

Utilizing Skyline to Rapidly Assign and Interpret IMS-(CID)-MS Data

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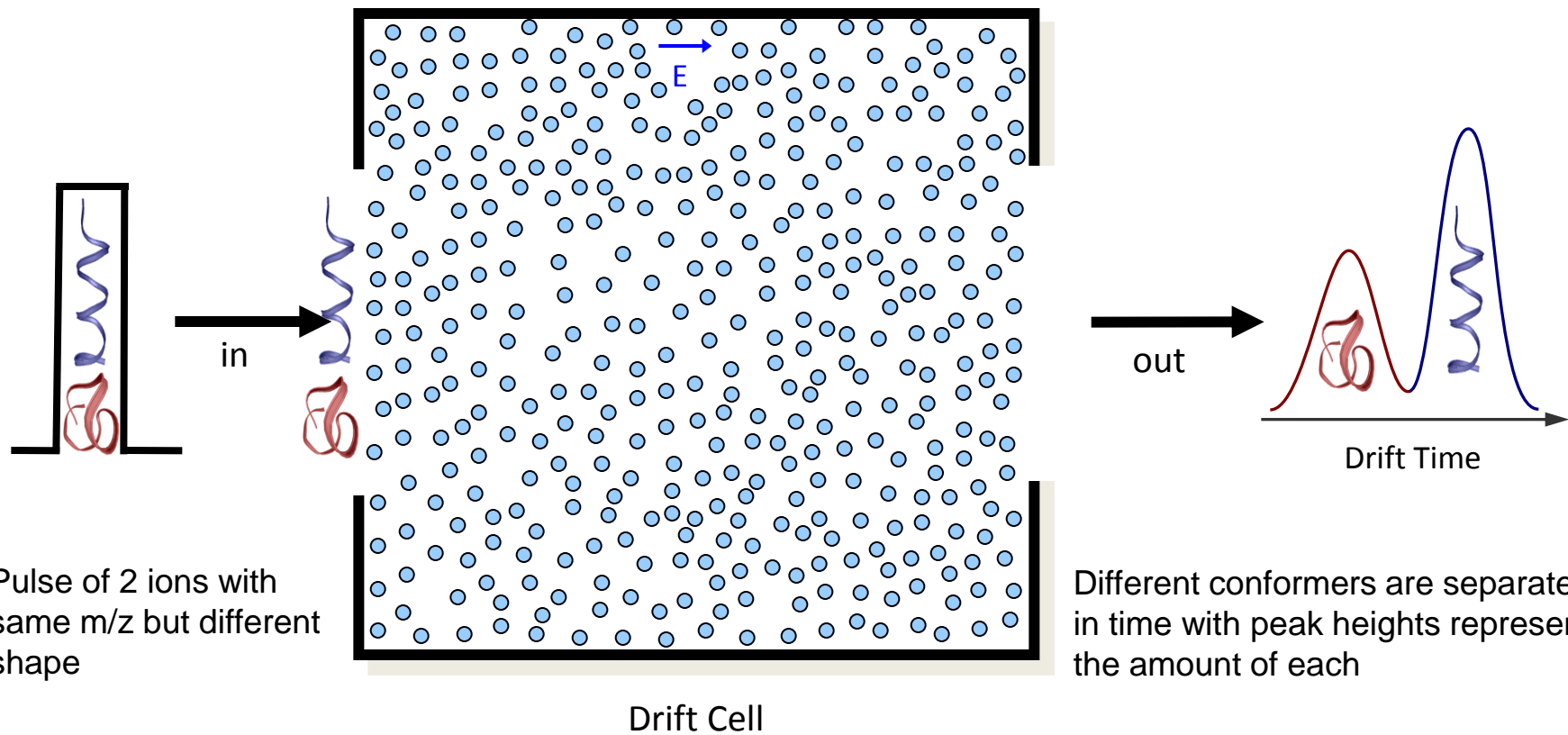
Pacific Northwest National Laboratory



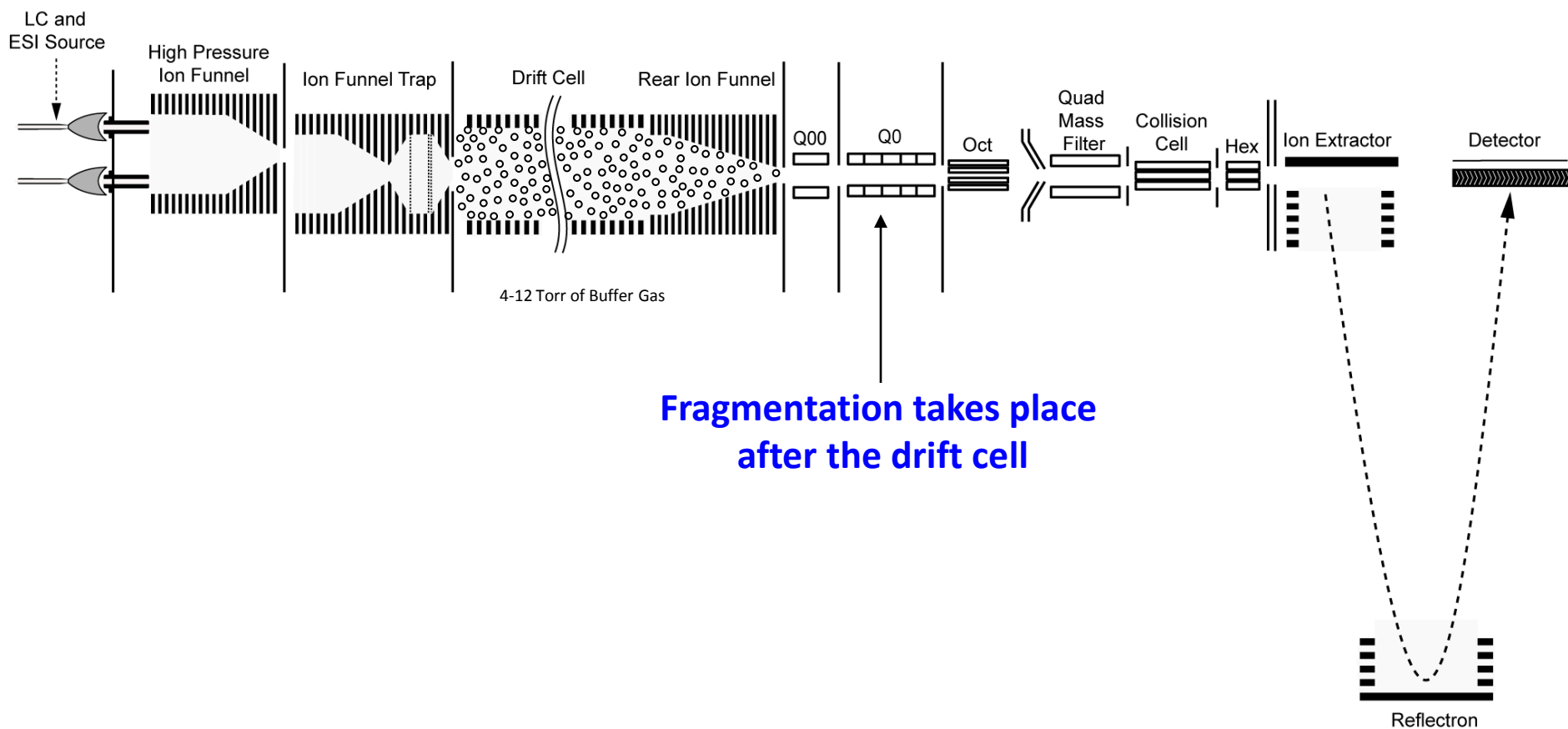
Why do we want to use Skyline to analyze our IMS-(CID)-MS data?

