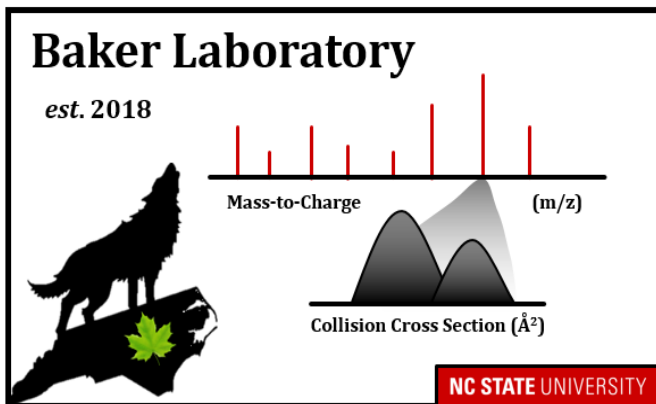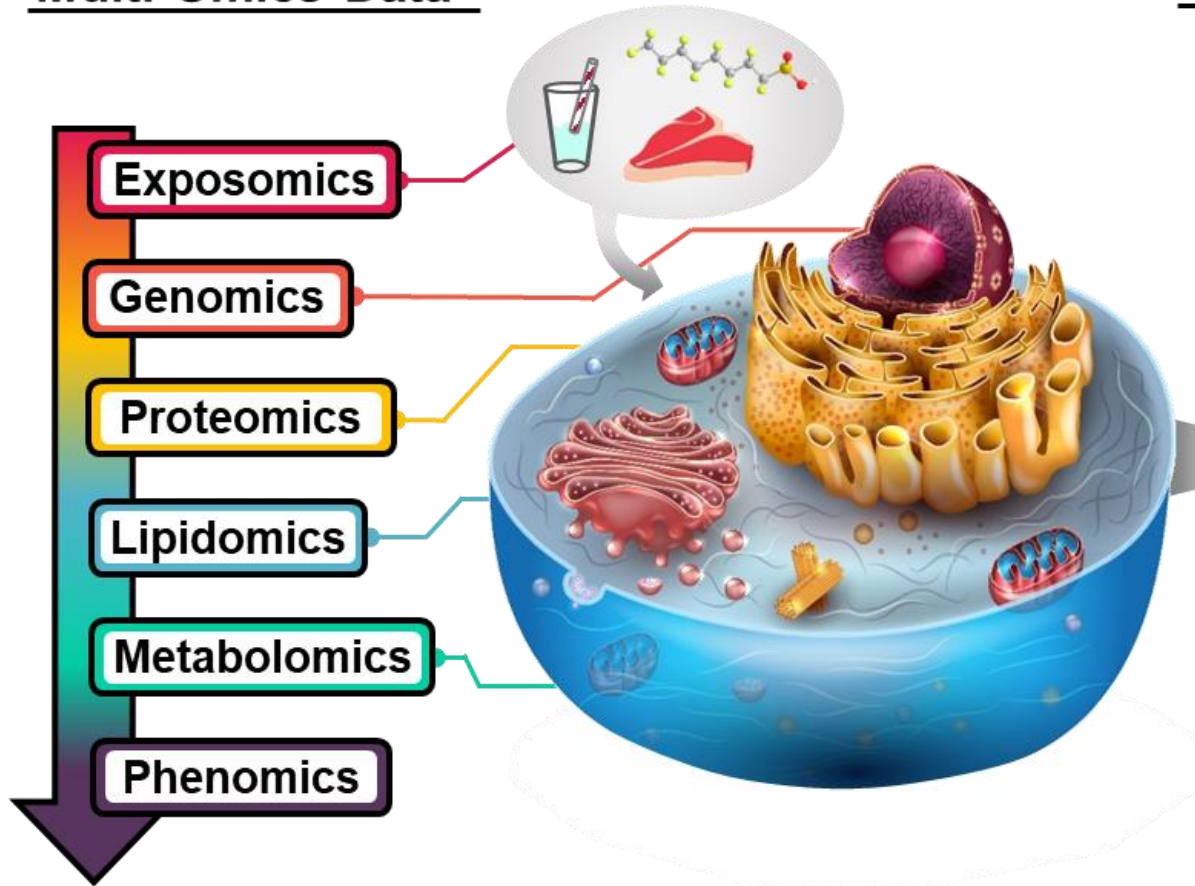# Ion Mobility Spectrometry: The Good, The Bad and The Ugly

Erin S. Baker

Department of Chemistry, NC State University

Multi-Omic Analyses
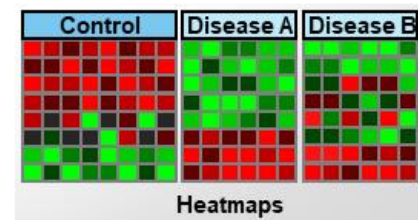
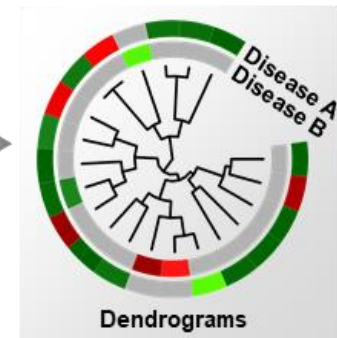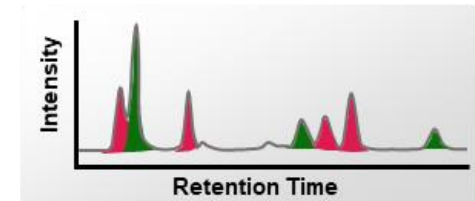- Only ~16% of deaths can be attributed solely to genetics

1) Rappaport, SM "Genetic Factors Are Not the Major Causes of Chronic Diseases" PLoS One 2016.
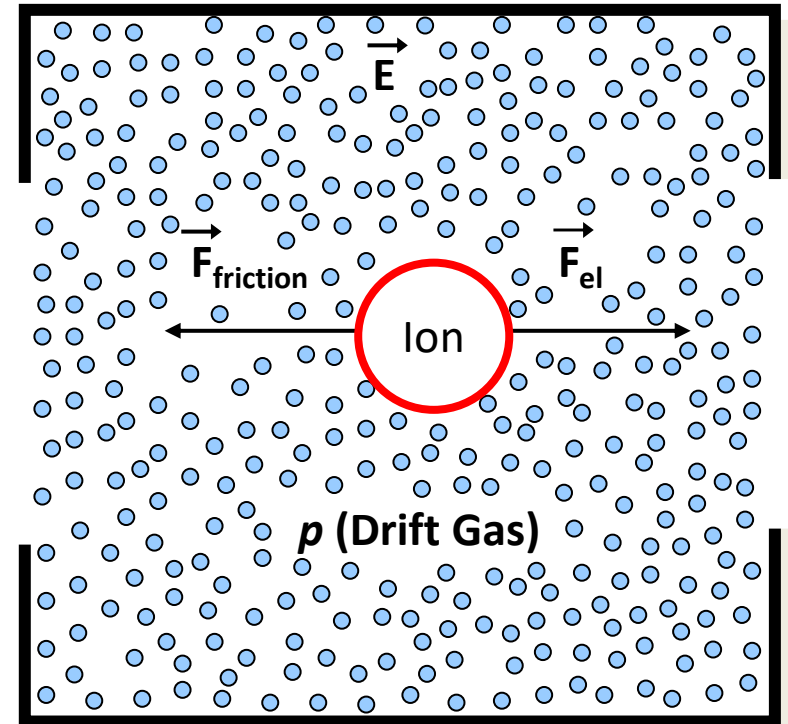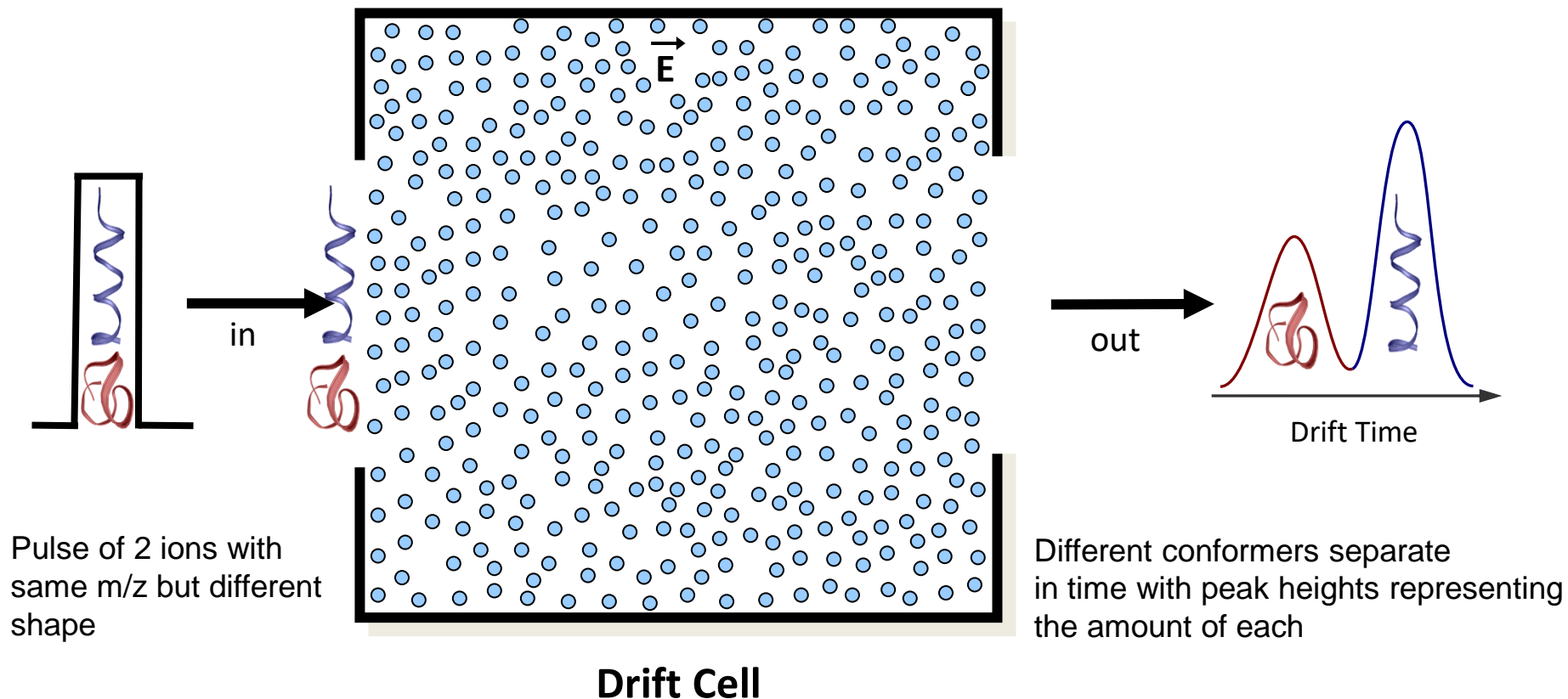
Ion Mobility Concept

