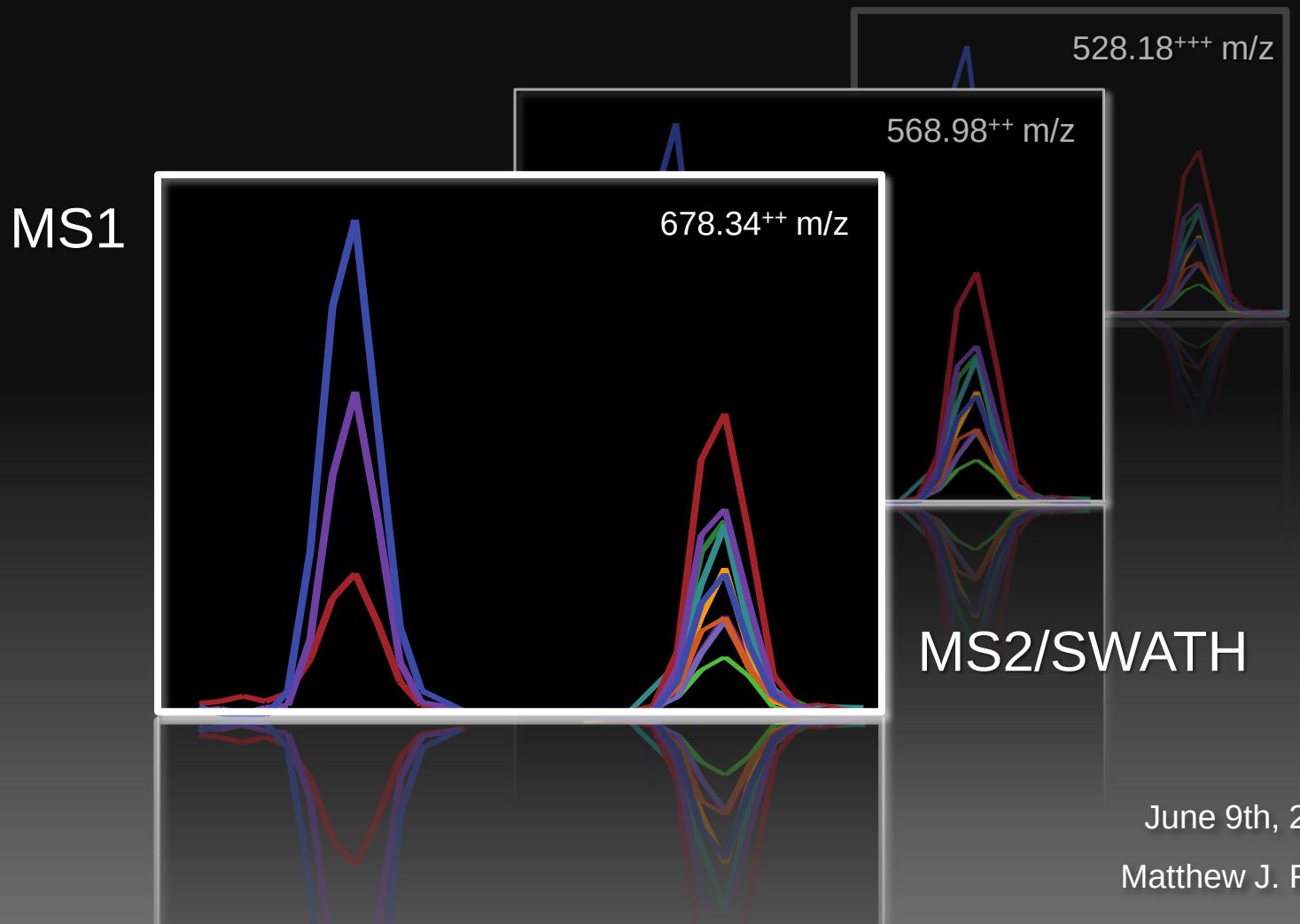
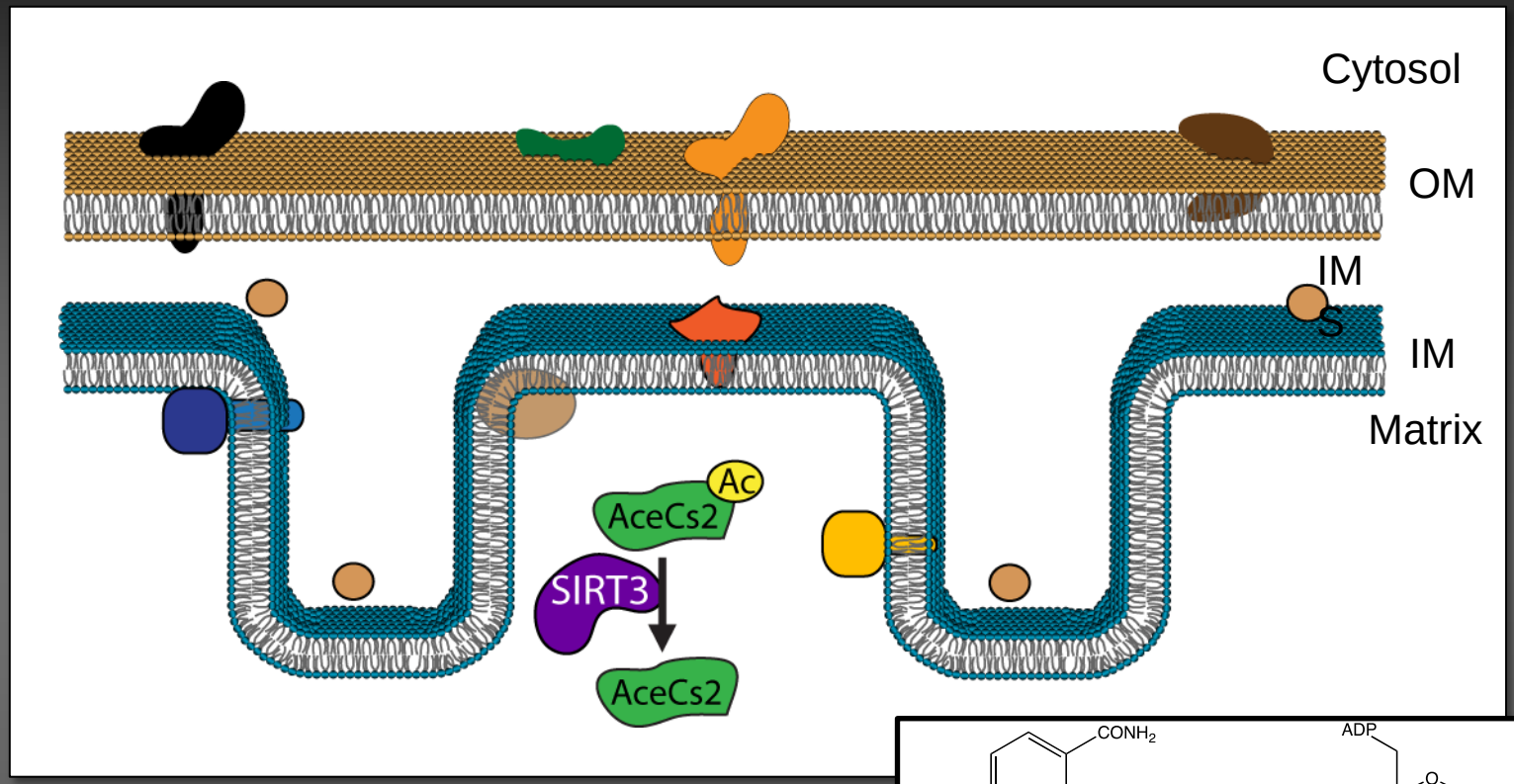
MS1 and MS2 crosstalk in label free quantitation of mass spectrometry data independent acquisitions