- Currently, all our IMS-(CID)-MS data is manually assigned which is very time consuming and limits our understanding of its benefits
- We need to understand the effect of the IMS separation on DIA identifications
- Skyline provides a rapid analysis tool to study MS and MS/MS quantitation with and without the IMS dimension

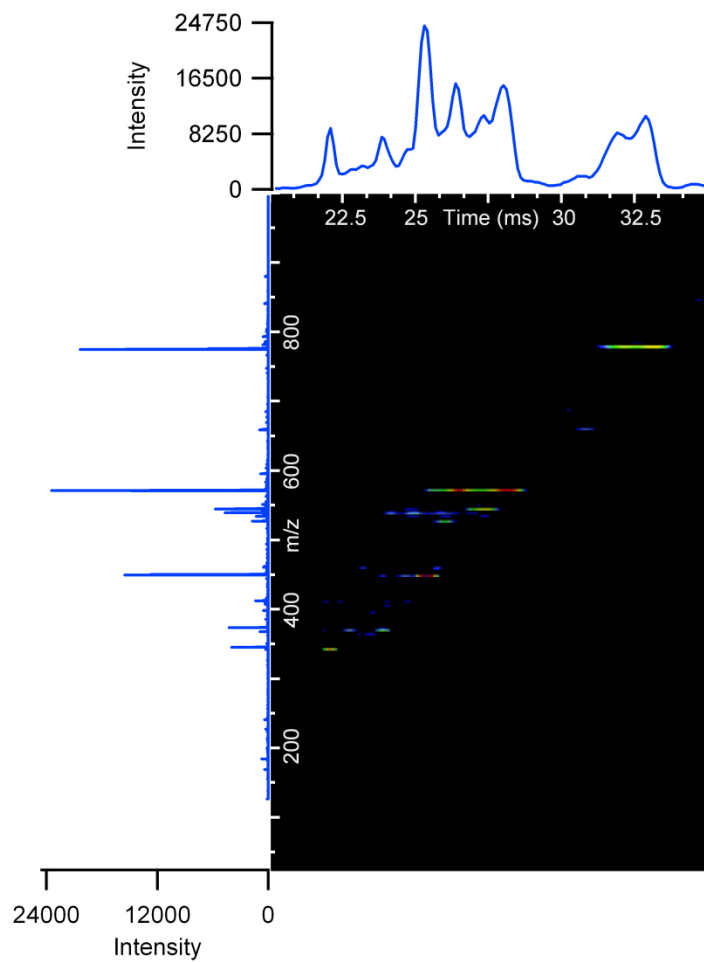
Ion mobility concept



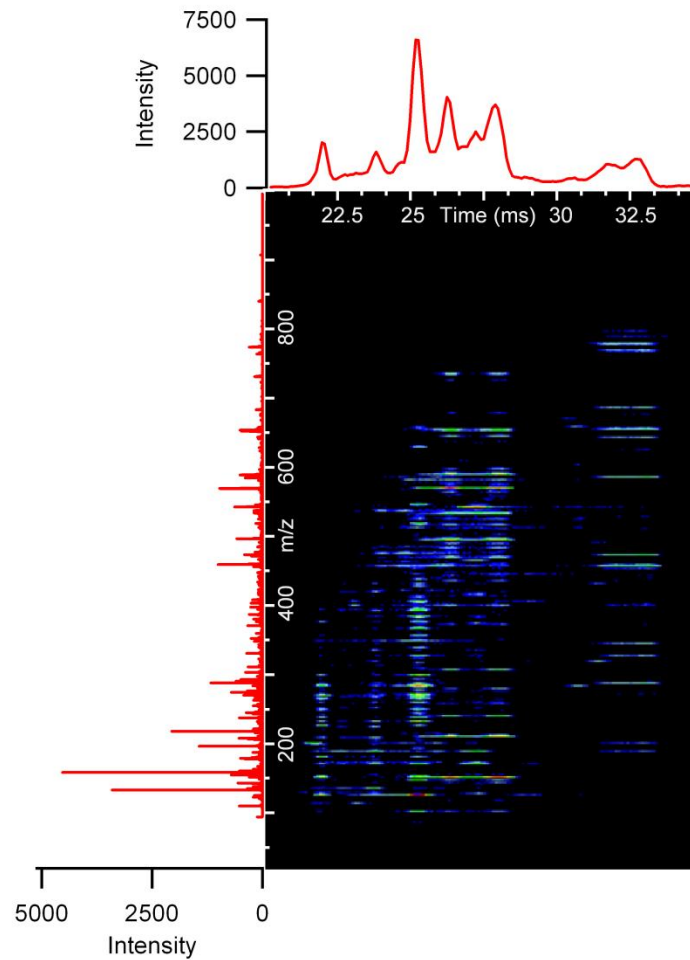
IMS-(CID)-MS data acquisition



Fragments have the same drift time as precursors

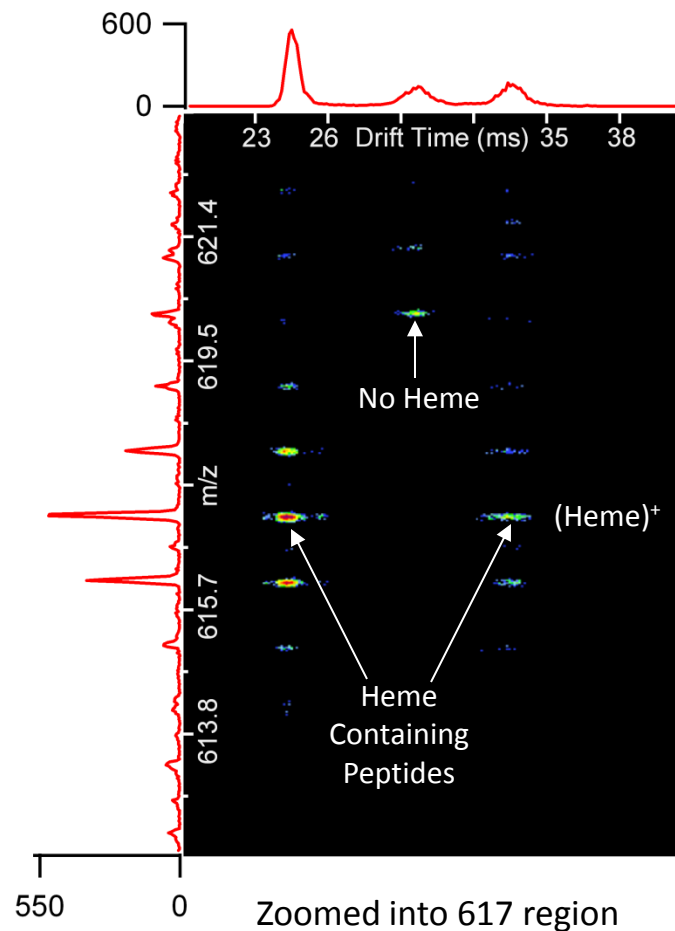
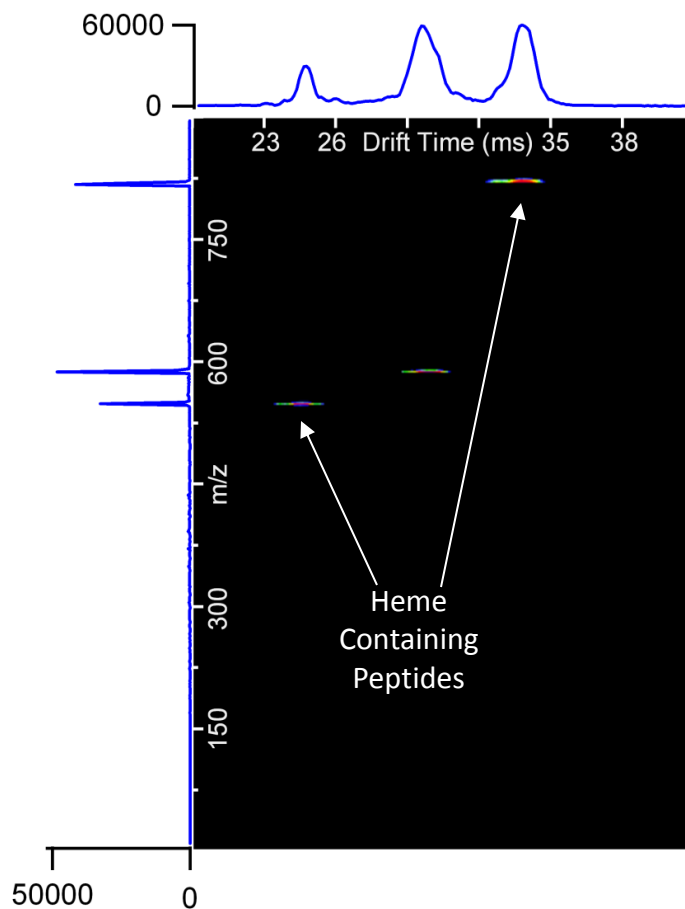


