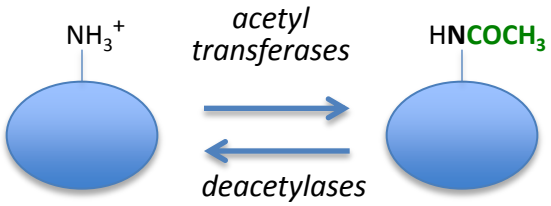
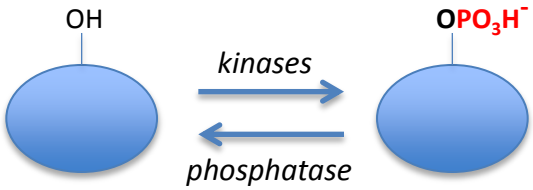
Platform Independent and Label-free Quantitation of Protein Acetylation and Phosphorylation using MS1 Extracted Ion Chromatograms in Skyline



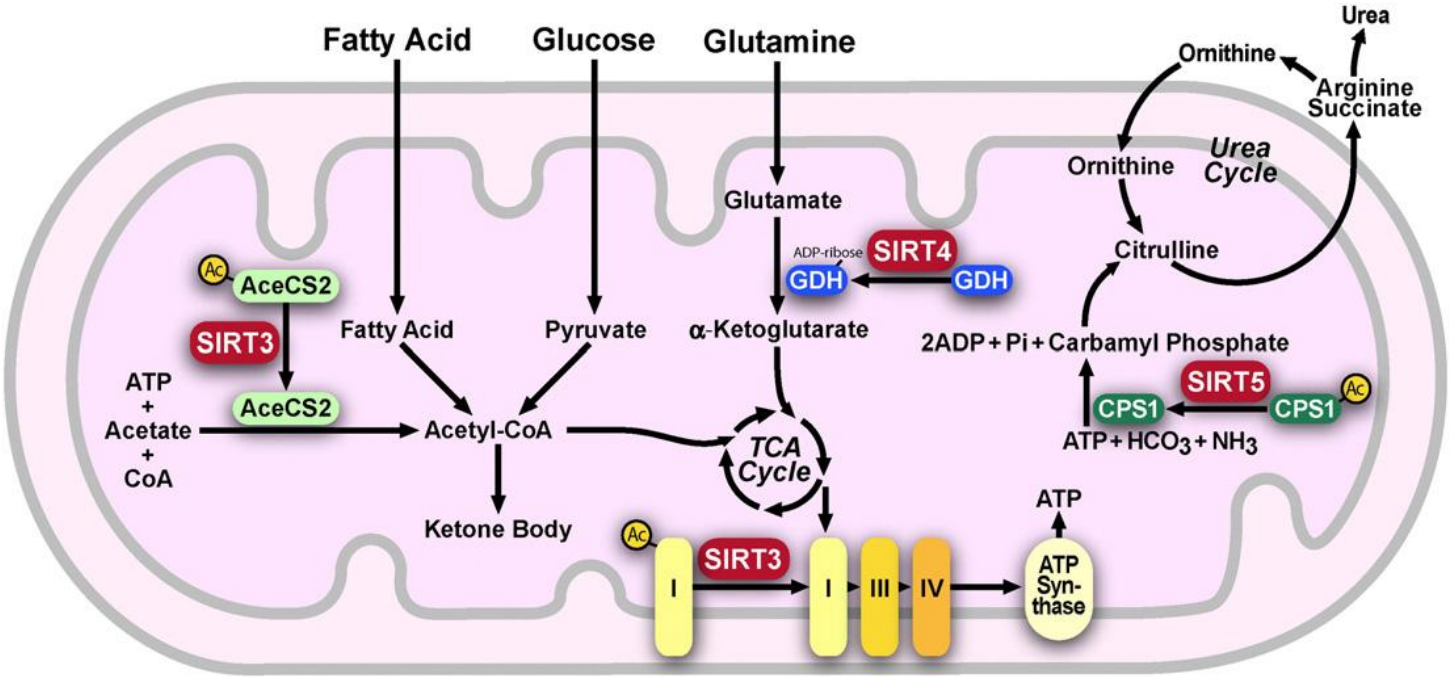
Birgit Schilling
Buck Institute for Research on Aging

