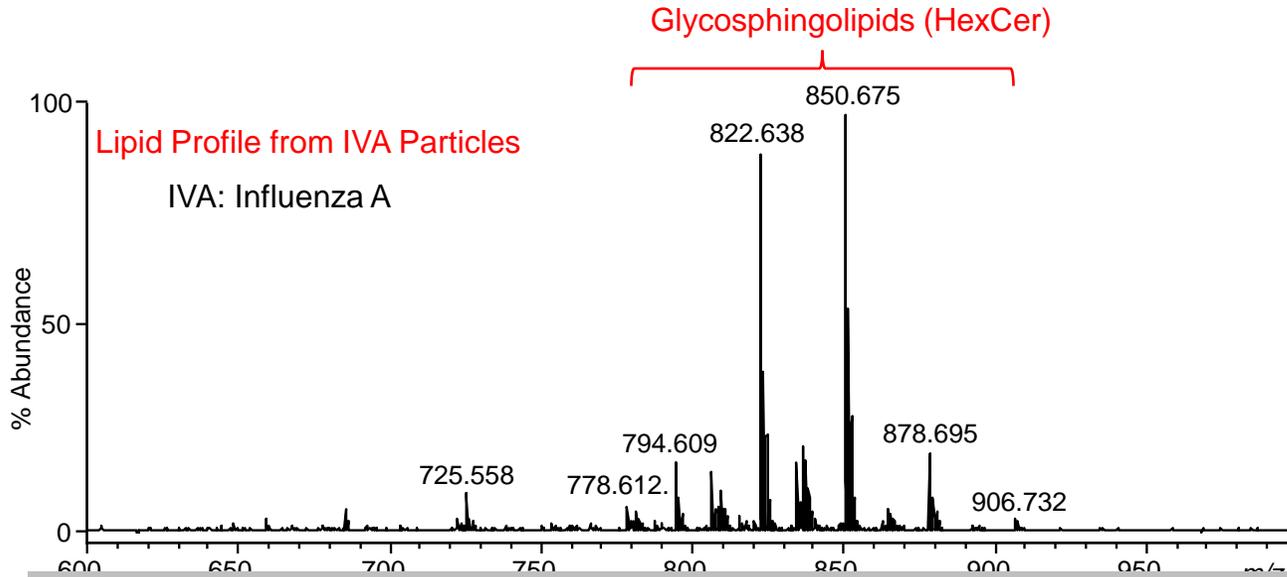


- Hexosylceramide: HexCer(d18:1/24:0-OH)**
- **GSL**
  - **Raft membrane lipid**

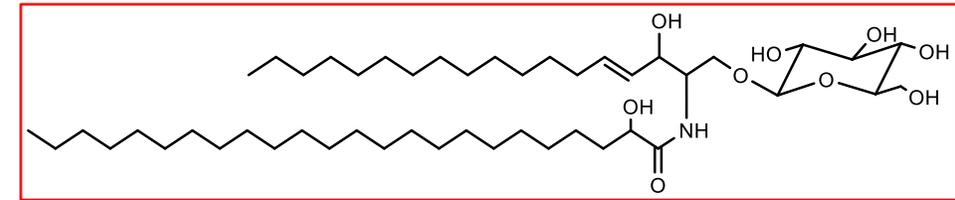


- Glycerophosphocholine: PC(16:0/18:1)**
- **Most abundant cellular membrane lipid**

# Rapid Lipid Detection: MALDI MS

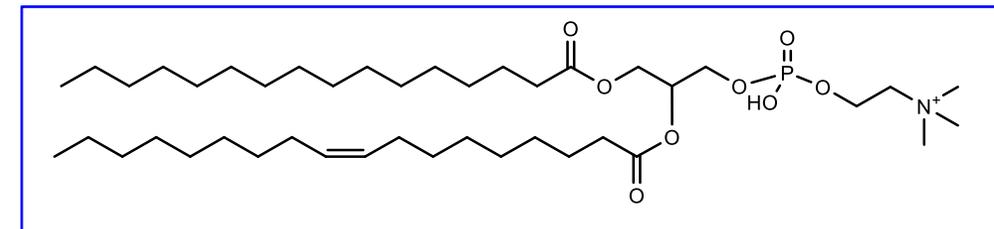
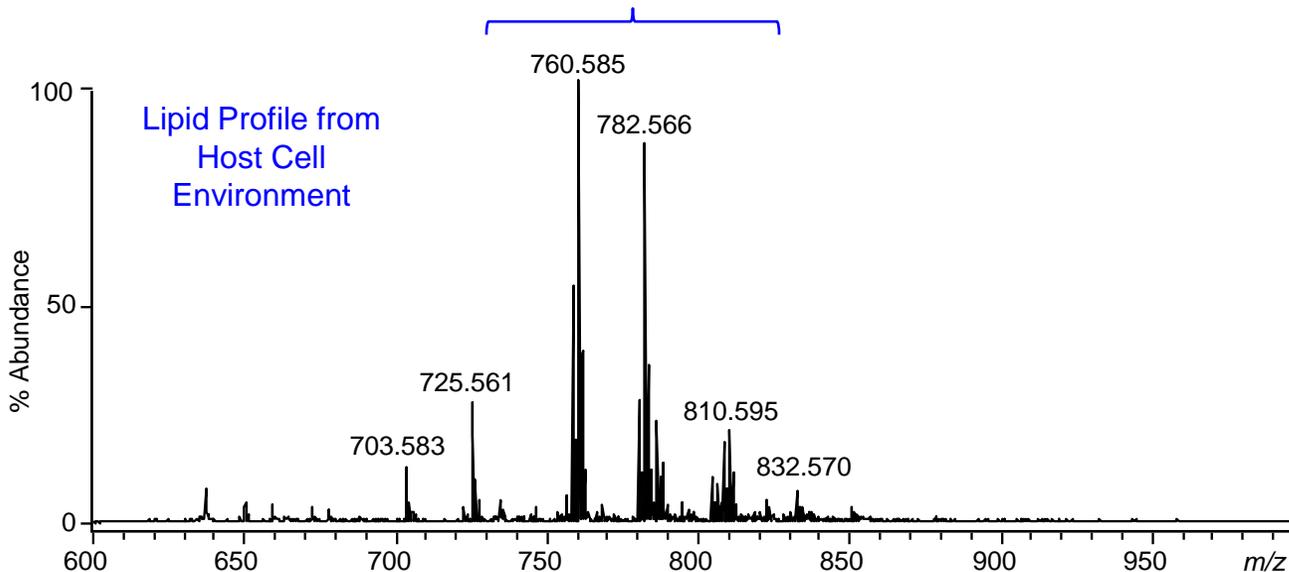


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- GSL
  - Raft membrane lipid

## Viral Lipid Composition Distinct from Host Environment

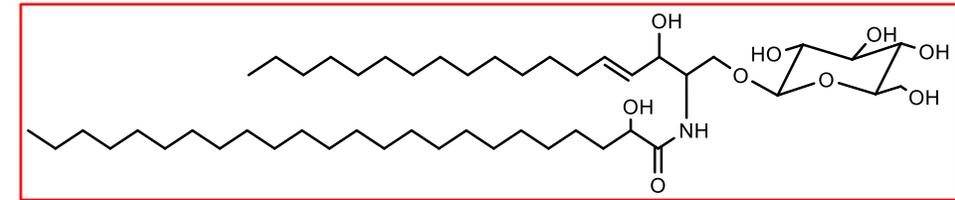
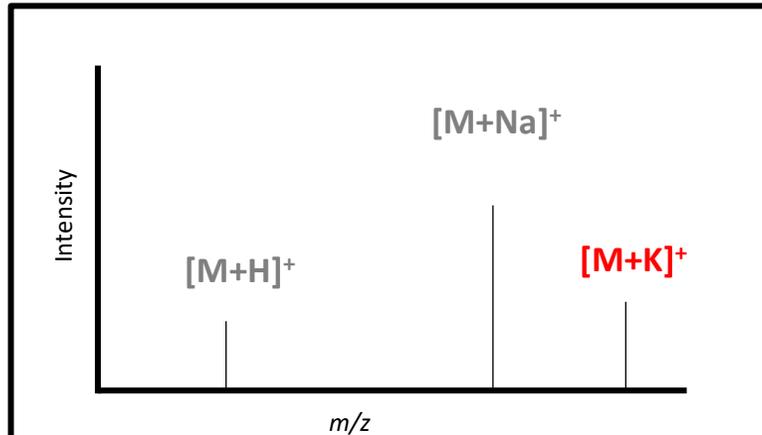
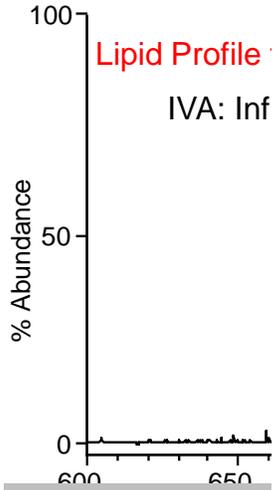