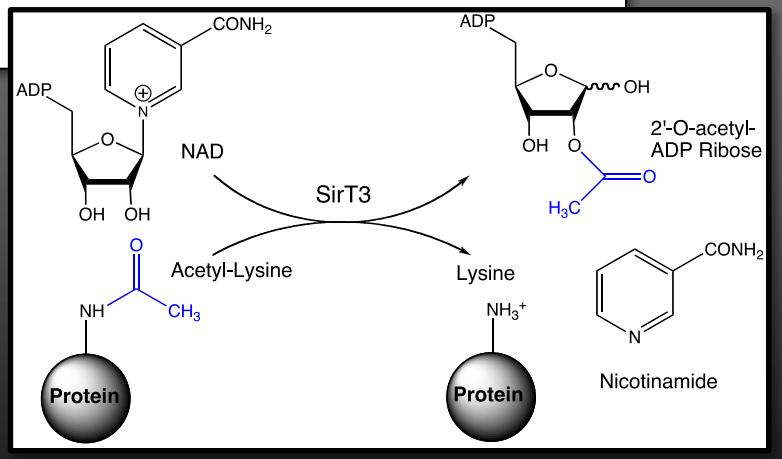
June 9th, 2013

Matthew J. Rardin

SIRT3 regulated mitochondrial lysine acetylation



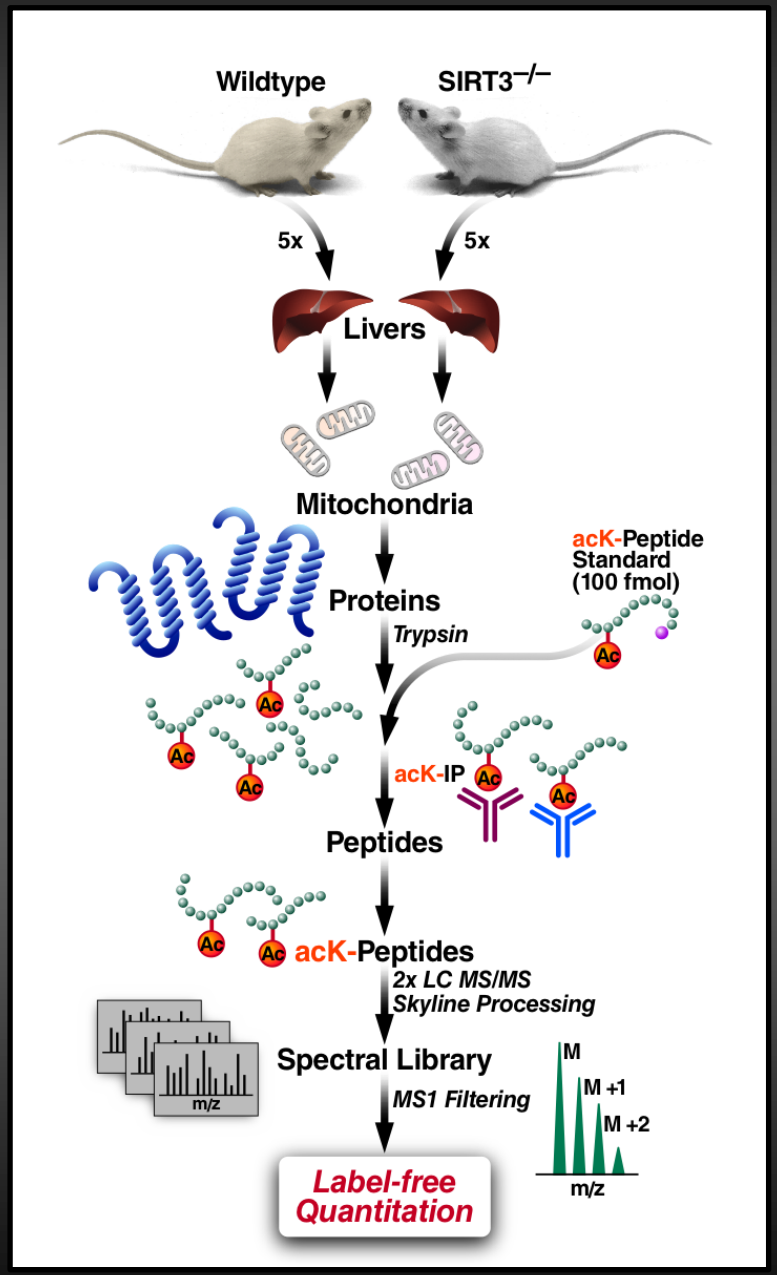
- Type 2 Diabetes
- Metabolic syndromes
- High/low-fat diet
- Aging
- Cancer



PNAS 2013 110 (16), 6601-6606.

Strategy for identification and quantitation of the SIRT3 regulated acetylome in mouse liver mitochondria

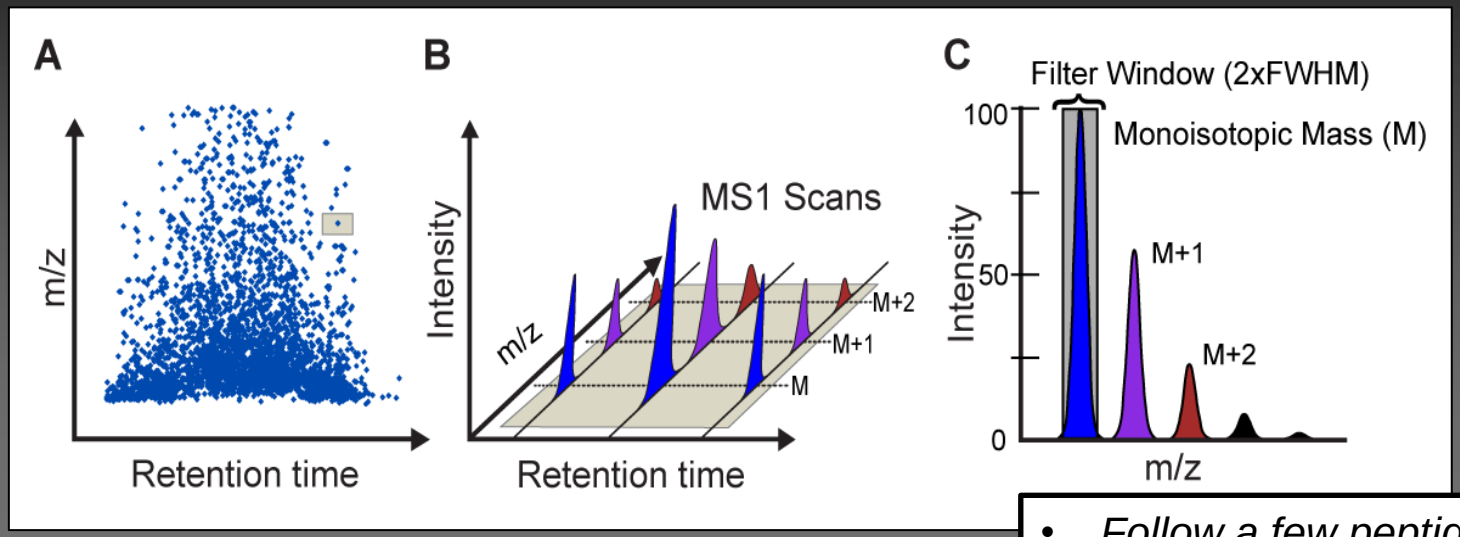
- Mitochondria were purified from 5WT and 5KO mice by differential centrifugation and normalized to total protein
- Perform a trypsin digest with two replicates per mitochondrial sample to control for process variability
- Spike-in acetyl standard LVSSVSDLP(acK)**R** as a sample process loading control
- Two polyclonal antibody combination for enrichment of lysine acetylated peptides
- Two injection replicates per sample were ran on the Triple TOF 5600
- Label free “relative” quantitation using MS1 Filtering
- Published spectral library information in Panorama



PNAS 2013 110 (16), 6601-6606.

