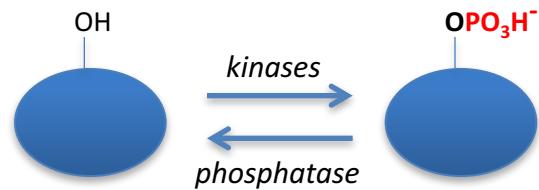
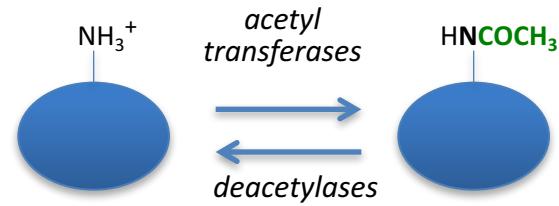


# From DDA to SRM, PRM and DIA



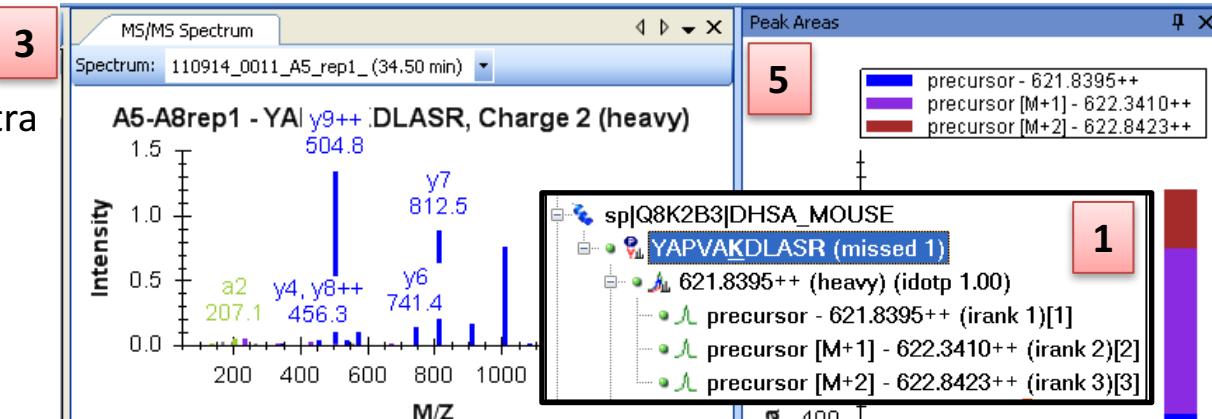
**Birgit Schilling**  
*Buck Institute for Research on Aging*



**US HUPO Skyline / Statistics Course, March 10-11, 2018**

# Skyline interface for MS1 filtering data

3) MS/MS spectra and ID

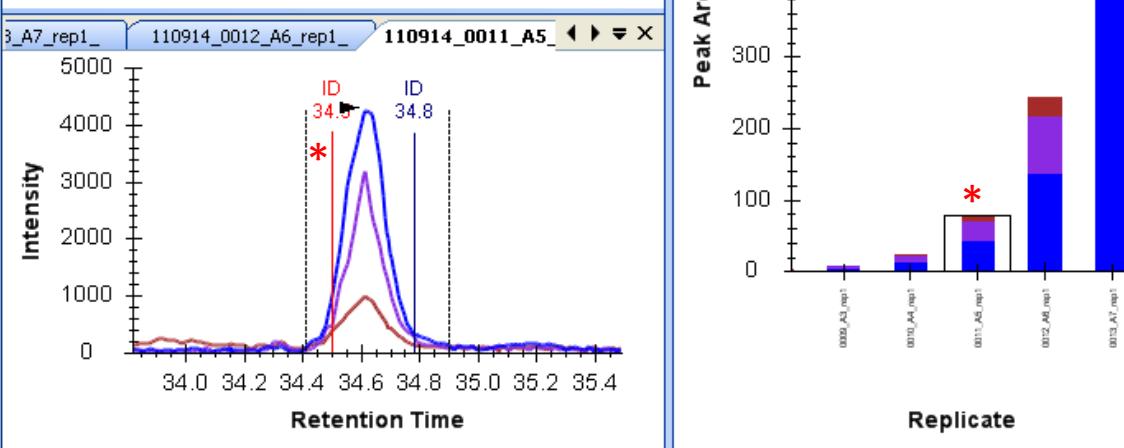


5) M, M+1,M+2 precursor peak areas

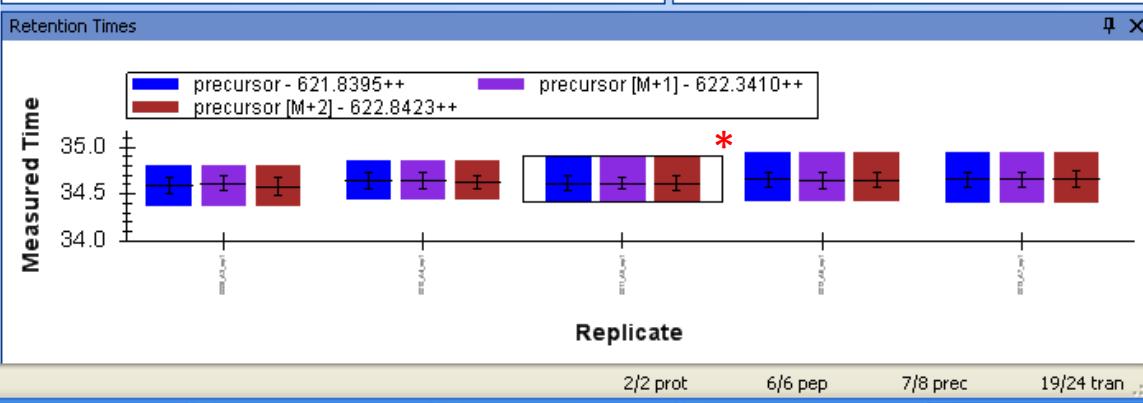
1) Peptide 'tree' with precursors

- irank
- idotp

2) RT and ID correlation; peak boundaries set for integration



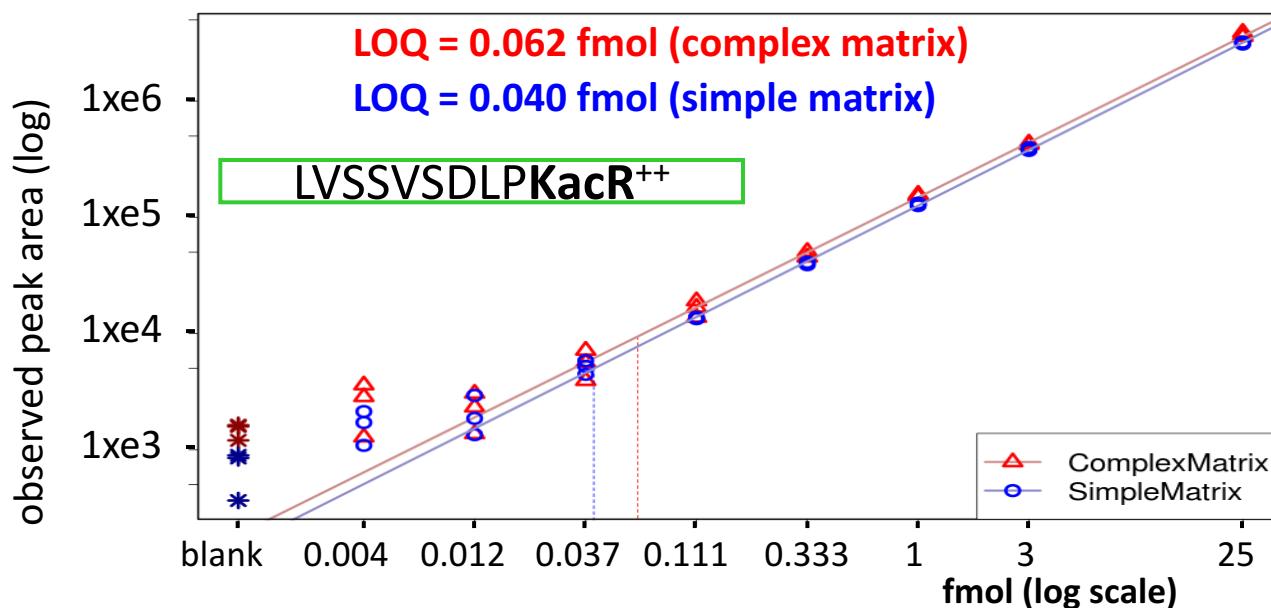
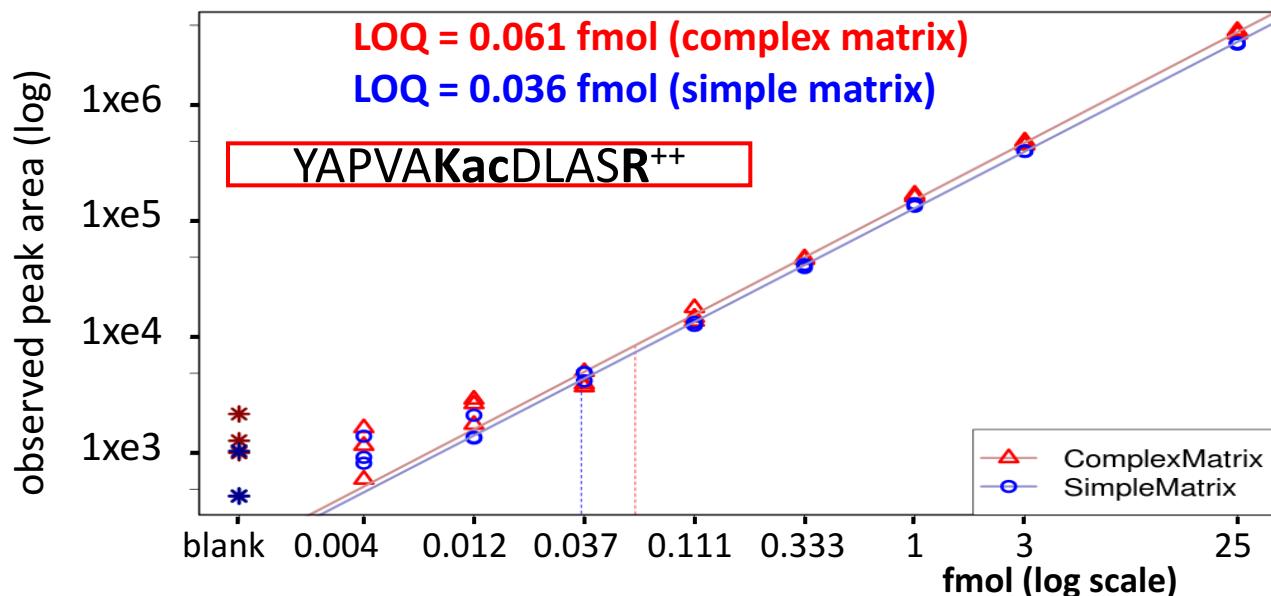
4) RT variation among peptides and replicates for each precursor isotope (M, M+1, M+2)



