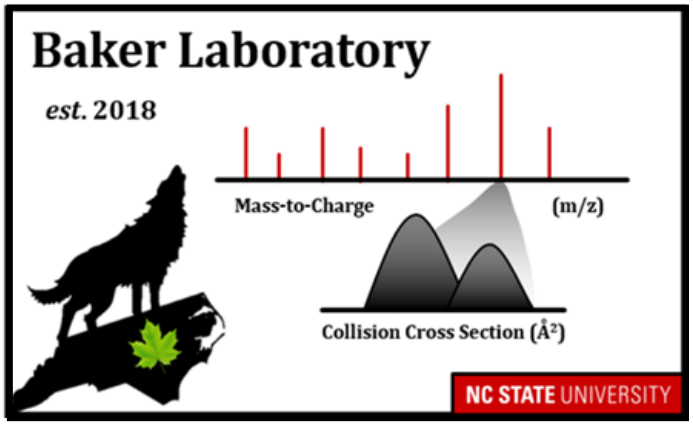
Developing Multidimensional Small Molecule Spectral Libraries for Rapid Lipid Detection and Quantitation



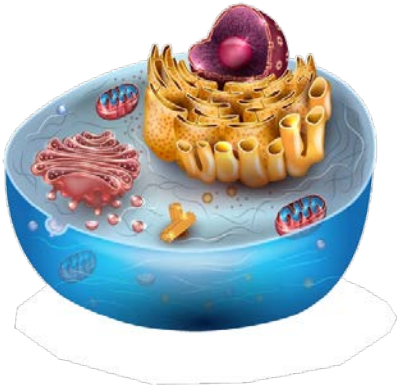
2020 Skyline User Group Meeting

Kaylie I. Kirkwood & Erin S. Baker

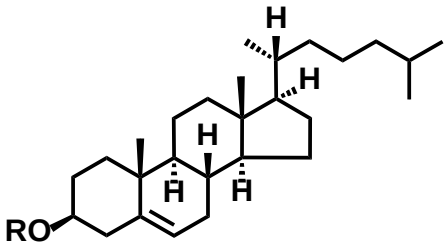
Lipid Introduction – Categories & Functions

Roles of Lipids

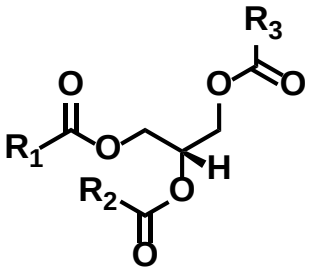
- Energy storage
- Signaling
- Structural components of cell membranes
- Precursors in hormone biosynthesis



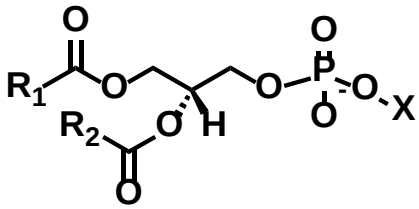
Sterol Lipids



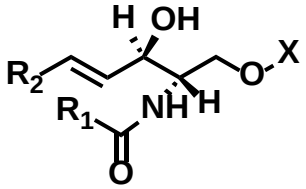
Glycerolipids



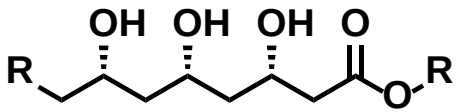
Glycerophospholipids



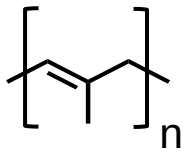
Sphingolipids



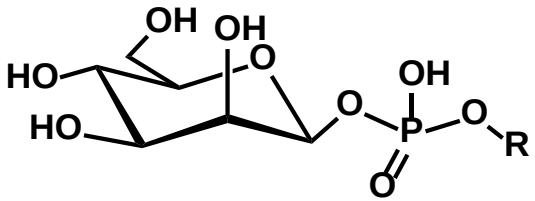
Polyketides



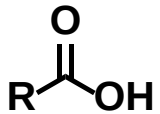
Prenol Lipids



Saccharolipids



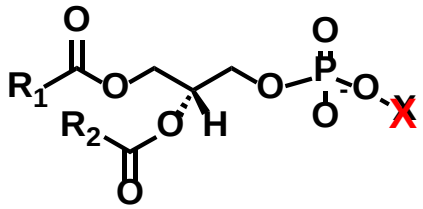
Fatty Acyls



Fahy, E., et. al. *J Lipid Res* **2009**, 50, S9-14.

Lipid Introduction – Classes & Structure

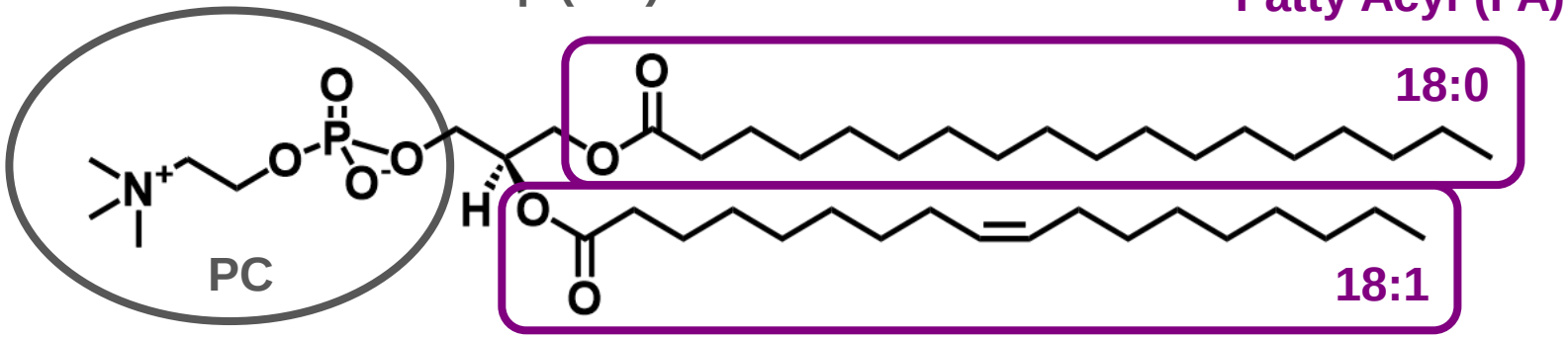
Glycerophospholipids



PC(18:0_18:1)

Head Group (HG)

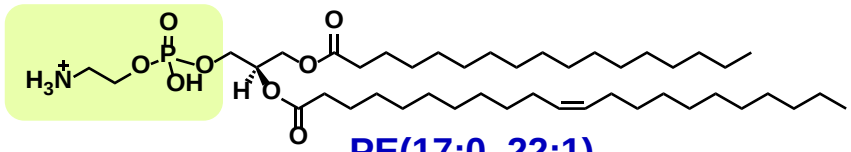
Fatty Acyl (FA)



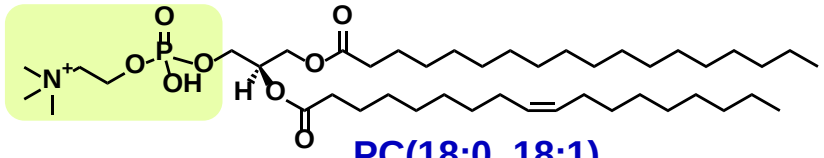
Complexity of Lipid Analysis – Isomers

[M+H]+ 788.6164 *m/z*

Class/head group change:

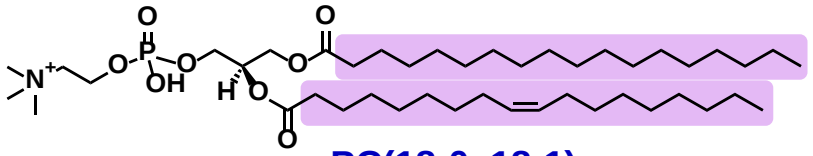


PE(17:0_22:1)

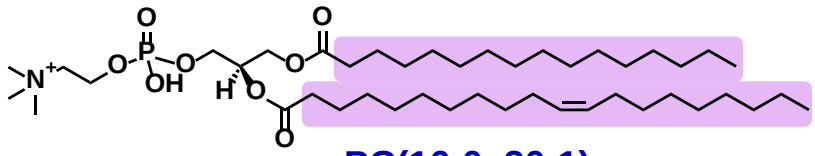


PC(18:0_18:1)

Fatty acyl tail composition change:

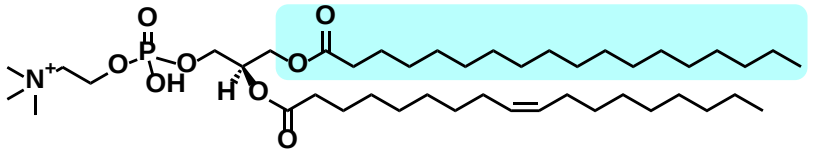


PC(18:0_18:1)