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- Most abundant cellular membrane lipid

# Rapid Lipid Detection: MALDI MS

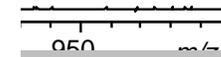
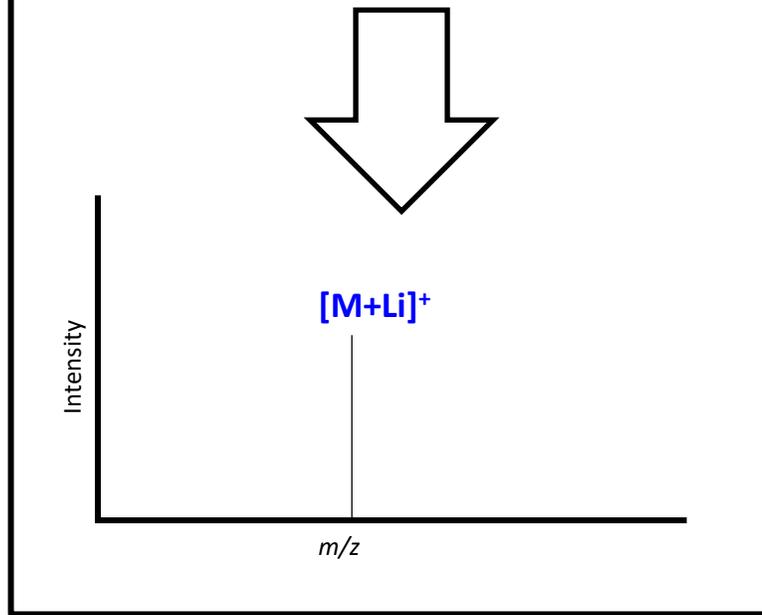
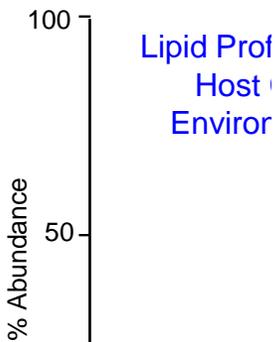
Glycosphingolipids (HexCer)

- Total lipid extract (MTBE)
- Matrix: DHB (1:1, MeOH:IPA)
- Positive ion mode
- MALDI TOF

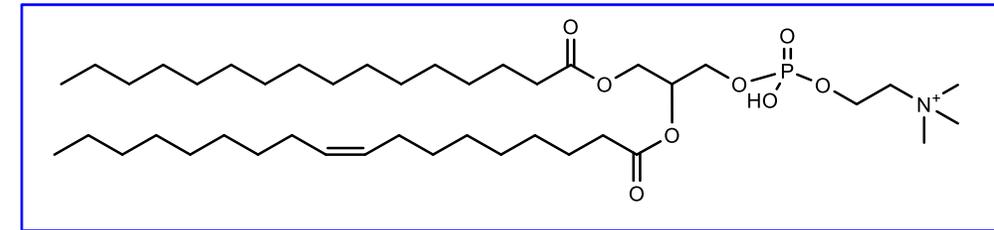


- Hexosylceramide: HexCer(d18:1/24:0-OH)
- GSL
  - Raft membrane lipid

Vir



distinct from Host Environment

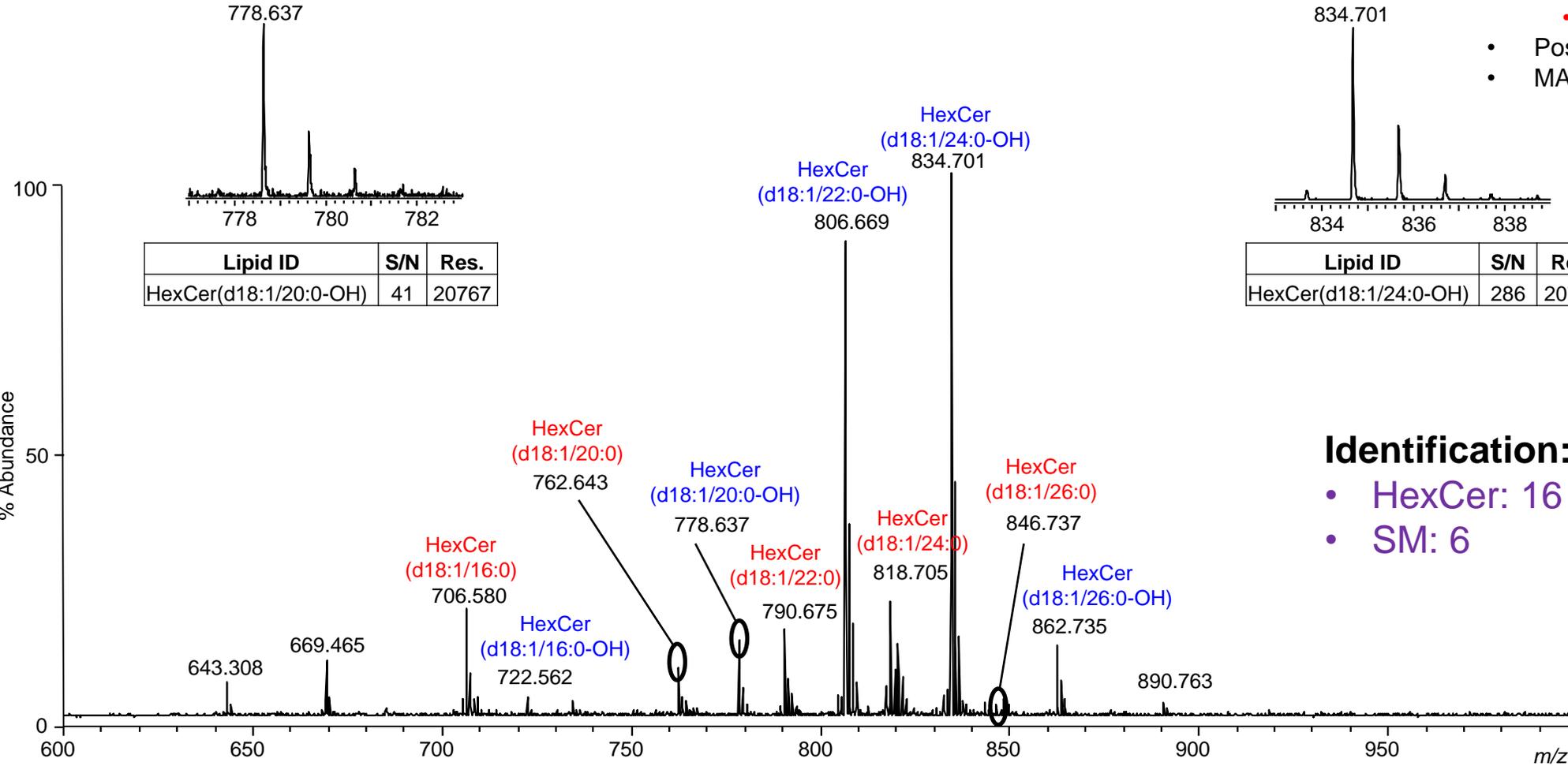
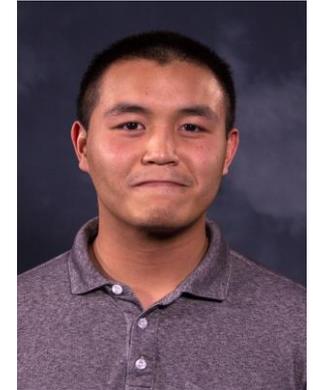