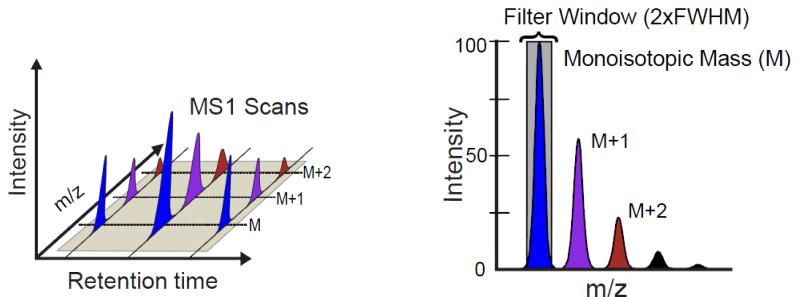
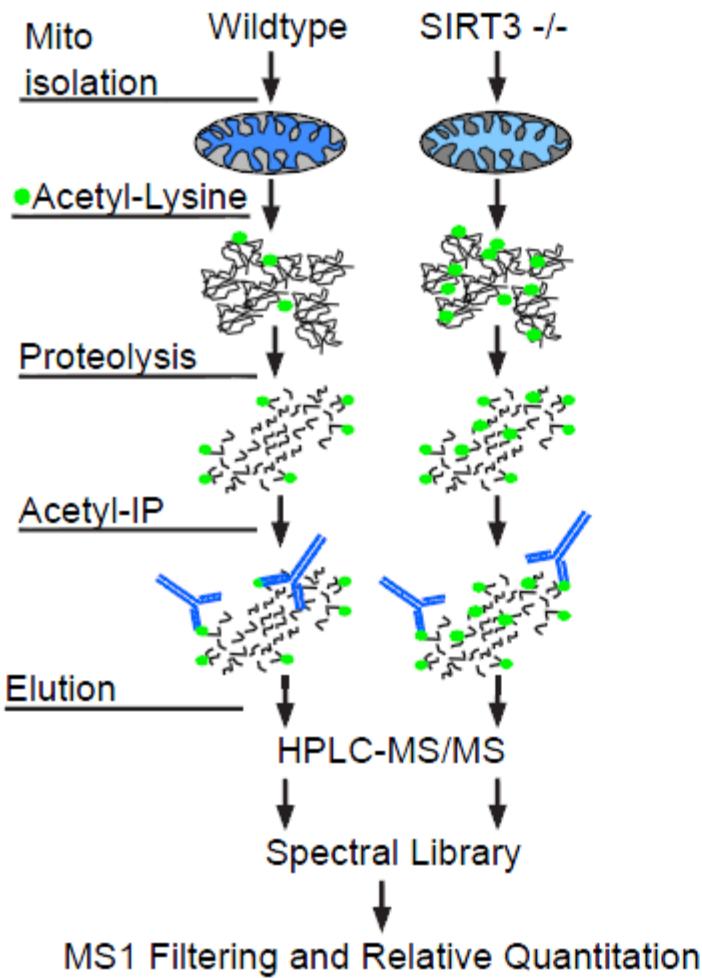
# MS1 Filtering Standard Concentration Curves for Lys-Ac Peptides

## TripleTOF 5600

- 6 peptide mix at 4 amol to 50 fmol
- Both simple and complex matrices
- Triplicate analysis +/- background matrices



# Development and Examples for MS1 Filtering Workflows



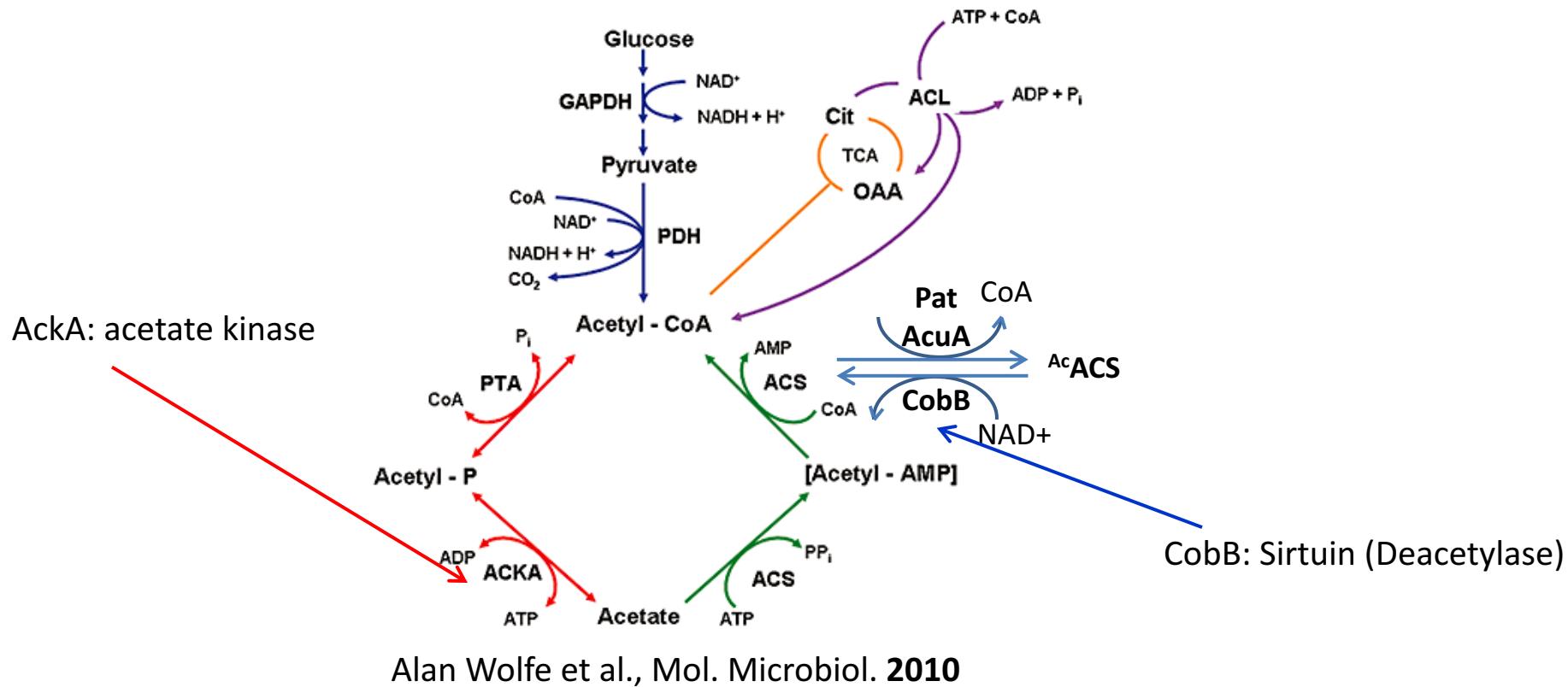
- Data-dependent acquisitions (discovery workflows) depend on dynamic sampling of MS/MS spectra.
- **MS1 scans** are truly **data-independent** and can be used for relative quantification.
- MS1 Filtering uses ion extracted chromatograms of peptide precursor ions for relative quantification
- Easy **interfacing** in Skyline with other data independent, **targeted workflows**, i.e. MRM, MRM-HR (PRM), SWATH-MS2.
- Interface with Panorama