Parent Spectrum



Fragmentation Spectrum

Due to time consuming manual analyses, until now we have only studied specific fragments

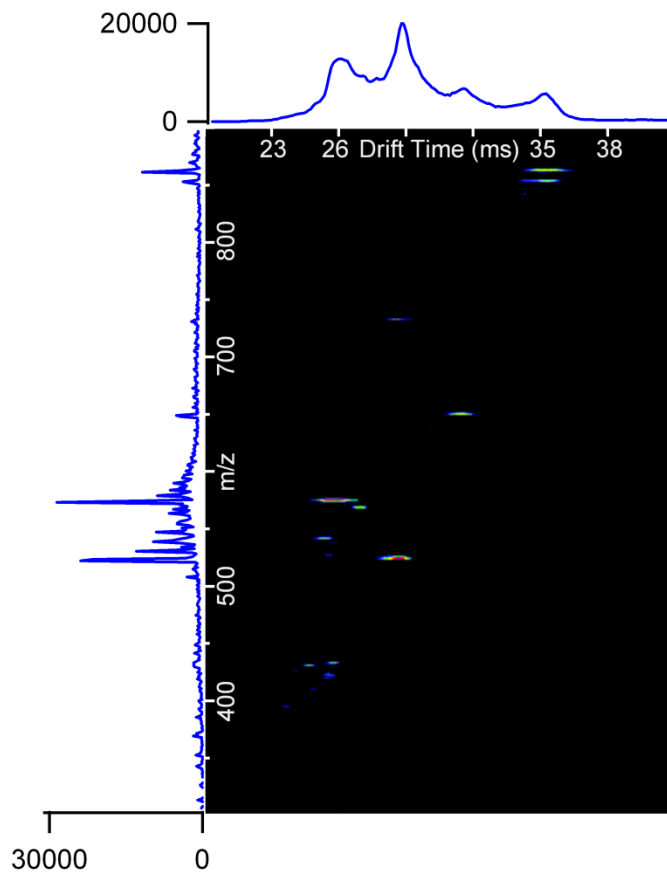


Parent Spectrum (3 Peptides Observed in 1 sec LC acquisition)
2 hemepeptides identified

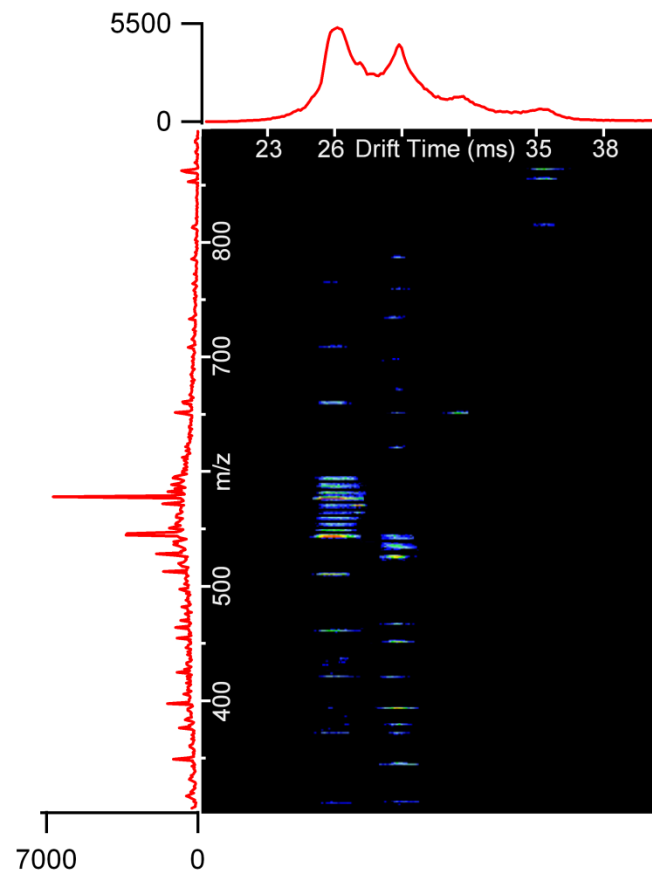
Fragmentation Spectrum

Find multiple hemepeptides simultaneously by looking for heme⁺ ion at 617

Due to time consuming manual analyses, until now we have only studied specific fragments



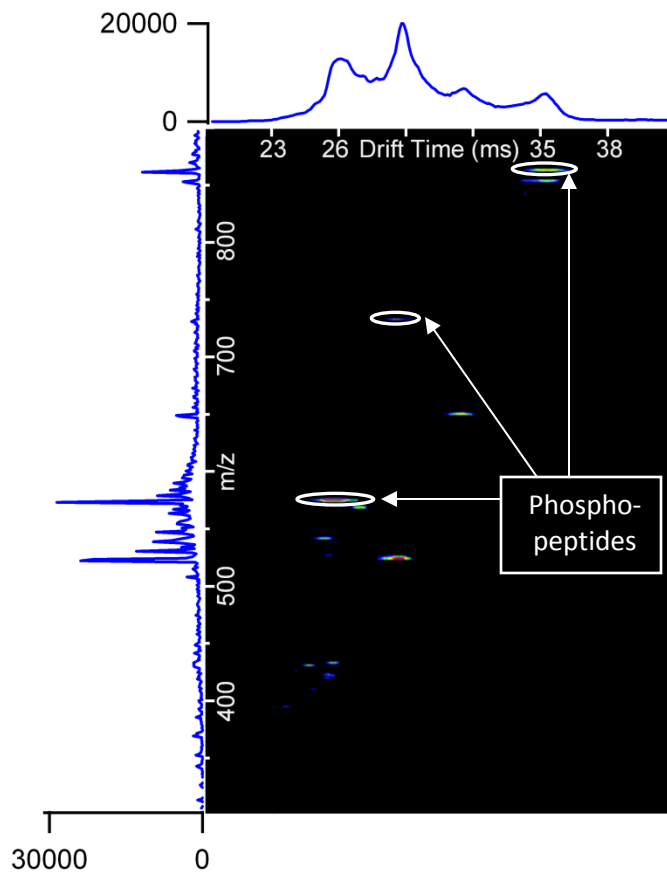
Parent Spectrum (11 Peptides Observed in 1 sec LC acquisition)