- Defines how an ion drifts through a gas under the influence of an electric field

- Separation based on the mass, charge, size & shape

- Variables in diverse IMS methods
  - Electric field
  - Pressure
  - Gas composition
  - Gas flow
 - Temperature

Drift Cell

Ion Mobility Concept

Drift Cell

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

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

Drift Time

E

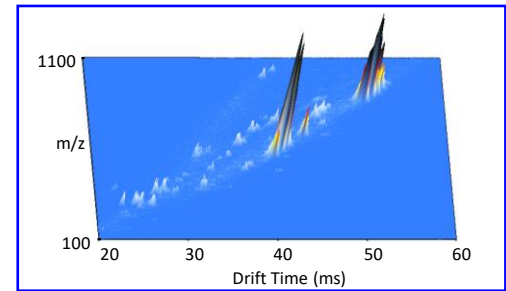in

out

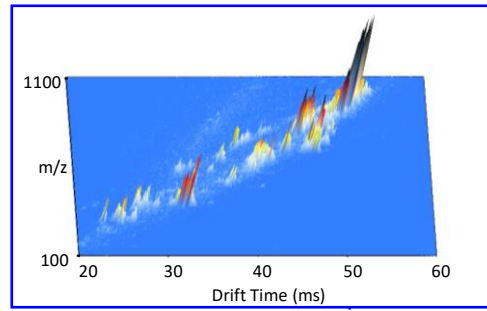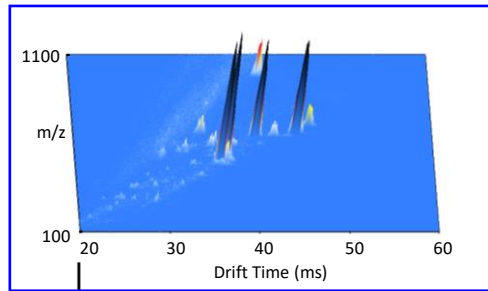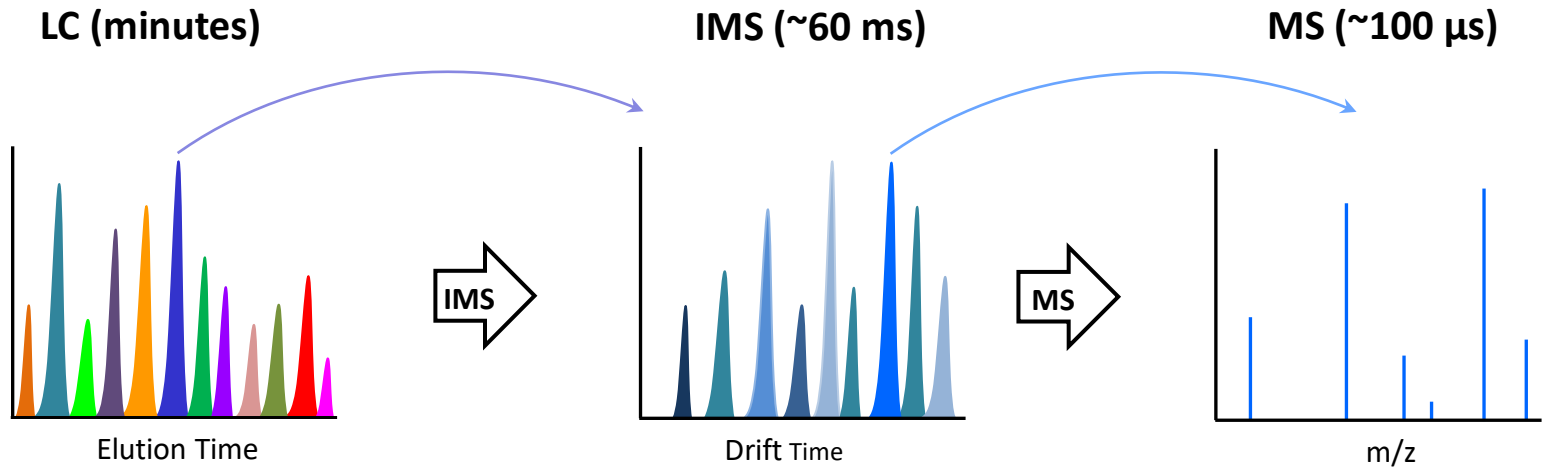**velocity is constant**

v = K E

K = ion mobility

Ion-Neutral Collision Cross Section

1. Area related to the size and shape of an ion

2. Robust physicochemical property

3. Can easily be compared between labs

4. Varies depending on drift gas

Slide courtesy of Professor Kevin Pagel and Waters
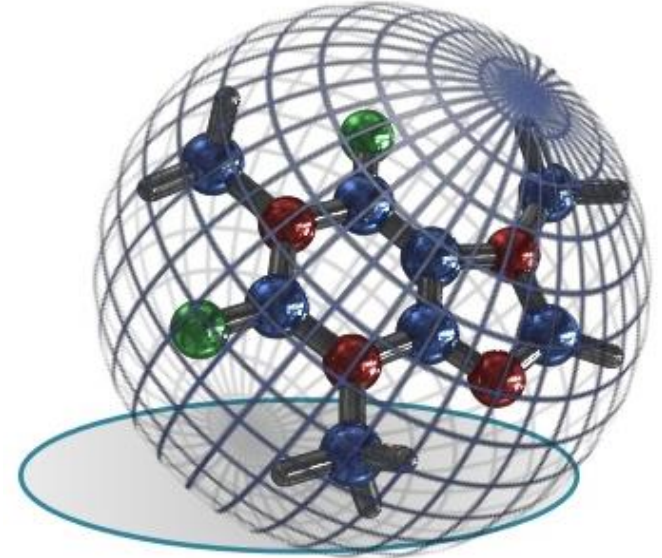
Multi-dimensional Analyses
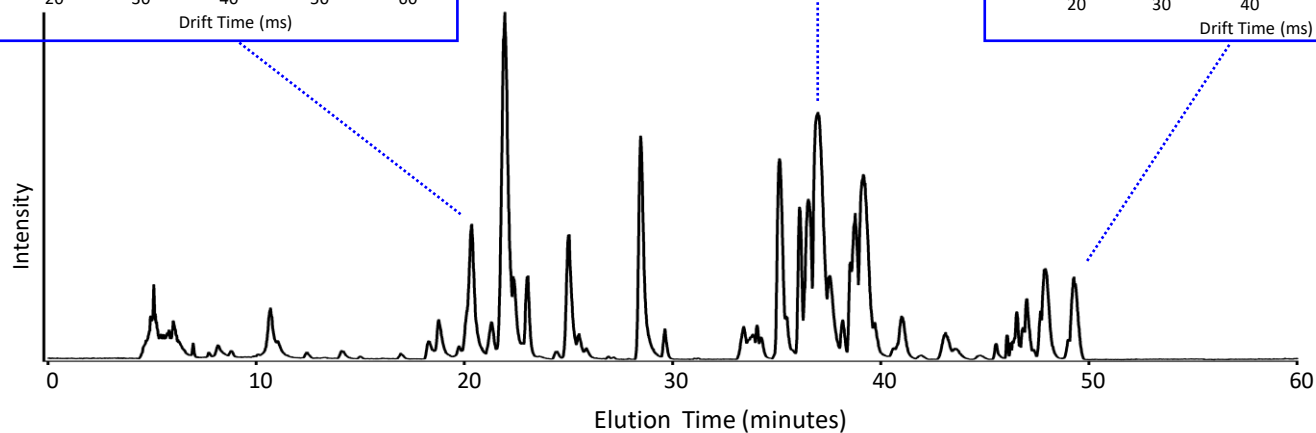
The Good: How Can IMS Help Multi-Omic Studies?