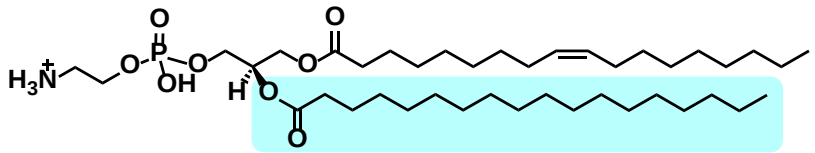


PC(16:0_20:1)

Fatty acyl tail orientation (*sn*-position) change:

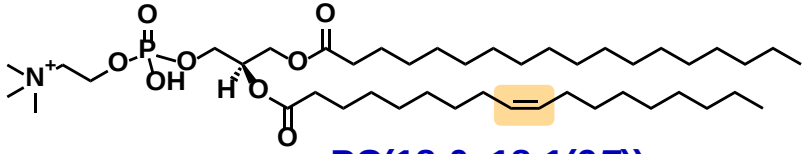


PC(18:0/18:1)

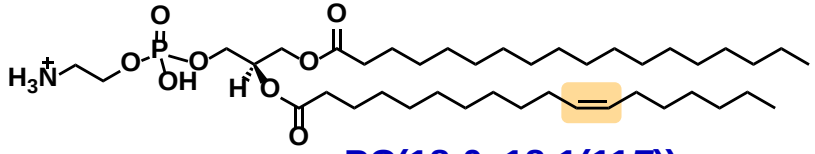


PC(18:1/18:0)

Double bond position change:

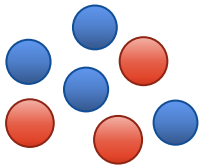


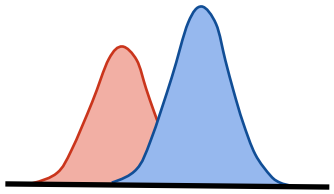
PC(18:0_18:1(9*Z*))

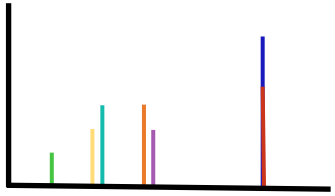


PC(18:0_18:1(11*Z*))

Multidimensional Separations & Ion Mobility





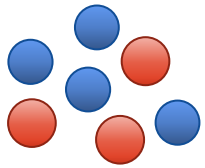


HPLC-C18
(Polarity, RT)

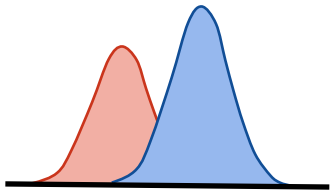
Ion Mobility
(Size, CCS)

CID-TOF
(Fragments, *m/z*)

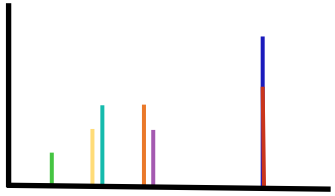
Multidimensional Separations & Ion Mobility



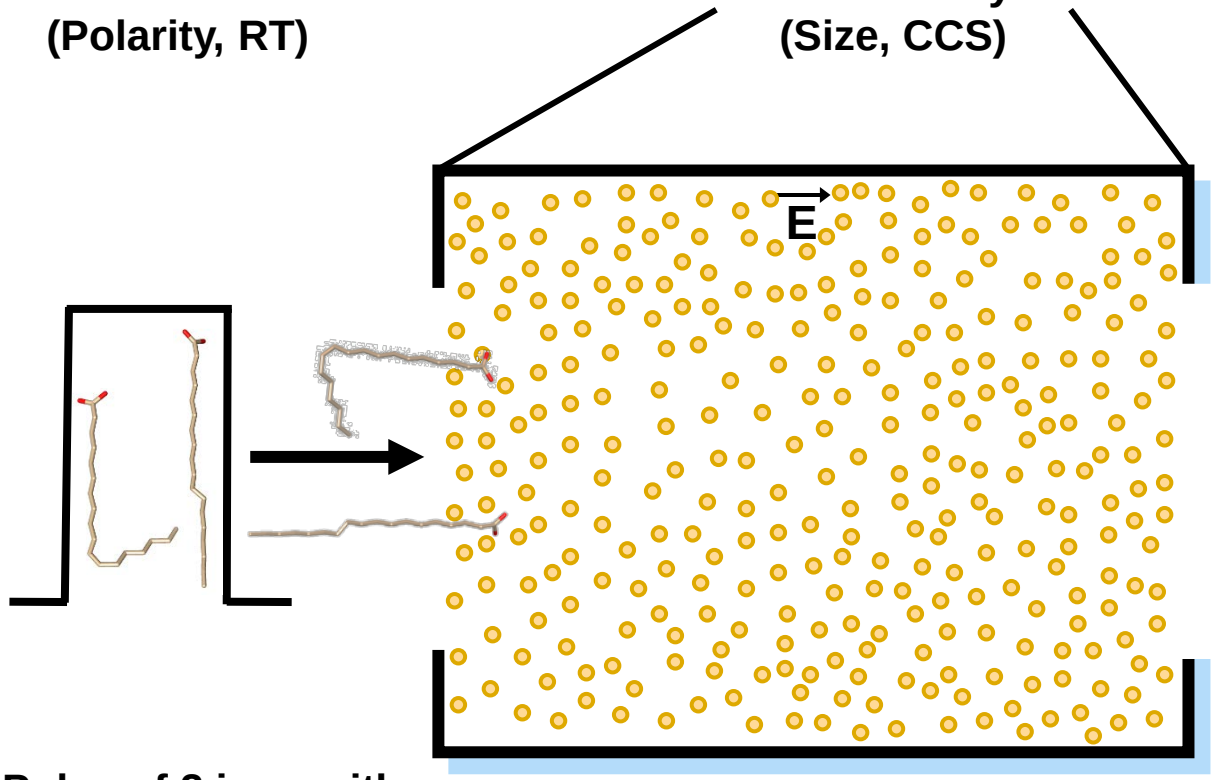
HPLC-C18
(Polarity, RT)



Ion Mobility
(Size, CCS)

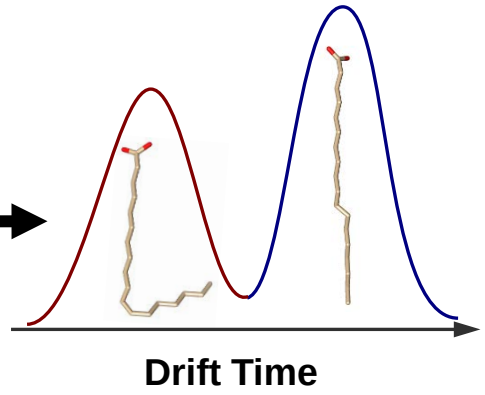


CID-TOF
(Fragments, *m/z*)



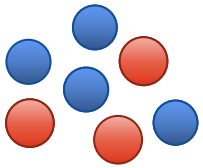
Pulse of 2 ions with same *m/z* but different shape

Drift Cell

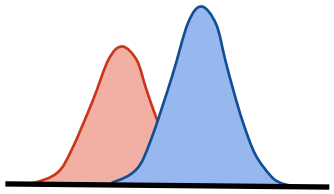


Different conformers separate in time with peak heights representing the amount of each

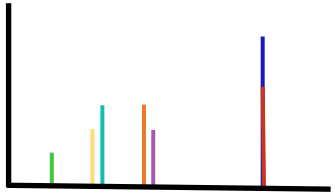
Multidimensional Separations & Ion Mobility



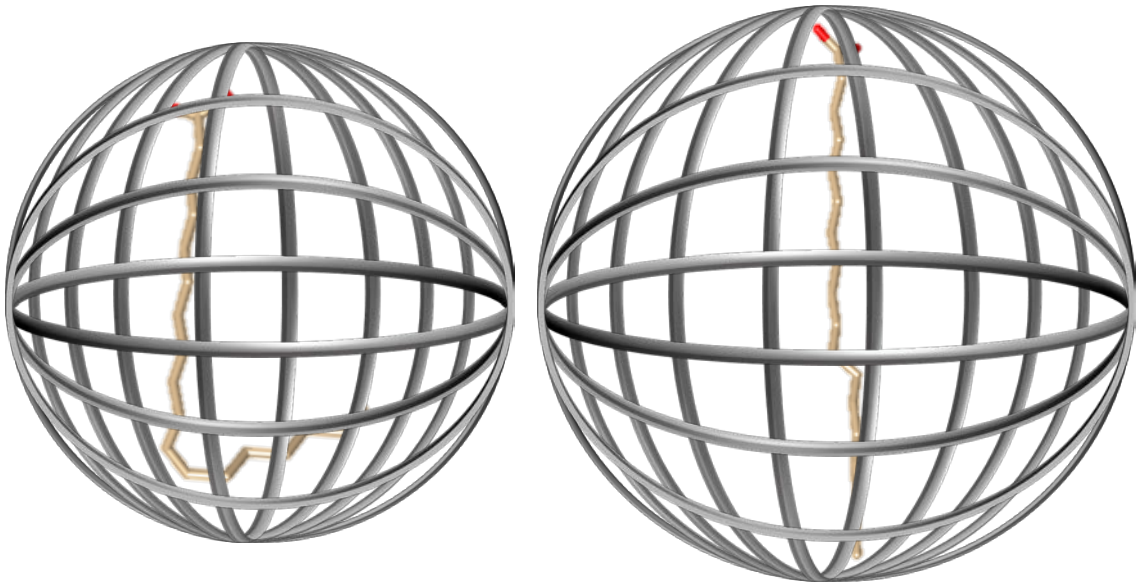
HPLC-C18
(Polarity, RT)