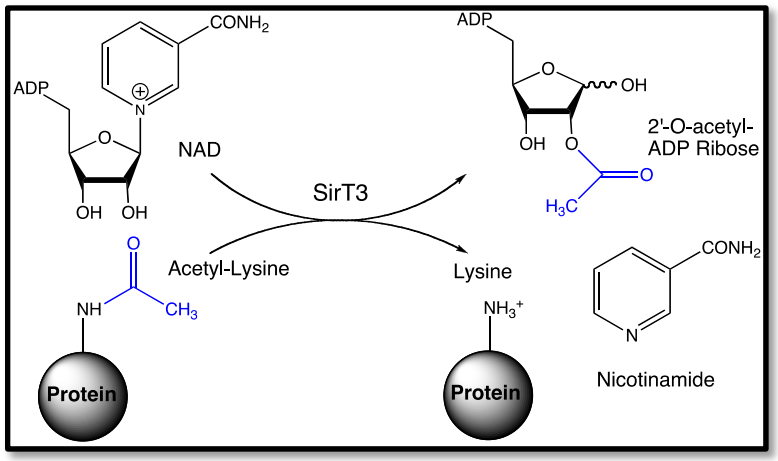


Skyline

Workshop ASMS 2012

Mitochondrial Sirtuins and Metabolic Regulation

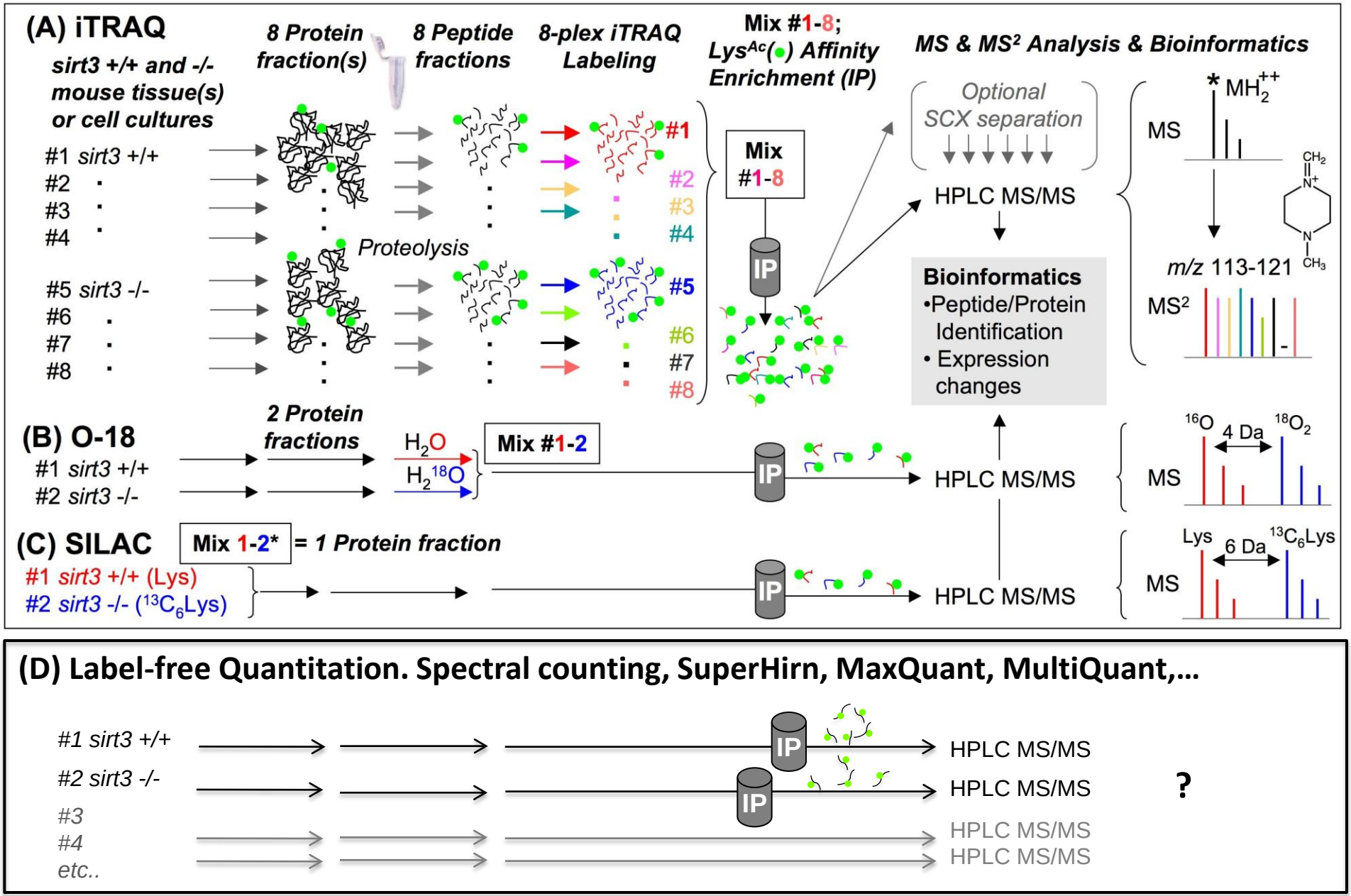




- ***Type 2 Diabetes***
- ***Metabolic syndromes***
- ***High/low-fat diet***
- ***Aging***
- ***Neurodegenerative diseases?***

MOH, 9:30 am : **M. Rardin** et al. “Quantitation of the Mitochondrial Lysine Acetylome in SIRT3 Knockout Animals using MS1 Filtering in Skyline.”

Designing a Quantitative Discovery Immunoaffinity-based 'Acetylproteome' Workflow



(D) Label-free Quantitation. Spectral counting, SuperHirn, MaxQuant, MultiQuant,…

Label-free, quantitative full scan filtering workflows in Skyline

MacLean et al., ASMS 2011 Poster:

"Skyline: Targeted Proteomics with Extracted Ion Chromatograms from Full-Scan Mass Spectra"

Buck Institute Workflows:

Using an AB SCIEX TripleTOF™ 5600 System to identify and quantitate posttranslationally modified peptides.

Ion chromatogram extraction (XICs) from **MS1 scans** that were acquired as part of data dependent acquisitions (DDA).

Skyline MS1 Filtering

Schilling et al., ASMS 2012, Tuesday TP03 Poster 081

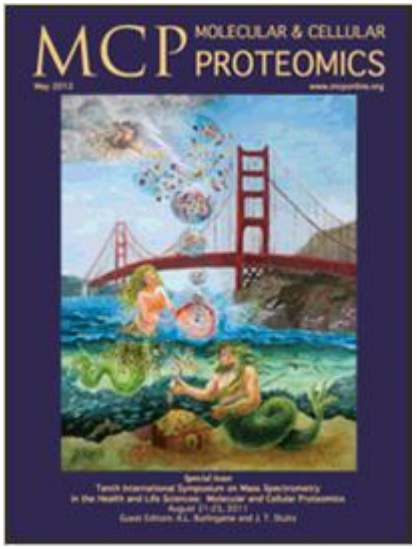
Author's Choice

Platform-independent and Label-free Quantitation of Proteomic Data Using MS1 Extracted Ion Chromatograms in Skyline

APPLICATION TO PROTEIN ACETYLATION AND PHOSPHORYLATION*[S]

Birgit Schilling‡§, Matthew J. Rardin‡§, Brendan X. MacLean§¶, Anna M. Zawadzka‡, Barbara E. Frewen¶, Michael P. Cusack‡, Dylan J. Sorensen‡, Michael S. Bereman¶, Enxuan Jing‖, Christine C. Wu, Eric Verdin‡‡, C. Ronald Kahn‖, Michael J. MacCoss¶§§, and Bradford W. Gibson‡¶¶¶**

MCP, May 2012



Page 202–214

Skyline > Start Page > Tutorials >

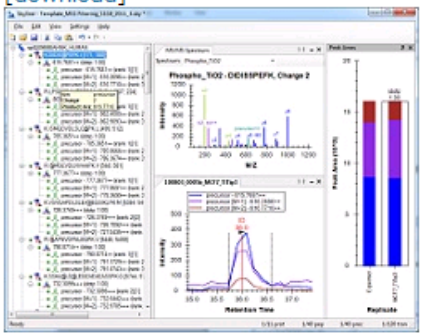
MS1 Full-Scan Filtering

Tutorial

PRINT

Get hands-on experience creating a Skyline document to measure quantitative differences in peptide expression using the MS1 scans from your data dependent acquisition (DDA) experiments. In this tutorial, you will generate a spectral library from a discovery data set, set up a Skyline document for MS1 filtering, import raw mass spectrometer data to extract precursor ion chromatograms from MS1 scans, with peak picking guided by MS/MS peptide identifications, and further process the resulting quantitative data in Skyline. If you are interested in label-free quantitative analysis of discovery data sets, this tutorial will give you a new tool set for your investigation. *(25 pages)*

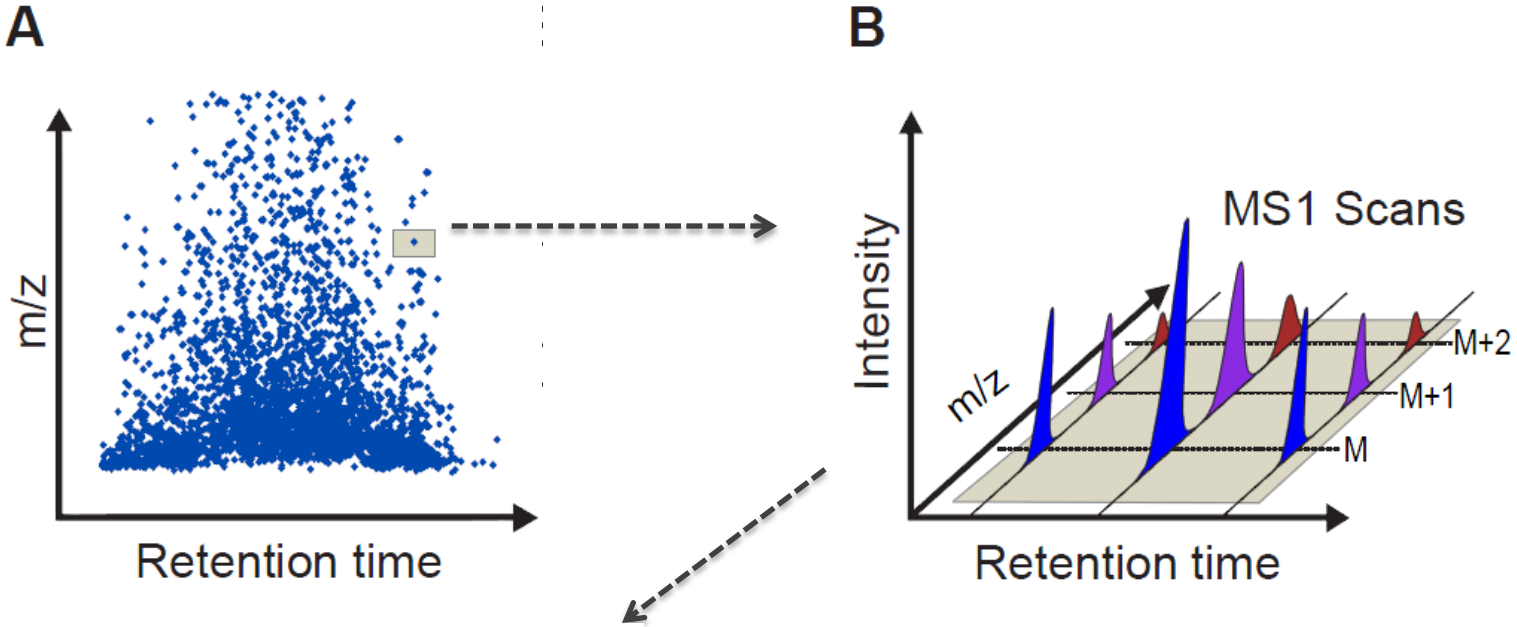
[download]