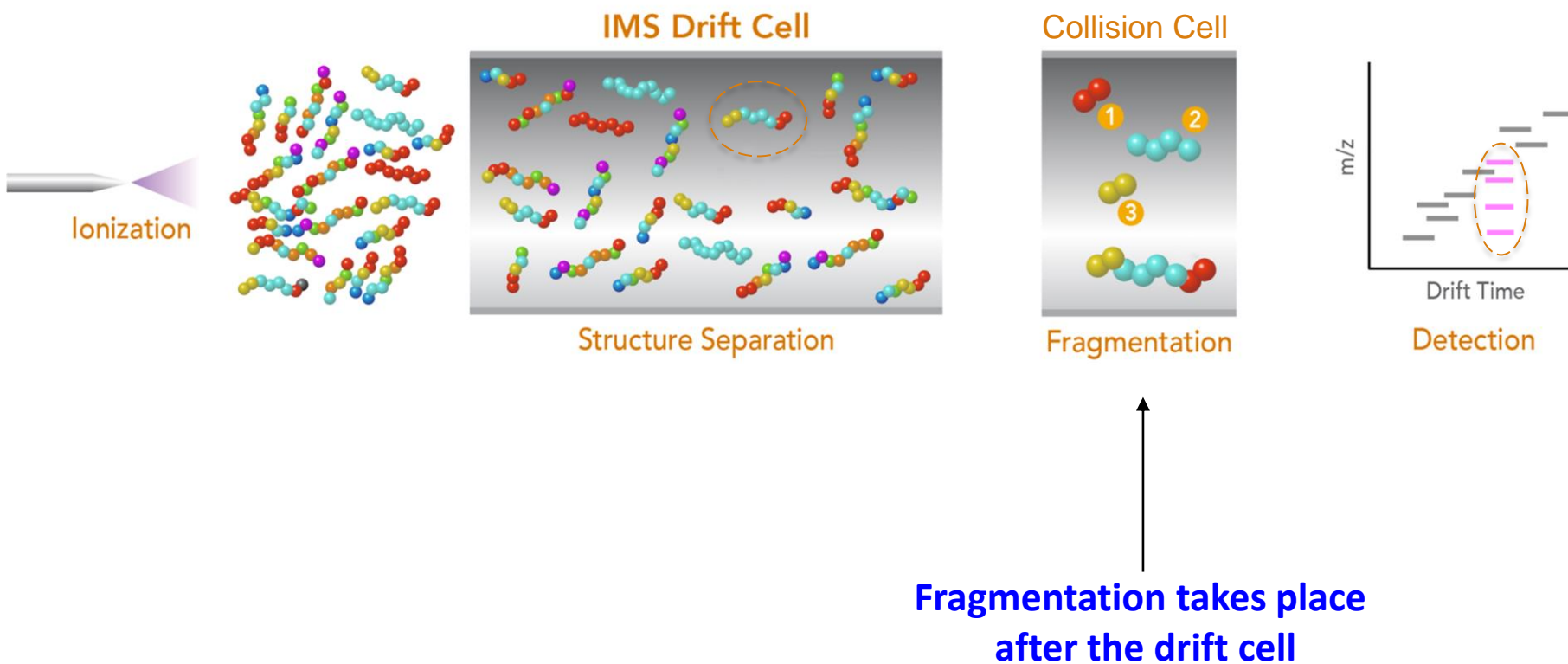1. IMS is fast (ms time scale) and easily coupled with front-end separations & MS

2. Allows evaluation of structural biomarker presence

3. Multi-dimensional analyses (such as LC-IMS-MS) provide more confident identifications

4. CCS values reduce potential molecular candidates in untargeted studies

5. Heavy labeled molecules have the same drift time as their endogenous versions, allowing pair analysis for absolute quantitation
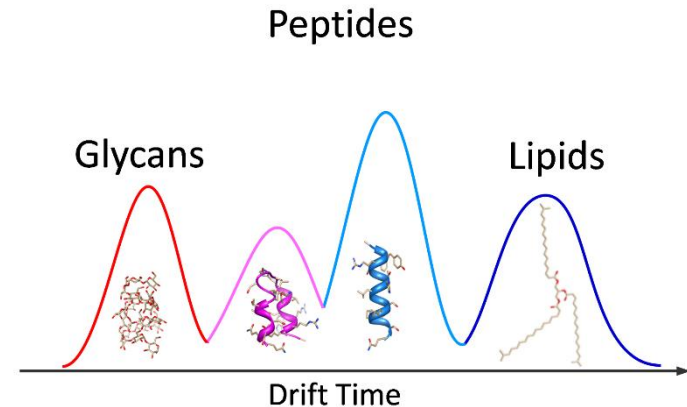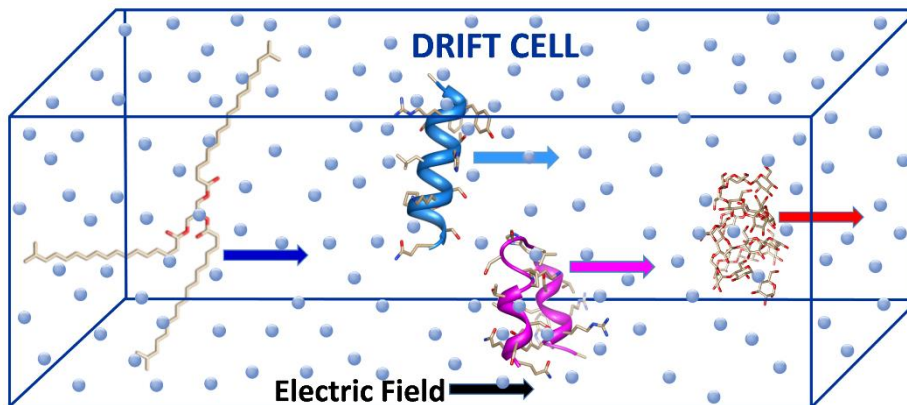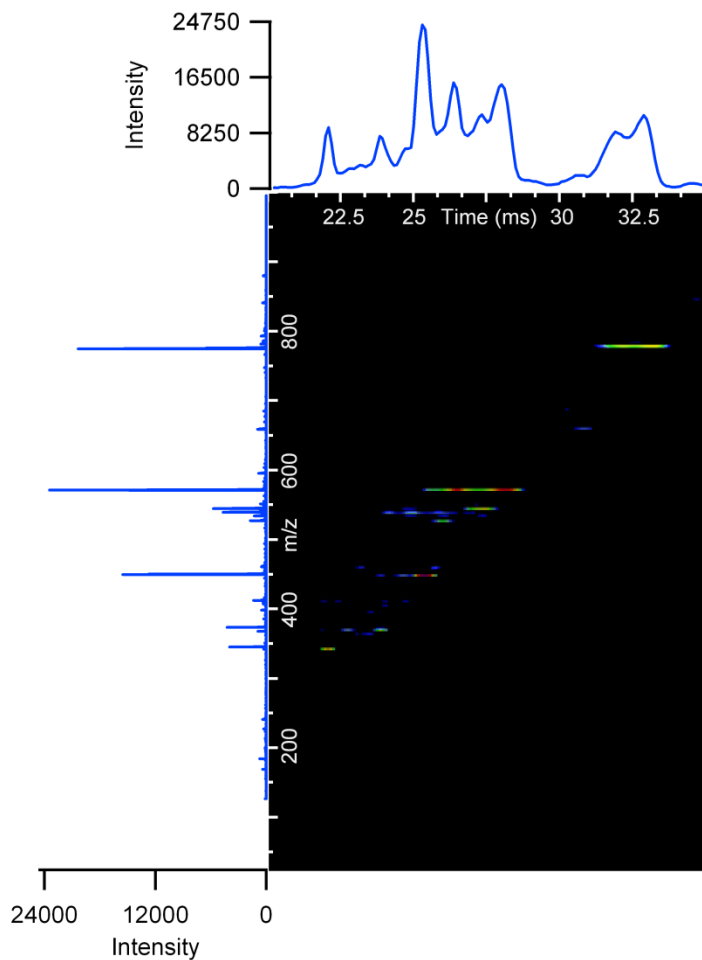
The Bad & The Ugly: Analyzing LC-IMS-(CID)-MS Data

- The Bad: LC-IMS-(CID)-MS data is large in complexity and file size

- The Ugly: Manual assignment is extremely time consuming, can only be done for a few targets and limits our ability to understand IMS benefits

- Need to address 2 questions:

  - Does IMS aid in identifications in DIA studies?