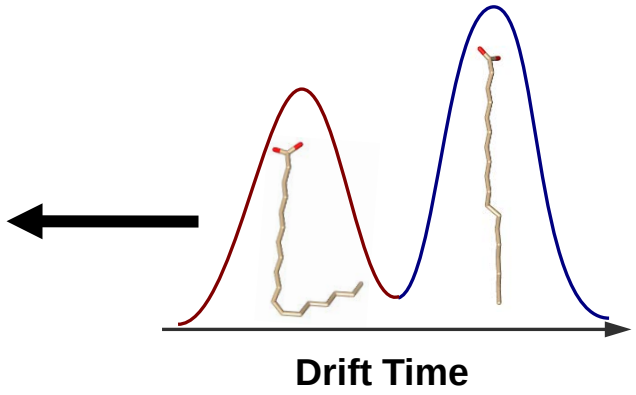


Ion Mobility
(Size, CCS)



CID-TOF
(Fragments, *m/z*)



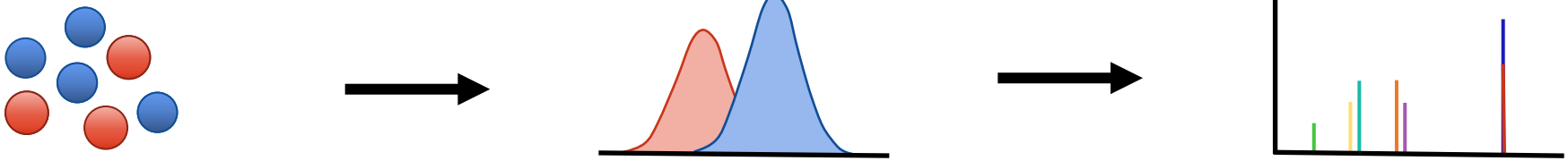


Higher drift time

Larger Collisional Cross Section (CCS, Å²)

Different conformers separate in time with peak heights representing the amount of each

Multidimensional Separations & Ion Mobility



HPLC-C18
(Polarity, RT)

Ion Mobility
(Size, CCS)

CID-TOF
(Fragments, *m/z*)

Challenges

- LC-IMS-(CID)-MS data is large in complexity and file size
- Manual assignment is time consuming and can only be done for a few targets & small datasets

Lipidomics Data Analysis – Choose Your Fighter

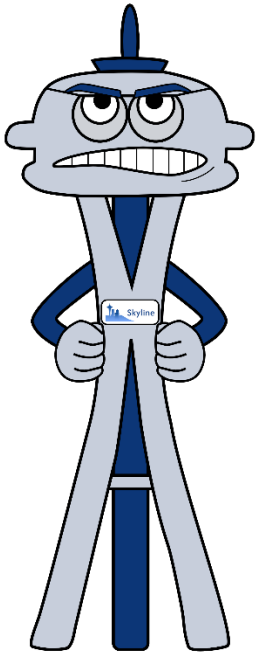
THE BLACK BOX

Strengths

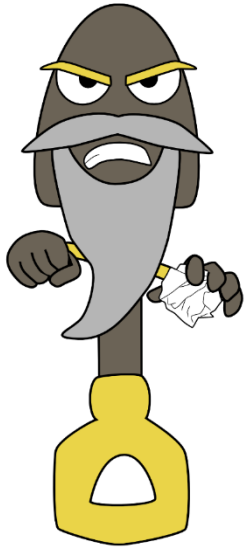
- Rapid analysis
- Good for large datasets
- Deep search

Weaknesses

- Little to no validation
- Over-annotation
- May sacrifice data (RT, CCS)
- Search may not be sample/species-specific



THE MANUAL LABORER



Strengths

- Confident annotations
- Utilizes all data
- Prioritization of biologically relevant lipids

Weaknesses

- Time-consuming
- Not sustainable for large datasets
- Shallow search

Skyline Small Molecule Workflows

Our initial workflow:

Create & Import Target List
Name, *m/z*, charge

→

Import Results
Filtered by *m/z*

→

Analyze & Visualize
View data, confirm IDs, compare peak areas & retention times

→

Export Report
Names, retention times, peak areas, mass errors, etc.

Skyline

Our proposed user workflow:

Import Spectral Library & iRT Calculator
Name, formula, adduct, CCS, fragments, neutral losses, iRT

→

Import Results
Filtered by *m/z*, drift time, predicted retention time

→

Analyze & Visualize
View data, confirm peak boundaries, compare peak areas & RTs/iRTs & library match

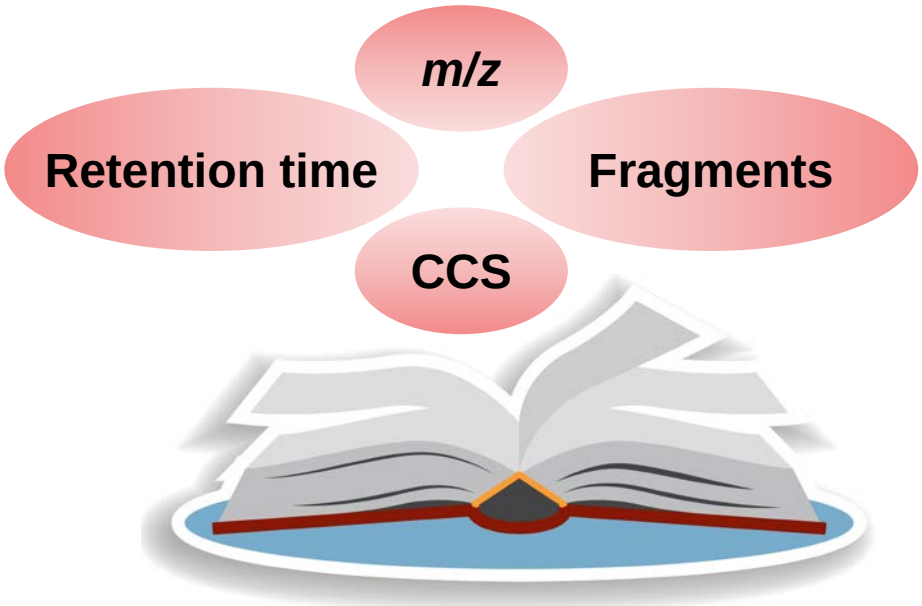
→

Quantify
Generate calibration curve from SIL standards

→

Export Report
Names, RT, peak areas, mass errors, CCS, predicted RT, concentrations or normalization (median, TIC)

Lipid Spectral Libraries in Skyline



Libraries contain hundreds of lipids from diverse classes

Fatty Acyls

Fatty acids, fatty esters

Sphingolipids

Cer, HexCer, SM, GM3

Sterol Lipids

Cholesterol esters

Glycerolipids

DG, TG, MG

Glycerophospholipids

PA, PC, PE, PG, PI, PS, CL

Adams, K.J., et. al. *J Proteome Res* **2020**, 19(4): 1447-1458.