Schilling, Rardin, MacLean et al., *MCP*, 2012

Rardin et al., *PNAS*, 2013

# Bacterial Protein Acetylation - Background

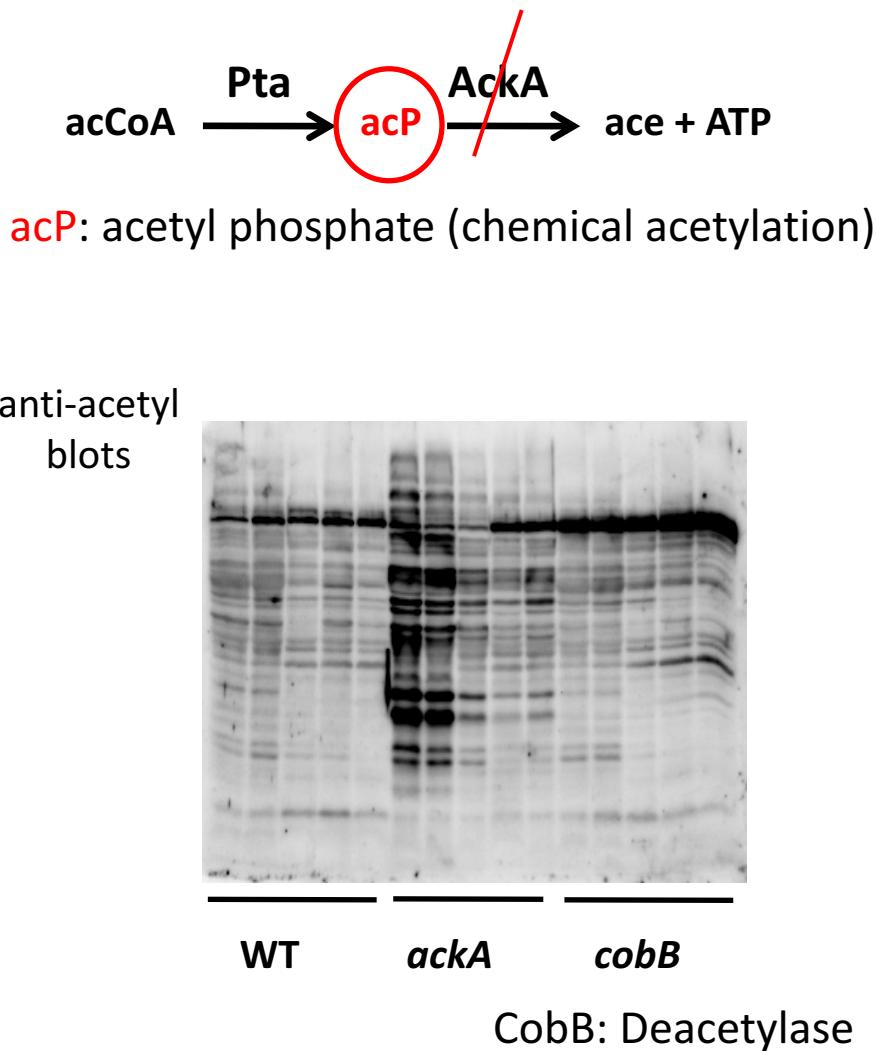
## Acetyl CoA and Acetyl phosphate (AcP)



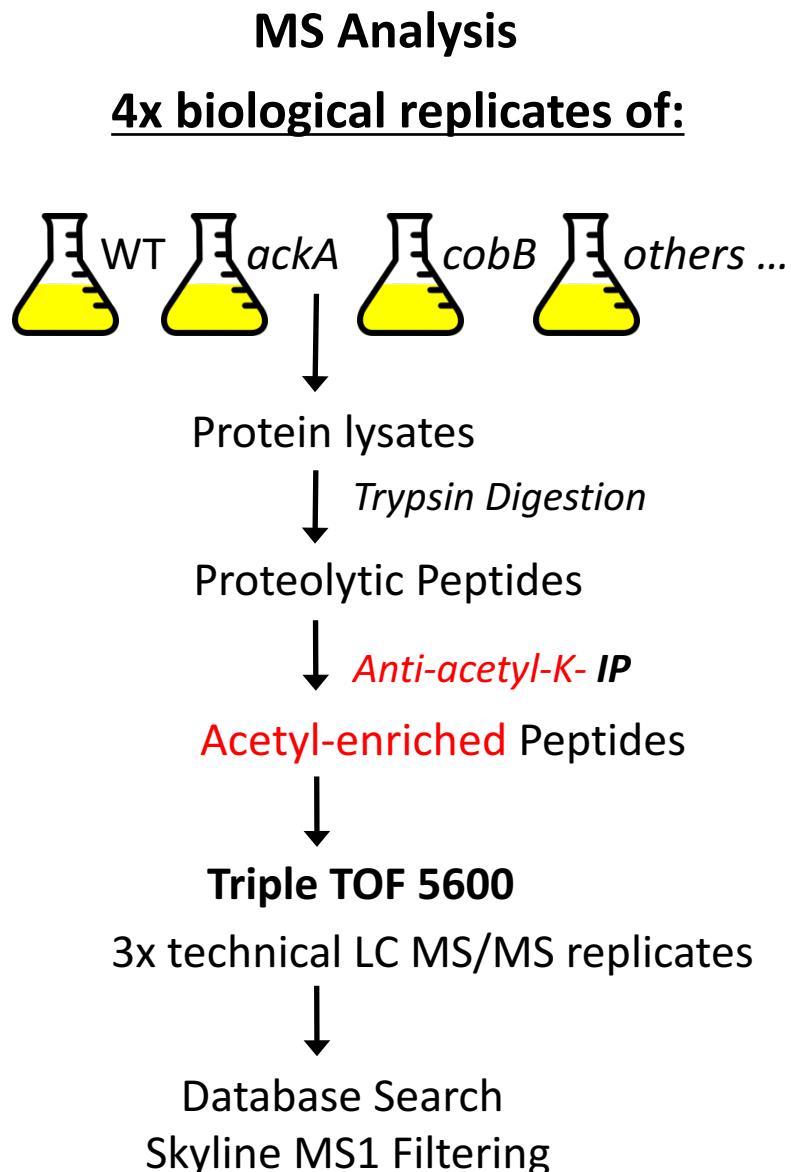
## Bacterial Acetylome studies:

*E. coli* (Yu et al., 2008, Zhang et al., 2009), *S. enterica* (Wang et al., 2010)

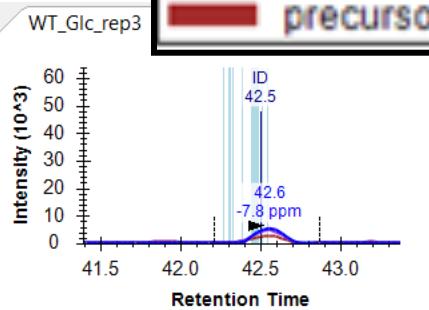
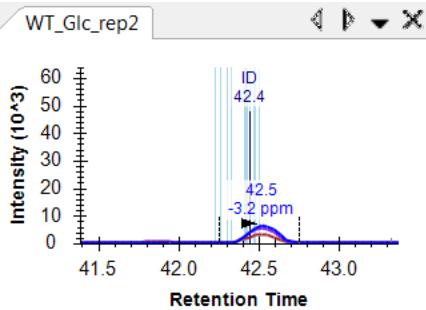
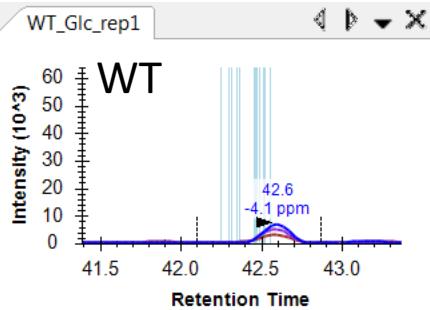
# *E. coli* acetylome comparing WT & mutants - Workflow



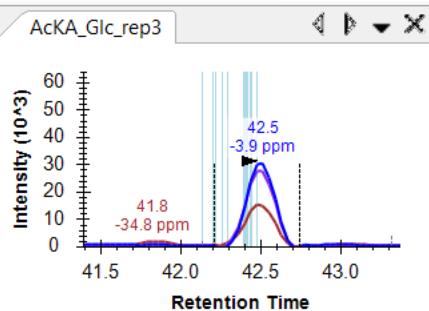
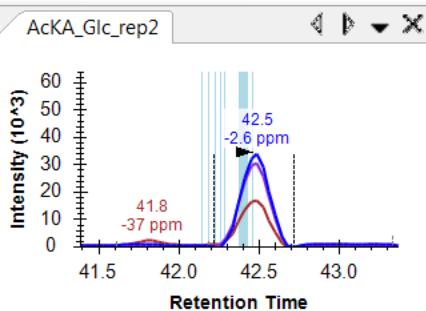
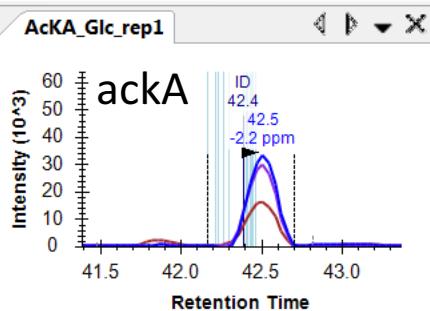
(Kuhn, Schilling, Gibson, Wolfe et al., *PlosOne* 2014)