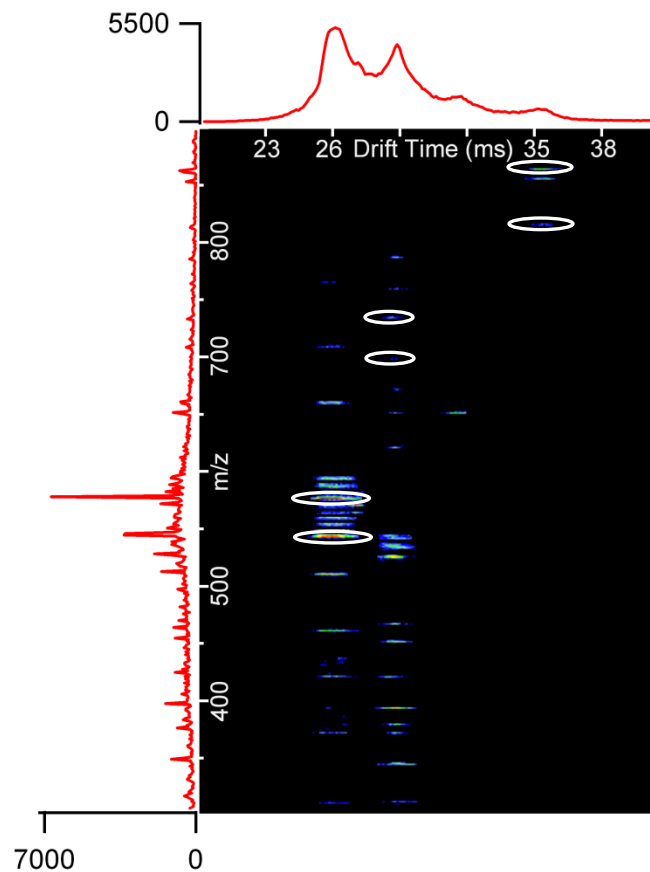
Fragmentation Spectrum

Find multiple phosphopeptides by looking for PO_3 or PO_4 mass difference

Due to time consuming manual analyses, until now we have only studied specific fragments



Parent Spectrum (11 Peptides Observed in 1 sec LC acquisition)
3 Phosphopeptides Identified

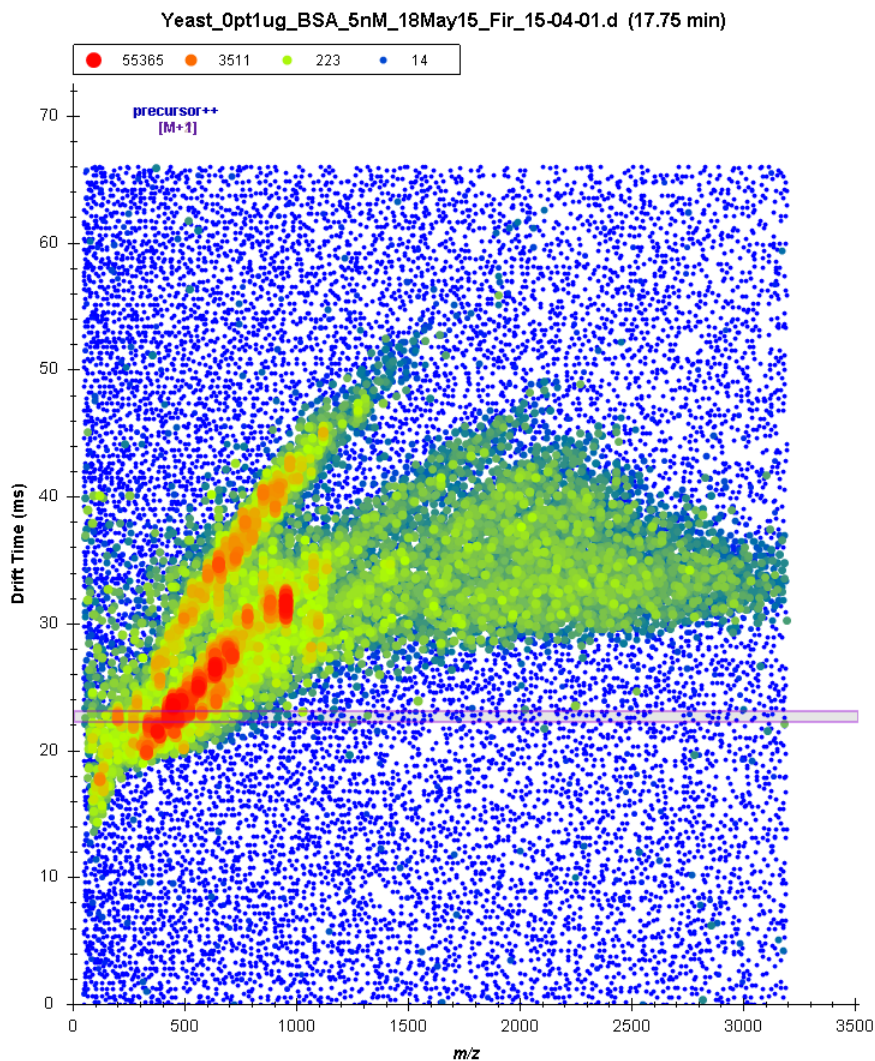


Fragmentation Spectrum

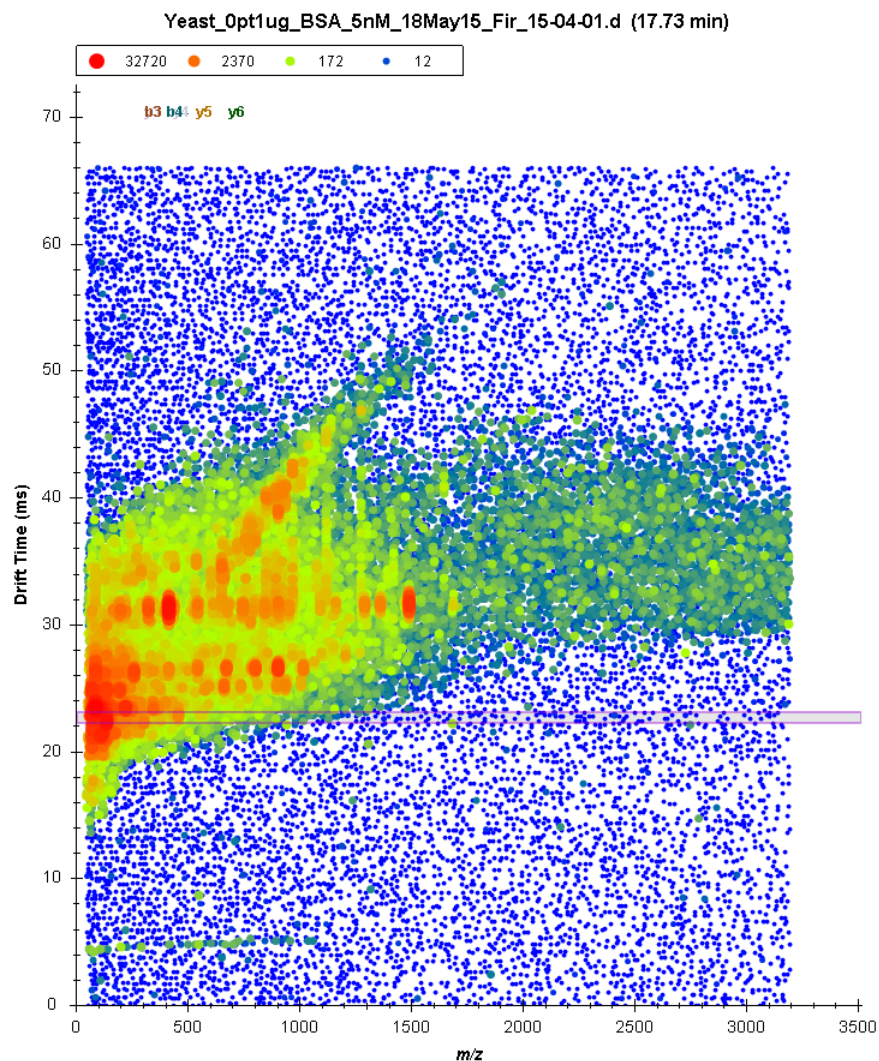
Find multiple phosphopeptides by looking for PO_3 or PO_4 mass difference