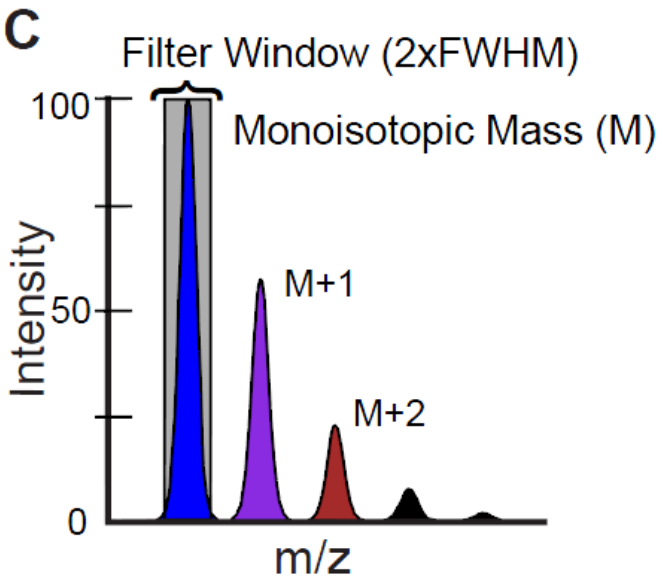


(http://proteome.gs.washington.edu/software/Skyline/tutorials/ms1filtering.html)

Skyline MS1 Filtering

A quantitative tool for discovery proteomics experiments

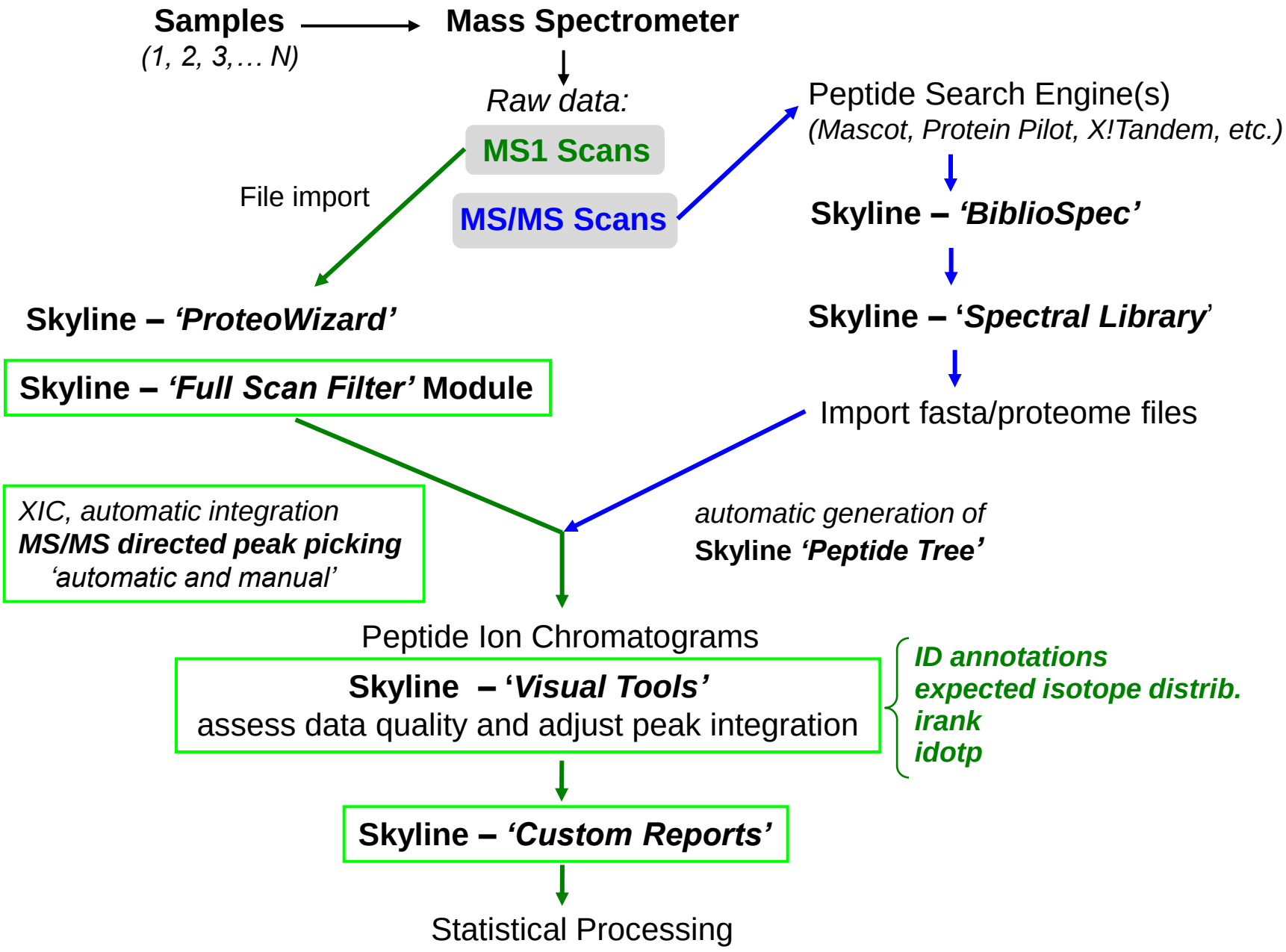




- *Follow a few peptide analytes to >3000 peptides*
- *Label-free*
- *Post analysis design*
- *Skyline interface and tools*
- *MS platform/manufacturer independent (QqTOF, FT and ITs)**

- *MacLean et al., ASMS abstract 2011*
- *Schilling et al., MCP, 11, **2012**, 202-214.*