Skyline MS1 Filtering

A quantitative tool for discovery proteomics experiments



- *Follow a few peptide analytes to >3000 peptides*
- *Label-free*
- *Skyline interface and tools*
- *MS platform/manufacturer independent (QqTOF, FT and ITs)**

Samples *(1, 2, 3,… N)*

Mass Spectrometer

Peptide Search Engine(s)

Redundant *Spectral Library*

Peptide Ion Chromatograms (raw data)

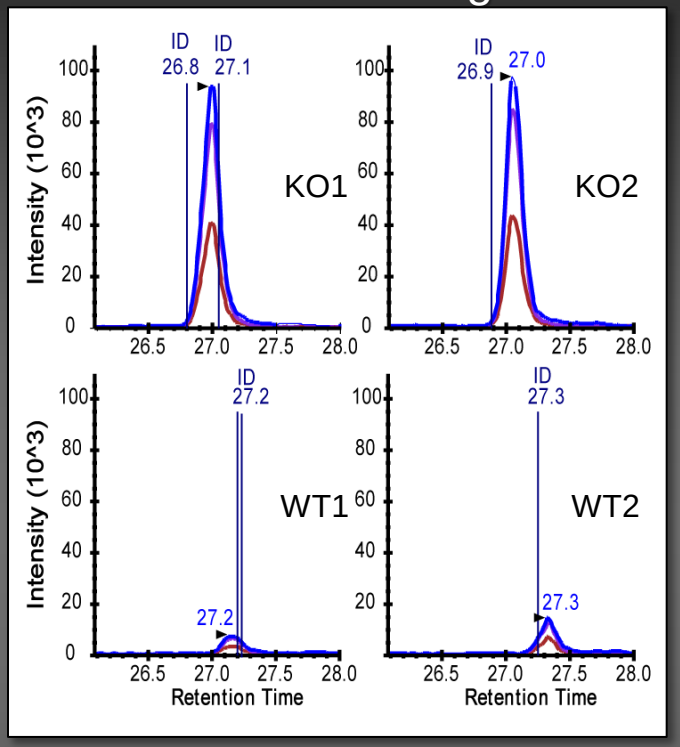
Peak Integration

Relative Quantitation

Mol Cell Proteomics 2012 11: 202-214.

Label free quantitation using MS1 Filtering in Skyline

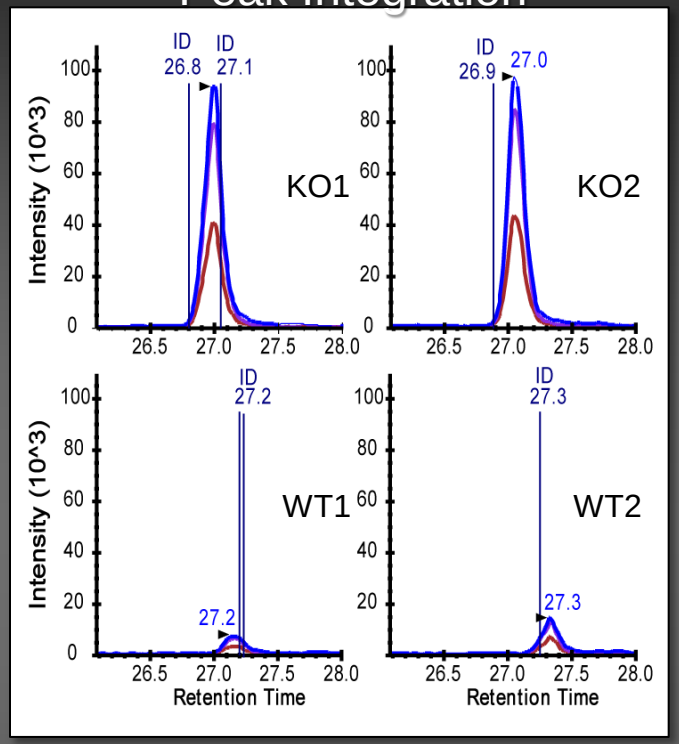
Peak Integration of the peptide ELQHHVK^{Ac}SVTAPYK^{+++}



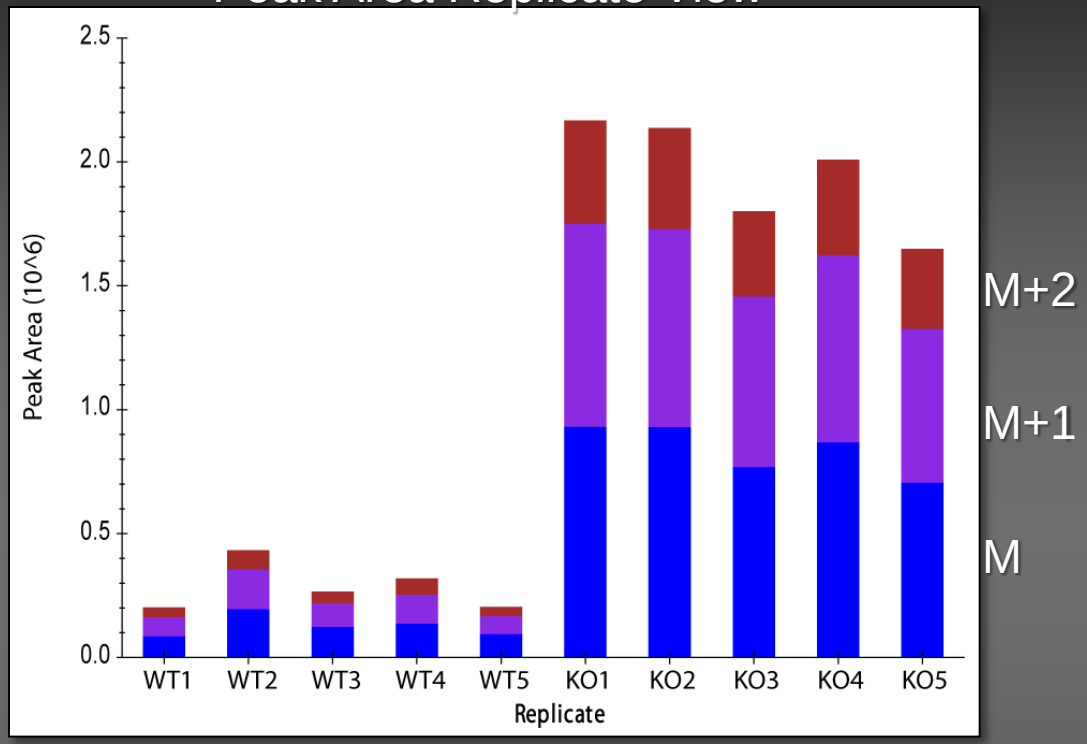
PNAS 2013 110 (16), 6601-6606.