IMS-MS and IMS-(CID)-MS were alternated

IMS-MS



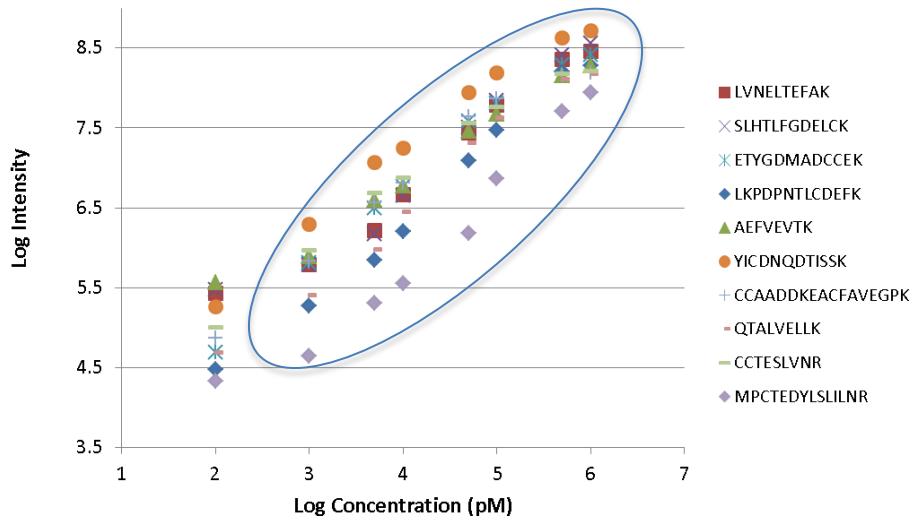
IMS-(CID)-MS (CE=29V)



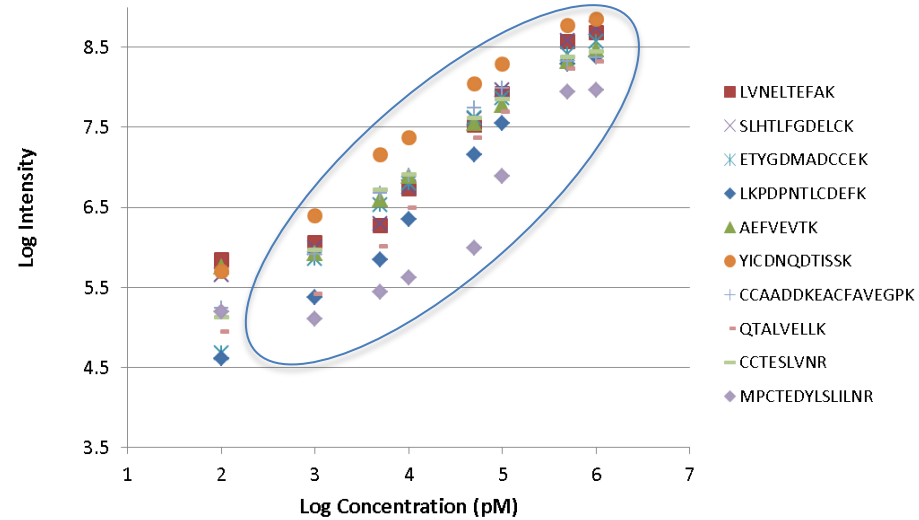
CE of 29 V was chosen since it fragmented 2+ ions well

Skyline provides the ability to perform fast DIA analyses

Drift Time Filtered MS Quantitation



MS Quantitation

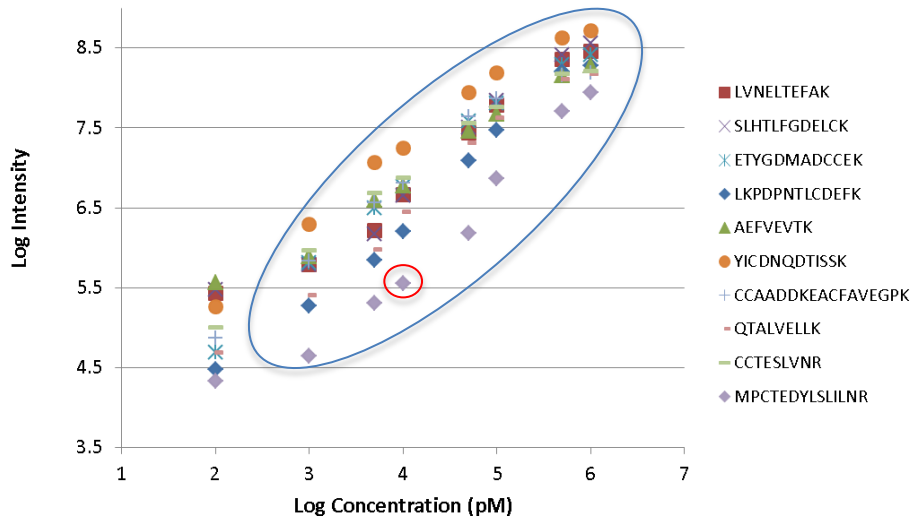


Good quantitation from 1 μ M to 1 nM for all peptides when drift time filtering was used

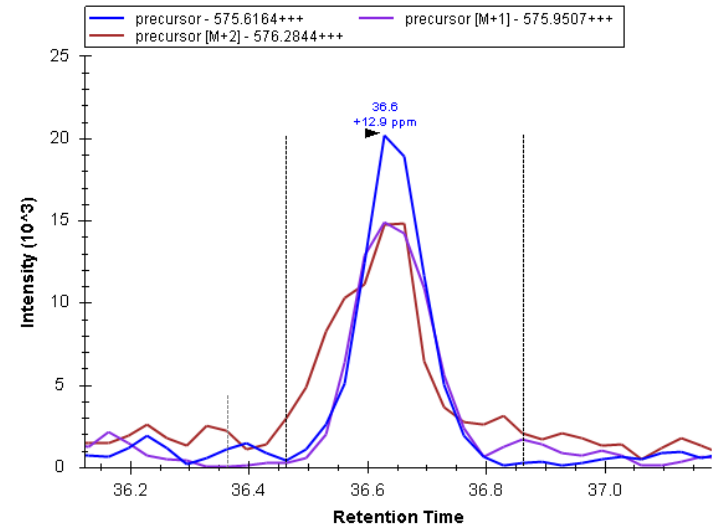
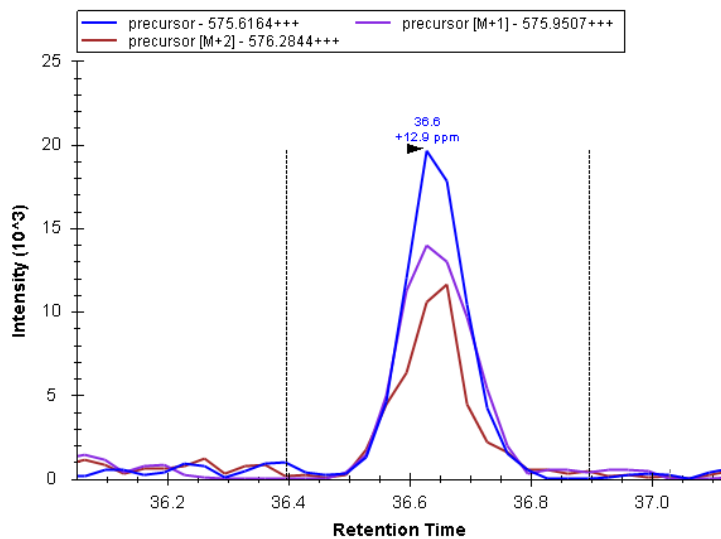
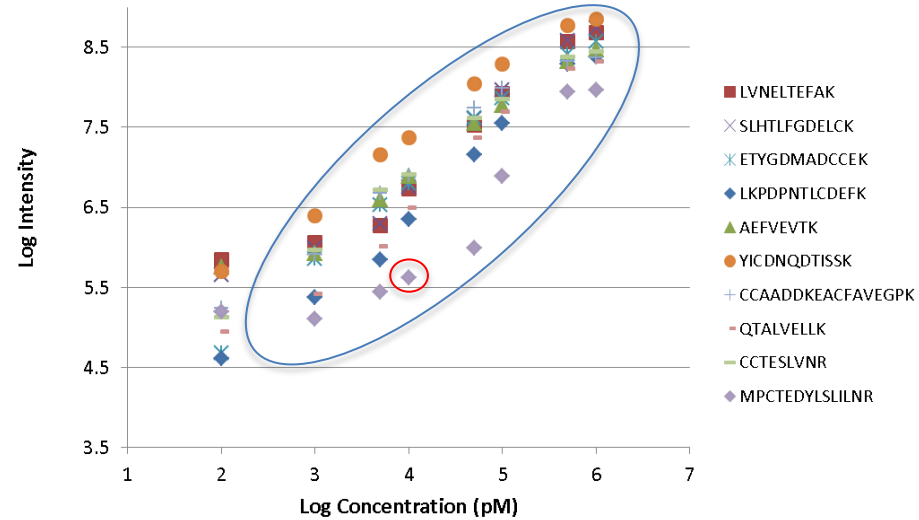
When drift time filtering wasn't used, the lower concentration peptide's quantitation wasn't as linear