Proteomic Data Flow in Skyline MS1 Filtering

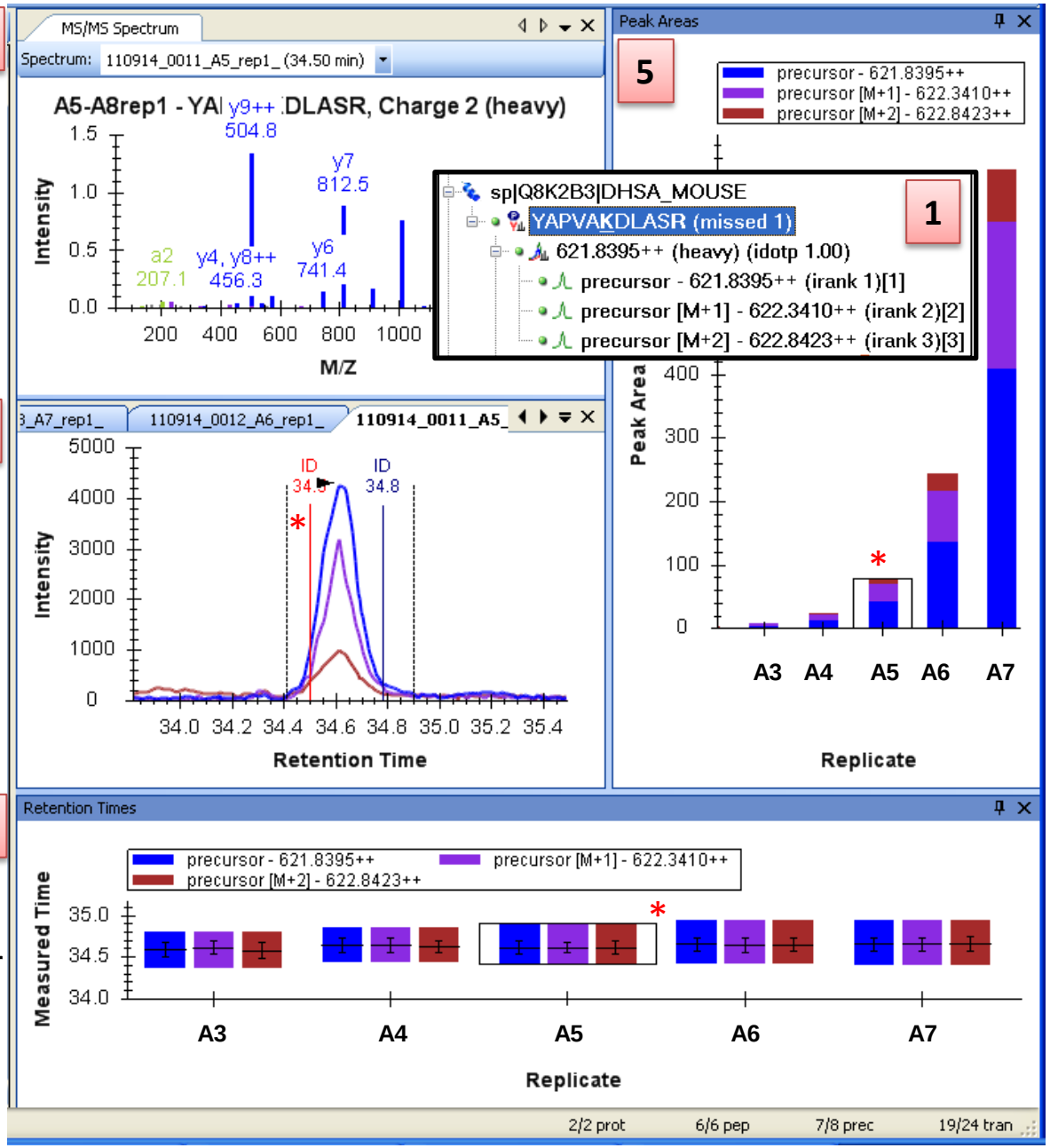


Skyline interface for MS1 filtering data

3) MS/MS spectra and ID

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

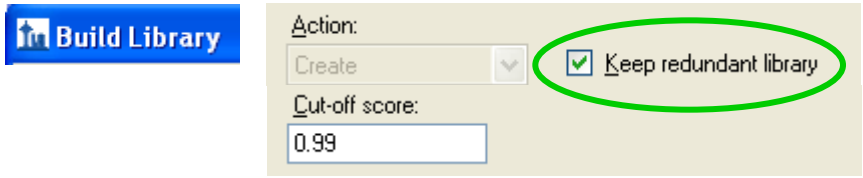
4) RT variation among peptides and replicates for each precursor isotope (M, M+1, M+2)



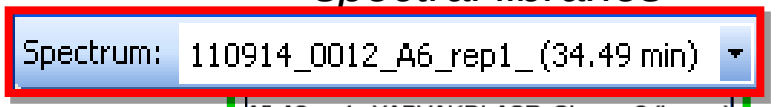
5) M, M+1,M+2 precursor peak areas

1) Peptide ‘tree’ with precursors
- irank
- idotp

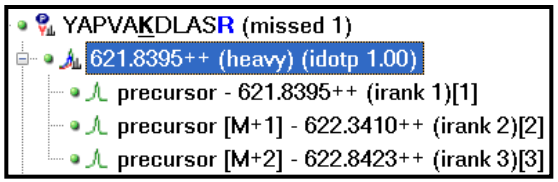
MS/MS (ID) directed Peak Picking for Skyline MS1 Filtering



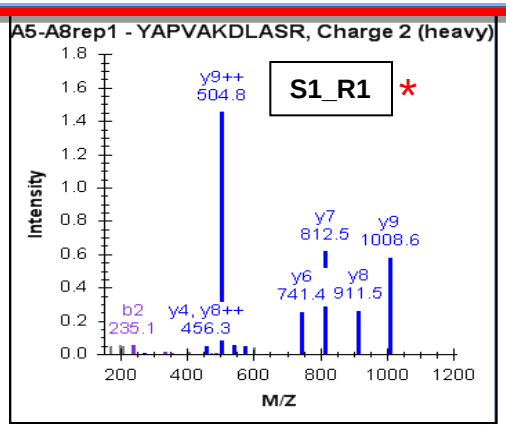
*MS/MS from **redundant** spectral libraries*



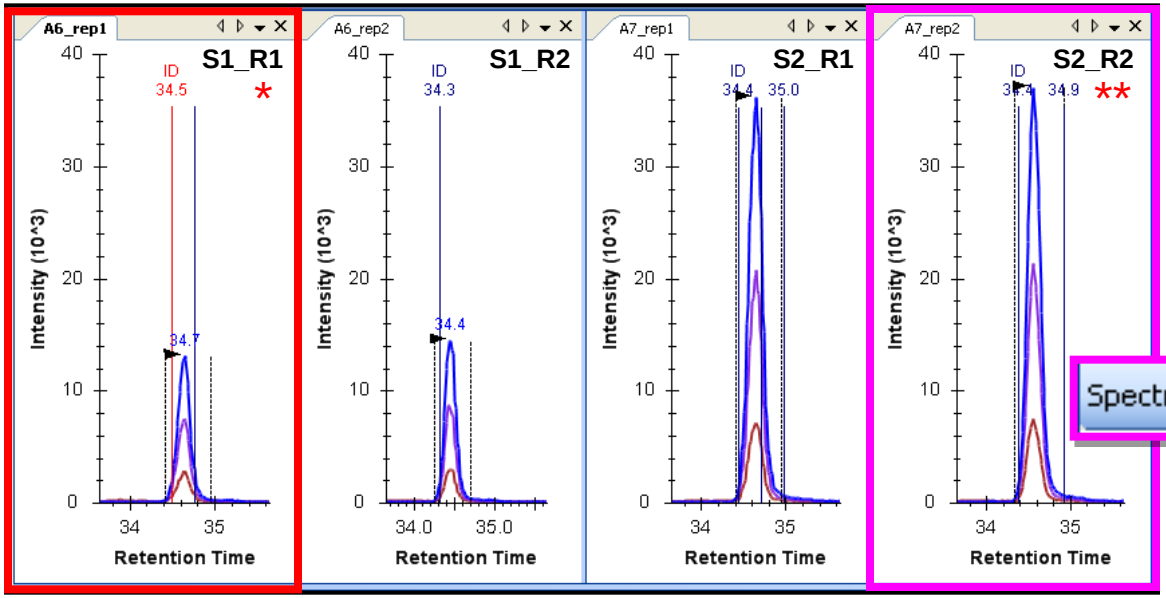
A

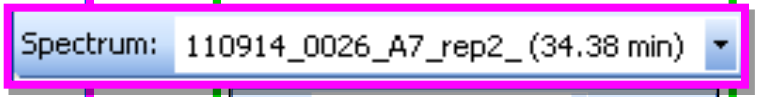


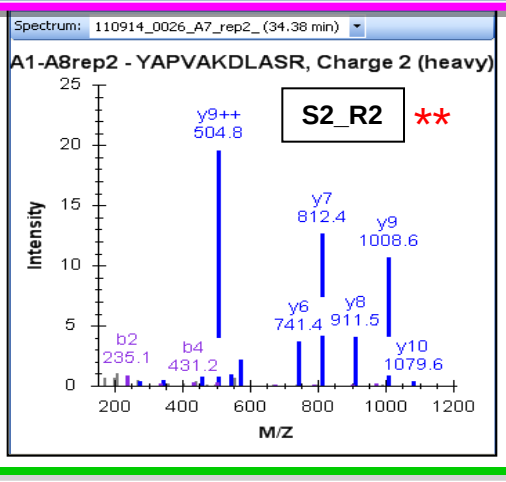
precursor M 621.8395++
precursor M+1 622.3410++
precursor M+2 622.8423++