Peak area replicates of ACSM1 K534

Peak Integration



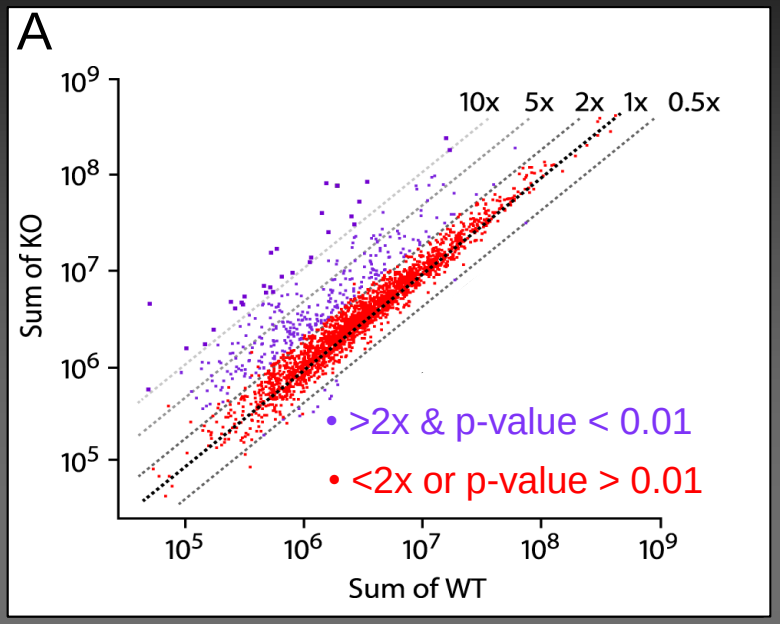
Peak Area Replicate View

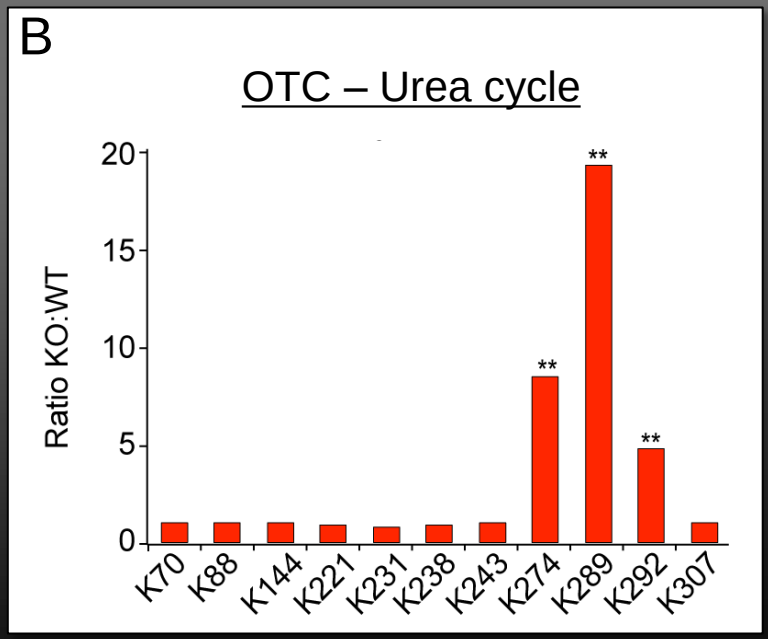


PNAS 2013 110 (16), 6601-6606.

Label free quantitation of K^{Ac} peptides in SIRT3-/-

- Quantitation of 2017 K^{Ac} sites
- Coefficient of variation of the peptide standard was 26% across 40 replicates
- Peptide area is normalized to spiked-in peptide standard
- Identified 266 sites on 136 proteins with greater than 2-fold increase in SIRT3-/- (p-value<0.01)
- Majority of sites were unchanged in the KO
- Quantitation of peptides from mitochondrial lysates showed no significant increase in protein expression

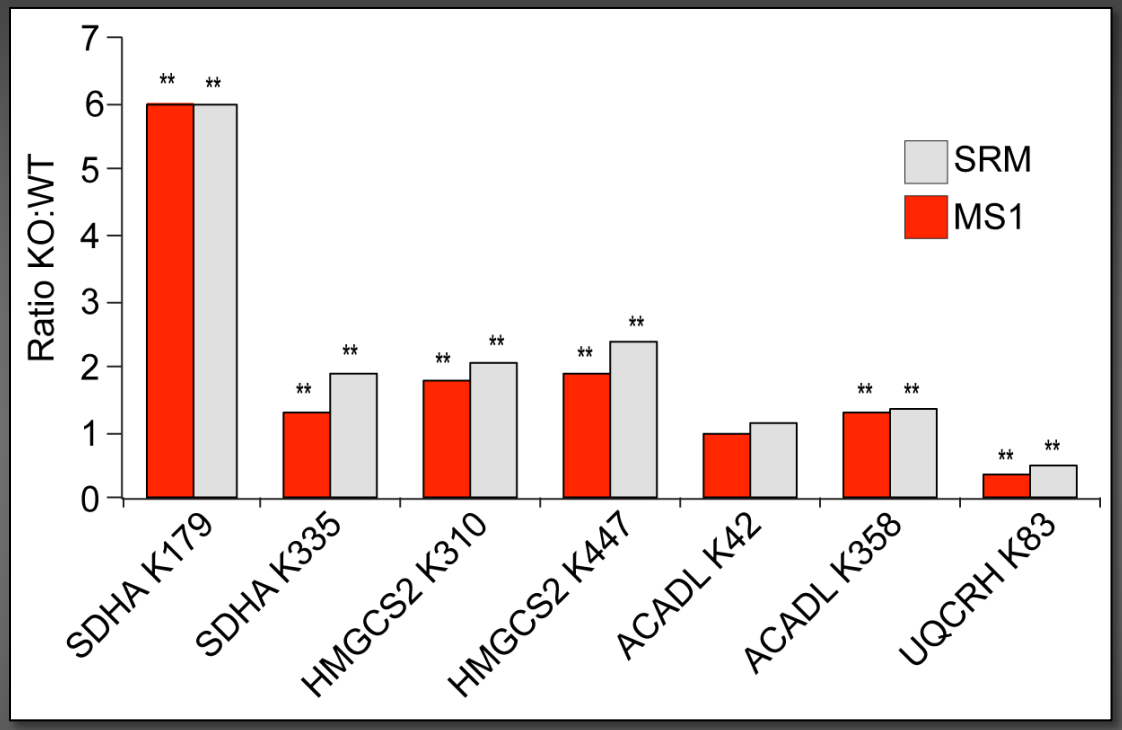




PNAS 2013 110 (16), 6601-6606.