Development of Spectral Libraries

1. Thousands of transitions are generated in LipidCreator

2. Lipid peak candidates are identified

3. CCS values are calculated using the Ion Mobility Predictor tool

4. Lipids are annotated based on drift time-aligned fragments

5. iRT calculator is calibrated from results

6. Spectral libraries are exported

Lipid included in spectral library

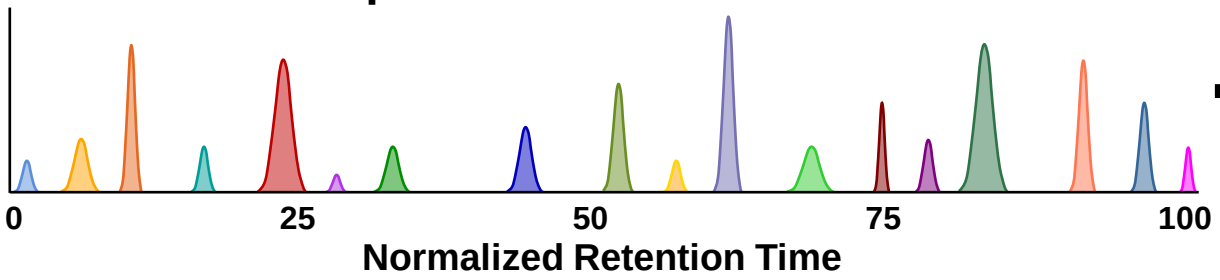
Confidence

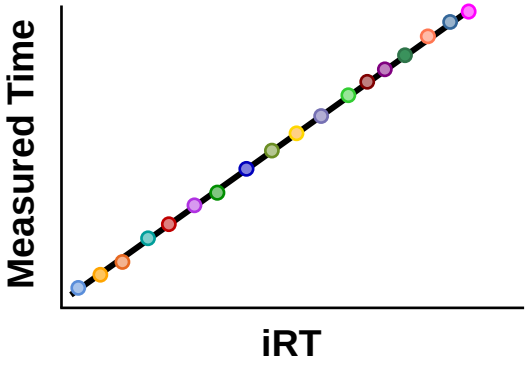
- ✓ **CCS within 2% of database value (if present)**
- ✓ **Drift time aligned fragments**
- ✓ **RT within expected class-specific window**
- ✓ **Multiple adducts co-elute (if present)**
- ✓ **≤ 5 ppm MMA**
- ✓ **Present in >1 sample**

Kopczynski, D. et. al. **2020**. http://doi.org/10.5281/zenodo.3529484

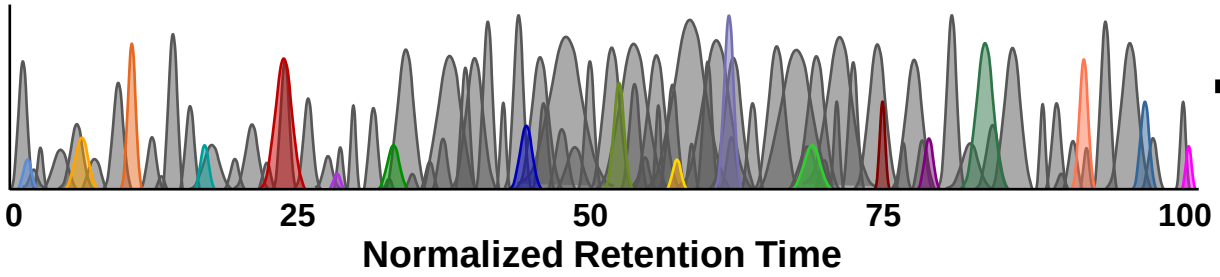
Retention Time Prediction with iRT

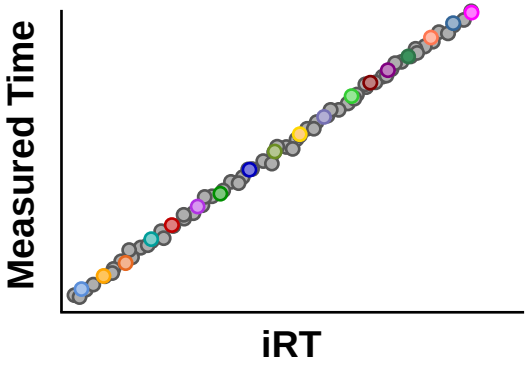
1. Set reference lipids and calibrate iRT calculator*



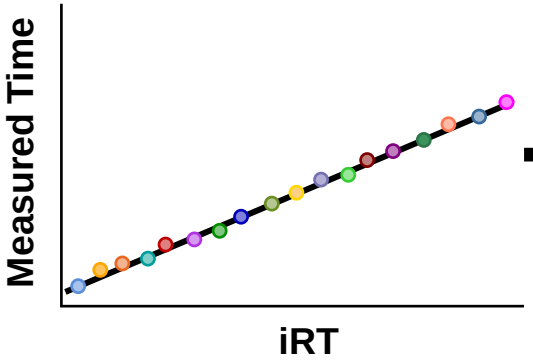


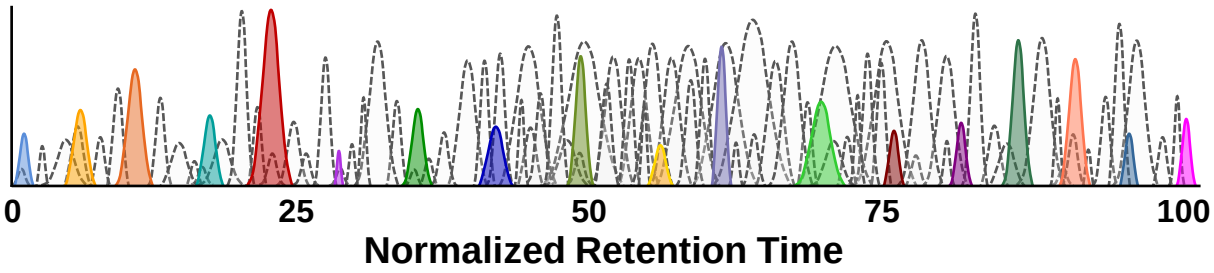
2. Add iRT values for new target lipids*





3. Predict retention times in new dataset

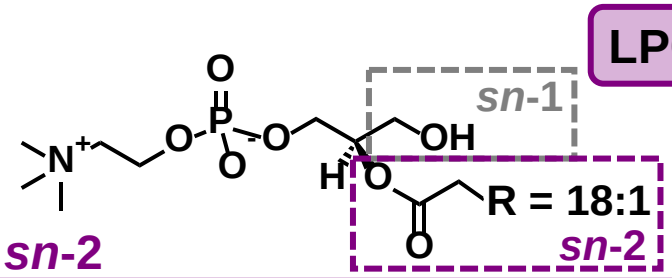


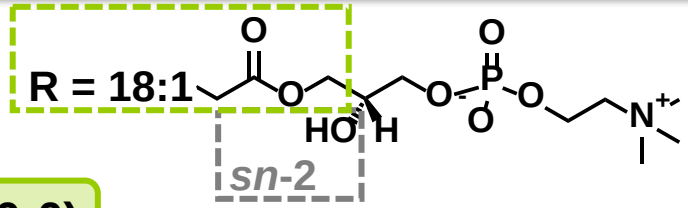


Escher, C. et. al. *Proteomics* **2012**, 12(8), 1111-1121.

*Completed during library development

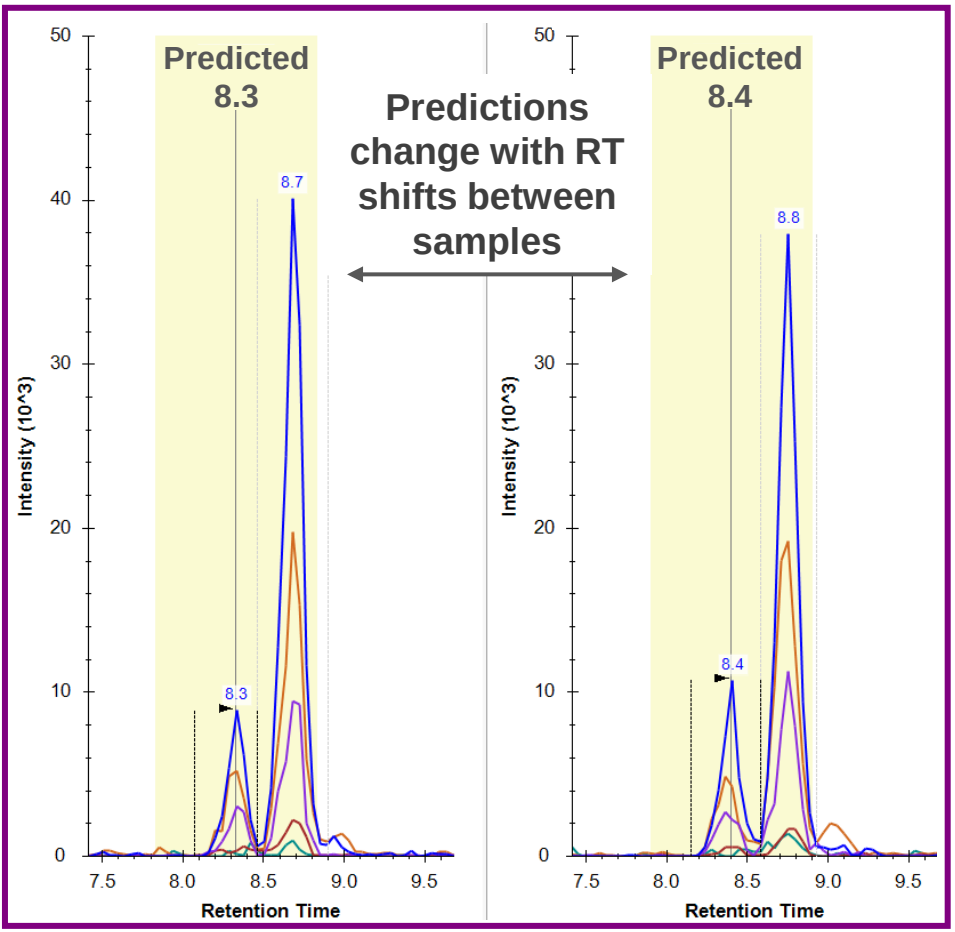
Library Features – RT Prediction with iRT