- Glycerophosphocholine: PC(16:0/18:1)
- Most abundant cellular membrane lipid

# Detection Enrichment with Lithium Adduct Consolidation

## Lipid Profiling of Virus Particles Using Novel Lithium Adduct Consolidation MALDI TOF MS: Selective Detection of Raft Membrane Glycosphingolipids

- Total lipid extract (MTBE)
- **Matrix: THAP** (1:1, MeOH:IPA)
  - **10mM LiCl**
- Positive ion mode
- MALDI TOF



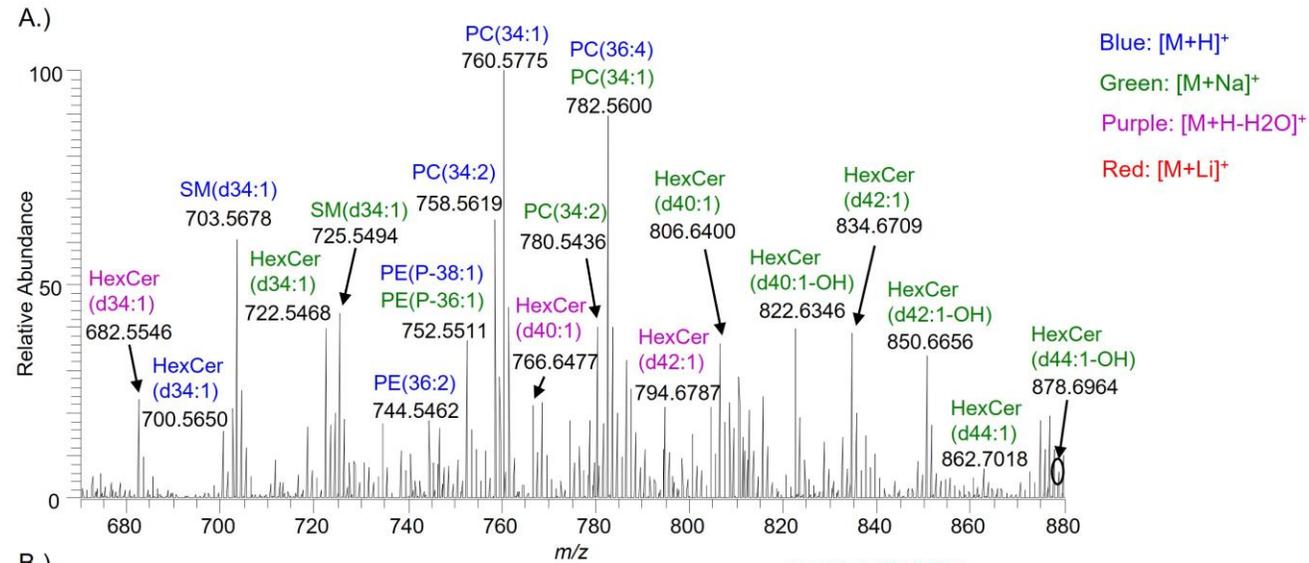
### Identification:

- HexCer: 16
- SM: 6

# High Resolution Mass Spectrometry: AP MALDI

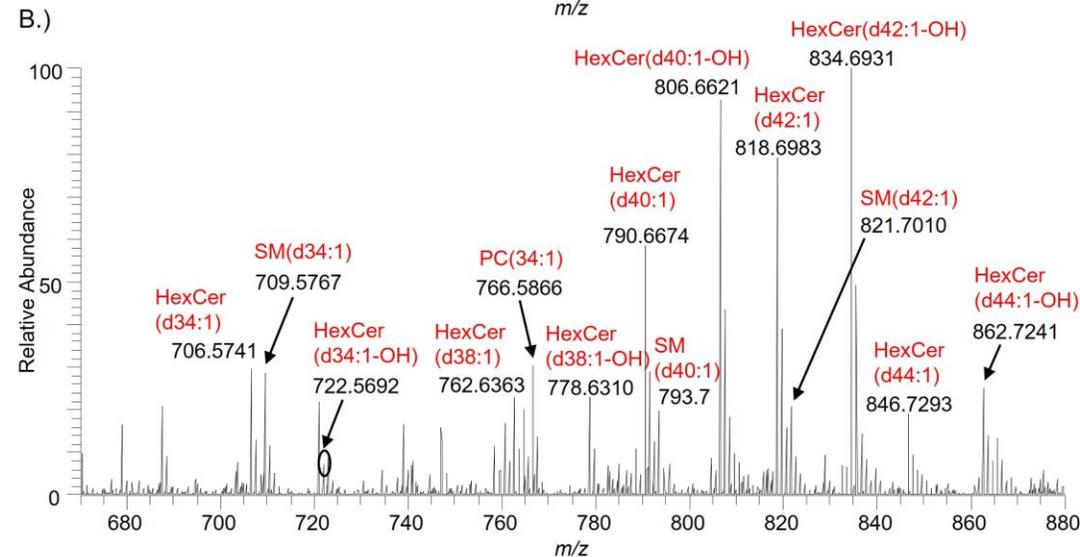


<http://apmaldi.com/>



## AP-MALDI HRMS with DHB

- Lipids from IVA virions



## AP-MALDI HRMS with THAP+Li

- Lipids from IVA virions

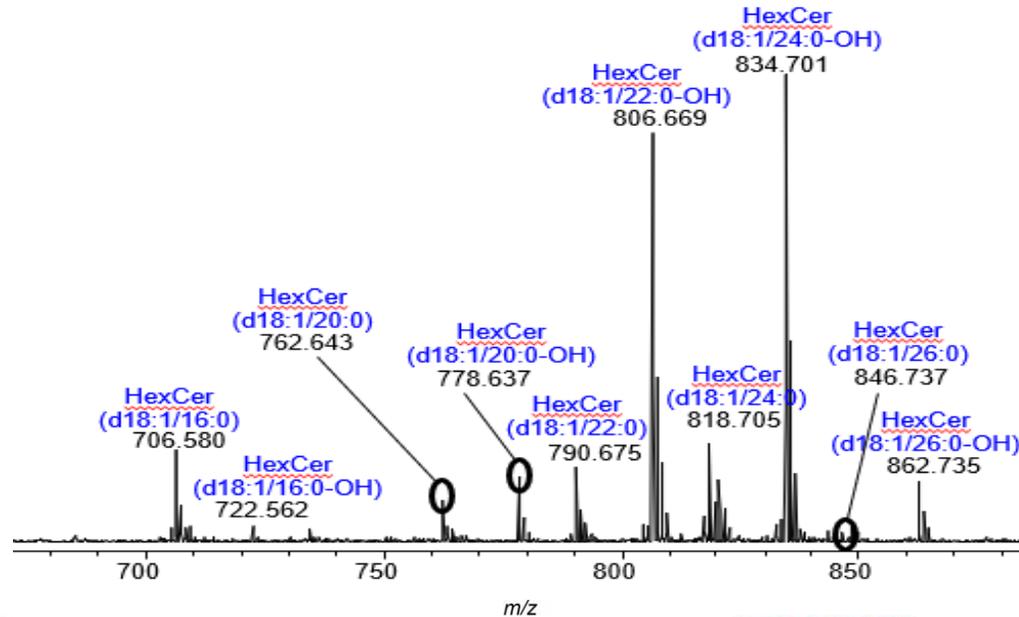
## Identification:

- HexCer: 44
- SM: 21

# High Resolution Mass Spectrometry: AP MALDI



<http://apmaldi.com/>

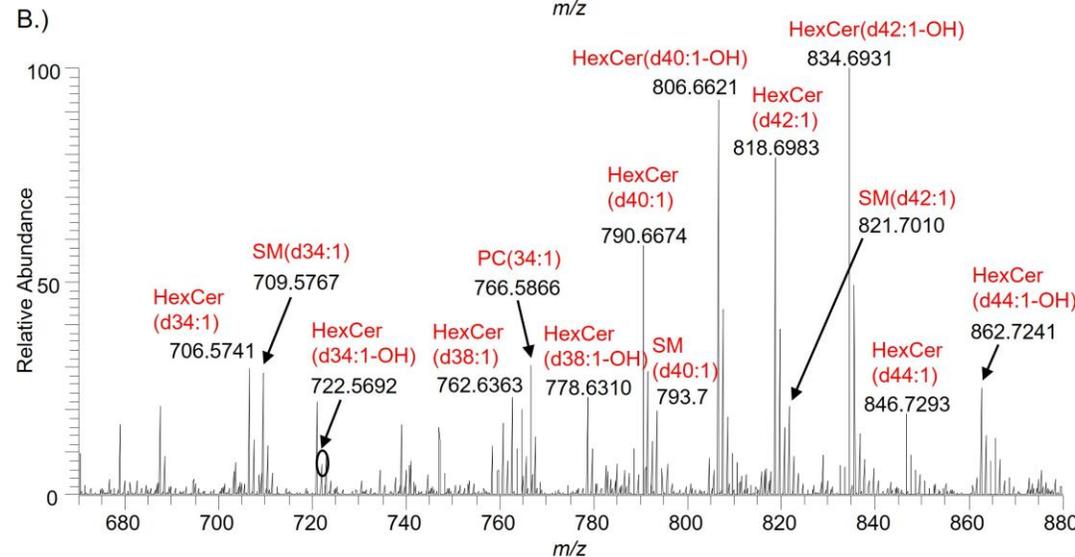


## vMALDI-TOF with THAP+Li

- Lipids from IVA virions

## Identification:

- HexCer: 16
- SM: 6



## AP-MALDI HRMS with THAP+Li

- Lipids from IVA virions

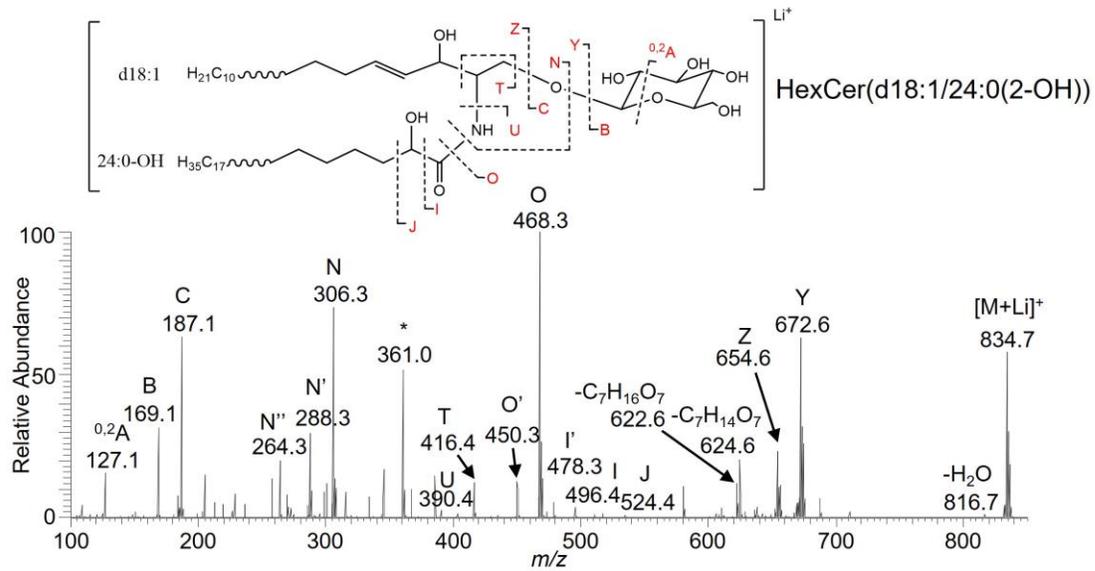
## Identification:

- HexCer: 44
- SM: 21

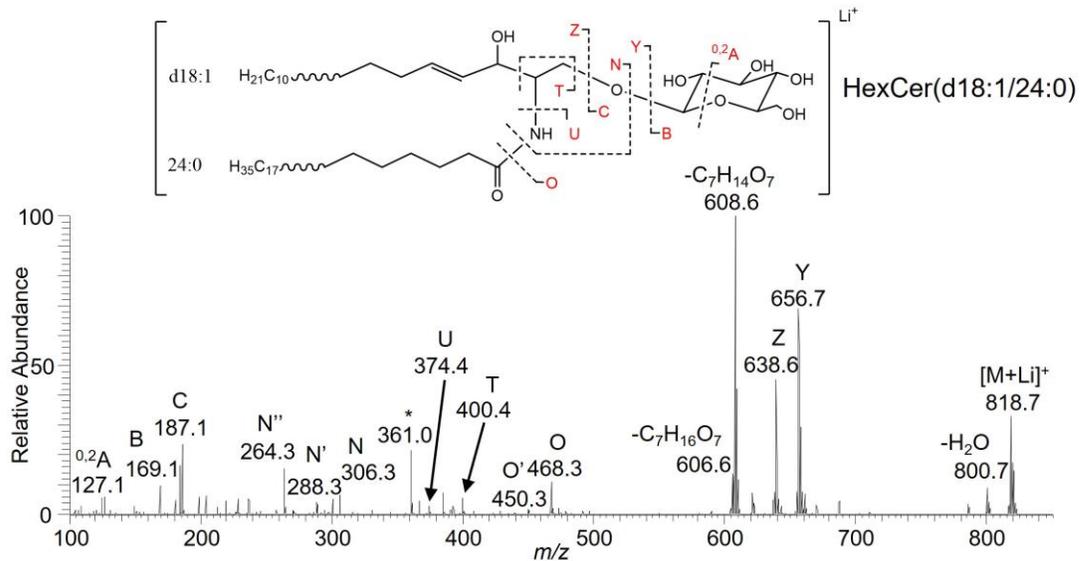


# Li Adducts provide extensive fragmentation via Tandem MS

## Tandem MS of $[M+Li]^+$ HexCer and hydroxylated HexCer



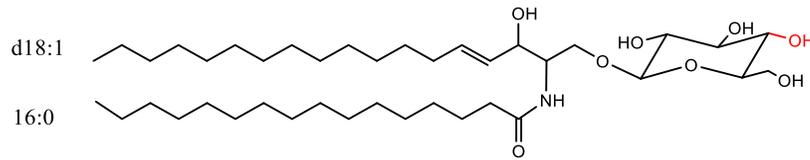
m/z	ID	Ion	Intensity	Lipid ID
722.5692	HexCer(d34:1-OH)	[M+Li] <sup>+</sup>	565	HexCer(d34:1-OH)*
778.6314	HexCer(d38:1-OH)	[M+Li] <sup>+</sup>	4389	HexCer(d18:1/20:0(2-OH))
806.6624	HexCer(d40:1-OH)	[M+Li] <sup>+</sup>	18733	HexCer(d18:1/22:0(2-OH))
820.6776	HexCer(d41:1-OH)	[M+Li] <sup>+</sup>	3278	HexCer(d18:1/23:0(2-OH))
834.6934	HexCer(d42:1-OH)	[M+Li] <sup>+</sup>	19231	HexCer(d18:1/24:0(2-OH))
862.7243	HexCer(d44:1-OH)	[M+Li] <sup>+</sup>	5110	HexCer(d18:1/26:0(2-OH))