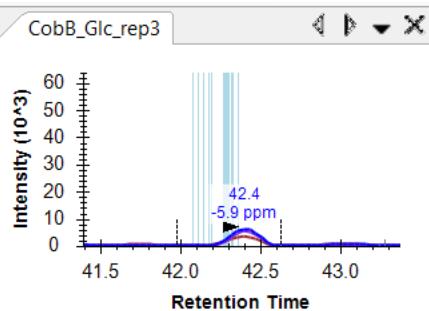
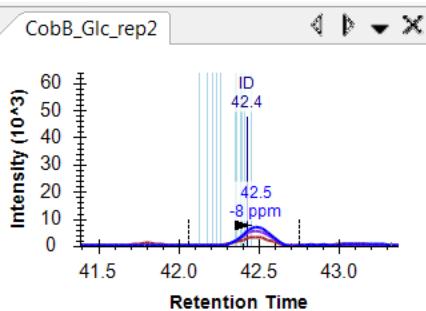
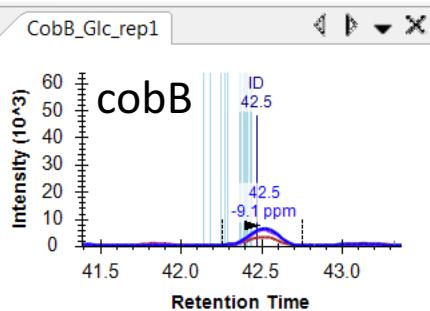
# How does this look in Skyline



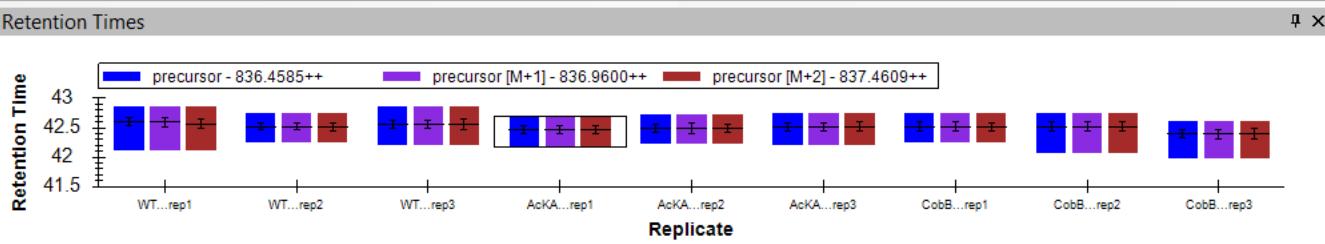
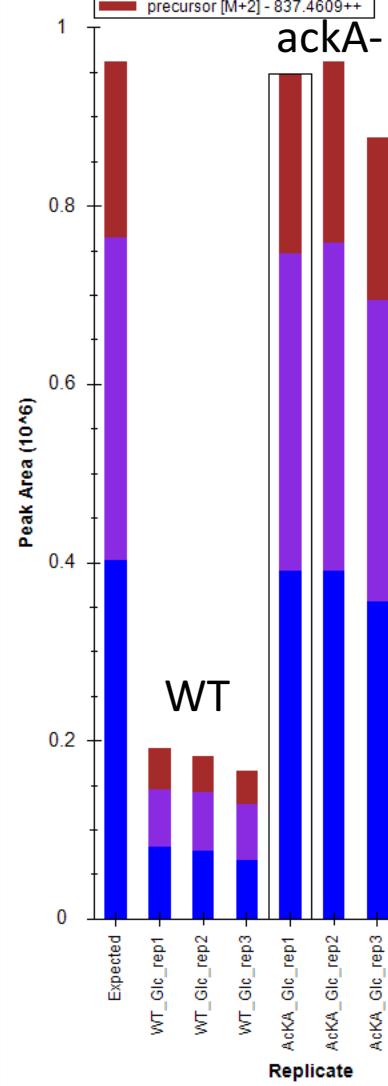
precursor - 836.4585++  
 precursor [M+1] - 836.9600++  
 precursor [M+2] - 837.4609++



precursor - 836.4585++  
 precursor [M+1] - 836.9600++  
 precursor [M+2] - 837.4609++



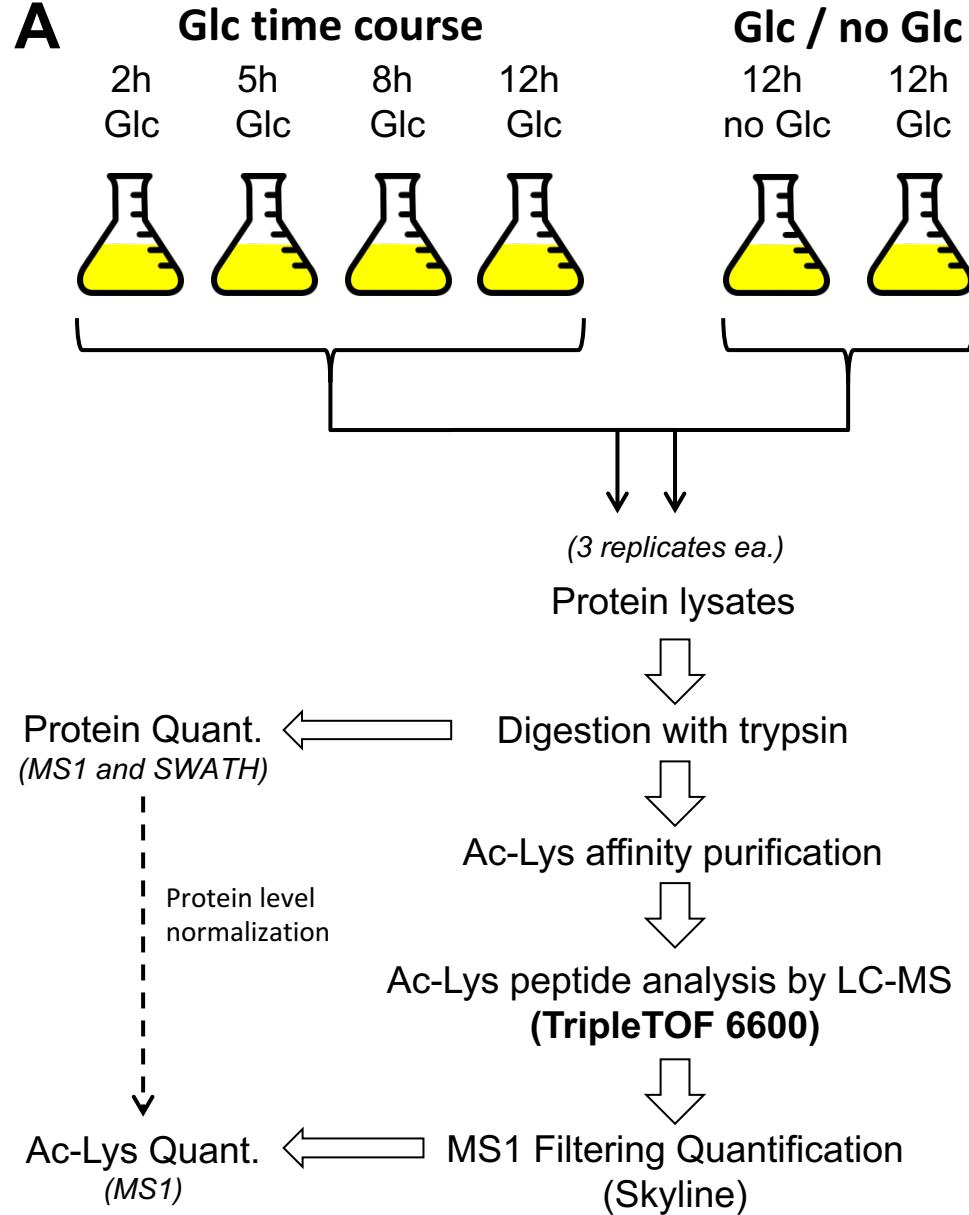
precursor - 836.4585++  
 precursor [M+1] - 836.9600++  
 precursor [M+2] - 837.4609++



Peak Areas Library Match

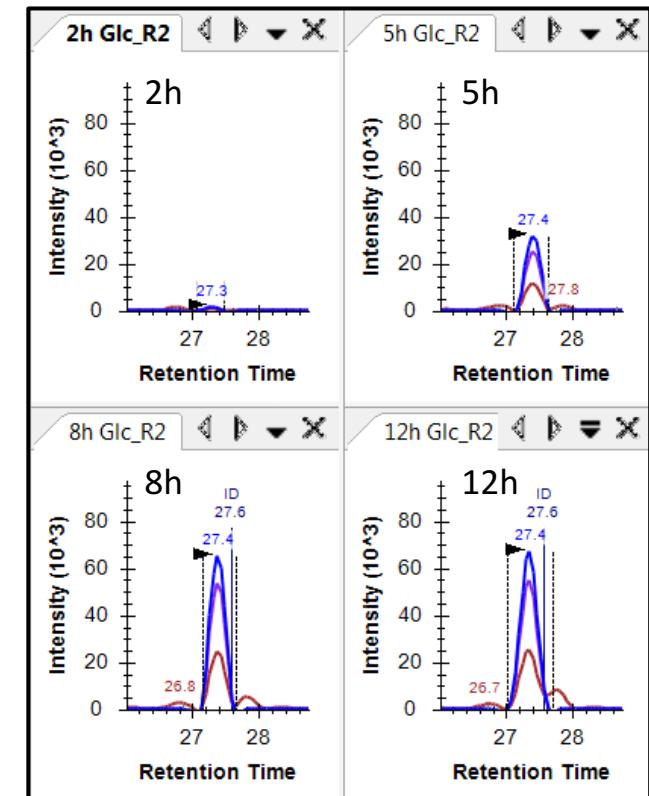
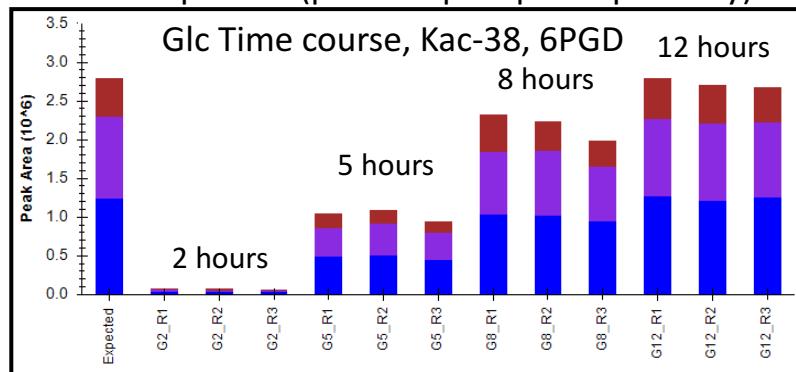
## *E. coli* Glucose dependent acetylation