Drift time removes interference in MS quantitation

Drift Time Filtered MS Quantitation

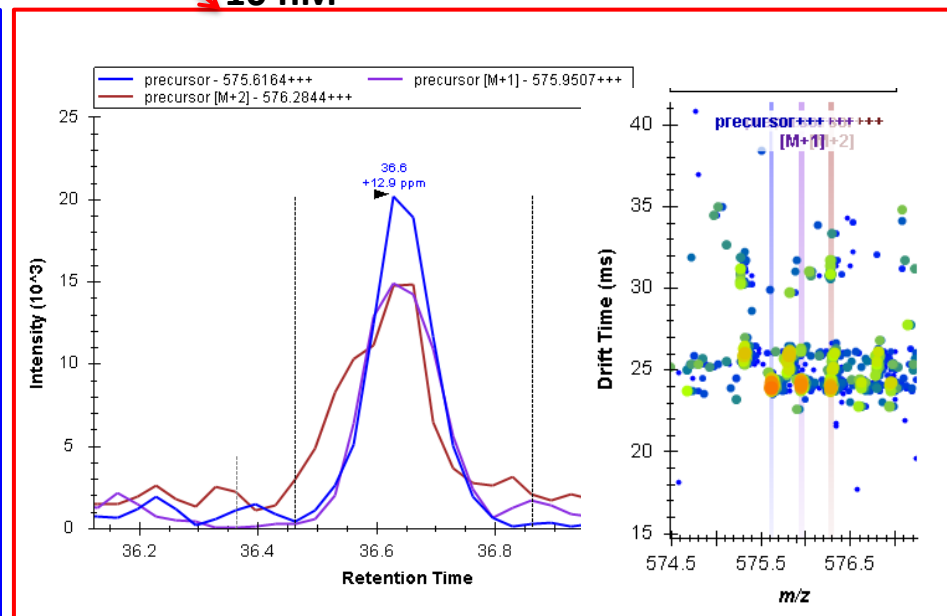
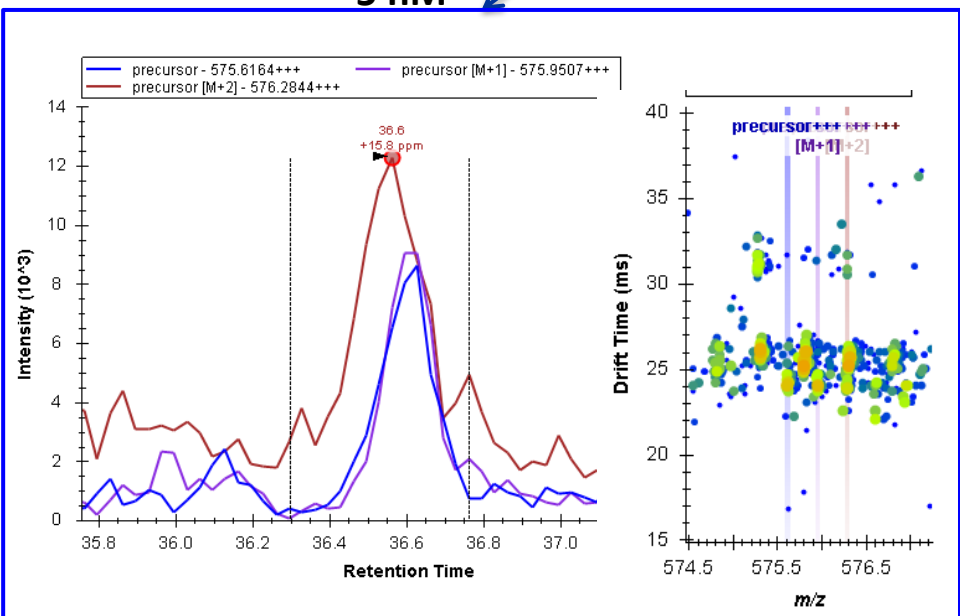
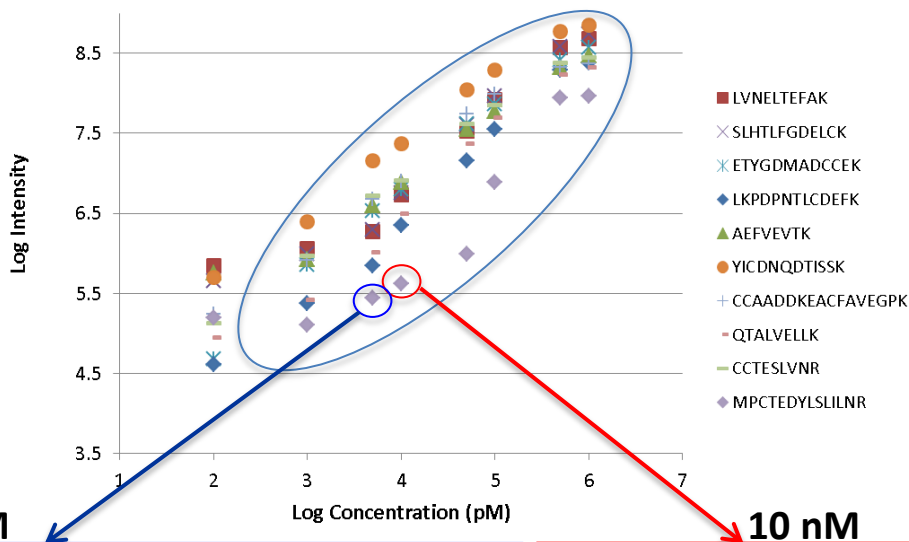