Targeted quantitation of acetyl peptides by Selected Reaction Monitoring (SRM) mass spectrometry

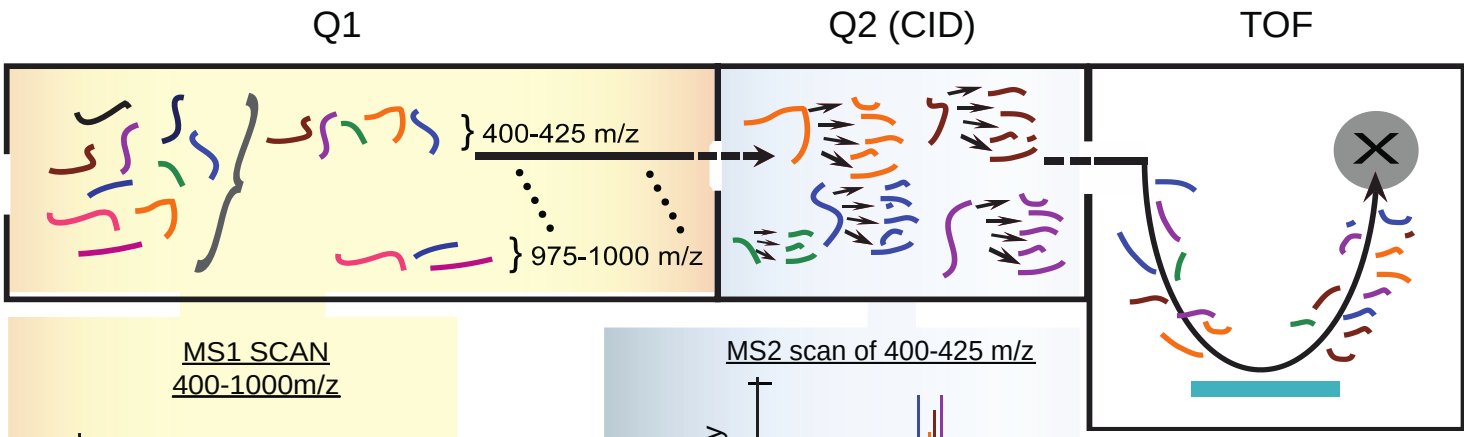
- Data collected on a 5500 QTRAP
- Spiked in 25 fmol of a heavy labeled synthetic peptide corresponding to peptides of interest
- SRM demonstrates similar results to MS1 Filtering

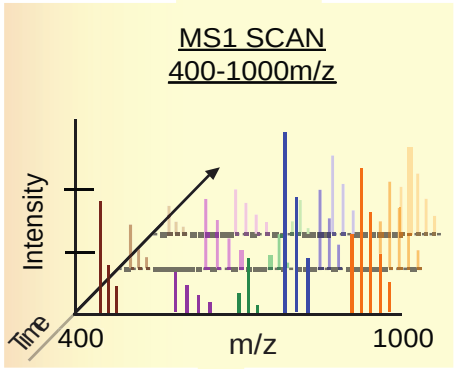


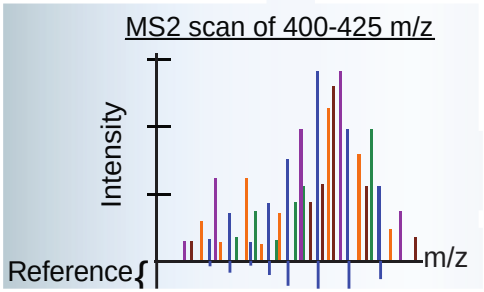
p-value < 0.05*, 0.01**

PNAS 2013 110 (16), 6601-6606.

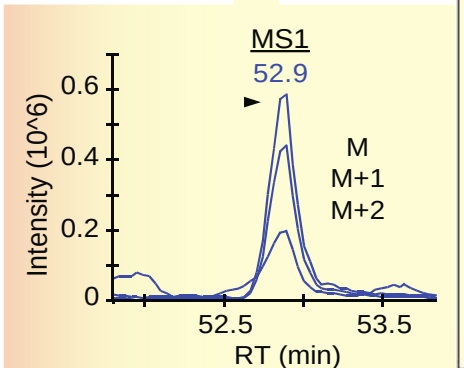
Data Independent Acquisition (DIA) on a Triple TOF 5600

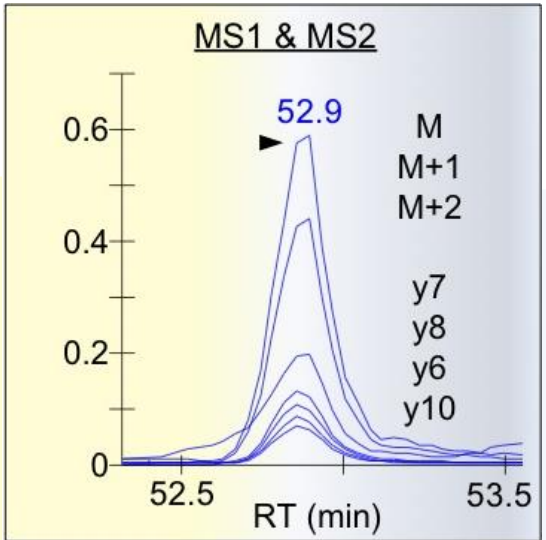




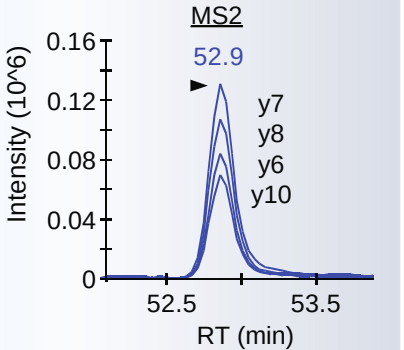


Extract precursor ion chromatograms





Extract top 4 product ion chromatograms



Adding MS1 quantitation to a DIA MS2 (SWATH) acquisition

1. What is the relationship between MS1 and MS2 quantitation in a DIA acquisition?
   -MS1 scan is acquired between each SWATH cycle

2. Is there value in combining MS1 analysis to SWATH MS2 data?

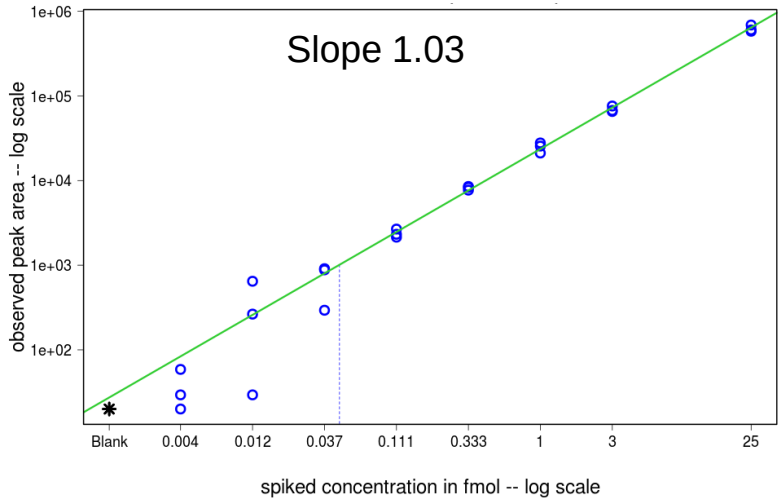
1. Are there cases when one method (MS1 or MS2) provides more accurate data than other
   When is one data set compromised?