  - Can Skyline be used as a rapid analysis tool to study the effect of the IMS dimension?
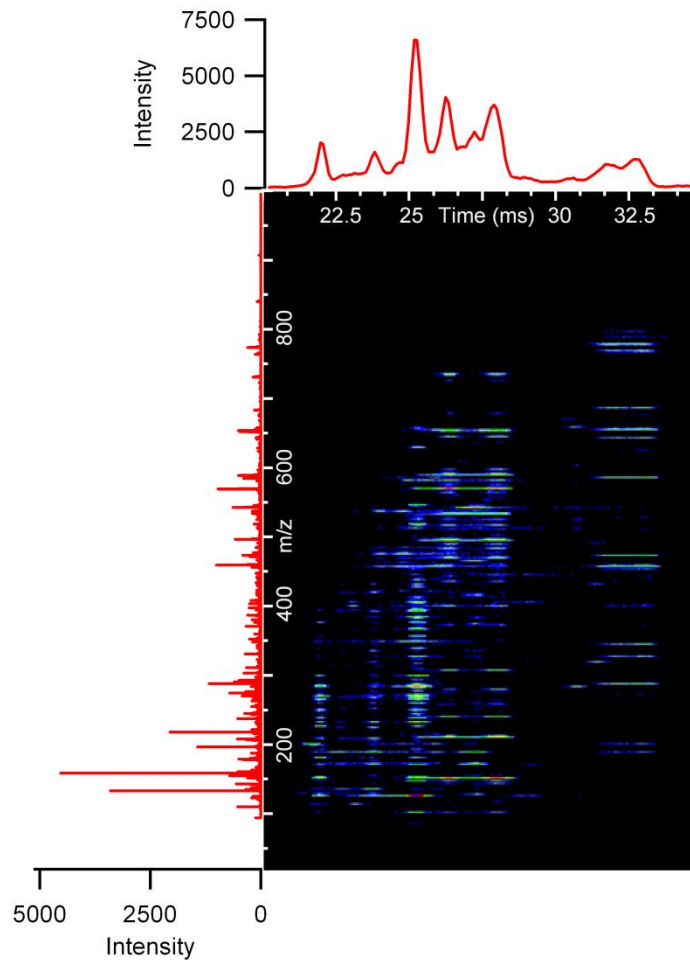
IMS-(CID)-MS Data Acquisition

Ionization

IMS Drift Cell

Structure Separation

Collision Cell

Fragmentation

Detection

m/z

Drift Time

Fragmentation takes place after the drift cell

Drift Time Alignment for Precursors & Fragments

Precursor Spectrum

Fragment Spectrum

LC-IMS-(CID)-MS Experiment

- Spiked 8 different concentrations of tryptic BSA digest (from 100 pM to 1 µM) into 0.1 µg/µL yeast digest

- Analyzed the effect of IMS on MS quantitation & all ions CID quantitation for the DIA data

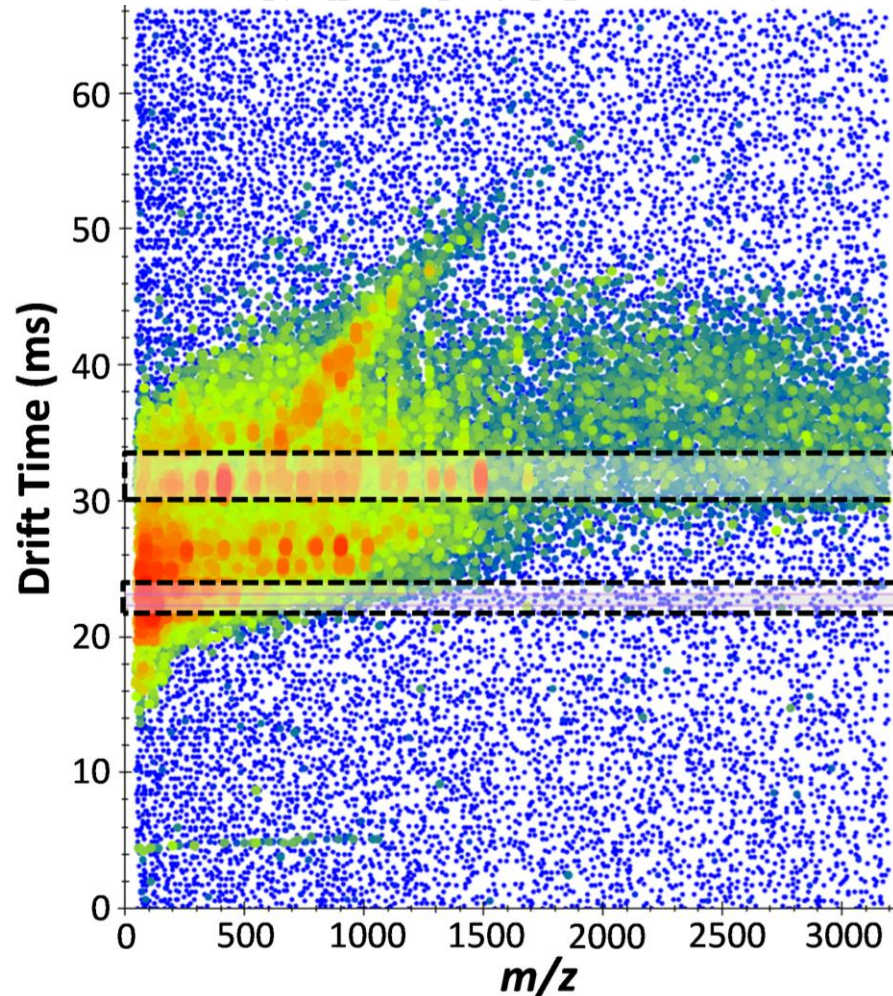|	BSA Concentration in Yeast							
	100 pM	1 nM	5 nM	10 nM	50 nM	100 nM	500 nM	1 µM
LC-MS								
LC-IMS-MS								
All ions fragmentation LC-(CID)-MS								
All ions fragmentation LC-IMS-(CID)-MS								

Alternating Precursor/Fragment Scans

IMS-MS

IMS-(CID)-MS (CE=29V)

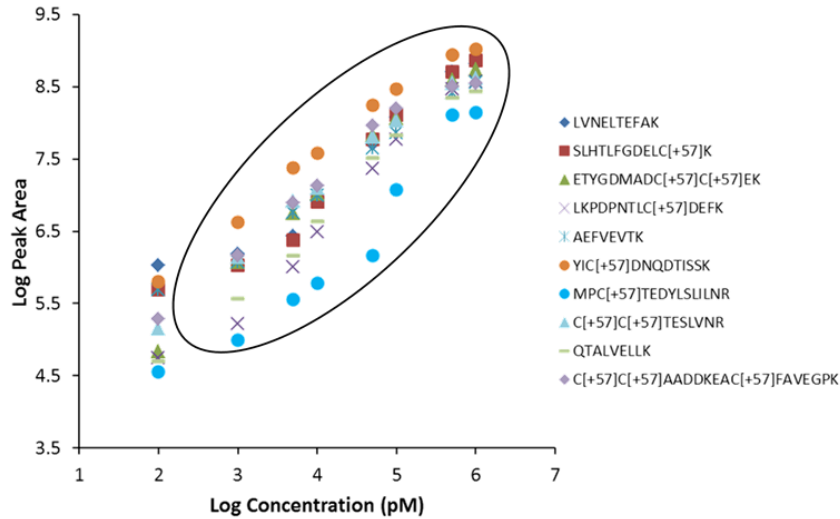Yeast_0pt1ug_BSA_5nM_18May15_Fir_15-04-01.d (17.75 min)
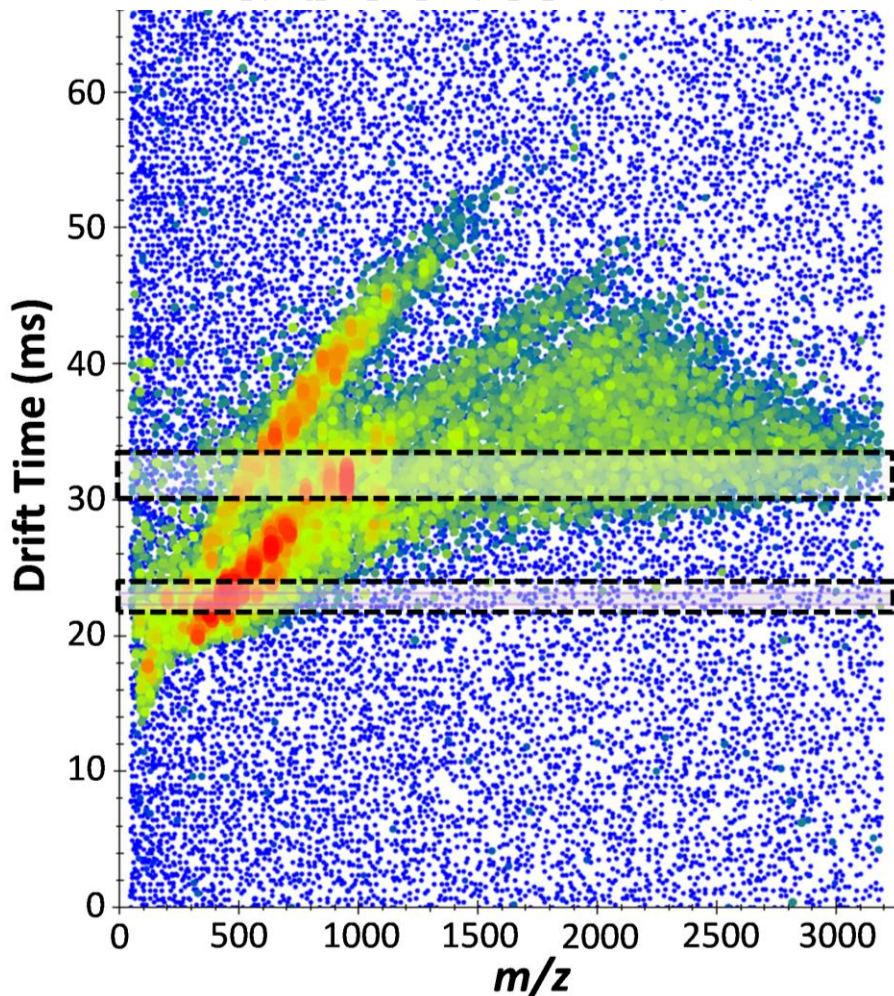
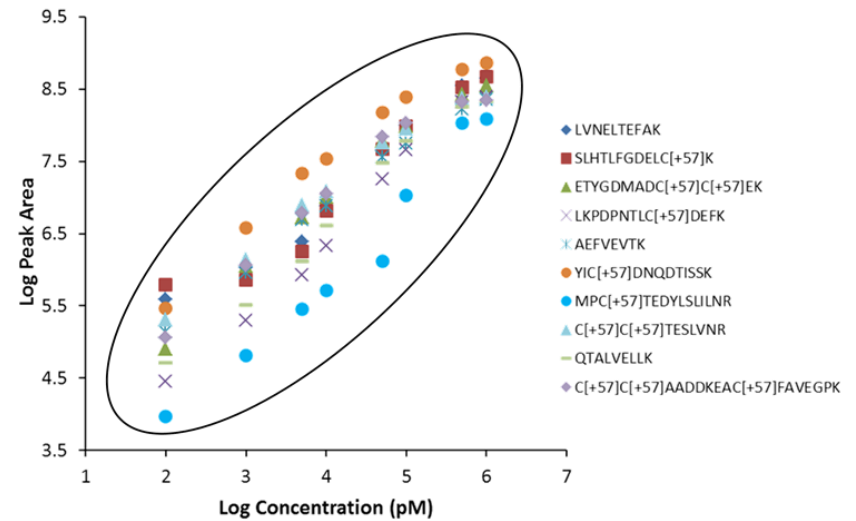Yeast_0pt1ug_BSA_5nM_18May15_Fir_15-04-01.d (17.73 min)