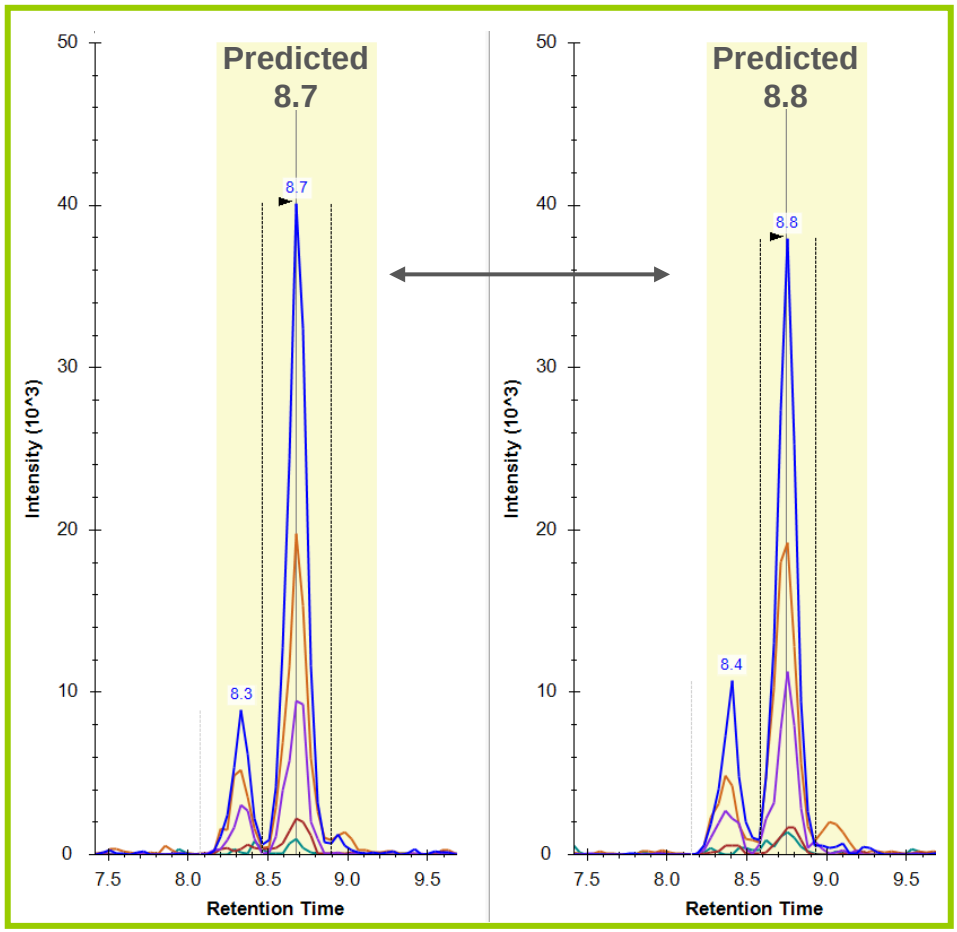




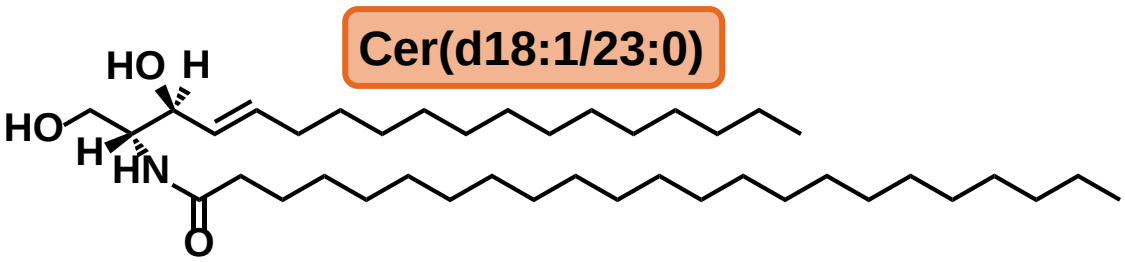
LPC(18:1/0:0)

sn-1

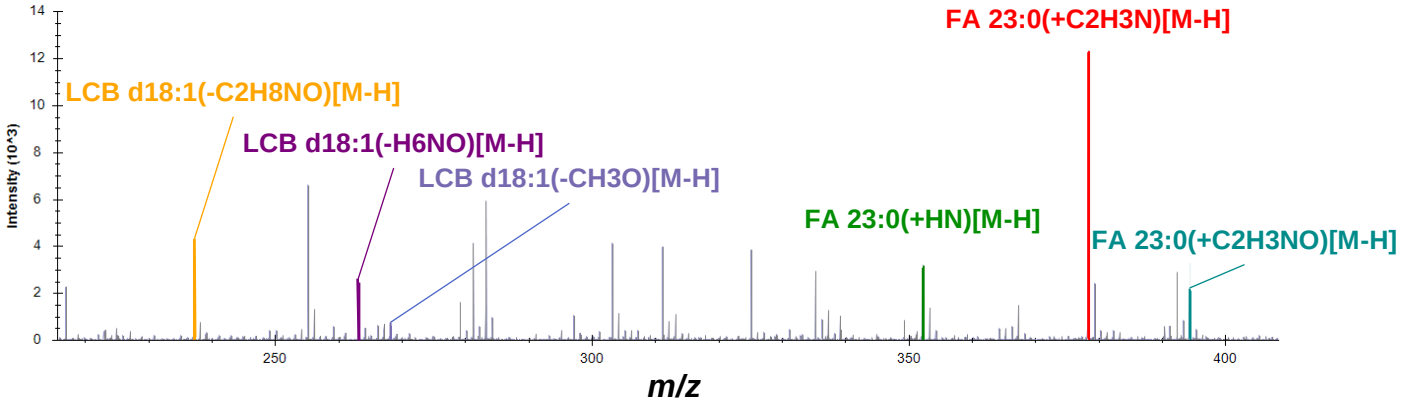




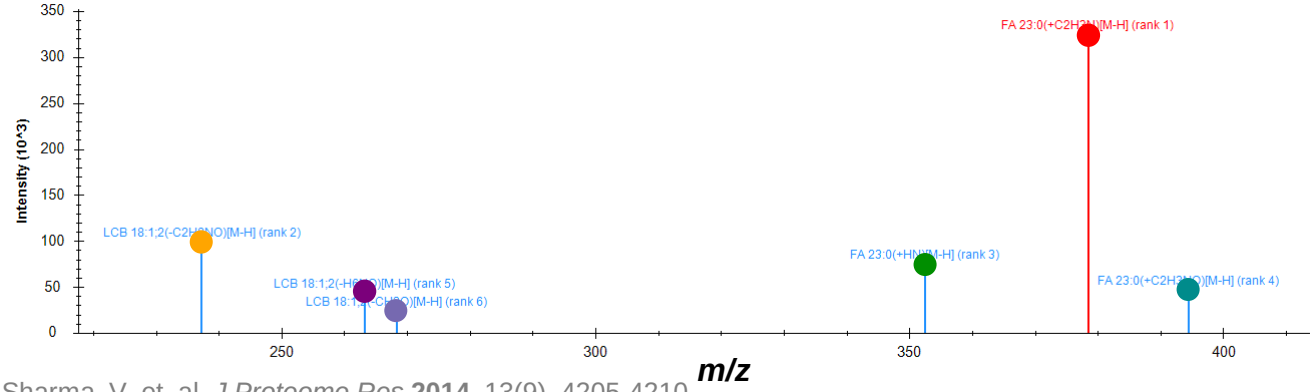
Library Features – Spectral Library Matching