MS1 Filtering vs SWATH in Simple and Complex Matrix

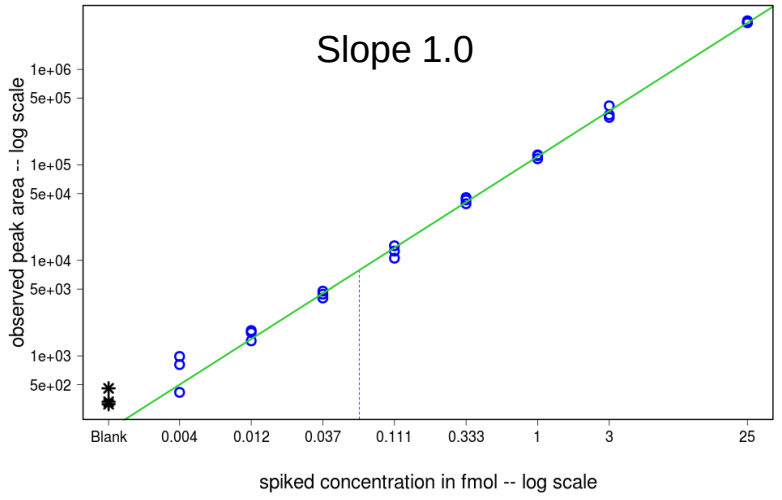
Std. Concentration Curve 4 amol – 25 fmol