**A**



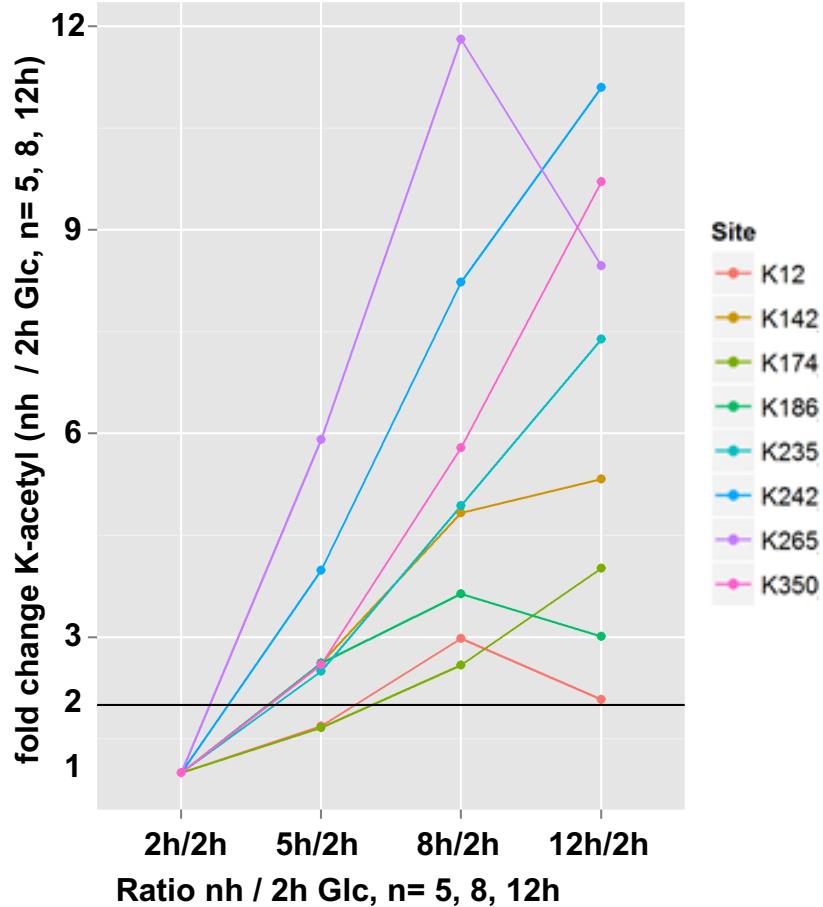
**B**

Example of glucose time course for **EKacTEEVIAENPGKK** (*m/z* 538.62+++), Kac-38 of 6PGD protein (pentose phosphate pathway)