MS Quantitation



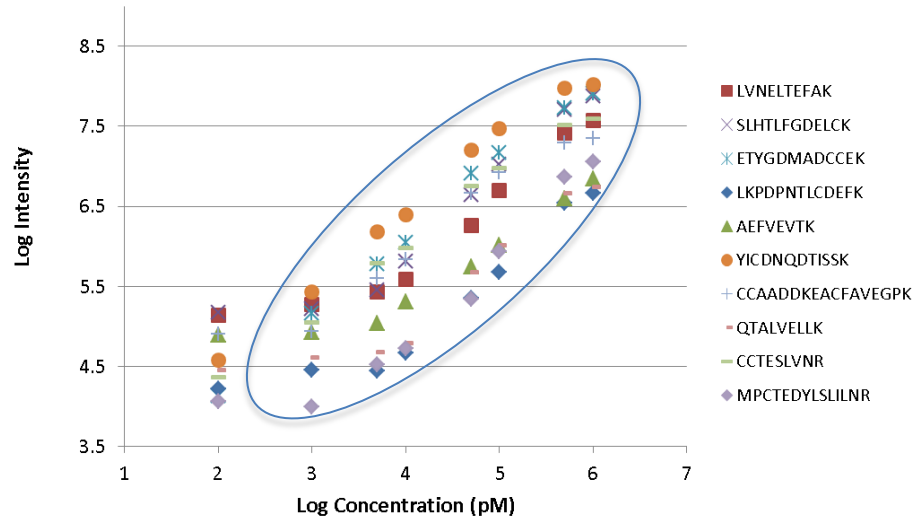
Another peak interferes when drift time filtering isn't performed

MS Quantitation

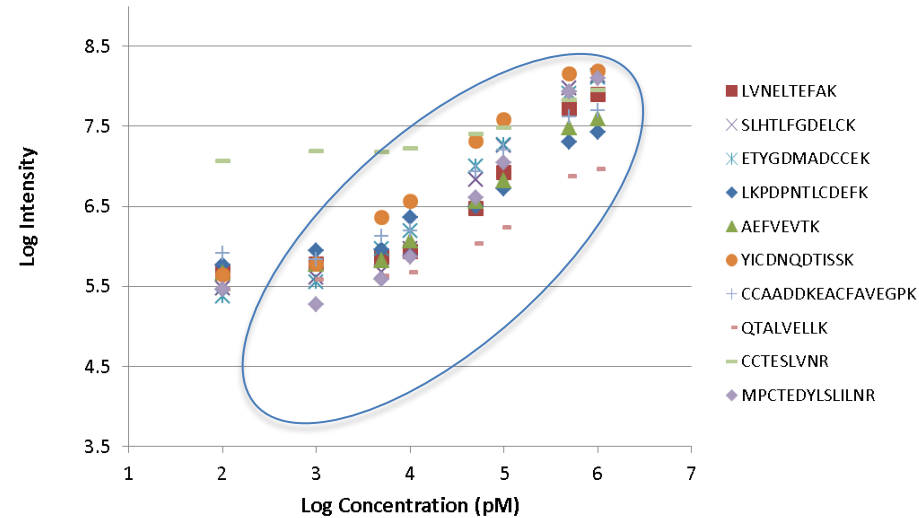


Drift time filtering removes interference in MS/MS quantitation

Drift Time Filtered MS/MS Quantitation



MS/MS Quantitation

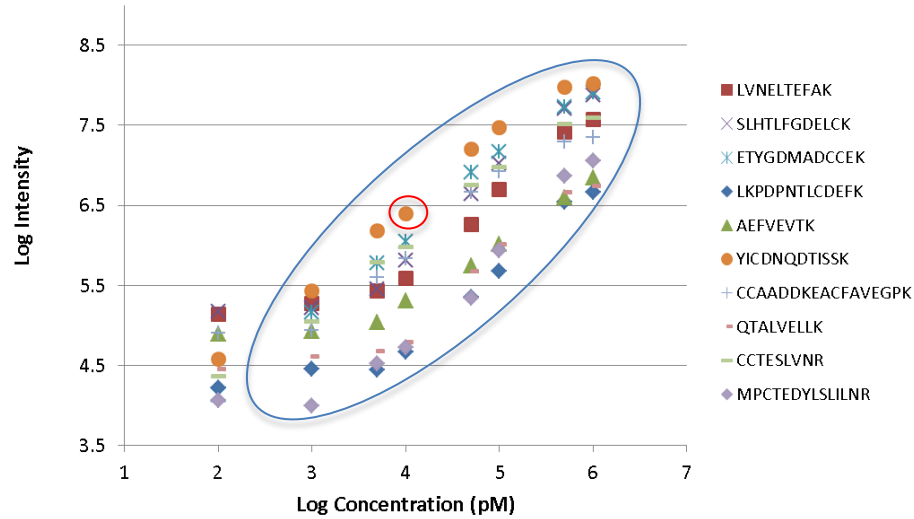


Good quantitation from 1 μ M to 1 nM for 7 of 10 peptides when drift time filtering and 10 for 10 from 1 μ M to 10 nM

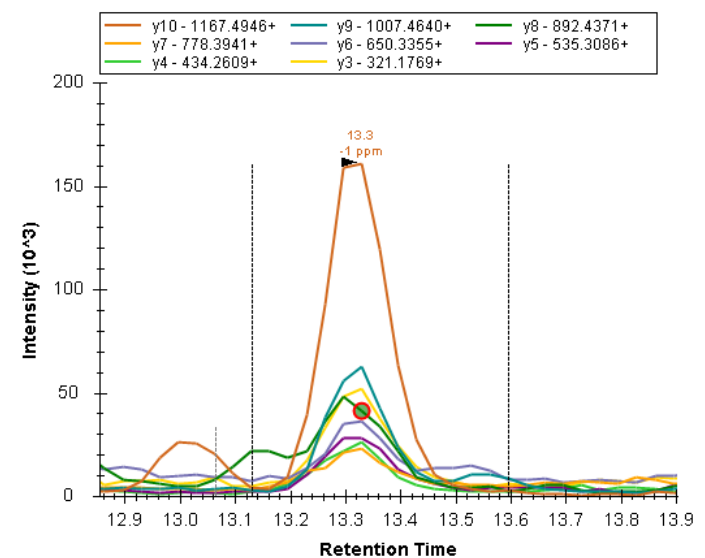
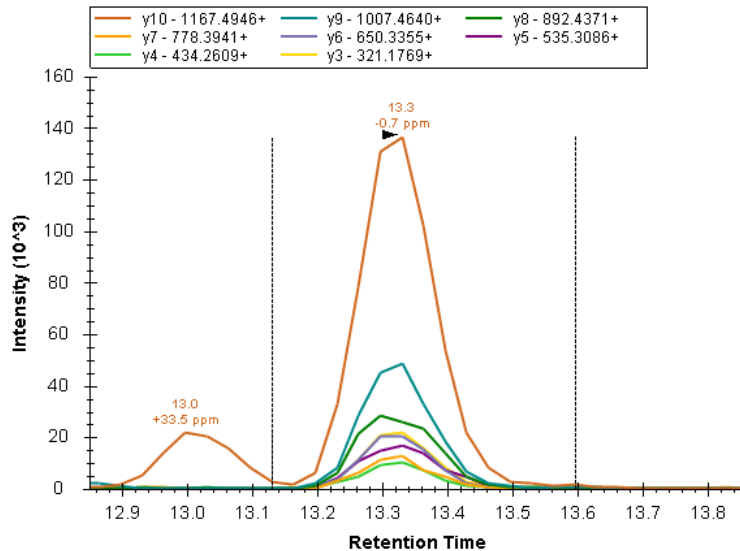
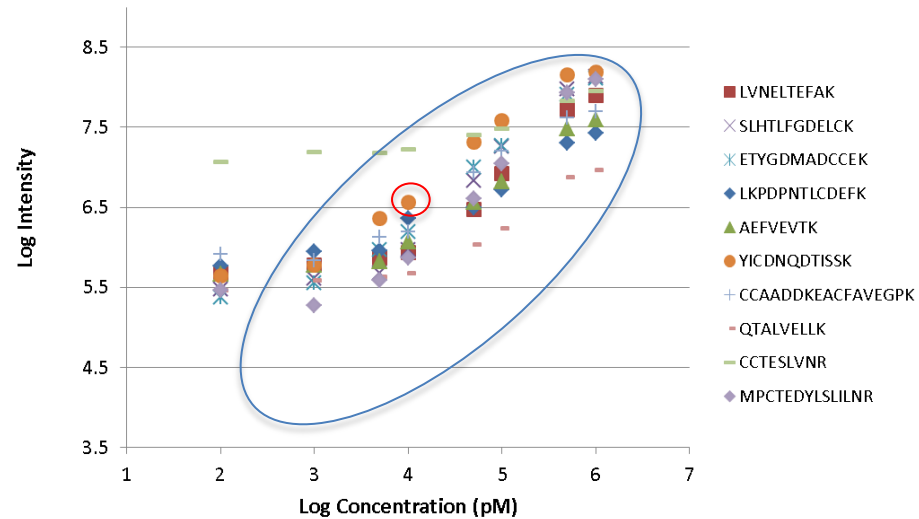
When drift time filtering wasn't used, many interferences caused bad quantitation