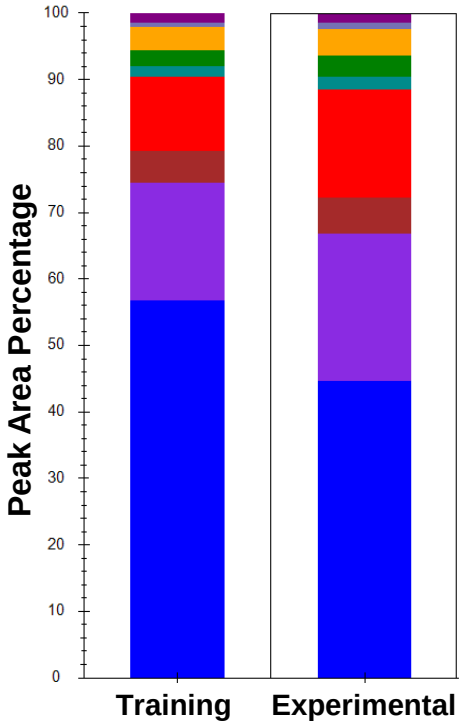


Spectrum from "Experimental" Dataset



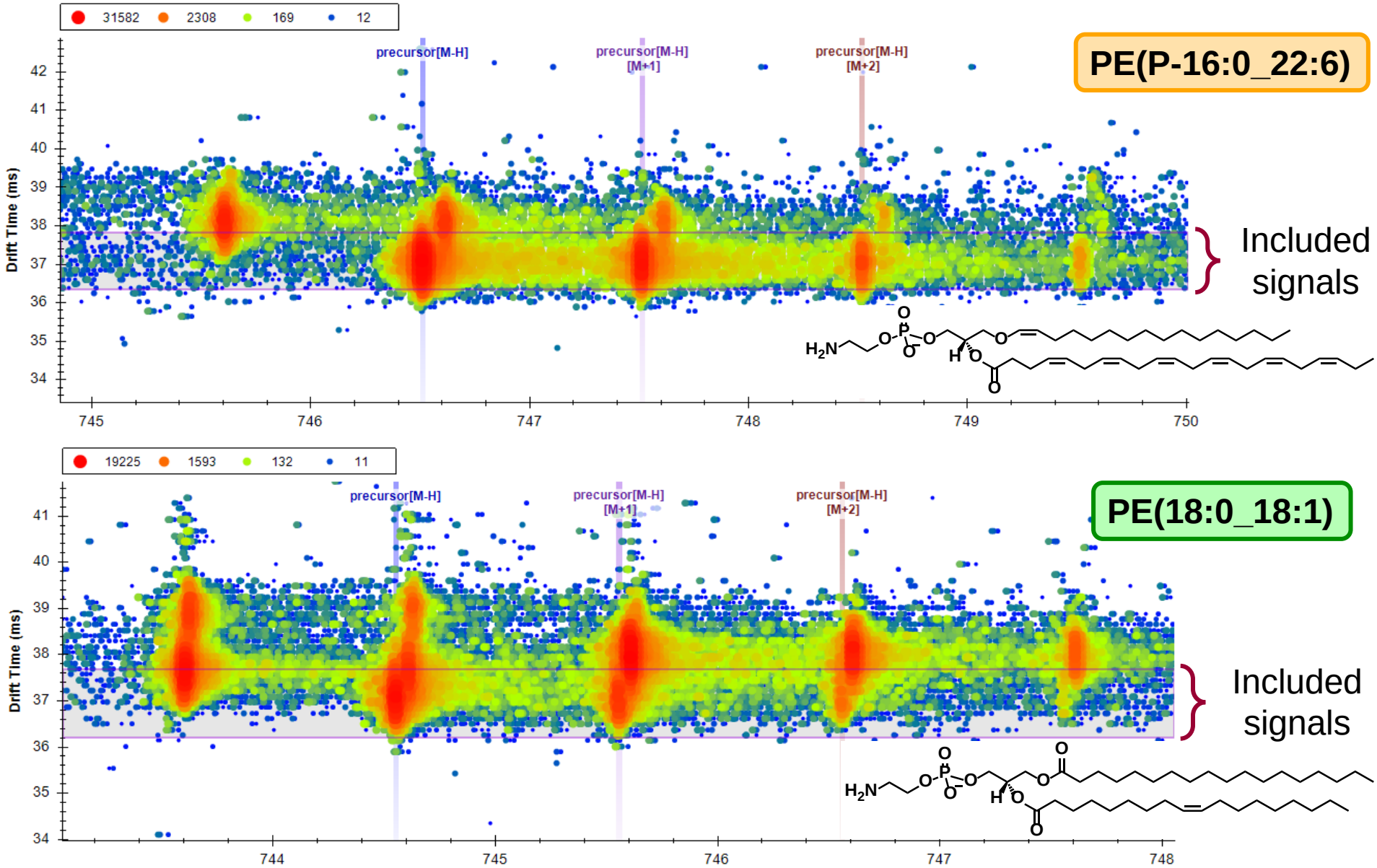
Library Match Spectrum from "Training" Dataset





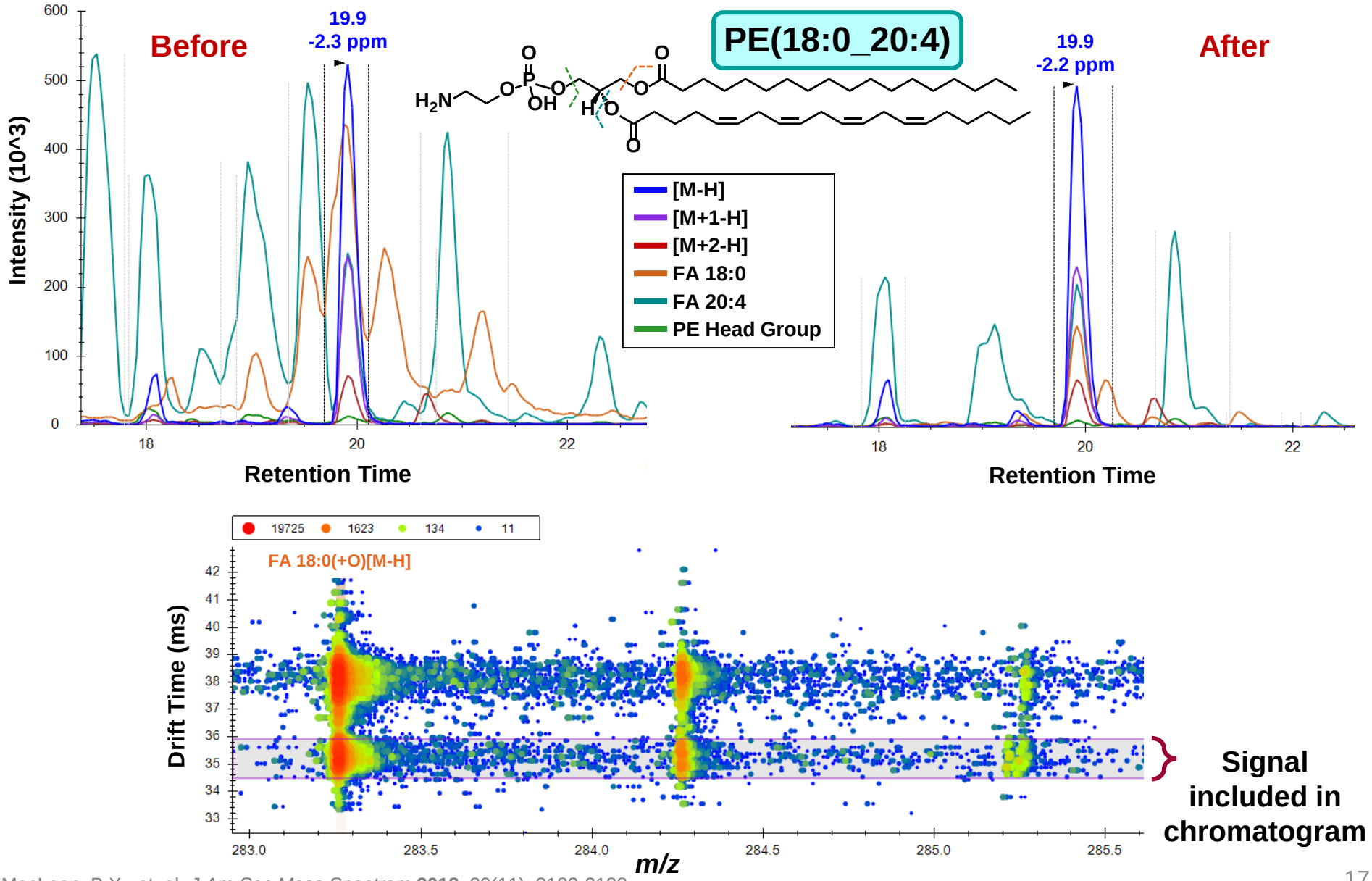
Sharma, V. et. al. *J Proteome Res* **2014**, 13(9), 4205-4210.

Library Features – Drift Time Filtering



MacLean, B.X., et. al. *J Am Soc Mass Spectrom* **2018**, 29(11): 2182-2188.