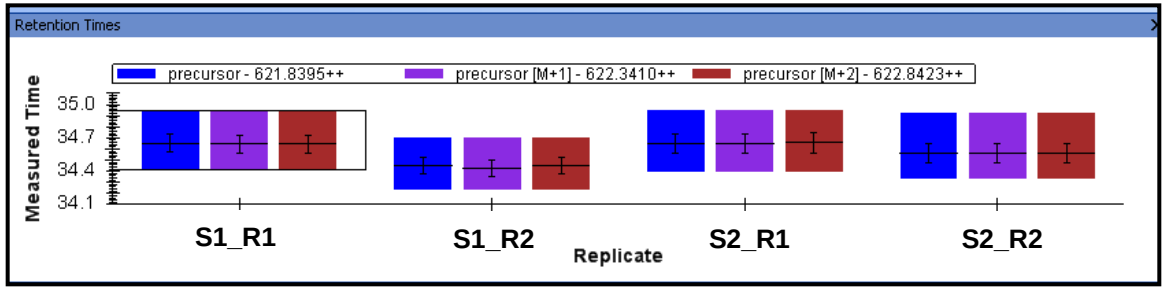
B





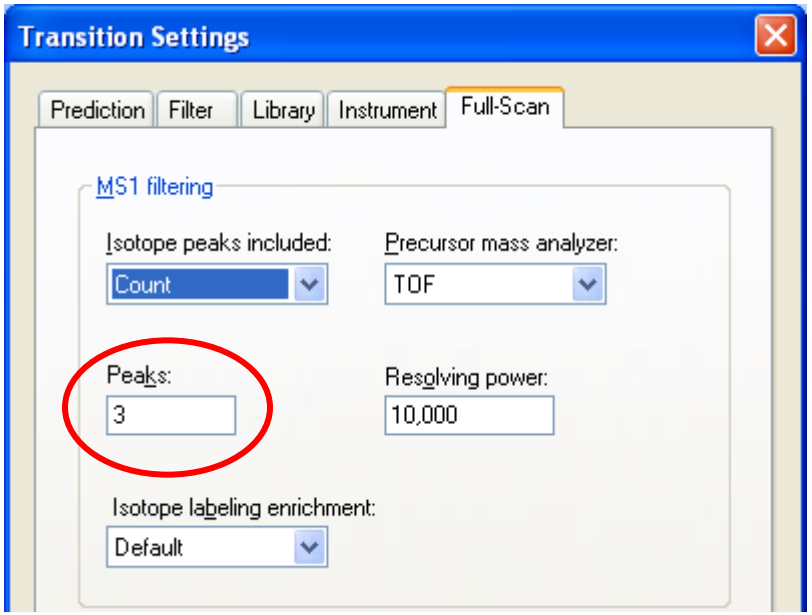


C



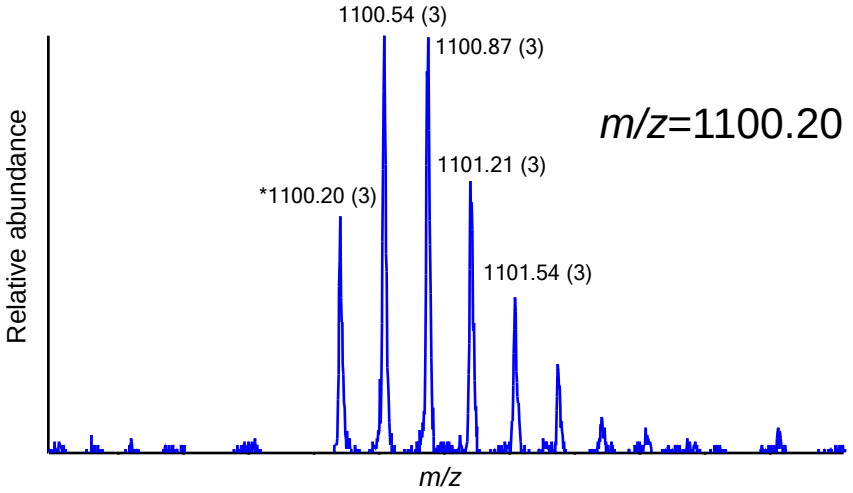
Advantage of quantitating multiple precursor isotopes, M, M+1, M+2