CE of 29 V was chosen since it fragmented 2+ ions well

Drift Time Filtering Effect on MS Quantitation
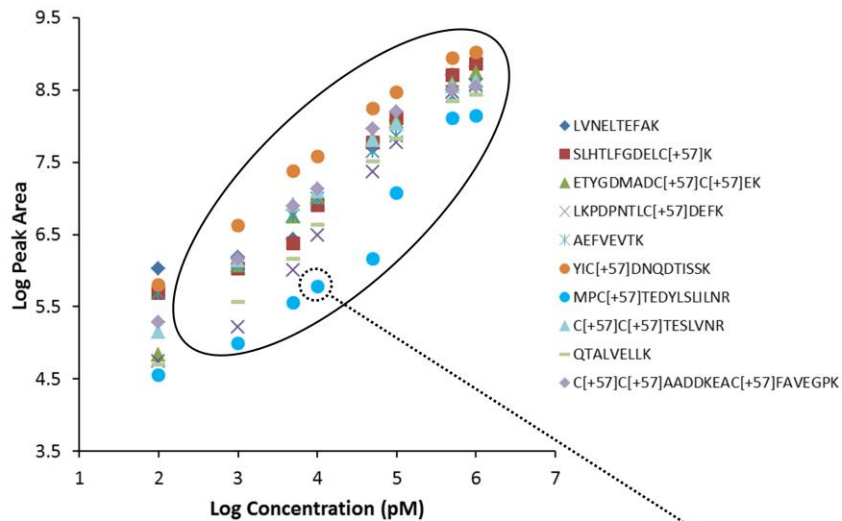
LC-MS Quantitation

LC-IMS-MS Quantitation

Good quantitation from 100 pM to 1 μM for all peptides when drift time filtering was used
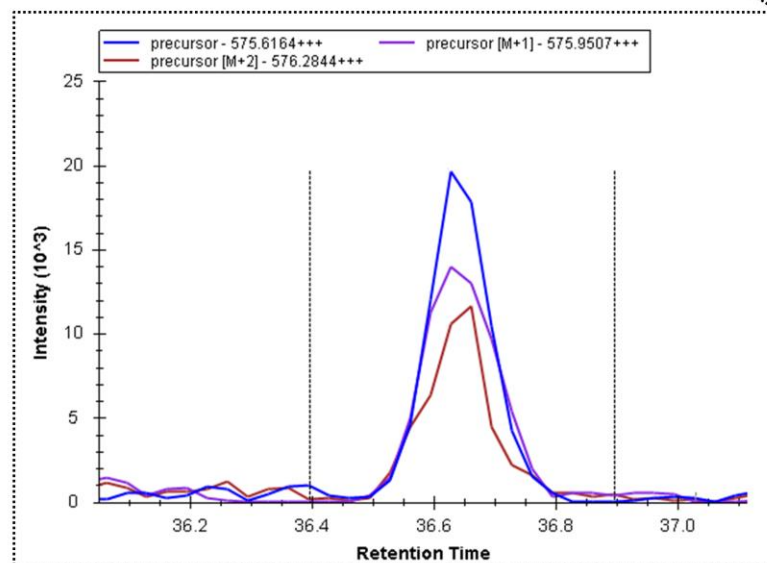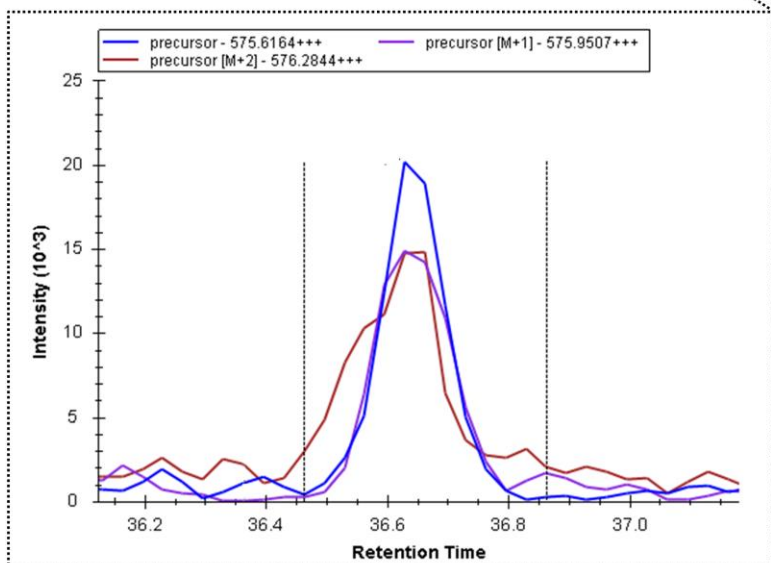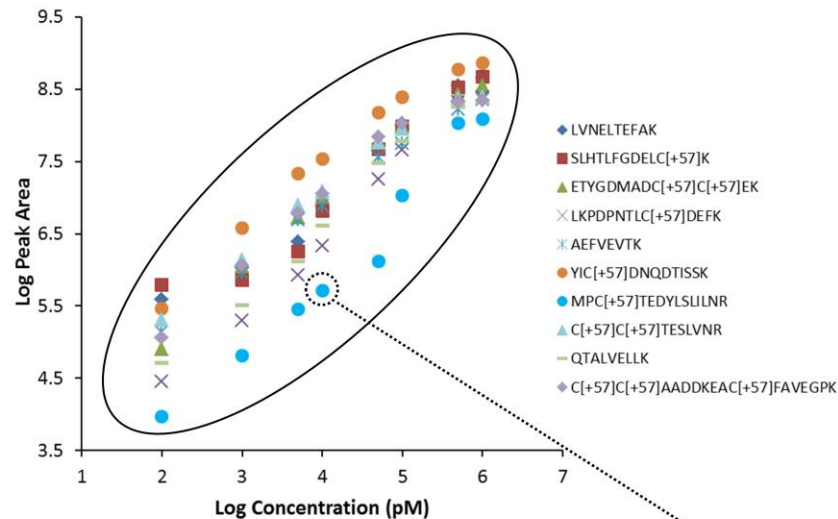
Without drift time filtering, lower concentration peptide quantitation was not as linear

Drift Time Filtering Effect on MS Quantitation
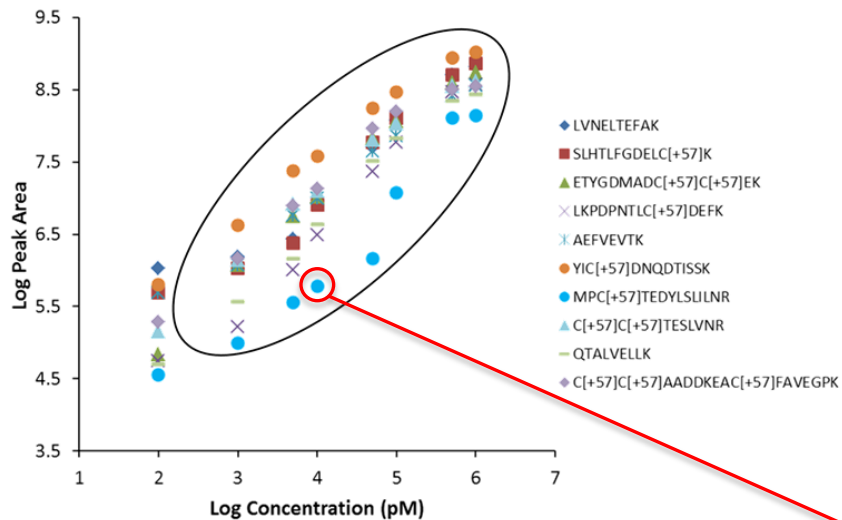