A Simple Matrix - 25fmol β-gal

SWATH - YAPVAK$_{Ac}$DLAS**R**

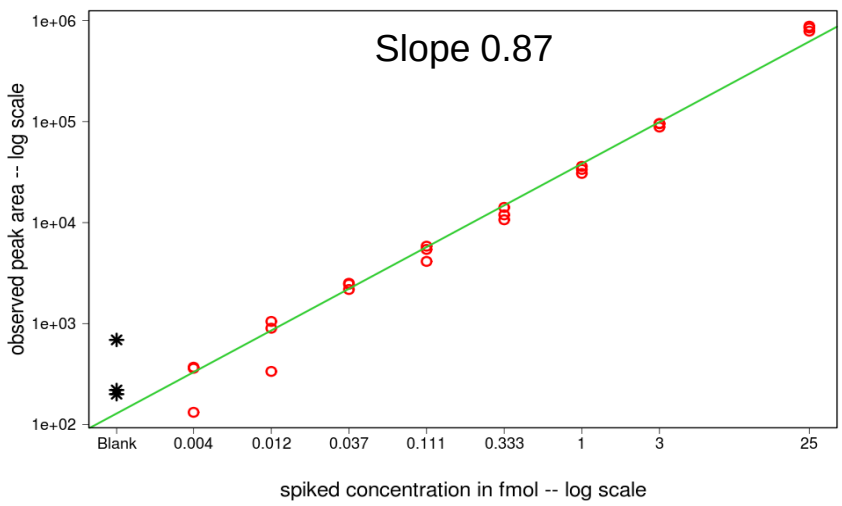


MS1 - YAPVAK$_{Ac}$DLAS**R**

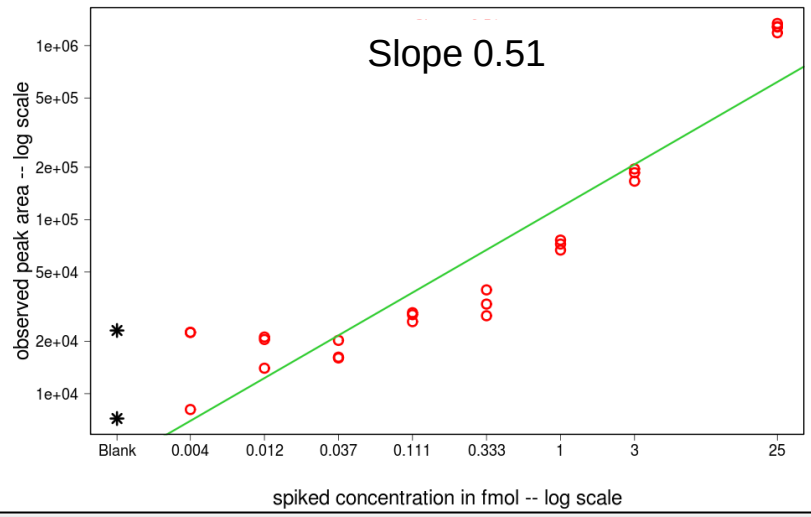


B Complex Matrix – 300 ng digest

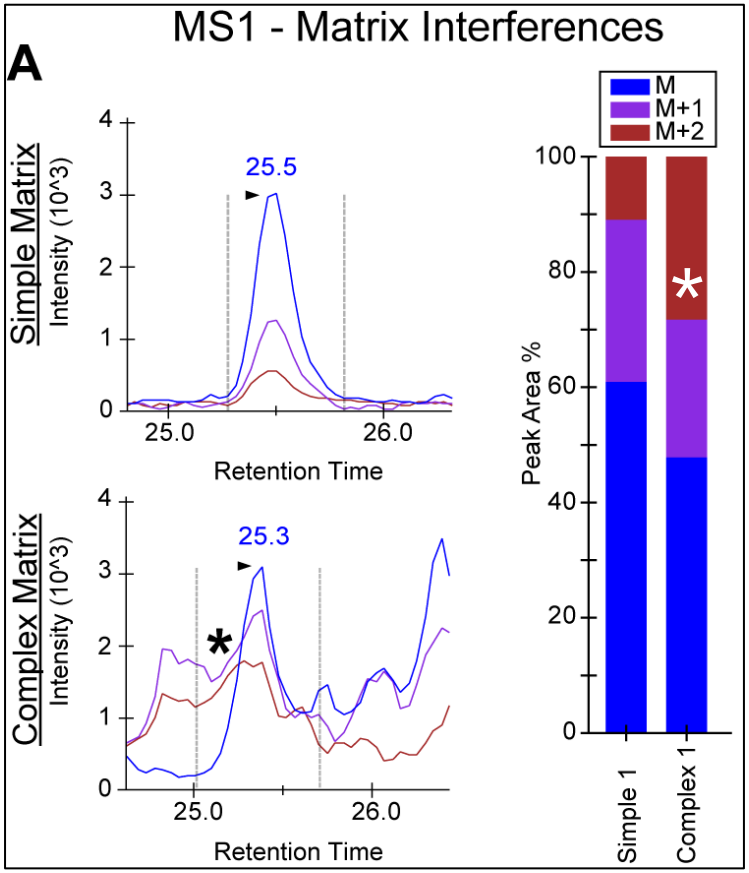
SWATH - MVQK$_{Ac}$SLA**R**

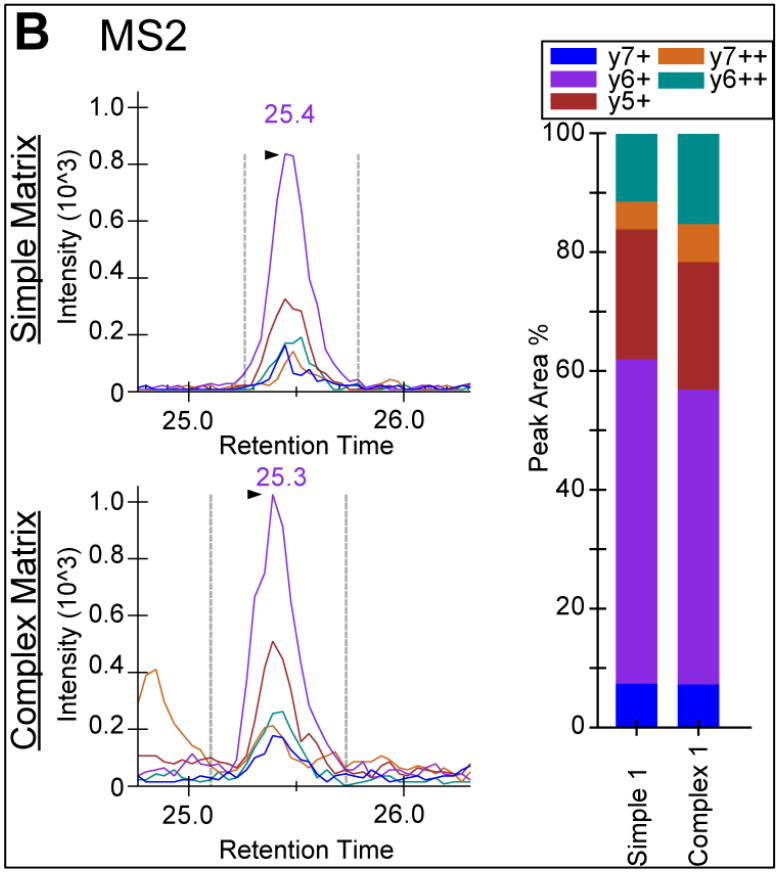


MS1 - MVQK$_{Ac}$SLA**R**



Inferences increase in the MS1 Signal with sample complexity



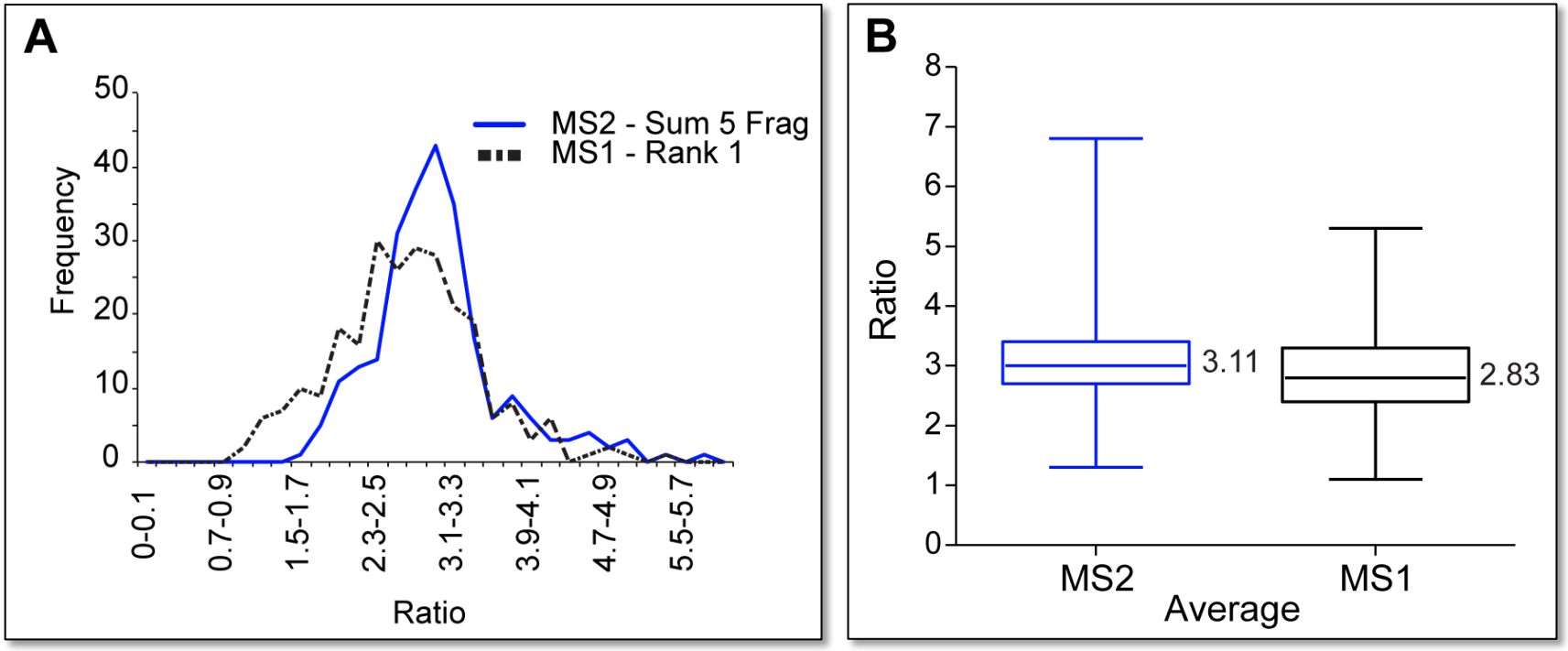


Simple Matrix – 25 fmol β–gal

Complex Matrix – 300 ng protein digest

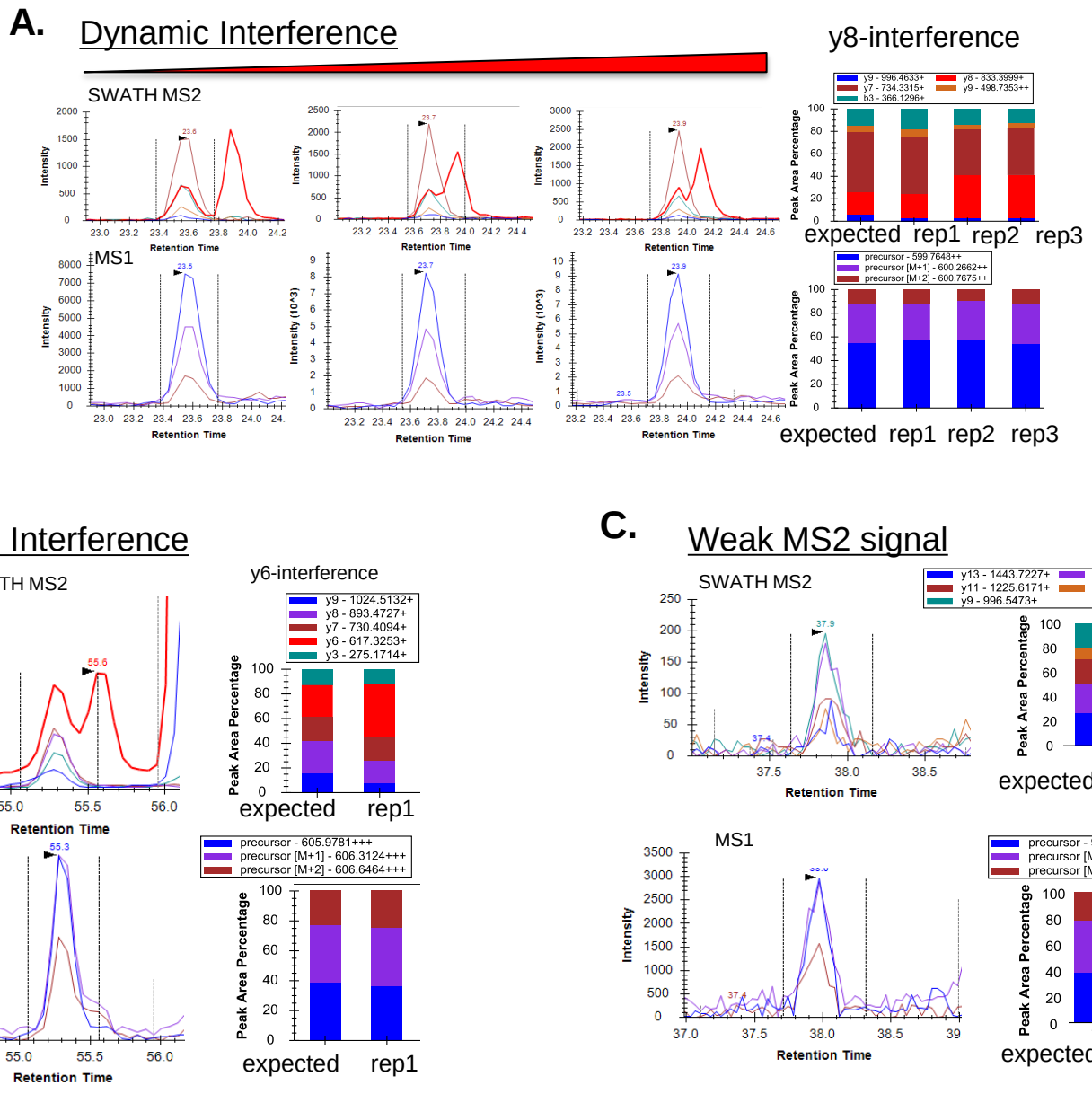
* - Interference

Quantitative comparison of 250 peptides at a fixed ratio using MS1 and SWATH

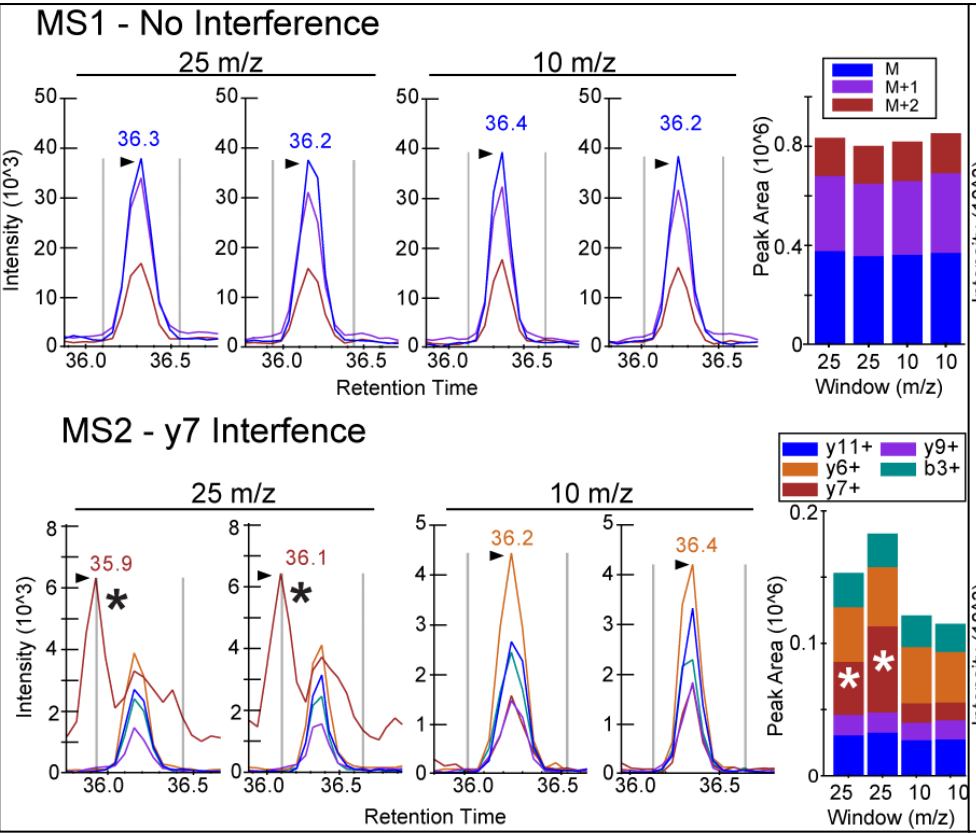


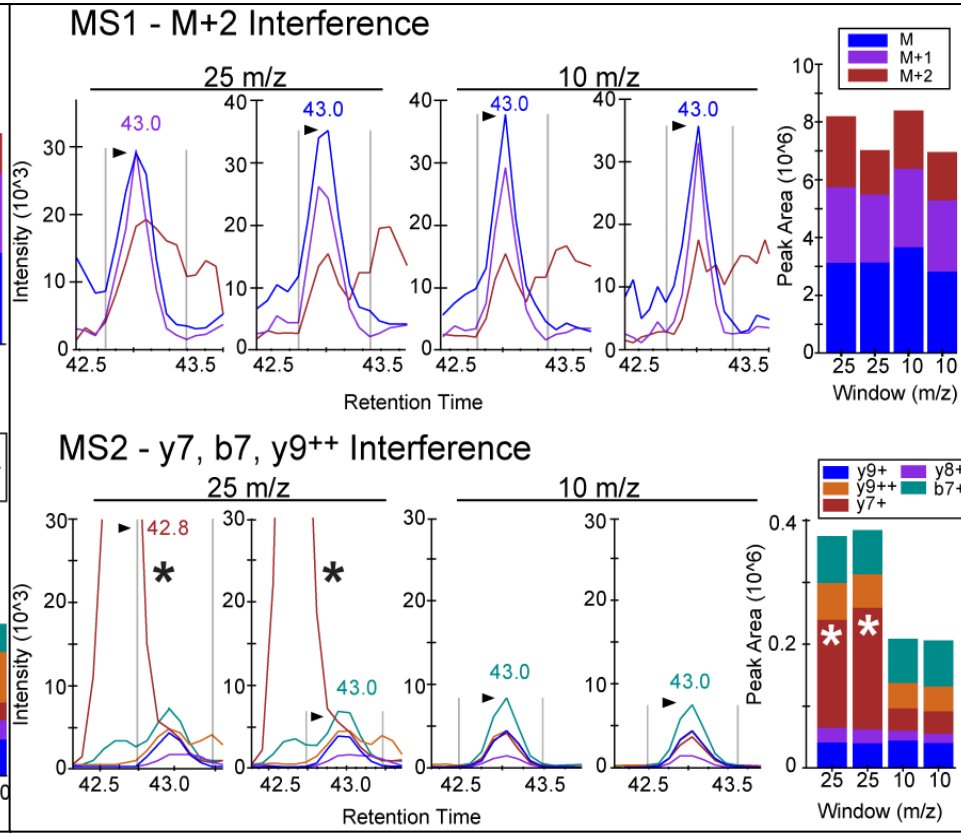
- 6 technical replicates were acquired using either 100ng or 300ng of a protein digest
- The frequency of the peptide ratios in MS2 was tighter than MS1
- The average ratio in the MS2 was closer to the actual ratio; 3.11 vs 2.83 in the MS1 Filtering
- Sum of fragment ions in MS2 dramatically improves the distribution of ratios and p-values
- SWATH performed better then MS1 Filtering, but not as dramatically as we expected

Inferences in the MS2 Signal