A

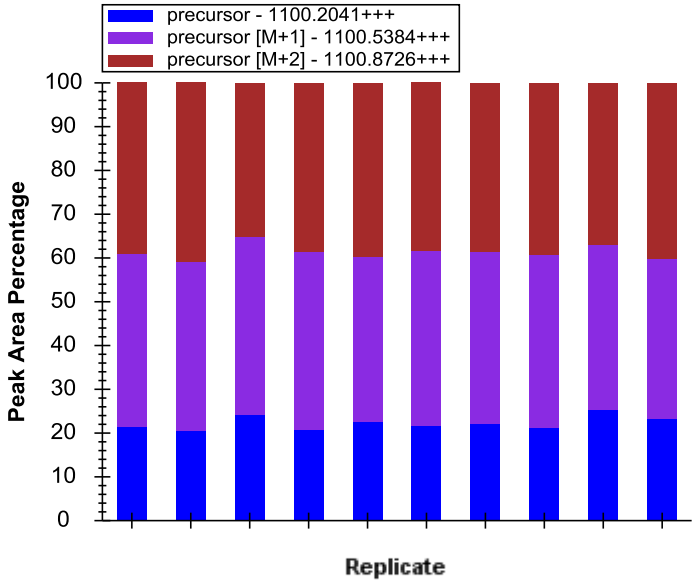


B Measured MS1 Peak Isotope distribution

GTLLYGTPTMFVDILNQPDFSSYDFTSIR 3+



C



D Observed peak area CV over 9 replicates

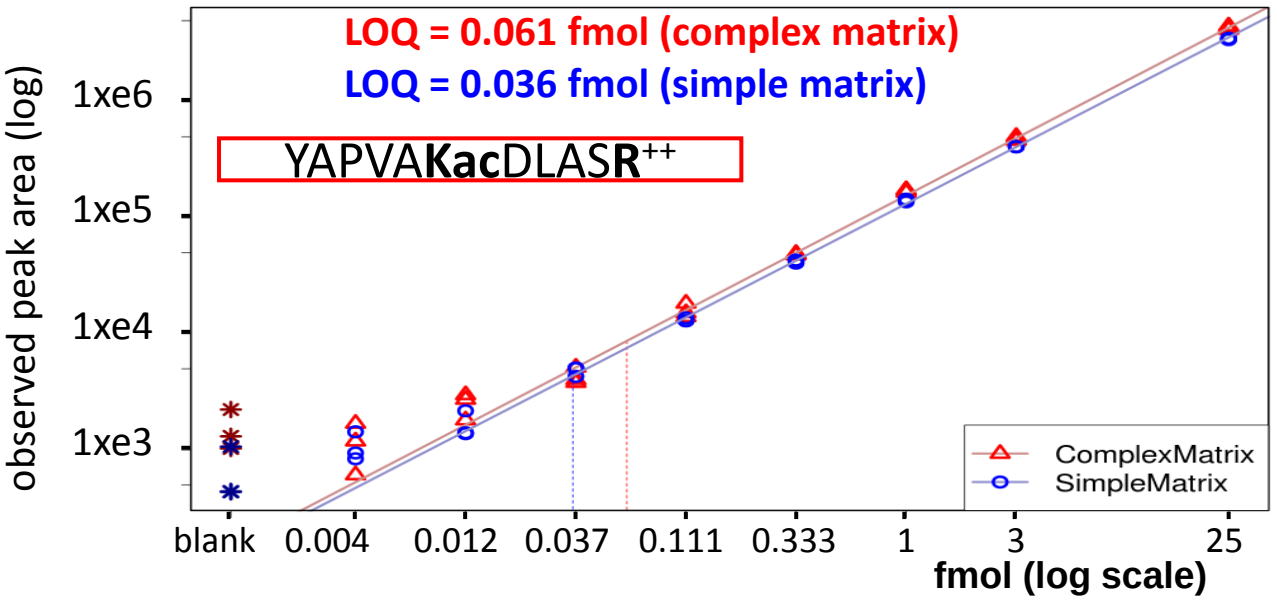
GTLLYGTPTMFVDILNQPDFSSYDFTSIR

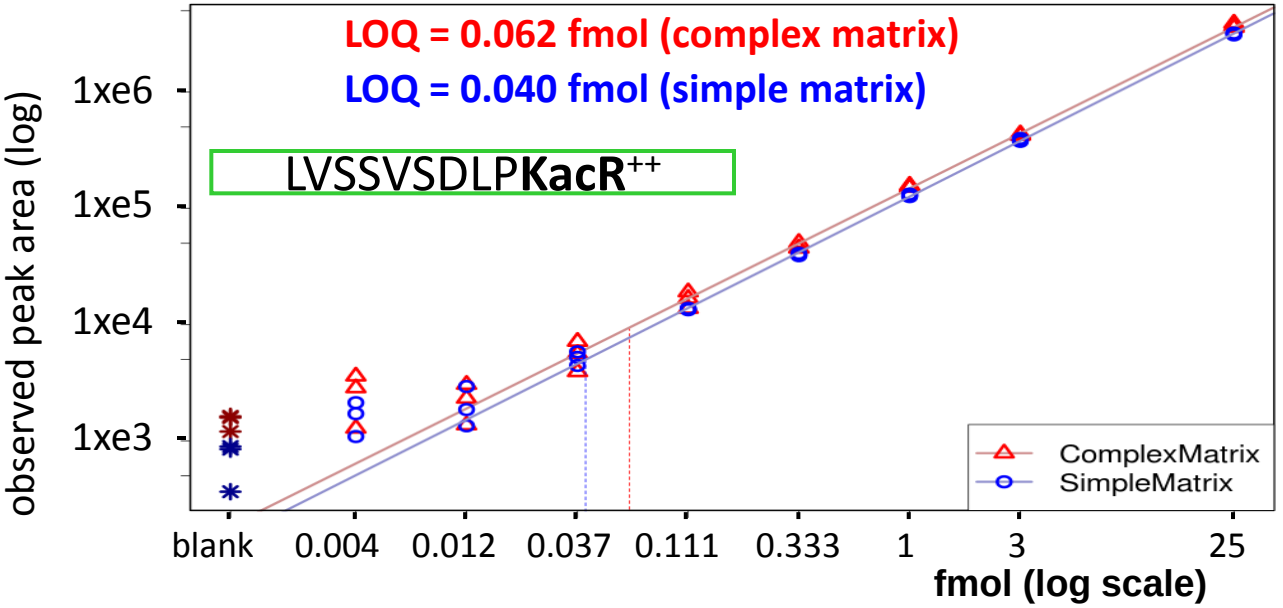
CvArea	ProductMz	FragmentIon	IsotopeDist Rank	IsotopeDist Proportion
16.20%	1100.2041	precursor	4	0.14
17.80%	1100.5384	precursor [M+1]	1	0.26
18.50%	1100.8726	precursor [M+2]	2	0.25

MS1 Filtering Standard Concentration Curves for Lys-Ac Peptides

TripleTOF 5600

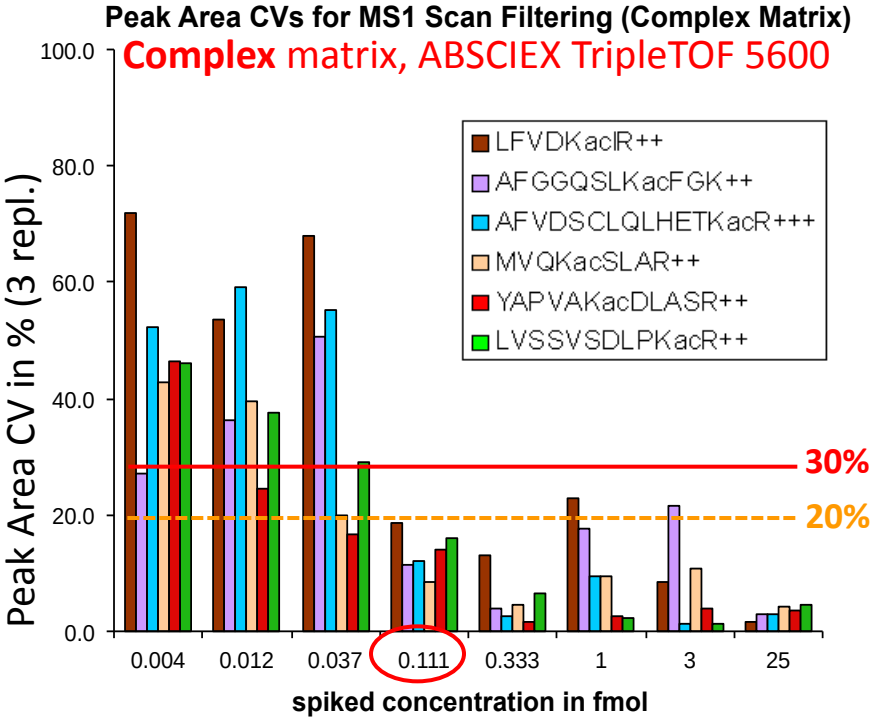
- 6 peptide mix at 4 amol to 50 fmol
- Both simple and complex matrices
- Triplicate analysis +/- background matrices