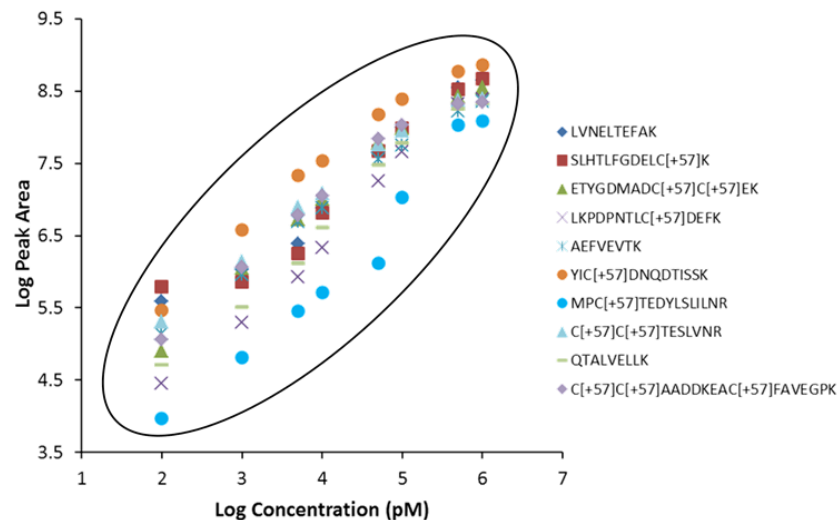**LC-MS Quantitation**

LC-IMS-MS Quantitation
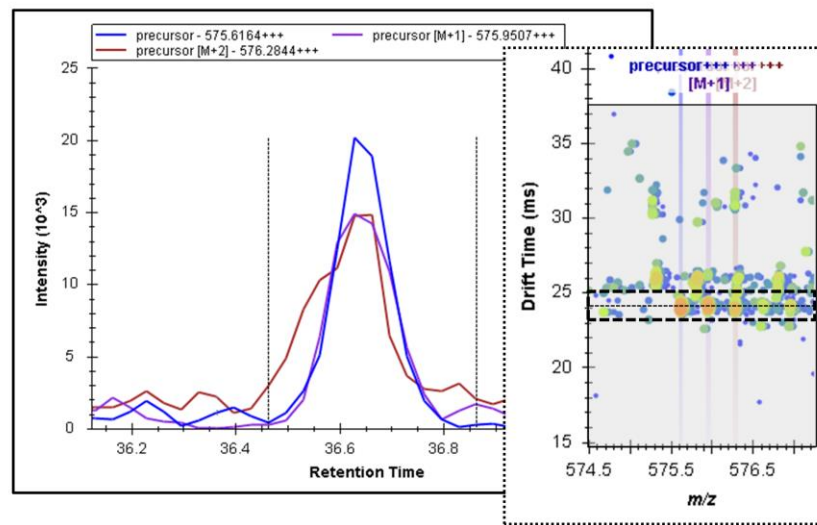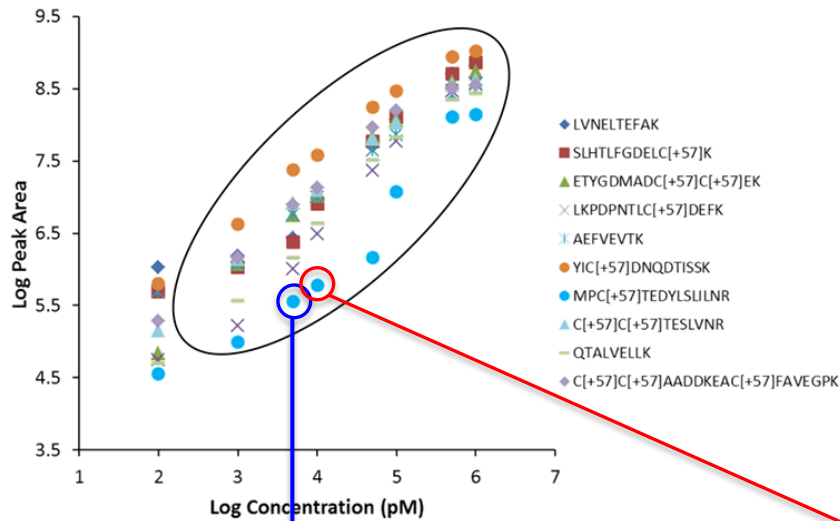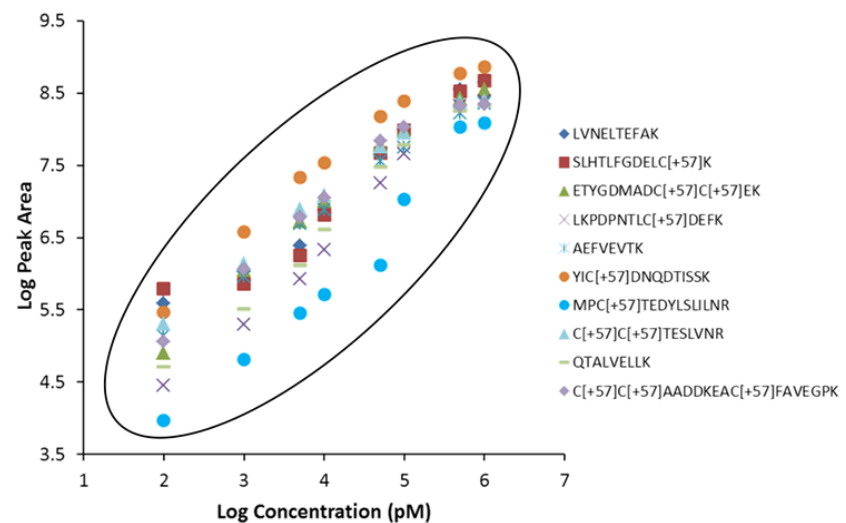
Peak Interferences Without Drift Time Filtering

LC-MS Quantitation

LC-IMS-MS Quantitation

Legend:
- LVNELTEFAK
- SLHTLFGDELC[+57]K
- ETYGDMADC[+57]C[+57]EK
- LKPDPNTLC[+57]DEFK
- AEFVEVTK
- YIC[+57]DNQDTISSK
- MPC[+57]TEDYLSLILNR
- C[+57]C[+57]TESLVNR
- QTALVELLK
- C[+57]C[+57]AADDKEAC[+57]FAVEGPK

10 nM Spiking

precursor - 575.6164+++   precursor [M+1] - 575.9507+++
precursor [M+2] - 576.2844+++
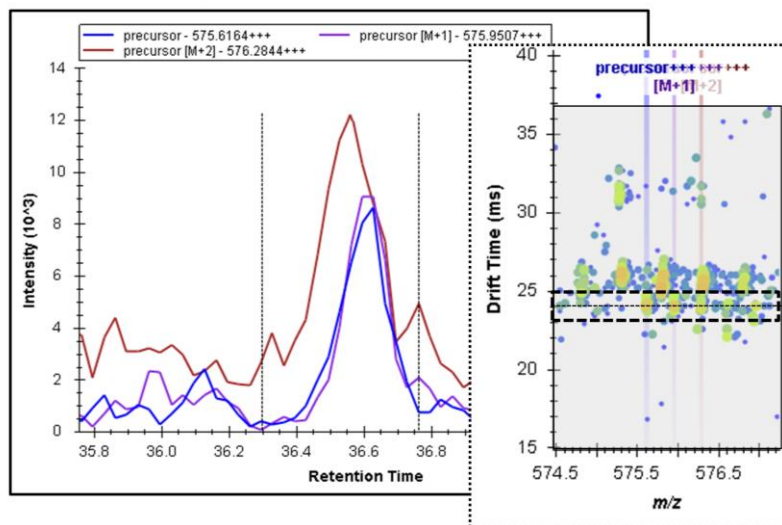
Peak Interferences Without Drift Time Filtering
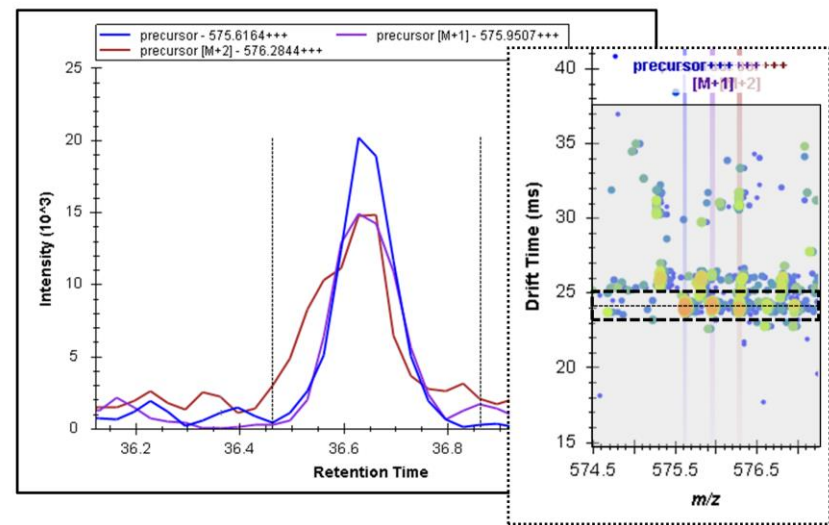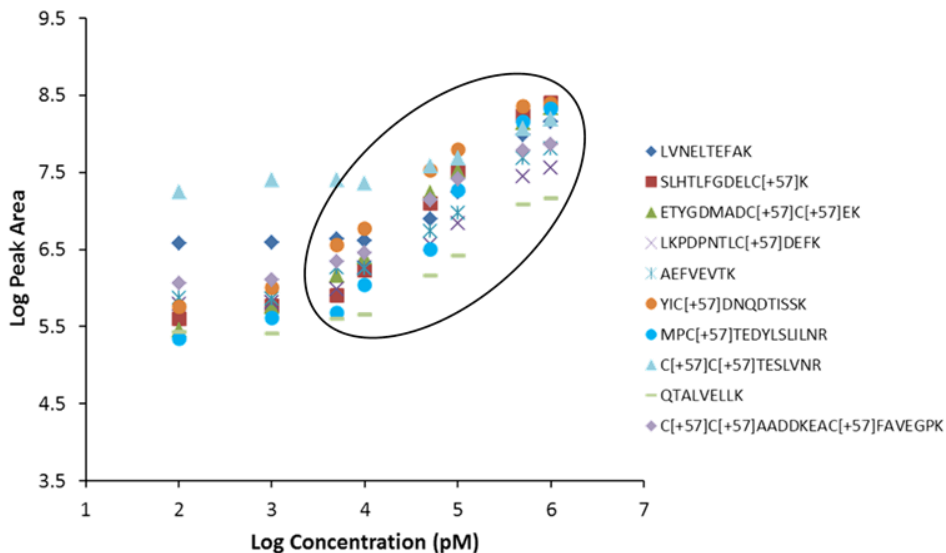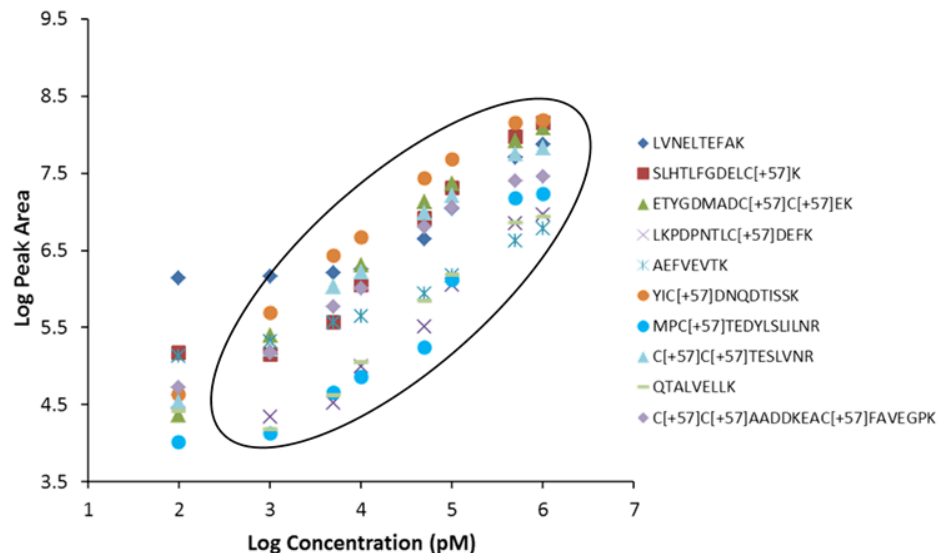
Drift Time Filtering Effect on Fragment Quantitation