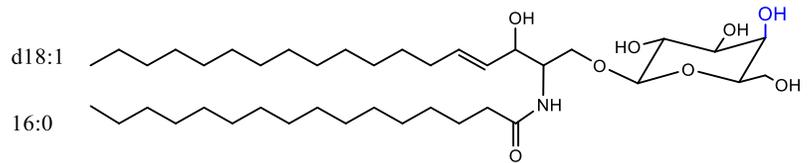
m/z	ID	Ion	Intensity	Lipid ID
706.5742	HexCer(d34:1)	[M+Li] <sup>+</sup>	7876	HexCer(d18:1/16:0)
762.6366	HexCer(d38:1)	[M+Li] <sup>+</sup>	5492	HexCer(d18:1/20:0)
790.6675	HexCer(d40:1)	[M+Li] <sup>+</sup>	13421	HexCer(d18:1/22:0)
804.6827	HexCer(d41:1)	[M+Li] <sup>+</sup>	1961	HexCer(d18:1/23:0)
818.6986	HexCer(d42:1)	[M+Li] <sup>+</sup>	16913	HexCer(d18:1/24:0)
846.7295	HexCer(d44:1)	[M+Li] <sup>+</sup>	3747	HexCer(d18:1/26:0)

# High resolution mass spectrometry doesn't resolve isomers

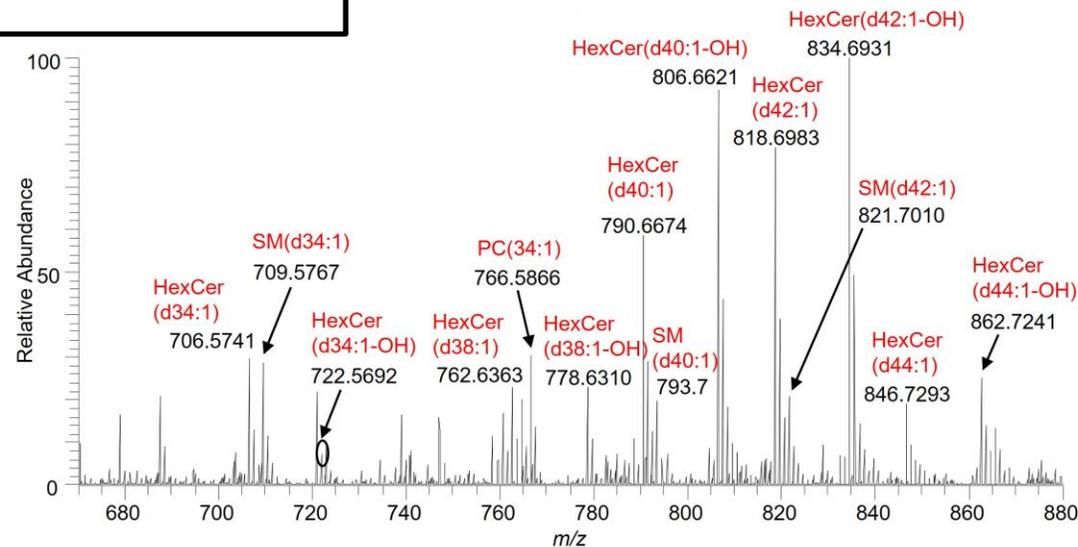
## Hexosylceramide



## Glucosylceramide



## Galactosylceramide



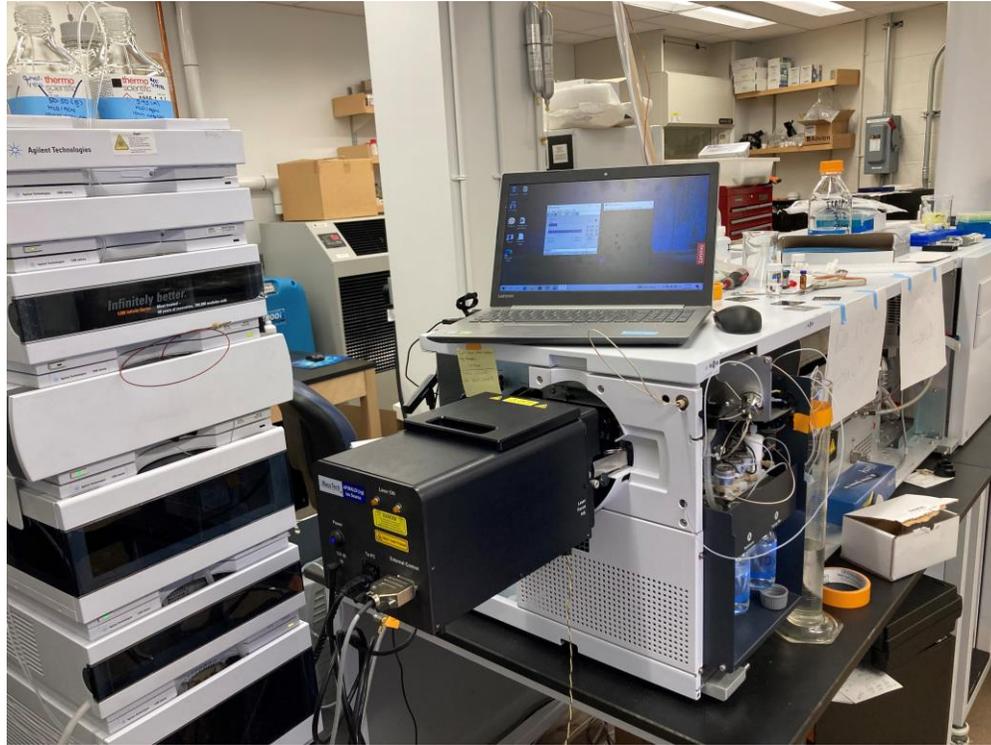
## AP-MALDI HRMS with THAP+Li

- Lipids from IVA virions

## Identification:

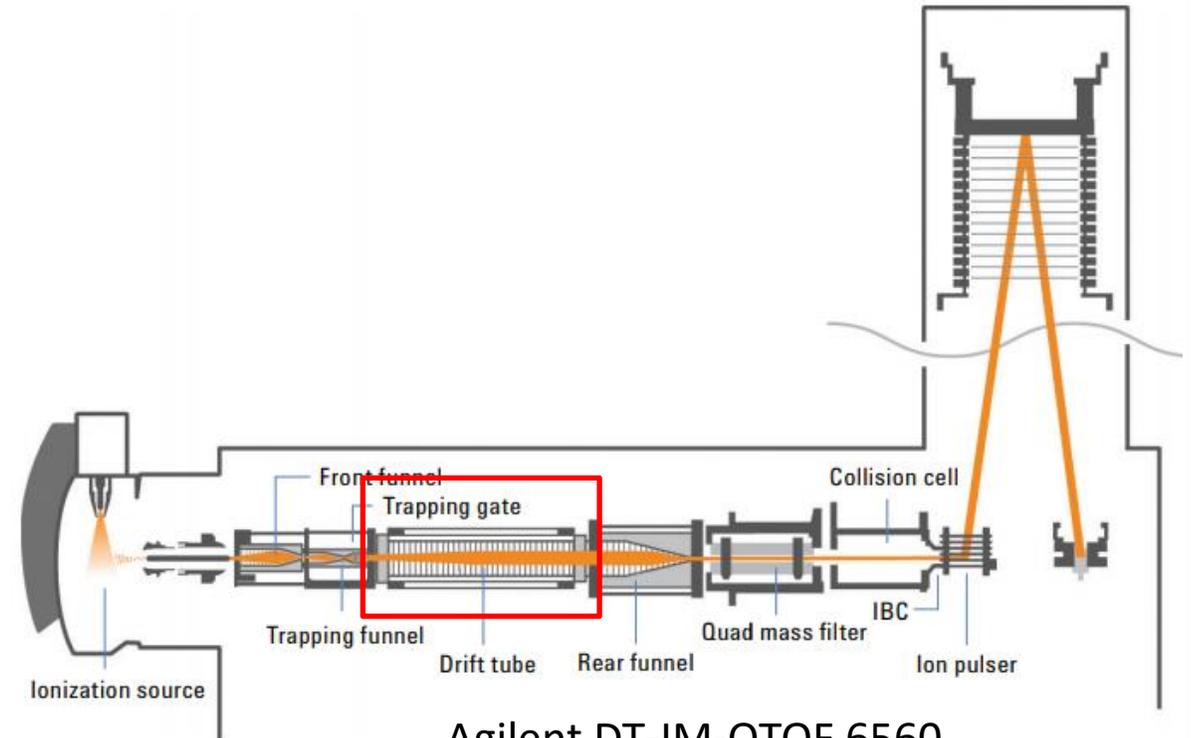
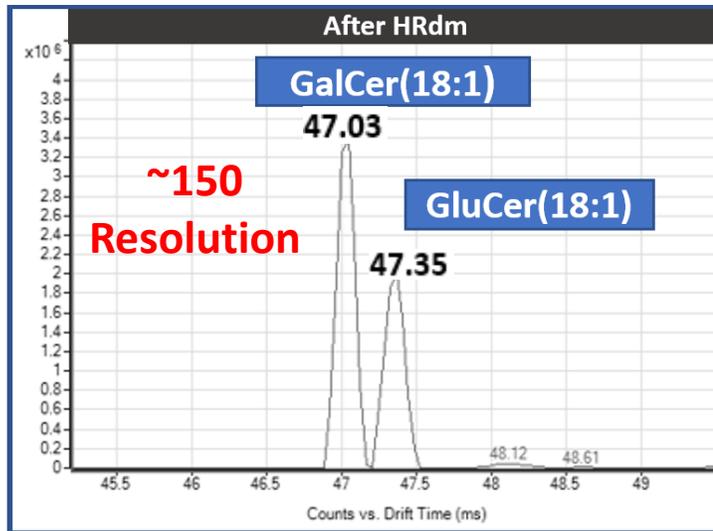
- HexCer: 44
- SM: 21