Reducing interferences in the MS2 scan during a SWATH DIA





SWATH 25 m/z	SWATH 10 m/z
MS1 scan – 250 msec	MS1 scan – 250 msec
MS2 scan – 125 msec	MS2 scan – 50 msec

SUMMARY

- During a SWATH DIA the MS1 scan is still acquired on the Triple TOF 5600.
- In Skyline, both the MS1 and MS2 scans can be extracted and used for quantification of peptide analytes
- In a simple matrix the MS1 scan appears to have greater sensitivity then the MS2 scan. However in more complex samples the MS2 scan outperforms the MS1 scan.
- The MS1 scan provides useful information and can be used as a secondary quantitation strategy
- Use of both scans allow for screening and identifying various types of interferences
- Reduction of the SWATH window in a complex sample reduces interferences without loss of sensitivity in the MS2 scan

Acknowledgements

Laboratory of Bradford W. Gibson

Birgit Schilling – MOA 3:50pm
MS1 Filtering for quantifying lysine acetylation in E. coli.

Alexandria D'Souza – TP521
External tool development in Skyline

Jason Held – WP555
Quantifying cysteine oxidation using MS1 Filtering and SWATH

Anna Zawadzka – THOD 8:50am
Breast cancer biomarker study using MS1 Filtering

University of Washington
Michael J. MacCoss
Brendan MacLean