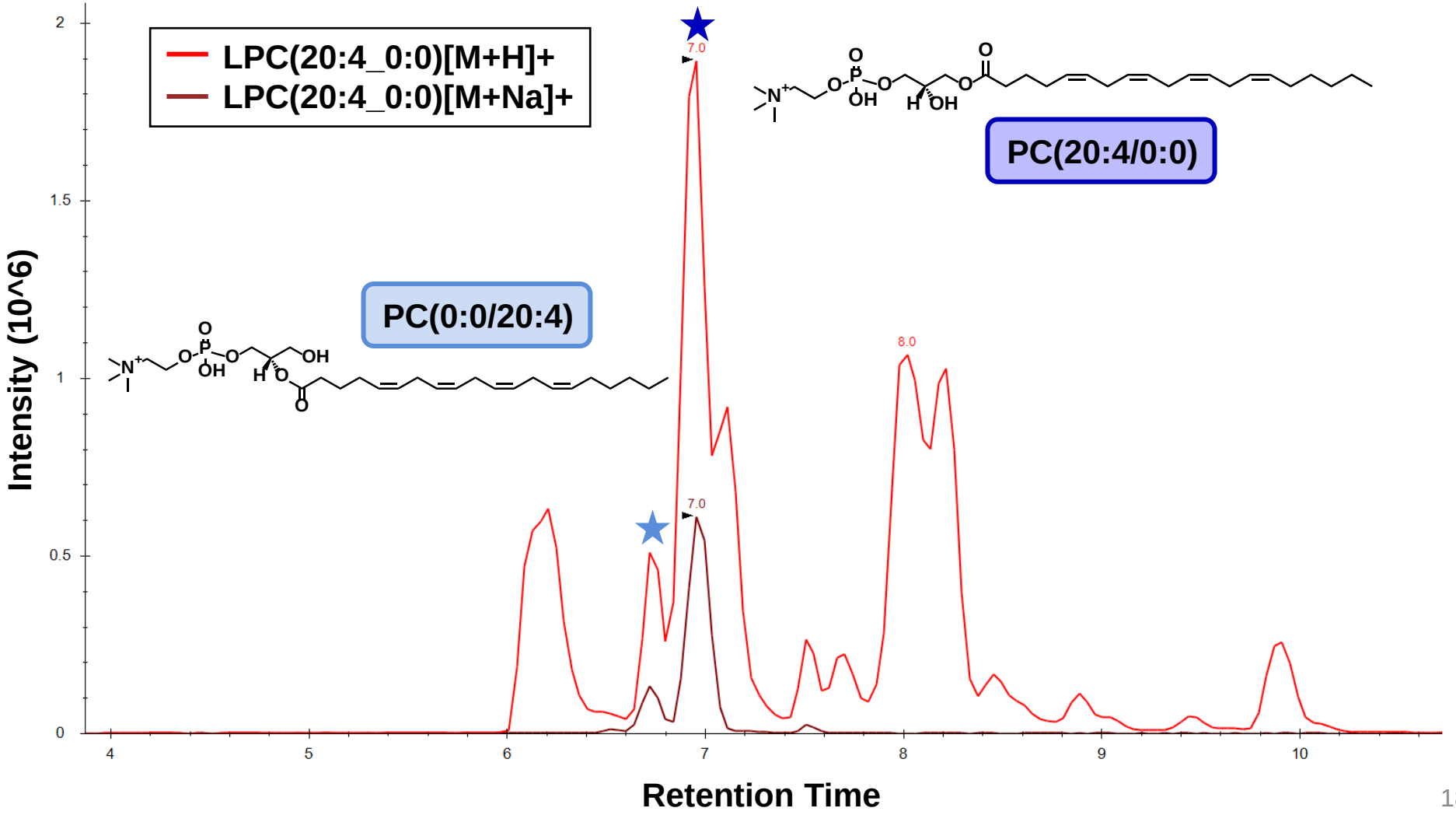
Library Features – Drift Time Filtering



MacLean, B.X., et. al. *J Am Soc Mass Spectrom* **2018**, 29(11): 2182-2188.

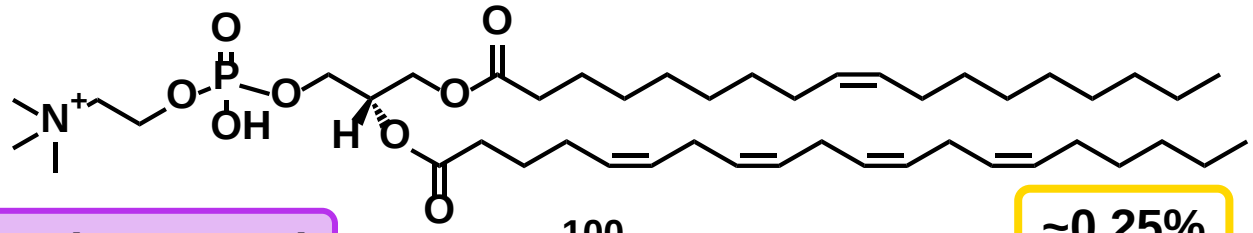
Library Features – Multiple Adducts

- PCs form highly abundant [M+H]+ ions
- CID produces extremely low abundance neutral loss fragments
- **[M+Na]+ ions can aid in filtering candidate peaks**



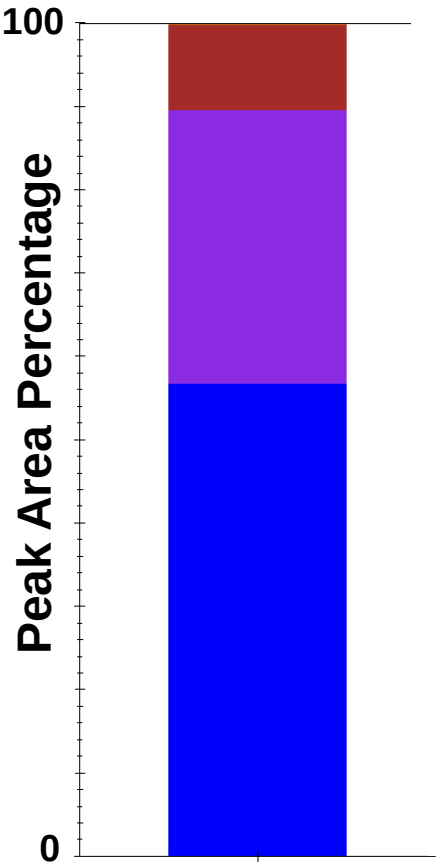
Library Features – Multiple Adducts

- PCs form highly abundant [M+H]+ ions
- CID produces extremely low abundance neutral loss fragments
- **[M+Na]+ ions can aid in fatty acyl assignment**



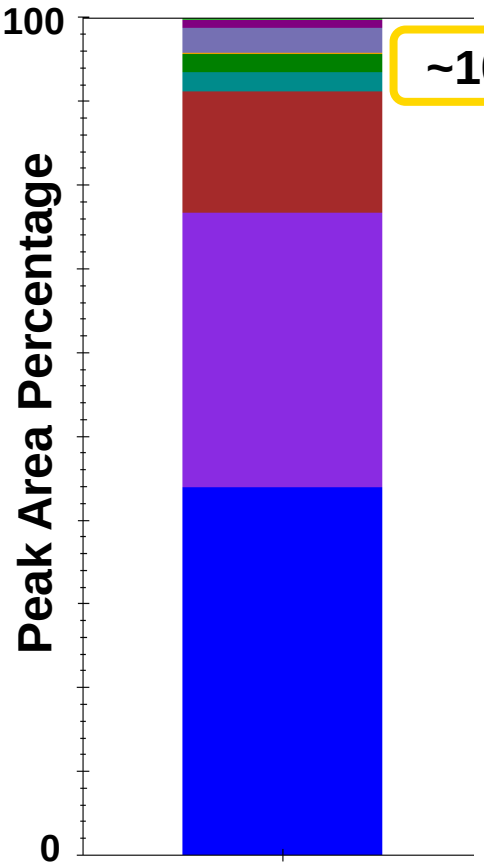
PC(18:1_20:4)

precursor - 808.5851[M+H]
precursor [M+1] - 809.5885[M+H]
precursor [M+2] - 810.5916[M+H]
M-18:1 - 526.3292[M+H]
M-20:4 - 504.3449[M+H]