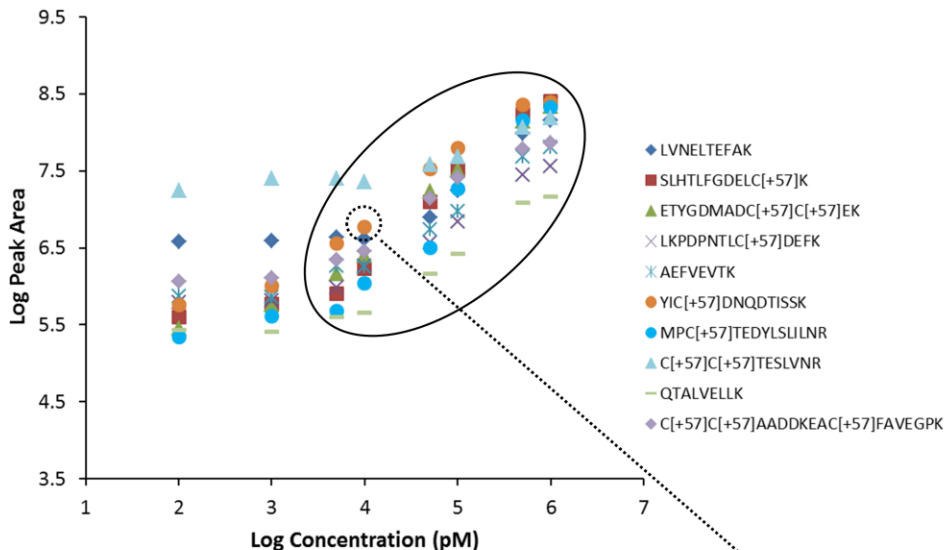**LC-(CID)-MS Quantitation**

LC-IMS-(CID)-MS Quantitation

Good quantitation from 1 nM to 1 µM for 8 of 10 peptides with drift time filtering and 10 for 10 from 10 nM to 1 µM
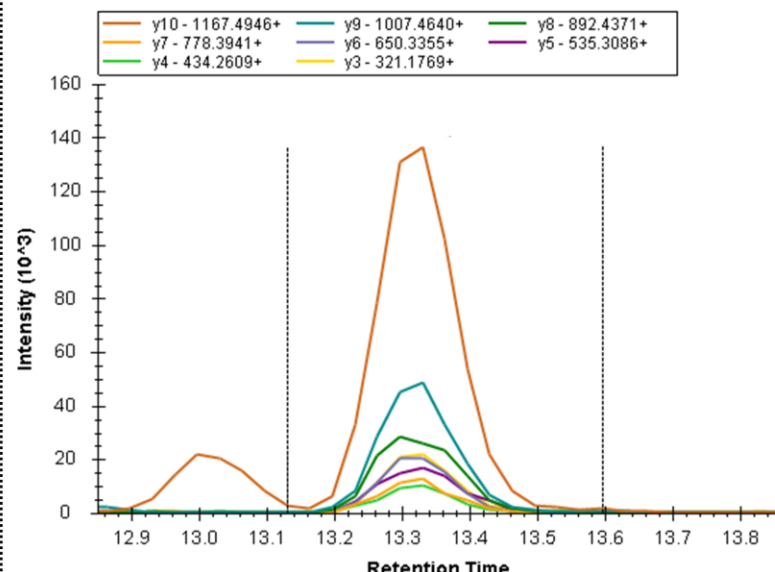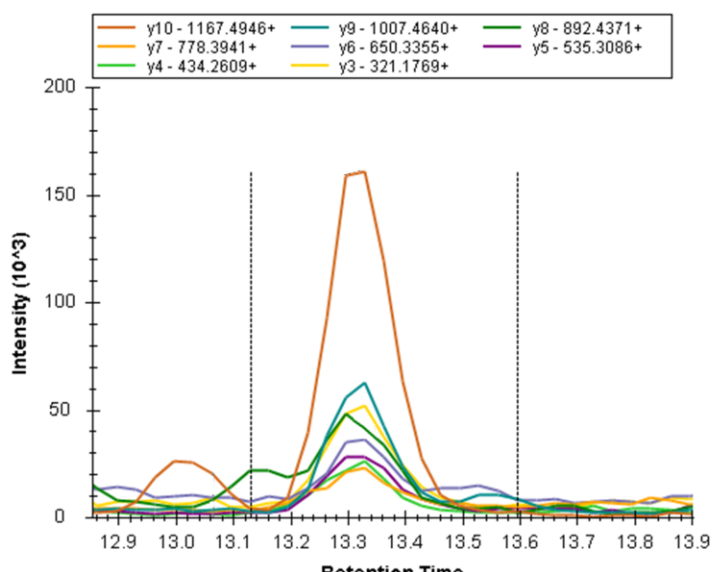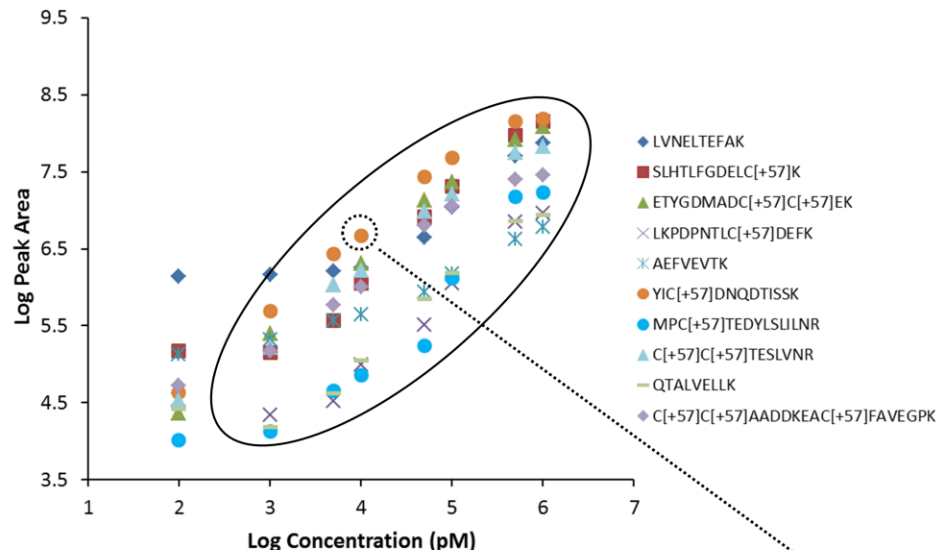
When drift time filtering was not used, many interferences caused bad quantitation at low concentration levels