# AP MALDI Configured to Agilent 6560: Drift Tube Ion Mobility



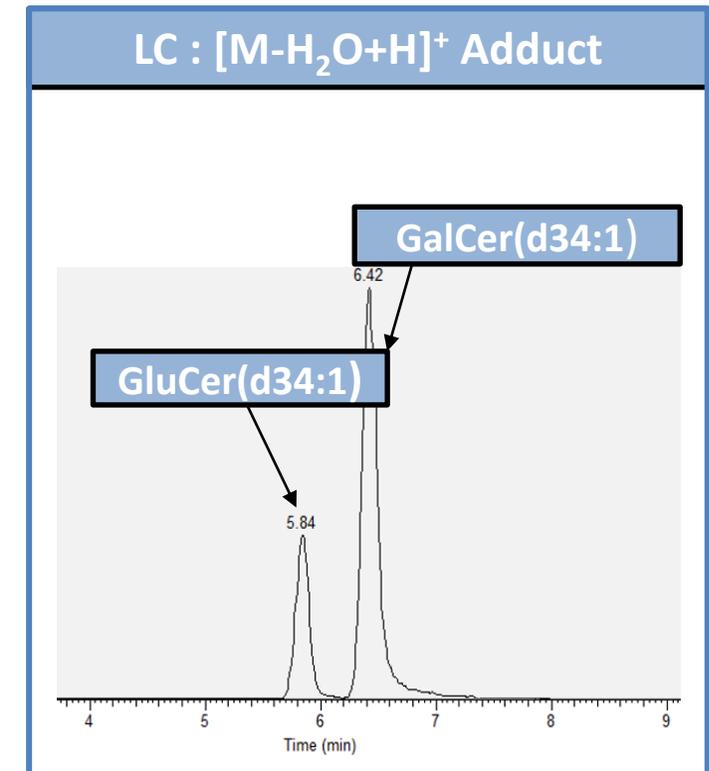
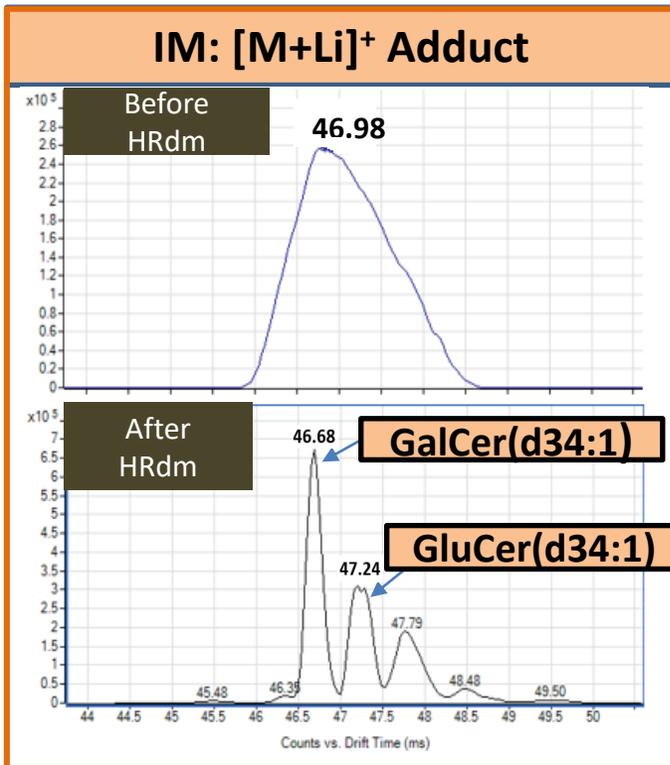
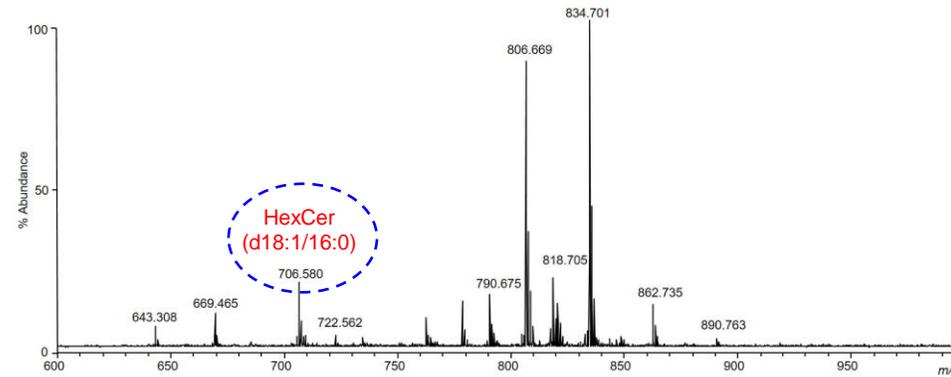
# HRdm enables gas-phase separation of isomeric HexCer

- Agilent DT-IM-QTOF 6560
- Separation based on collision cross section (CCS) and charge
- Direct CCS calculation
- Resolution: ~150 (with HRdm)