~0.25%

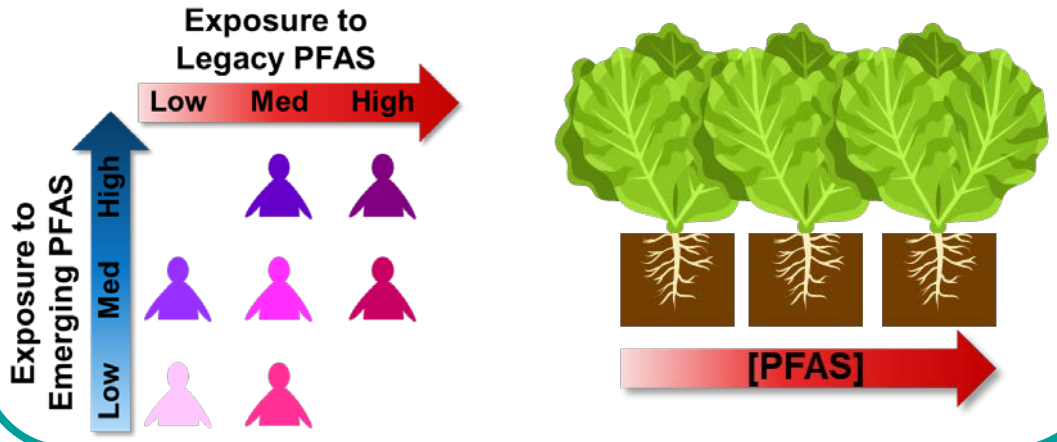
precursor - 830.5670[M+Na]
precursor [M+1] - 831.5704[M+Na]
precursor [M+2] - 832.5735[M+Na]
M+Na-18:1 - 548.3111[M+Na]
M+Na-20:4 - 526.3268[M+Na]
M+Na-TMA-18:1 - 489.2376[M+Na]
M+Na-TMA-20:4 - 467.2533[M+Na]
M-18:1 - 526.3292[M+H]
M-20:4 - 504.3449[M+H]
M-TMA-18:1 - 467.2557[M+H]
M-TMA-20:4 - 445.2714[M+H]



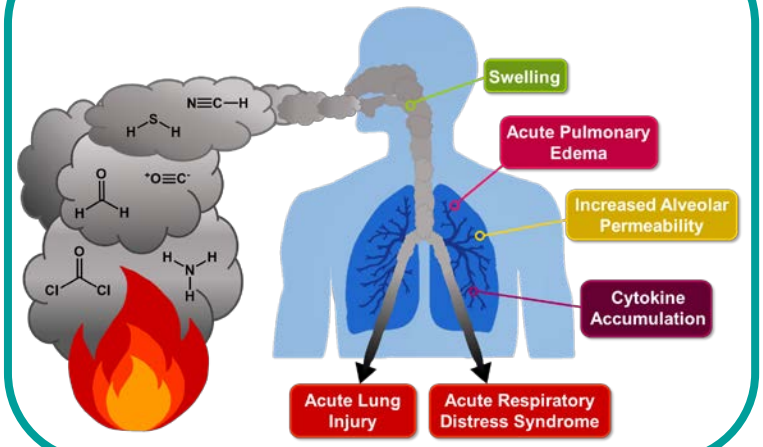
~10%

Applications

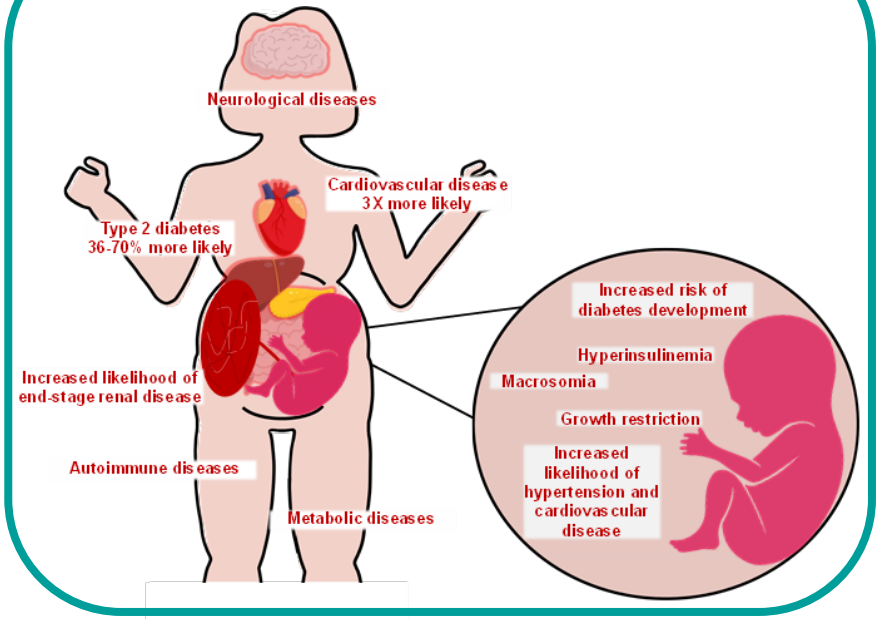
PerFluoroAlkyl Substances (PFAS) exposure



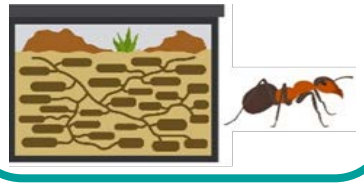
Severe smoke inhalation



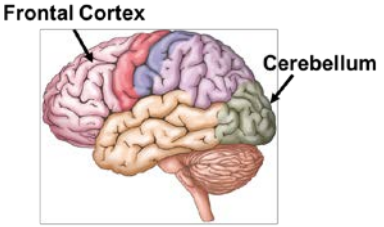
Pregnancy disorders



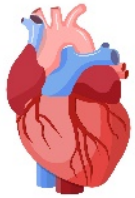
Soil bacteria decomposition



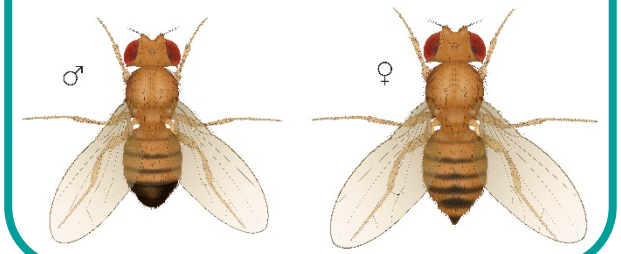
Alzheimer's Disease



Myocardial infarction

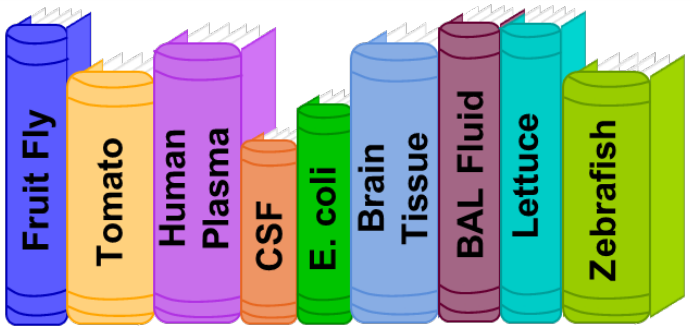


Fruit fly sex differences

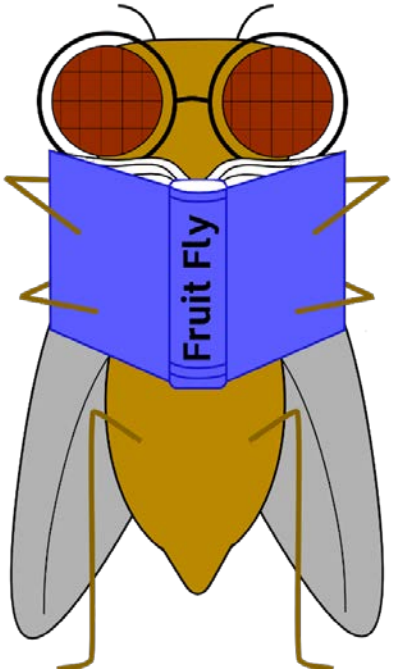


Conclusions

Skyline is the ideal software to develop and share our lipid spectral libraries



- Rapid, targeted data processing
- Support for large target lists with multiple transitions and adducts
- LipidCreator plugin for generating initial target lists
- Support for ion mobility data and retention time prediction
- Simple export, import, editing and sharing of spectral libraries through Panorama
- Helpful visualization and quantitation tools
- Vendor-independent and freely available



Skyline

Acknowledgements

Baker Lab Members

- Prof. Erin Baker
- Dr. James Dodds
- Karen Butler
- Melanie Odenkirk
- Allison Stewart
- MaKayla Foster
- Caitlin Hodges
- Nancy Abdelrahman

@BakerLabNCSU & @KaylieKirkwood

Sources of Funding

METRIC

Agilent

NIH NIEHS

NIH

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

NC STATE UNIVERSITY

NIMBL

And the Skyline team, especially

- Brendan MacLean
- Brian Pratt
- Kaipo Tamura
- Nick Shulman