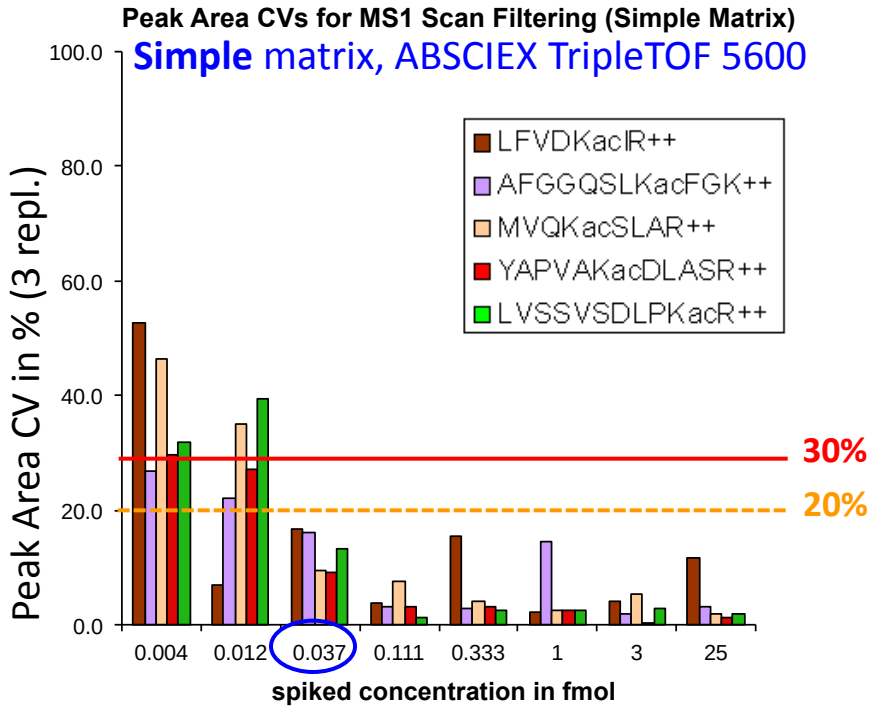


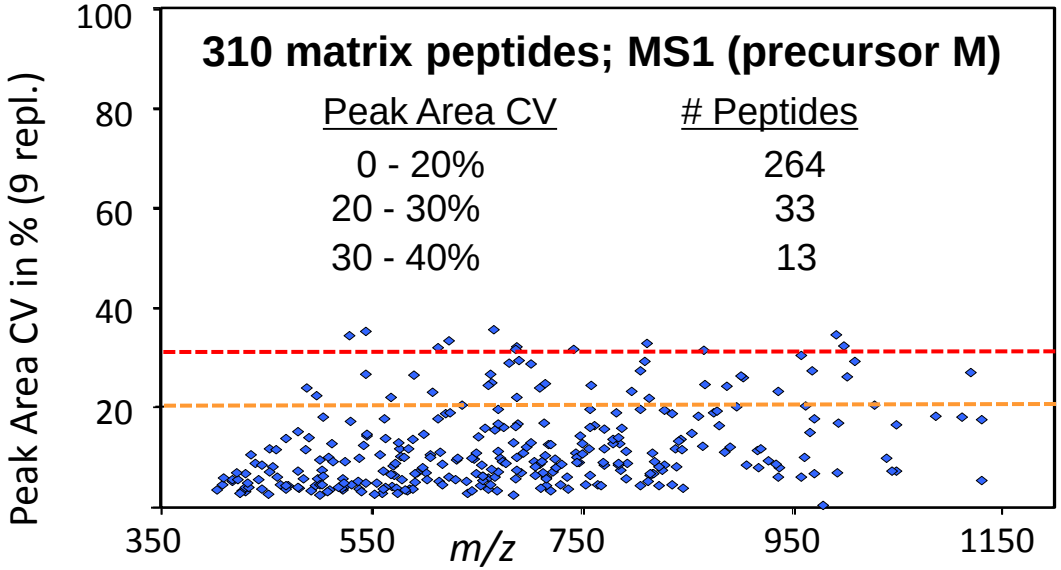


regression slopes:
1.03 & 1.03 for YAP
0.99 & 1.00 for LVS

Reproducibility for MS1 Scan Filtering



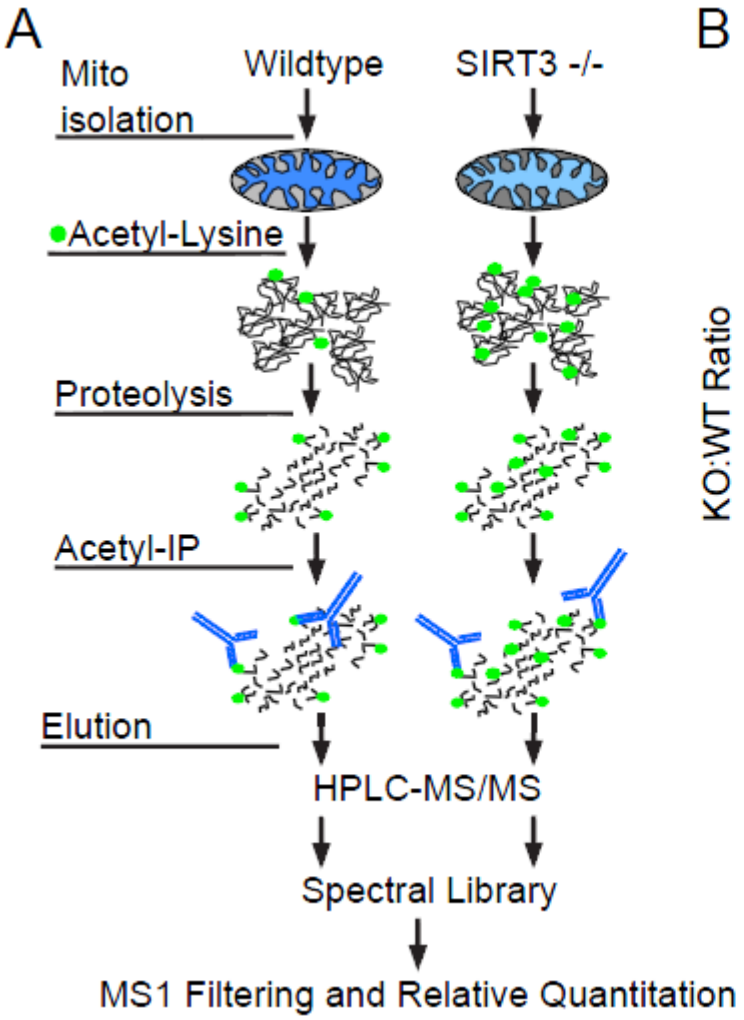


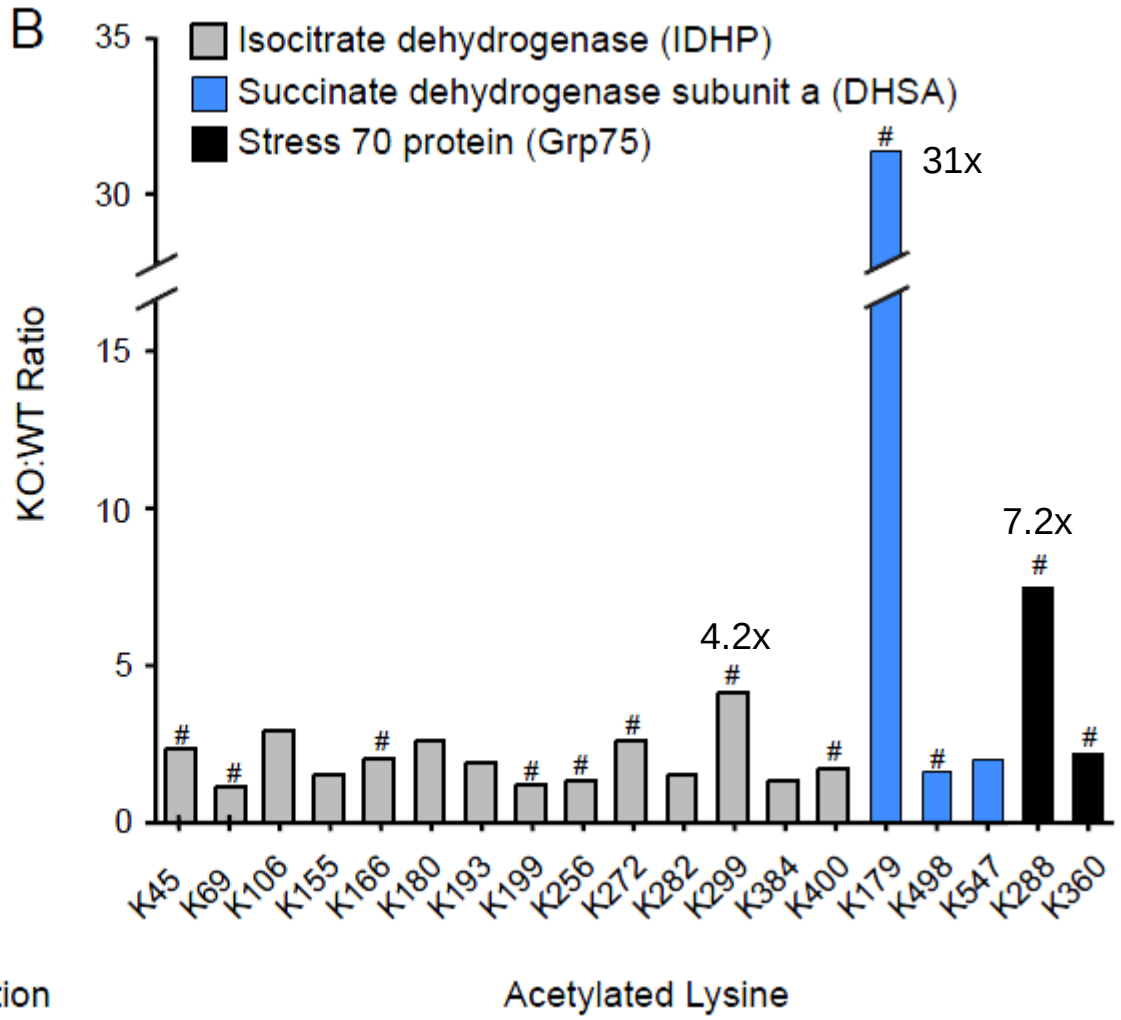


85% with CV<20%

Identification of SIRT3 Substrates in Mouse Mitochondria

Example, Skeletal muscle

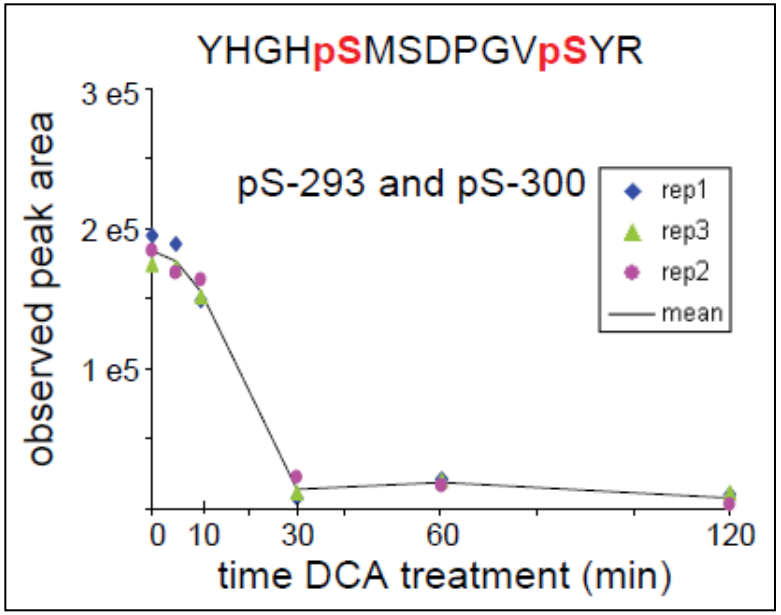


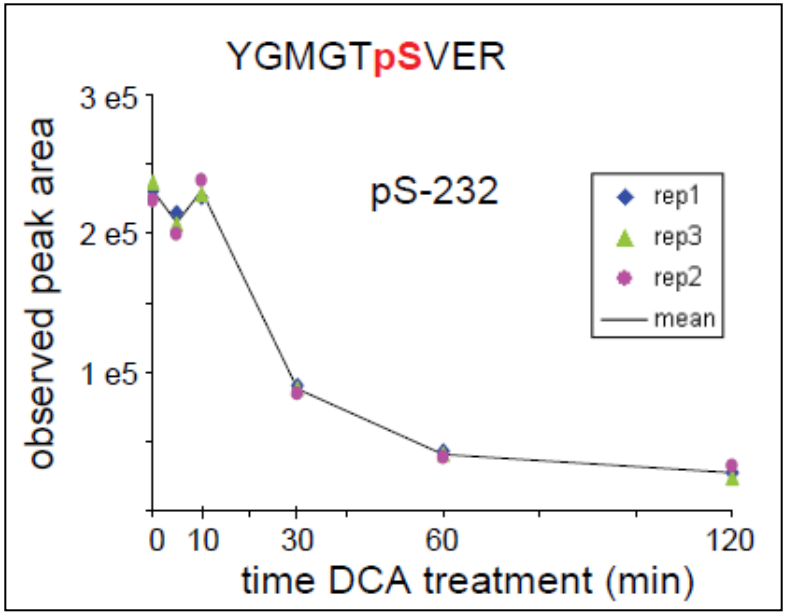