Agilent DT-IM-QTOF 6560

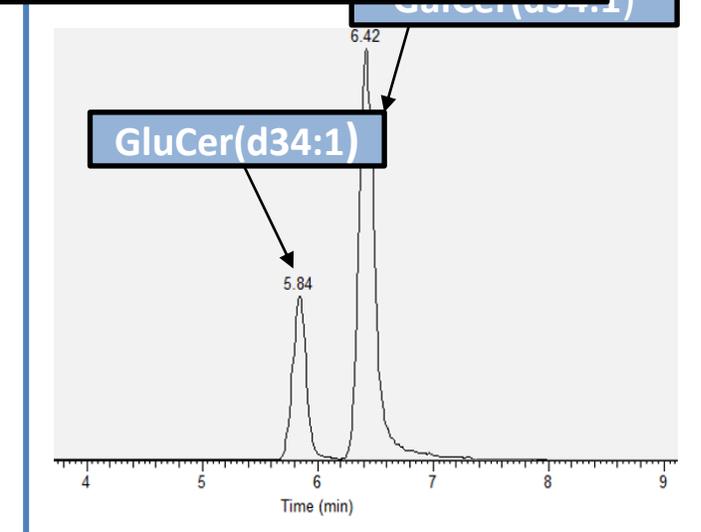
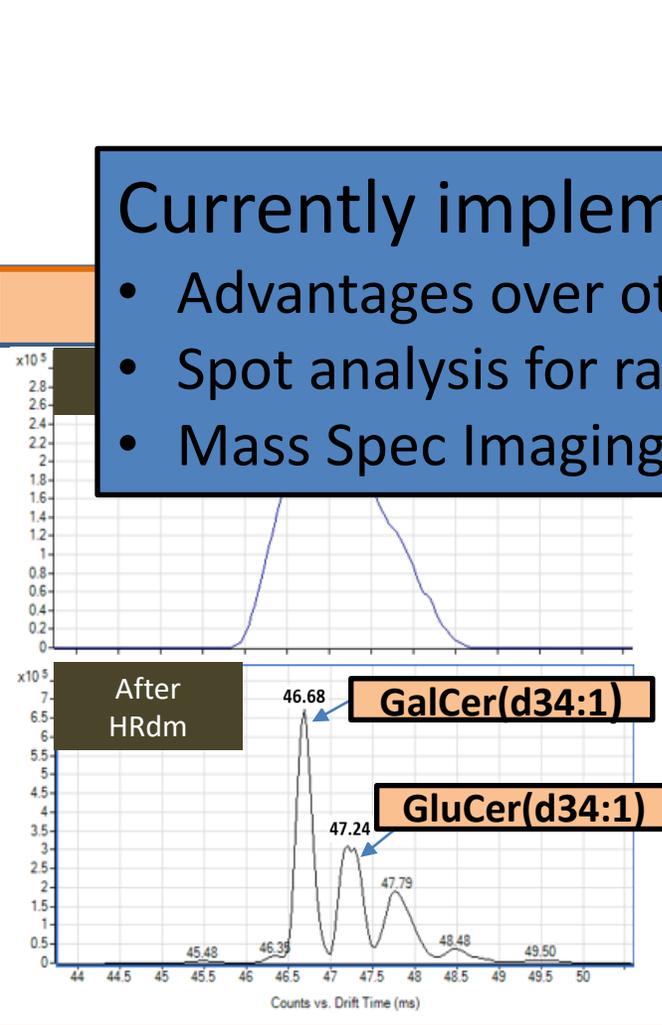
# HRdm enables gas-phase separation of isomeric HexCer from IVA virions



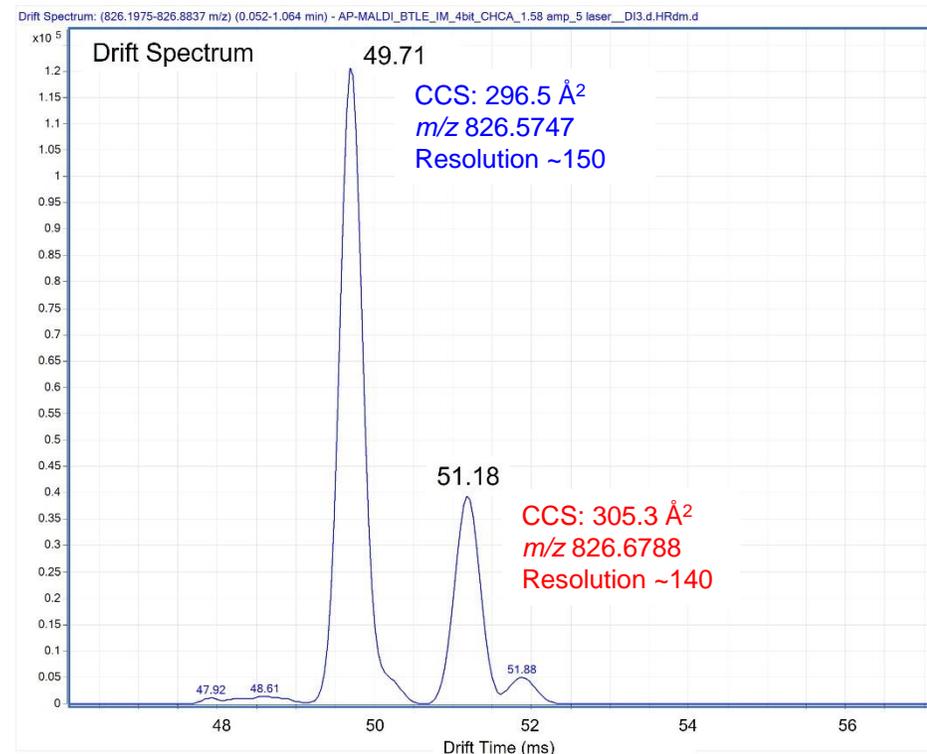
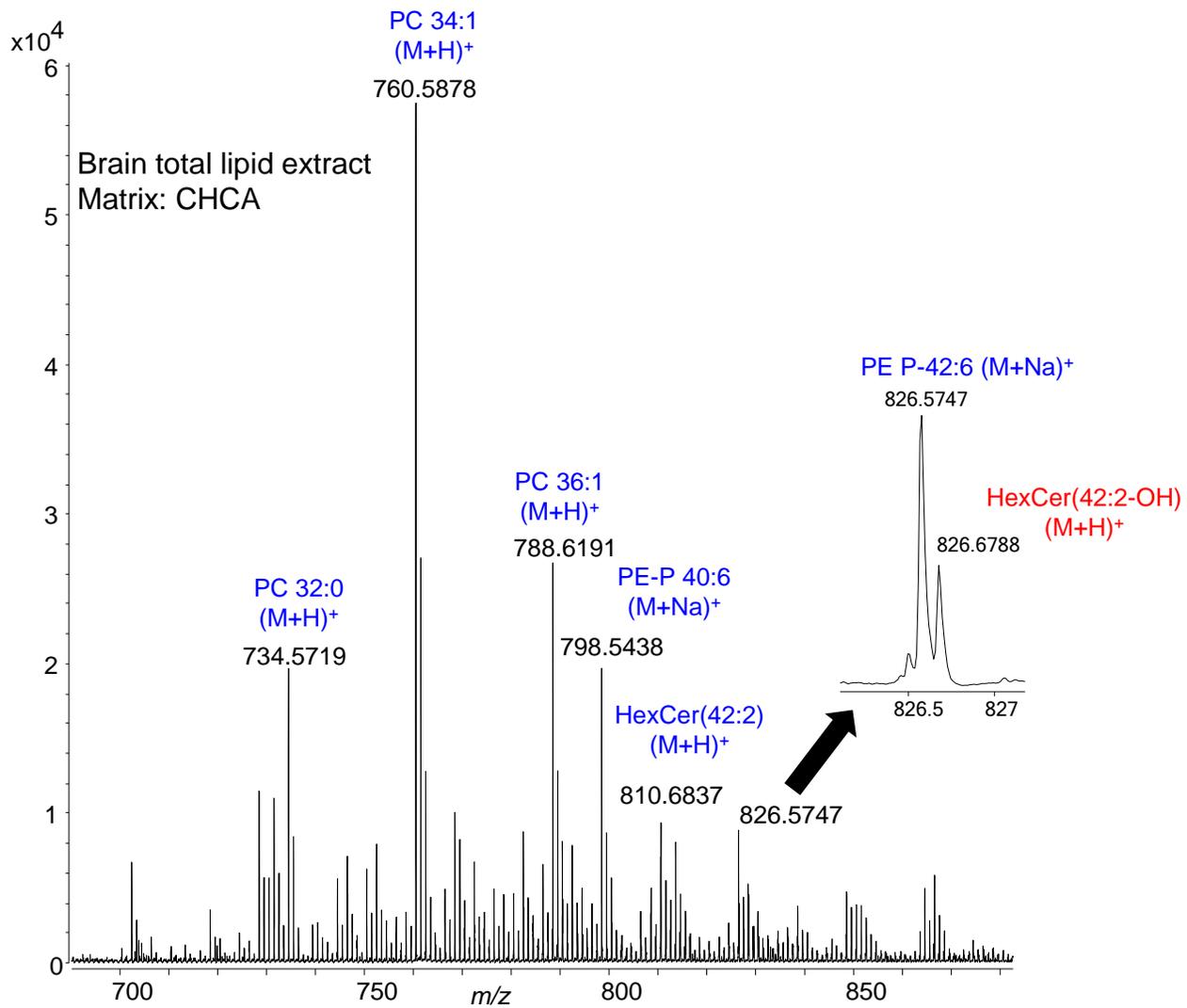
# HRdm enables gas-phase separation of isomeric HexCer from IVA virions

Currently implementing AP-MALDI on our 6560

- Advantages over other platforms
- Spot analysis for rapid profiling of lipid composition
- Mass Spec Imaging (MSI) applications: use of HRdm for gas-phase separation