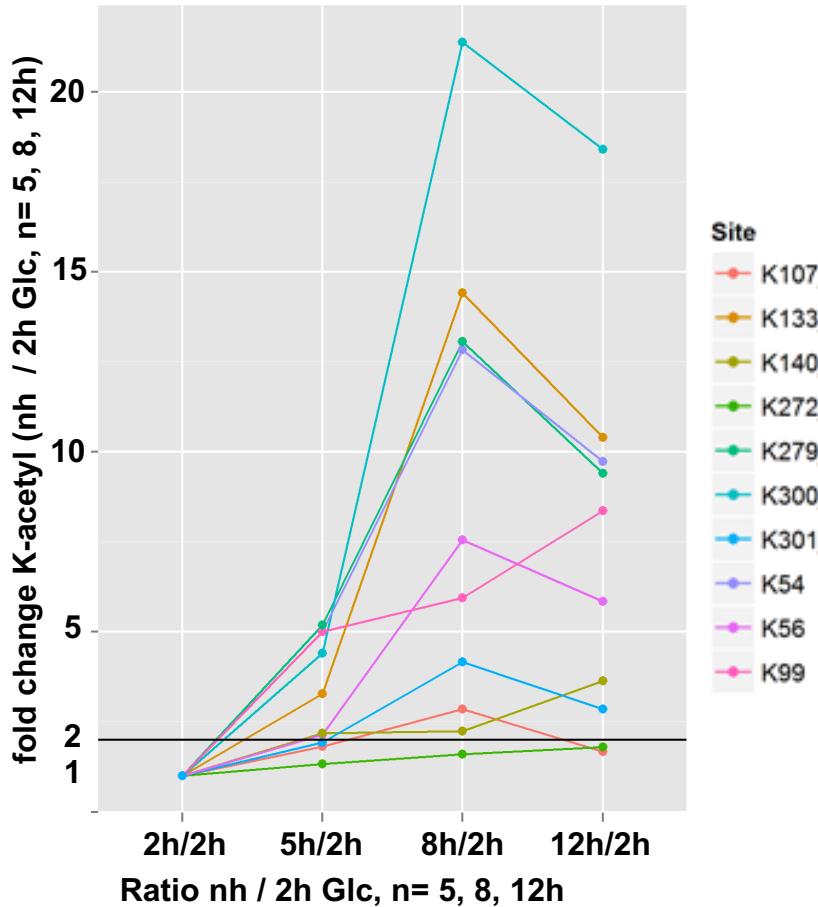


# Following acetylated peptides throughout a time-course, nh / 2h Glc

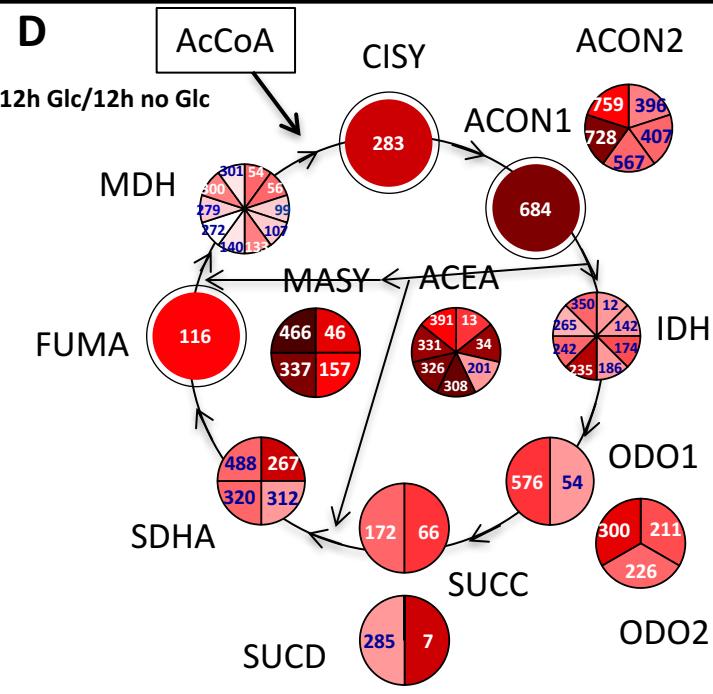
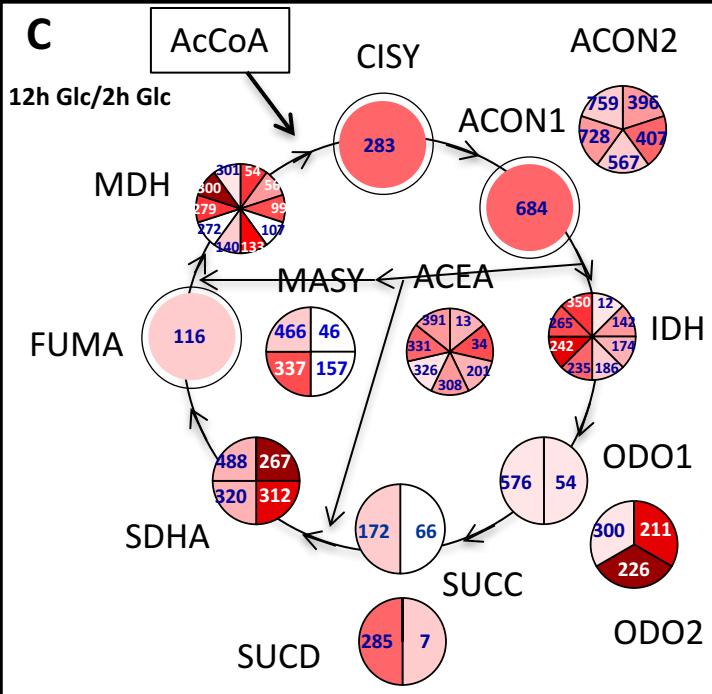
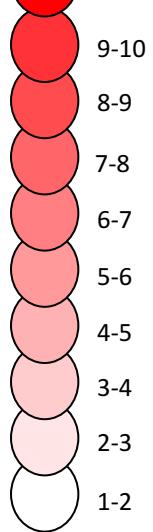
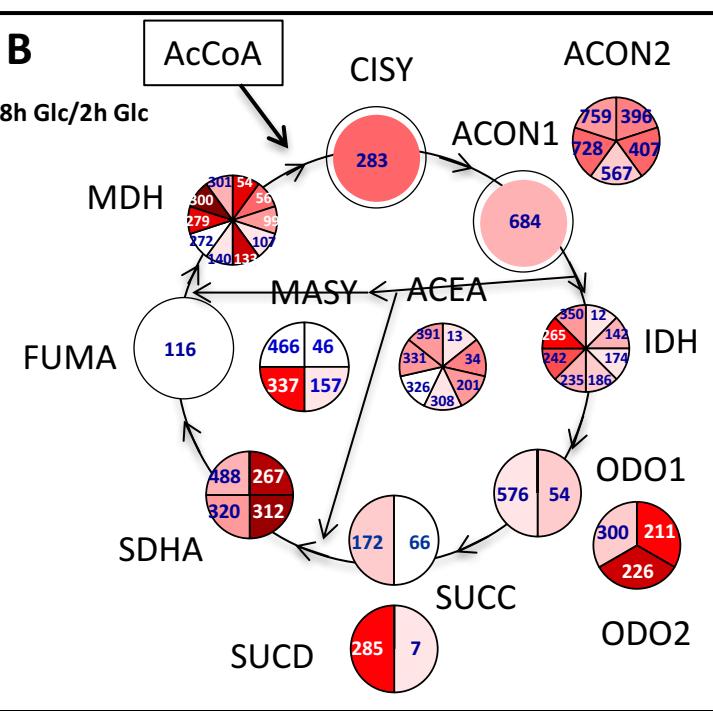
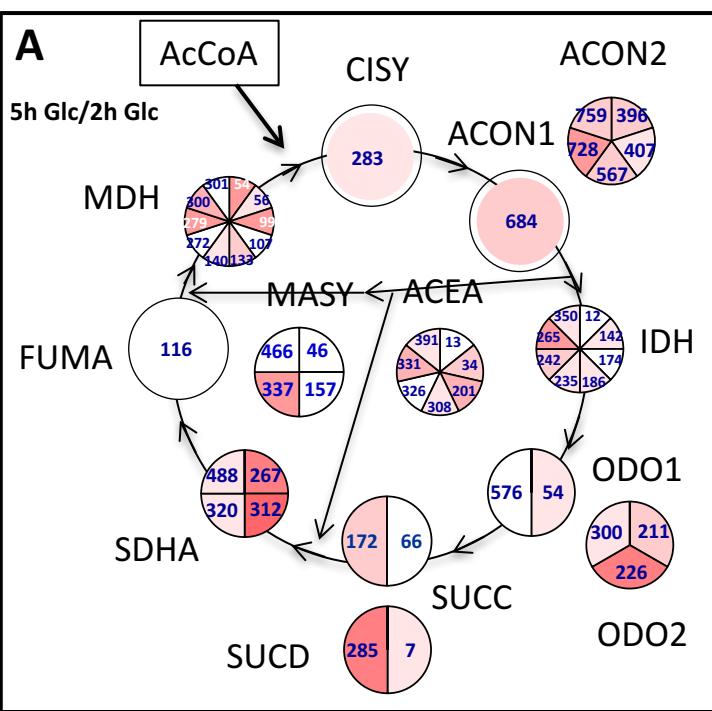
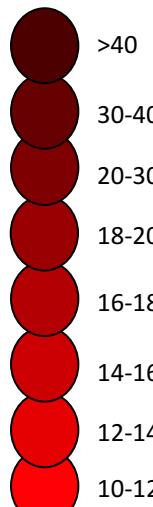
## A Isocitrate dehydrogenase (IDH)



## B Malate dehydrogenase (MDH)

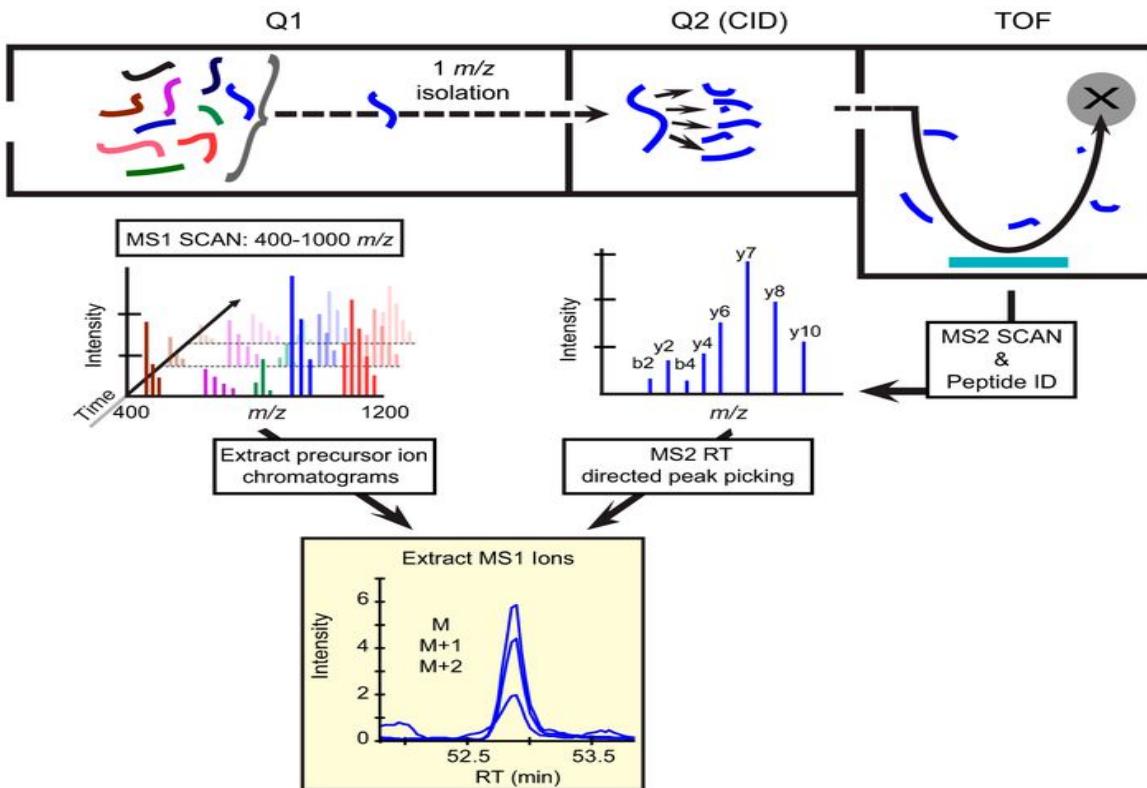


Fold change



# MS1 Scan Extraction on a Triple TOF 6600 Mass Spectrometer

## A. DDA acquisition (top 30 MS/MS)



### EVENTS

1. Peptides enter the MS from HPLC
2. MS1 scans all intact peptides in a mass range (every 3s)
3. One selected peptide is then fragmented by collision induced fragmentation (CID)
4. All fragments are analyzed in the MS2 scan (repeat steps 3-4 x 30)
5. Fragment ions provide sequence information for peptide identification
6. Extract ion intensity chromatograms
7. Retention time information ensures proper peak integration

# From Discovery data sets (MS1 Filtering) to Targeted (SRM) assays

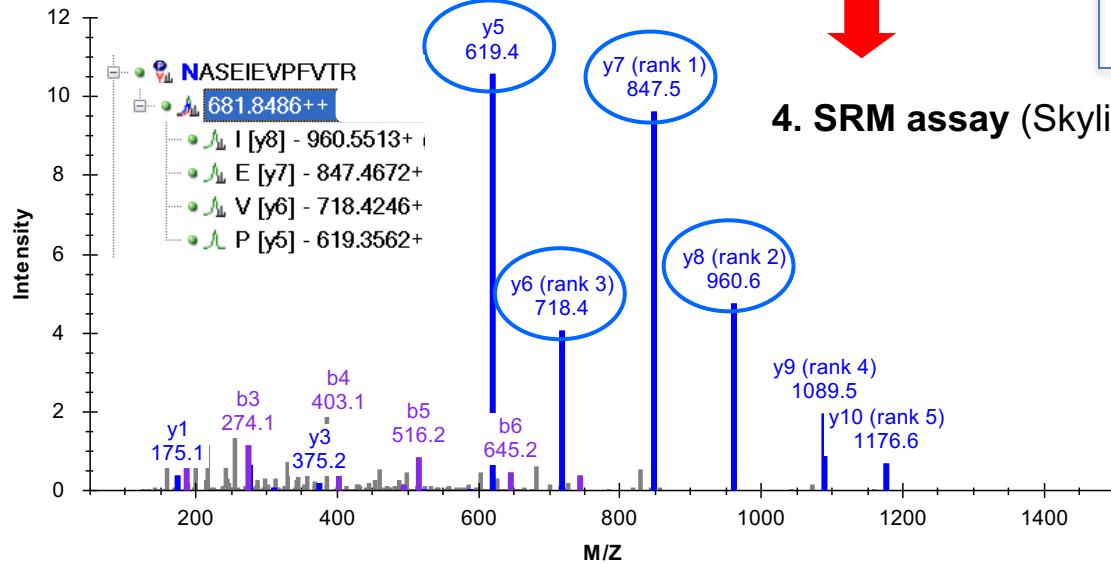
## 1. Discovery of de-glycopeptides (DDA)