Drift Time Filtering Removes Fragment Interferences
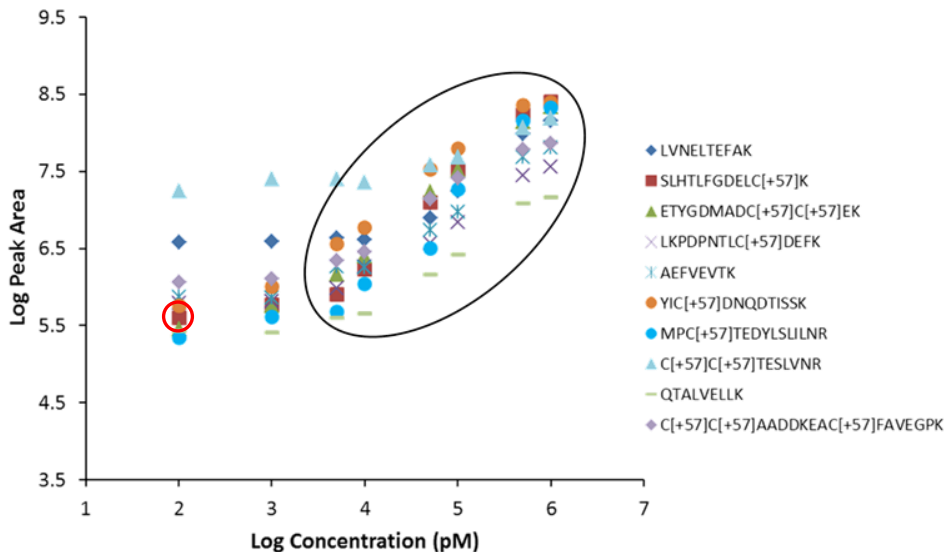
LC-(CID)-MS Quantitation

LC-IMS-(CID)-MS Quantitation

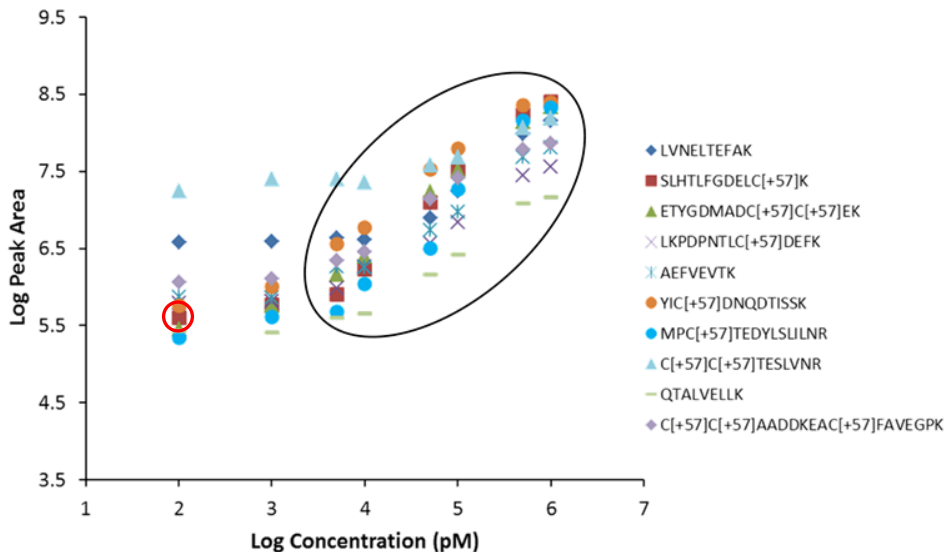# Drift Time Filtering Removes Fragment Interferences

Drift Time Filtering Removes Fragment Interferences
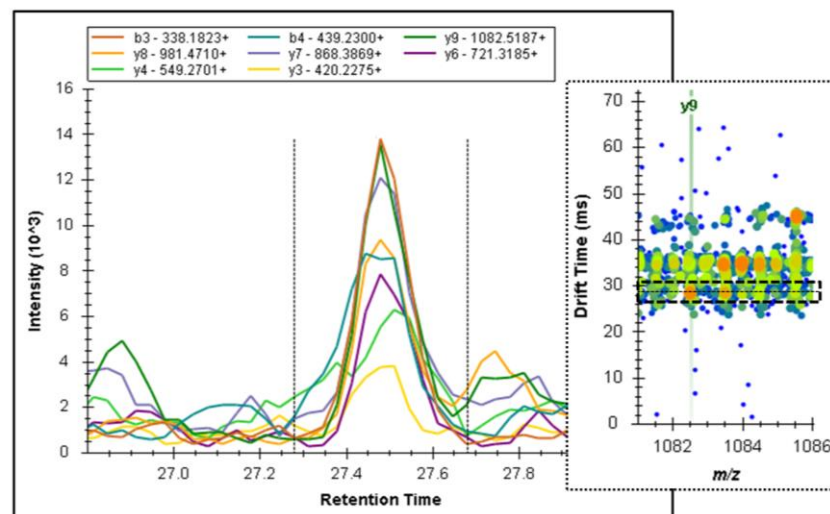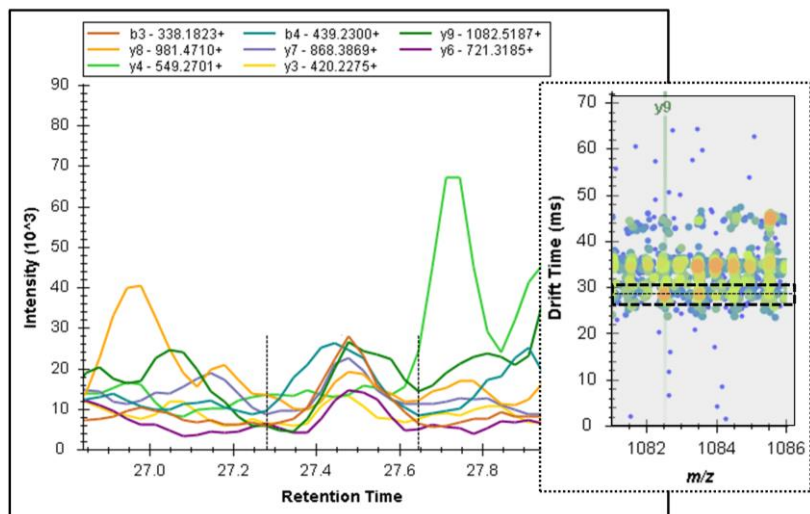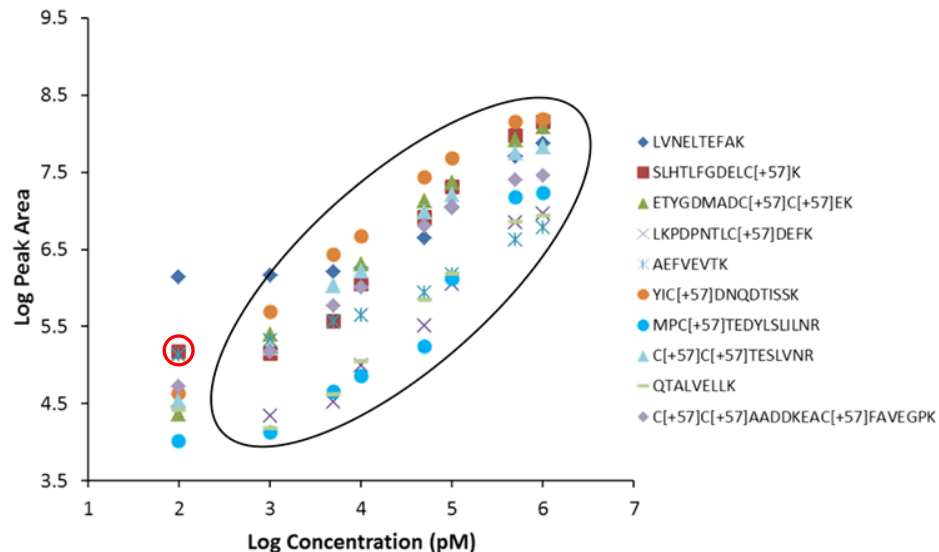
Summary

Skyline:

- Quickly analyzed the LC-IMS-(CID)-MS data

- Showed that IMS removed interfering peaks in LC-MS and fragmentation data

- Enabling further studies to better understand the effect of IMS on numerous analysis types (e.g. other omics)

Skyline Lipidomics Workflow

Kaylie Kirkwood

Basic

Import Target List	Import Data Files	Analyze & Visualize	Export Report
Name, m/z, charge	Vendor-neutral	View filtered data, confirm IDs, compare peak areas and retention times	All target list information + retention times, peak areas, mass errors, etc.

Skyline

Advanced

Import Target List	Import Data FIles	Normalized RT Calculator	Analyze & Visualize	Quantify Lipids	Export Report
Basic + formulas, adducts, CCS, RT, fragments	Basic	Predict RTs based on linear or Lowess regressions	Basic + compare to spectral library, calculate CCS	Generate calibration curve from SIL standards	Basic + concentrations, normalized retention times

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- Allison Stewart
- Kaylie Kirkwood
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- Caitlin Hodges
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Nat Brace
Mike MacCoss

Texas A&M U
Ivan Rusyn

METRIC