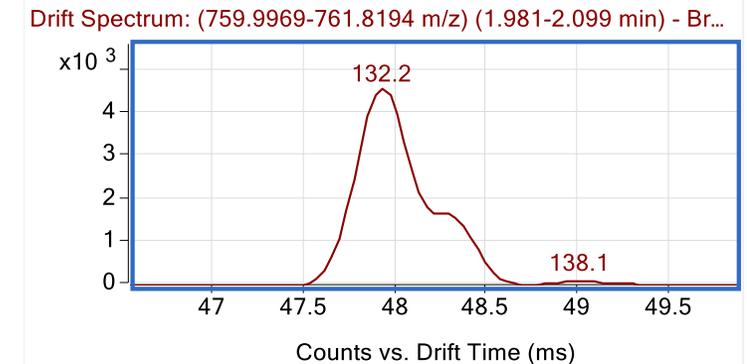
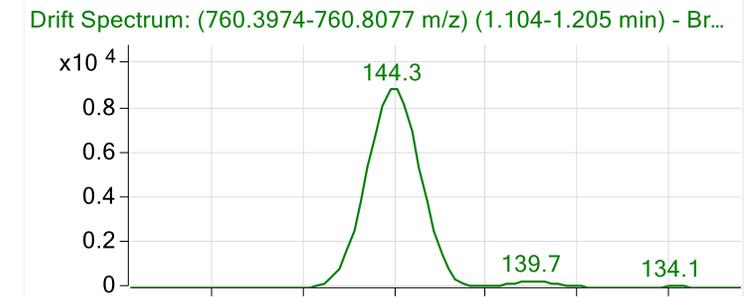
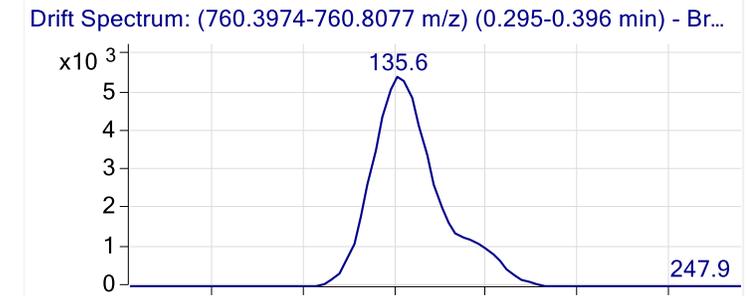
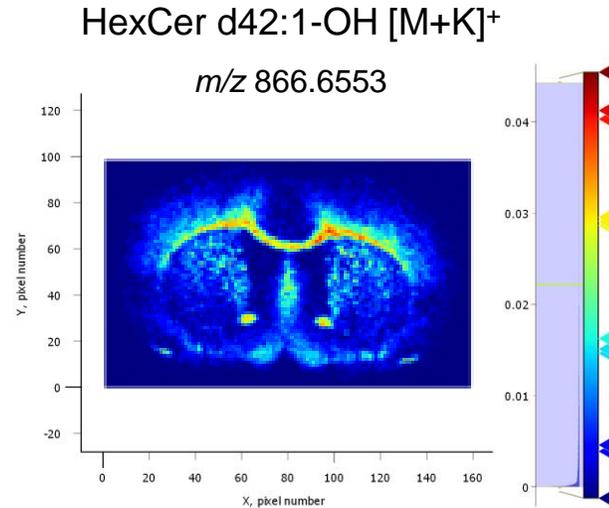
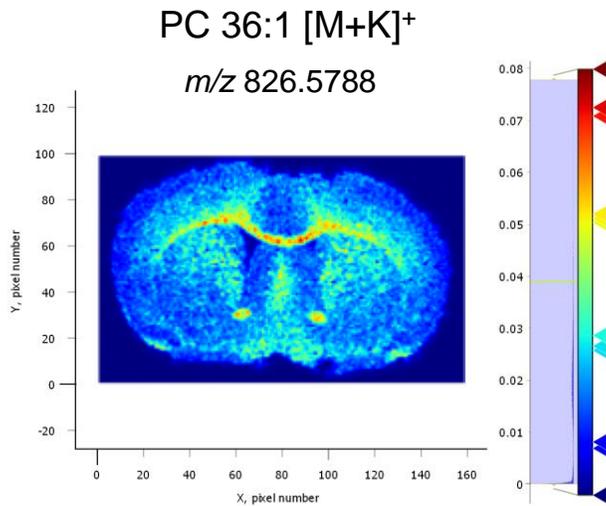
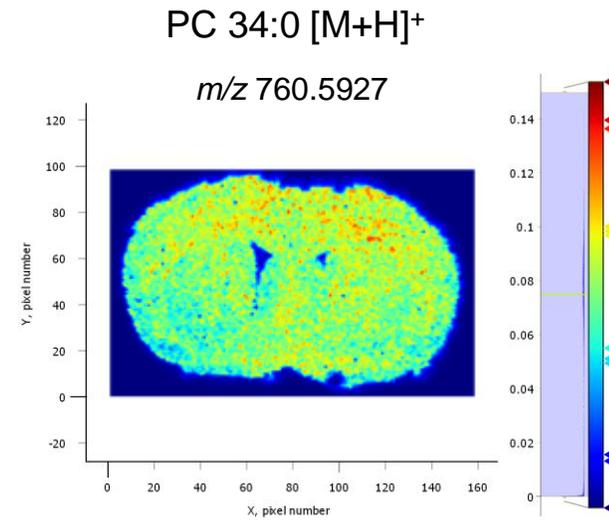
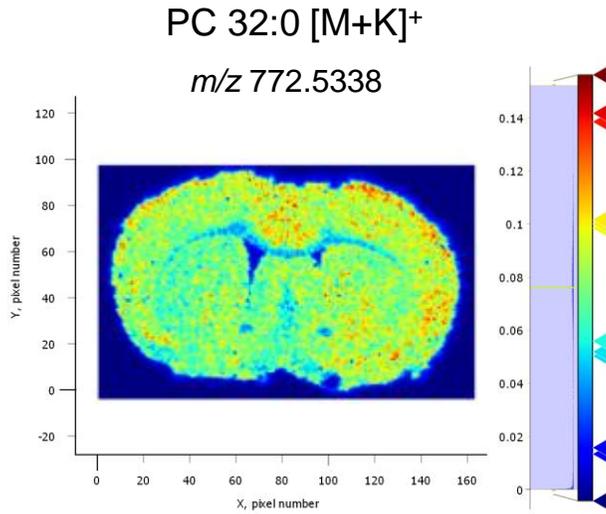


# AP MALDI-6560 Spot Analysis Example



# AP MALDI-6560 Mass Spec Imaging Example

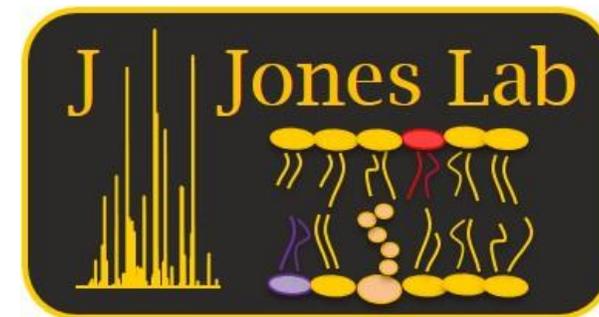
Drift spectra across tissue detailing differential spatial localization of isomeric species ( $m/z$  760.5927)  
IM Resolution > 130



# Acknowledgements

## Jones Laboratory (UMB SOP)

Anh Tran  
Yulemni Morel  
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Ahmed M. Abdel-Megied Ali (former post-doc)



[www.jacewjoneslab.com](http://www.jacewjoneslab.com)



Poster WP276: Imaging with AP-MALDI on 3Q and IM-QTOF

Poster TP349: Y Morel, Characterization of Oxidized phospholipids

Poster TP351: A Tran, Structural analysis of sphingolipids



## MassTech Inc.

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Nivedita Bhattacharya  
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John Sausen  
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- MassTech Research Contract