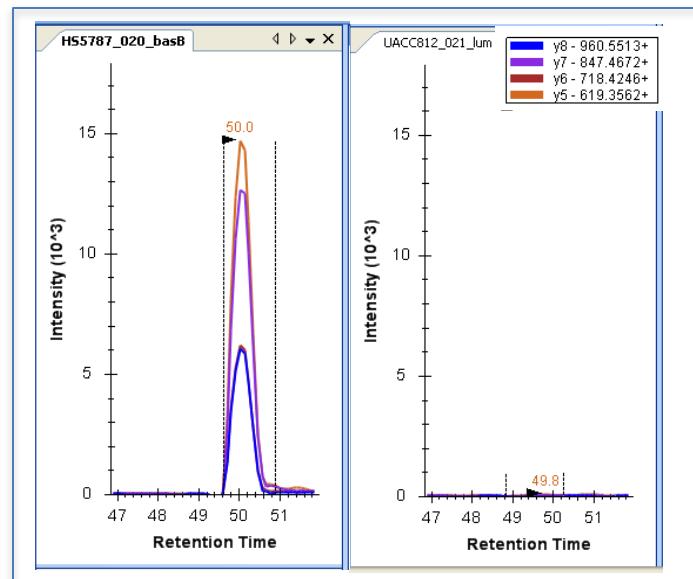
## 2. Label-free Quantitation with MS1 Filtering



## 3. Spectral Library in Skyline for SRM selection



## 4. SRM assay (Skyline)



## 5. LC-SRM MS Assessment (QQQ)



## 6. Multiplexing of LC-SRM MS Assay



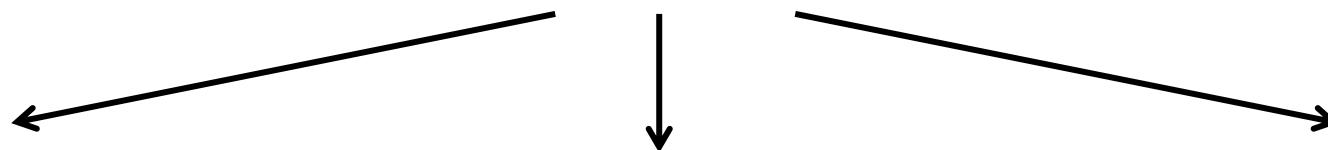
## 7. Plasma LC-SRM MS Assay

# Dynamic Workflows in Skyline

Data Dependent Acquisitions (DDA) using MS1 Filtering



Data Independent Acquisitions (DIA) :  
(targeted assays)



**SRM**

Selected reaction monitoring

**MRM-HR / PRM**

High-resolution MRM

Parallel Reaction Monitoring

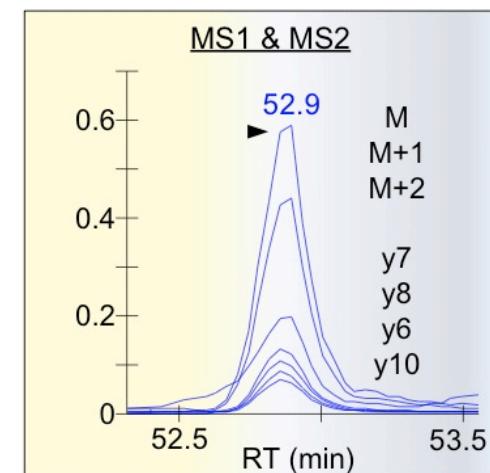
**SWATH-like DIA**

## Additional Considerations:

MS1 Peptide Ion Intensity Chromatograms in MS2 (SWATH)

Data Independent Acquisitions. Improving Post Acquisition Analysis of Proteomic Experiments.

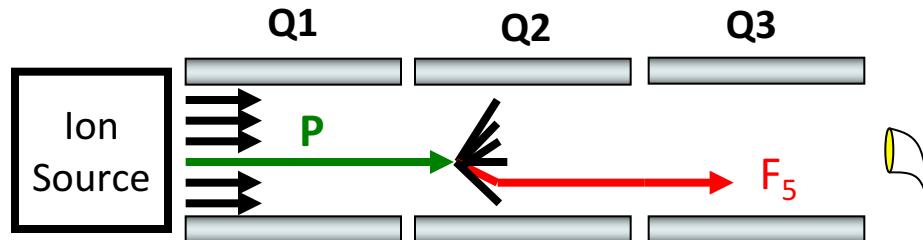
Rardin, Schilling et al. *MCP* under revision



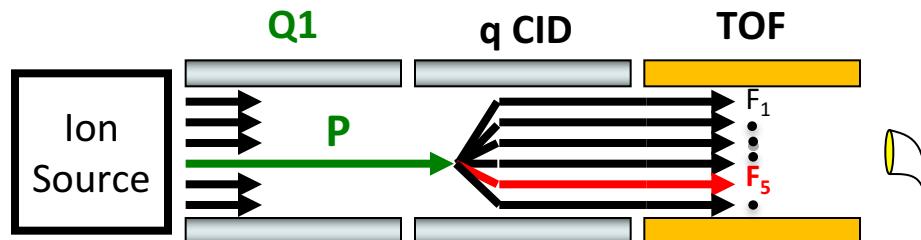
# Skyline 'MS2 Filtering' (Pseudo-MRM, MRM-HR, PRM)

PRM: Parallel Reaction Monitoring

## SRM-MS with triple quadrupoles



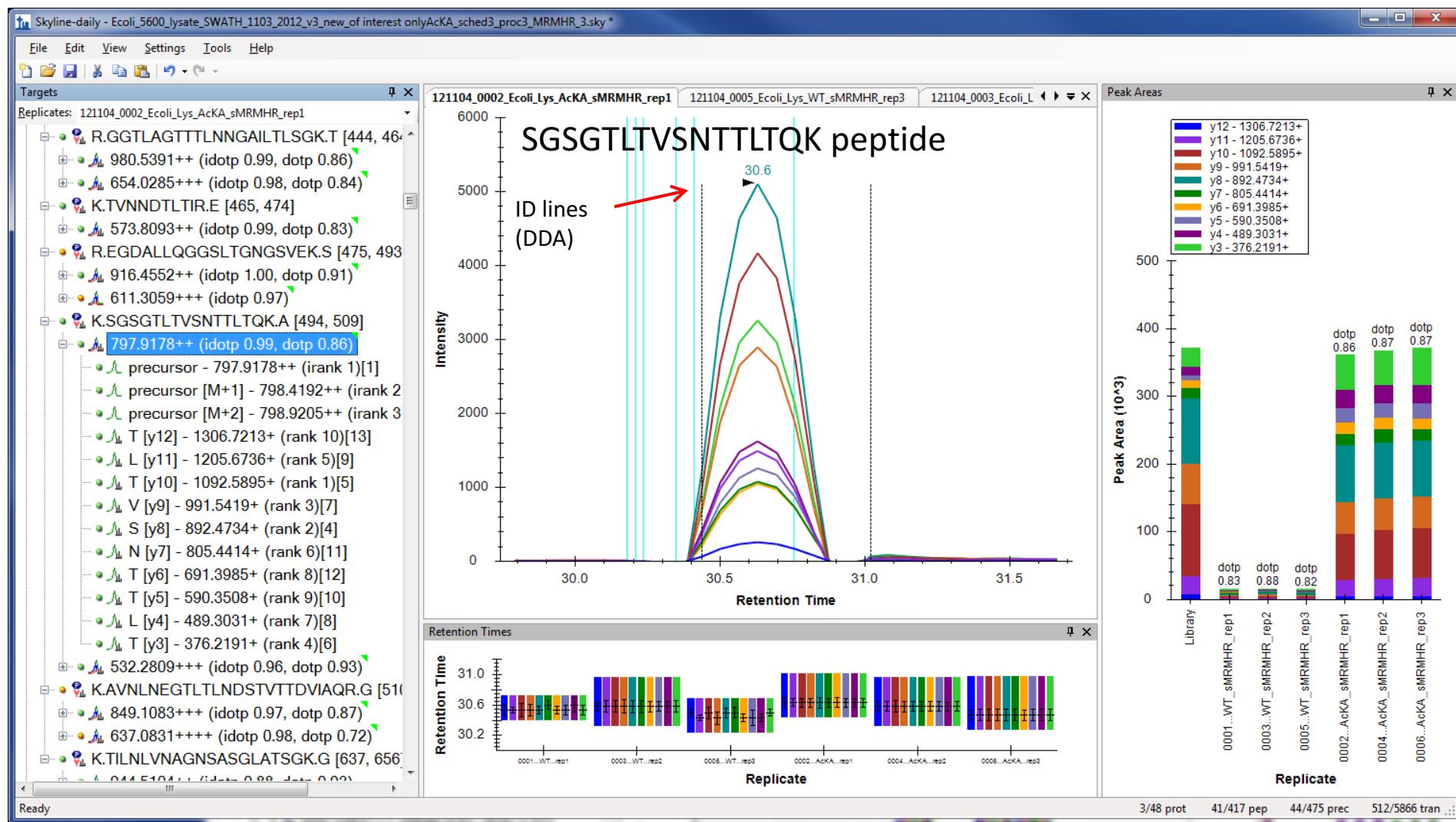
## PRM with QqTOF (and other high resolution MS platforms\*)