Baseline noise increases when drift time isn't used

Drift Time Filtered MS/MS Quantitation

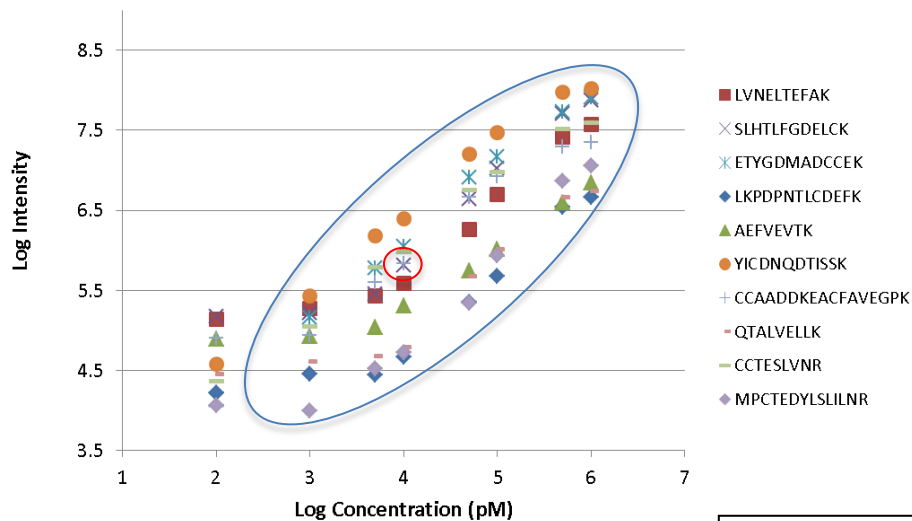


MS/MS Quantitation

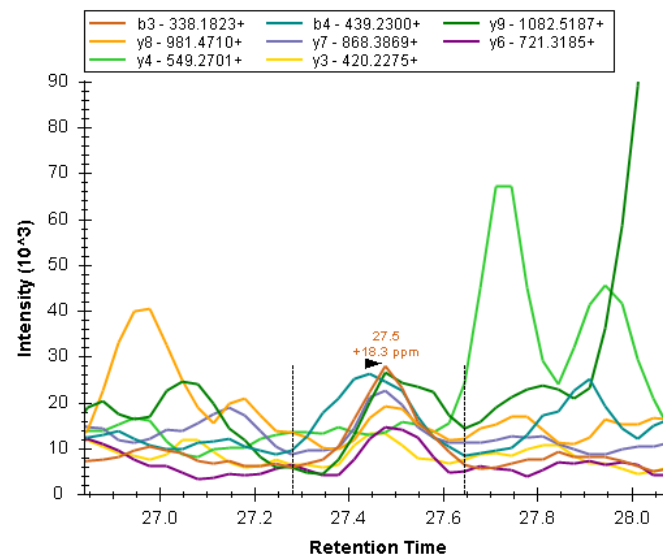
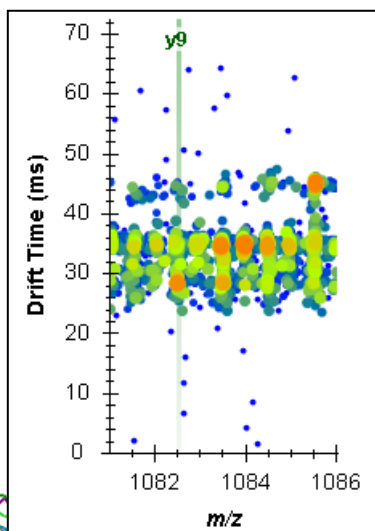
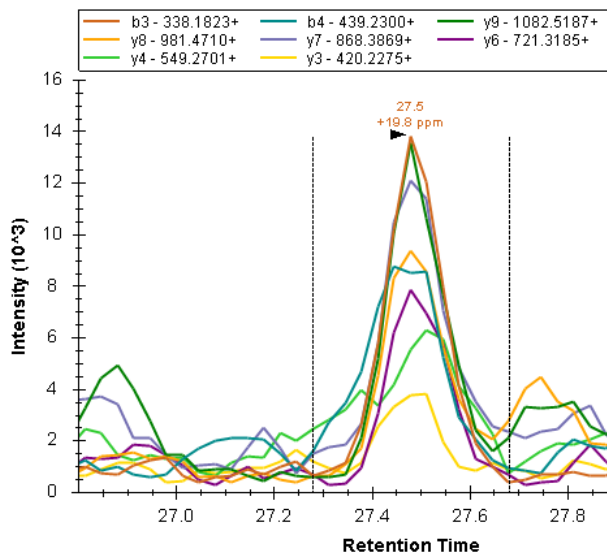
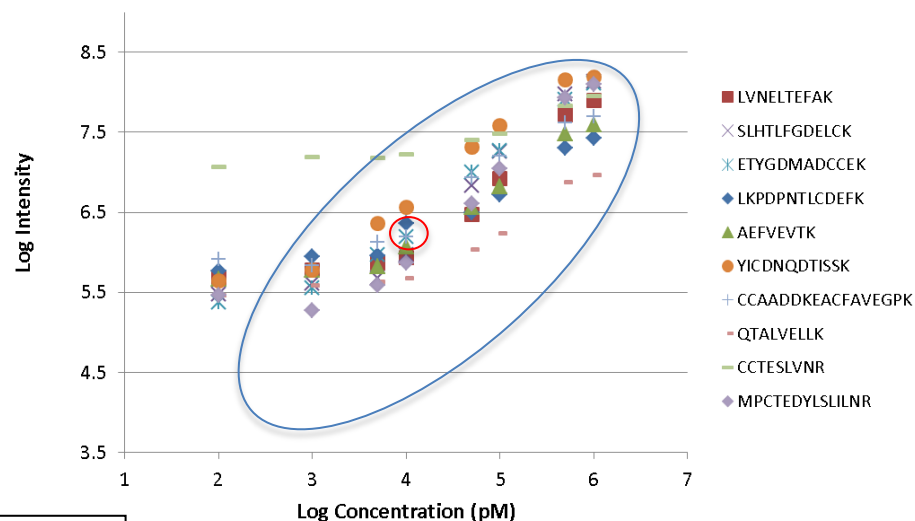


Interfering peaks cause quantitation problems when drift time isn't used

Drift Time Filtered MS/MS Quantitation



MS/MS Quantitation



Summary

Skyline:

- Quickly analyzed the IMS-(CID)-MS data
- Showed that IMS drift time allows better MS and MS/MS quantitation by removing interfering peaks
- Enables further studies to better understand the effect of IMS on other types of DIA data (such as SWATH)

Acknowledgements

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