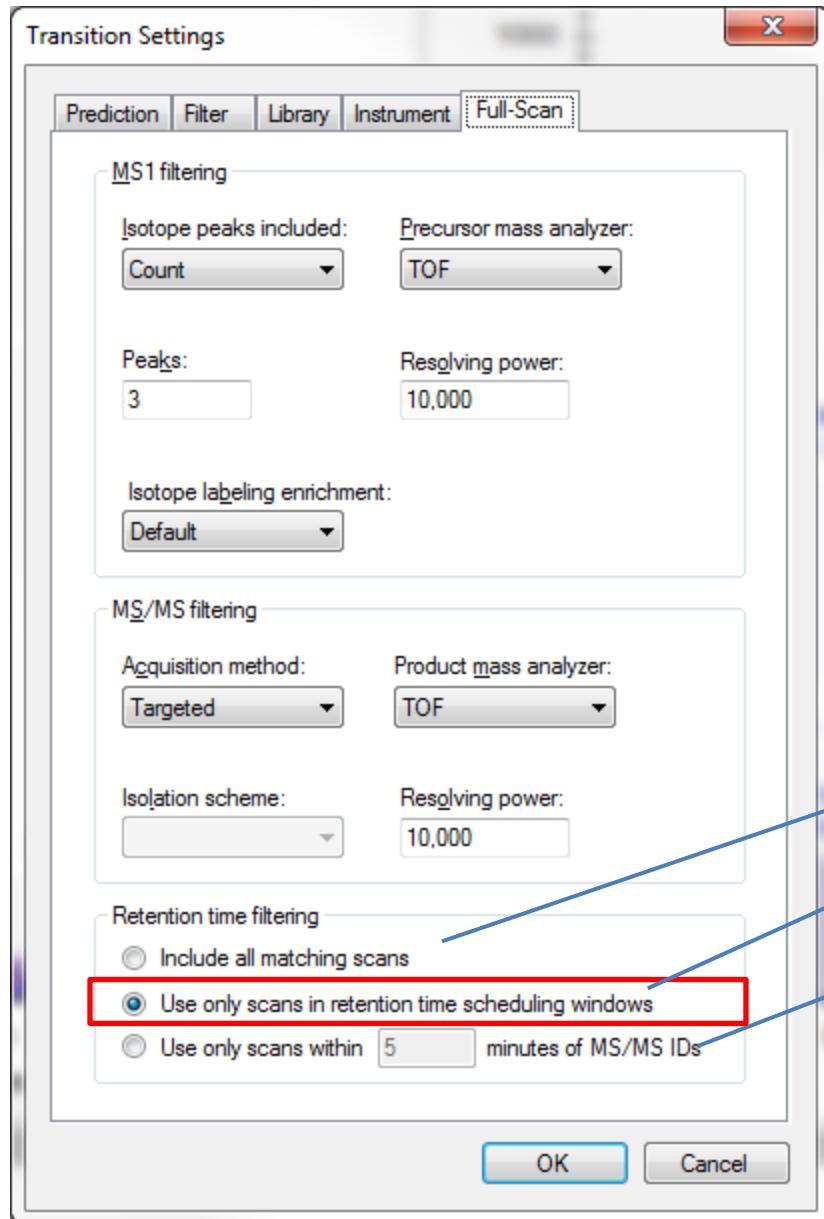
\*Frequently used platforms for PRM: QExactive, Fusion

# How does PRM Data look in Skyline - example

Targeted PRM acquisitions, *E. coli*: 3x WT, 3x mutant, MS2 Quantitation  
Fragment ions y3-y12



# Setup of Skyline for file import of PRM files

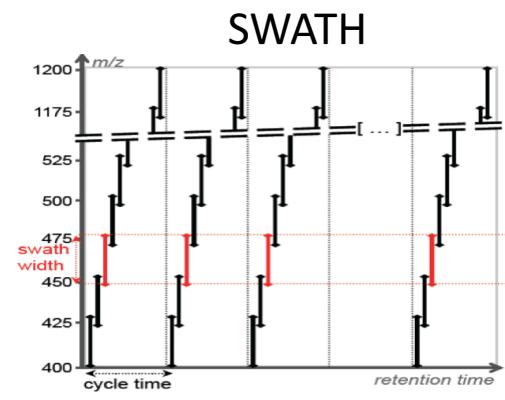
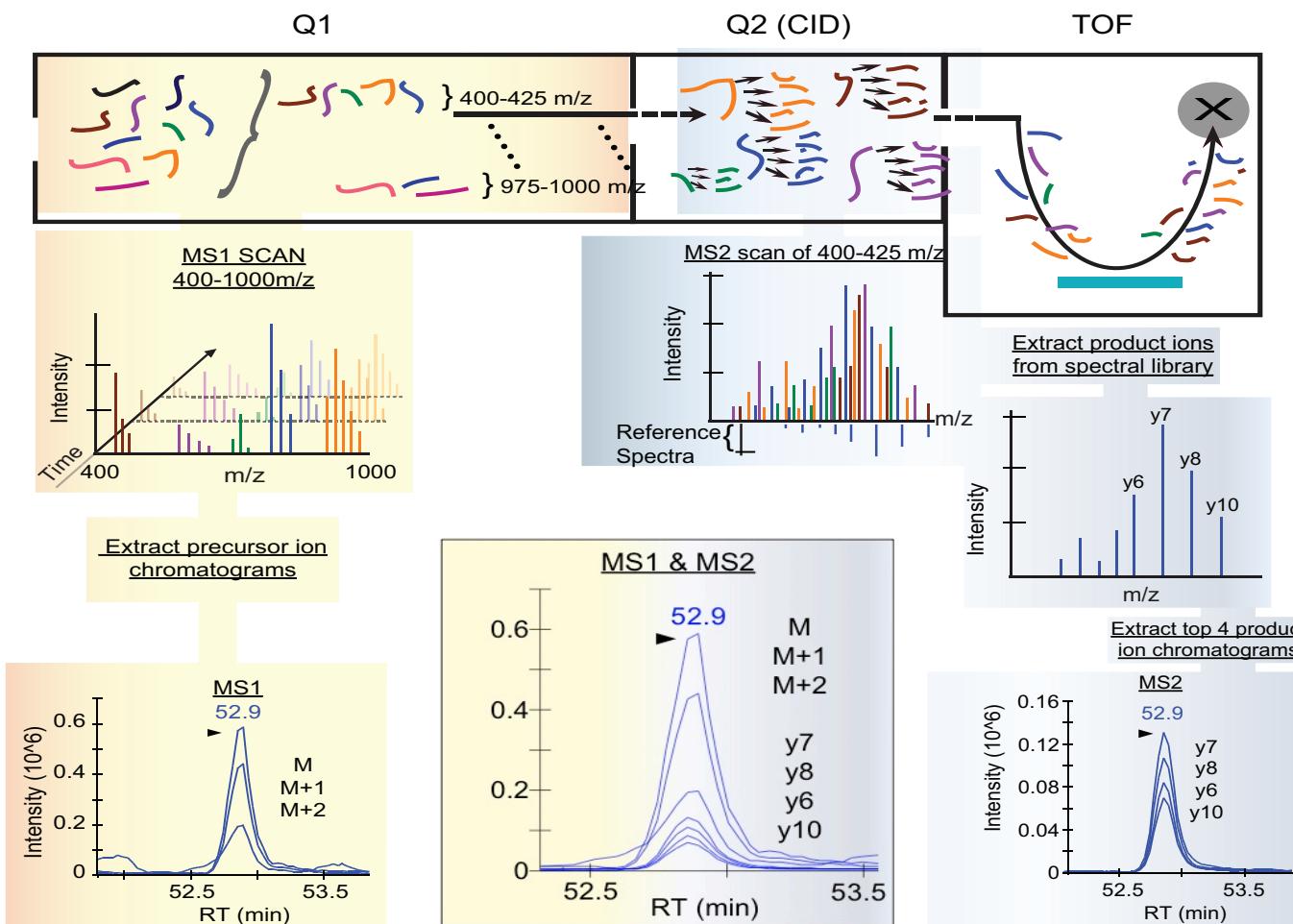


Make sure your peptide tree is populated with added fragment ions based on spectral library fragmentation and “File - Transition Settings – Filter Tab and Library Tab” settings

Either use full chromatogram import  
**Typical setting chosen for sPRM files**

Note: sPRM data sets can be searched through regular database search engines, then build a spectral library in Skyline, activate the library, then import sPRM runs, when checking this field the peak picking upon file import will be directed by the identified MS/MS in the spectral library.

# Data Independent Acquisition (DIA) - TripleTOF 6600



Rardin, Schilling  
MCP 2015