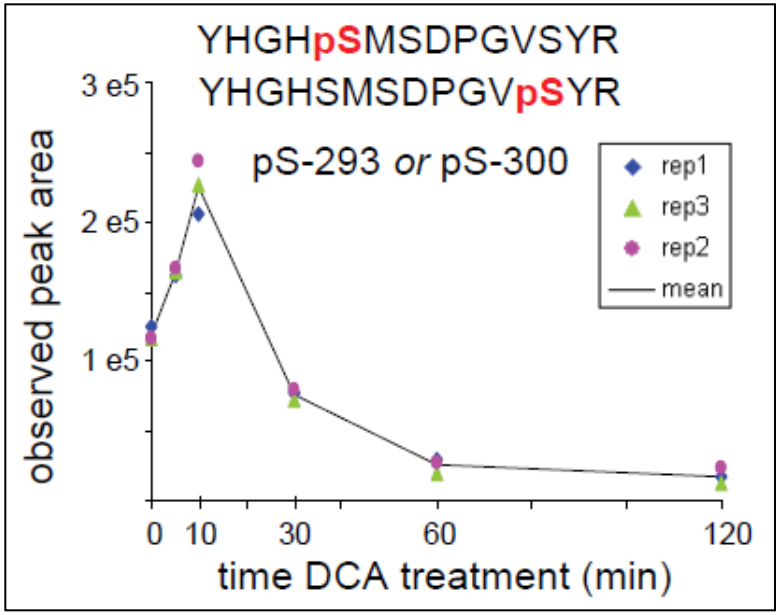
Rardin et al., MS1 Quantitation of >2,000 acetyllysine peptides

Mouse Mitochondrial Liver– DCA treatment (Kinase inhibitor)

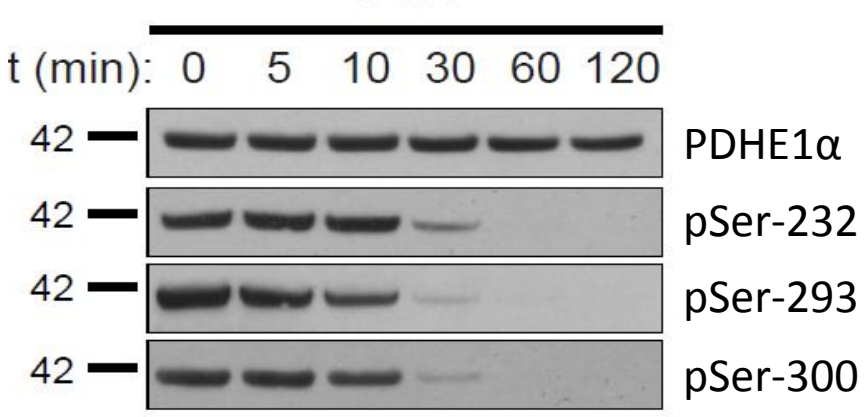
MS1 Filtering for 3 phosphopeptides, Pyruvate DehydrogenaseE1α, 0-120 min







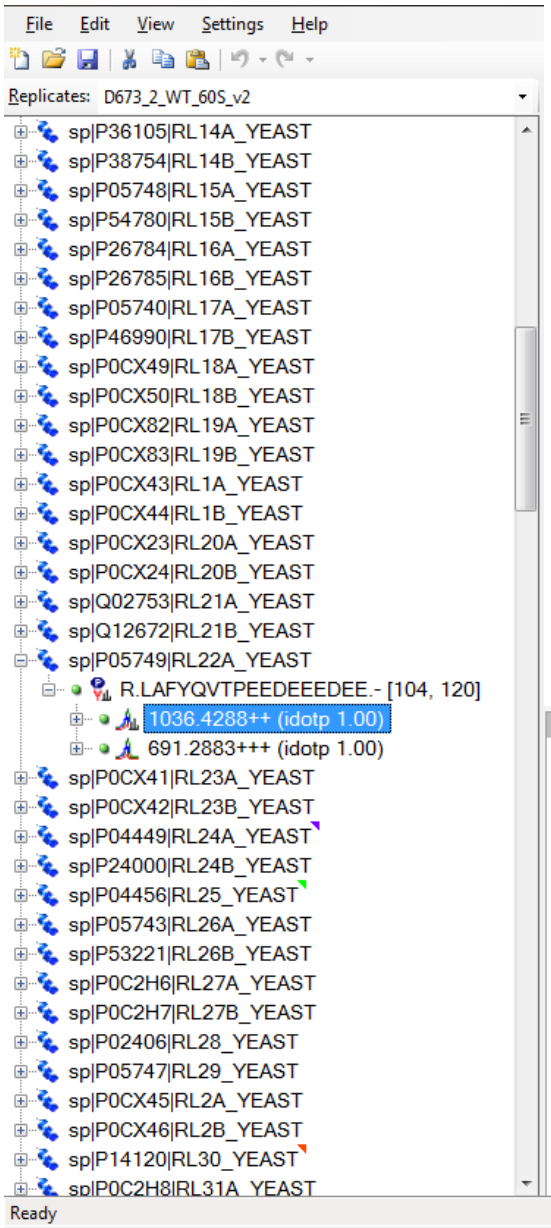
Western blot for comparison



DCA: dichloroacetate (pyruvate analog, inhibitor)

Polysome Changes - High Throughput MS1 Filtering

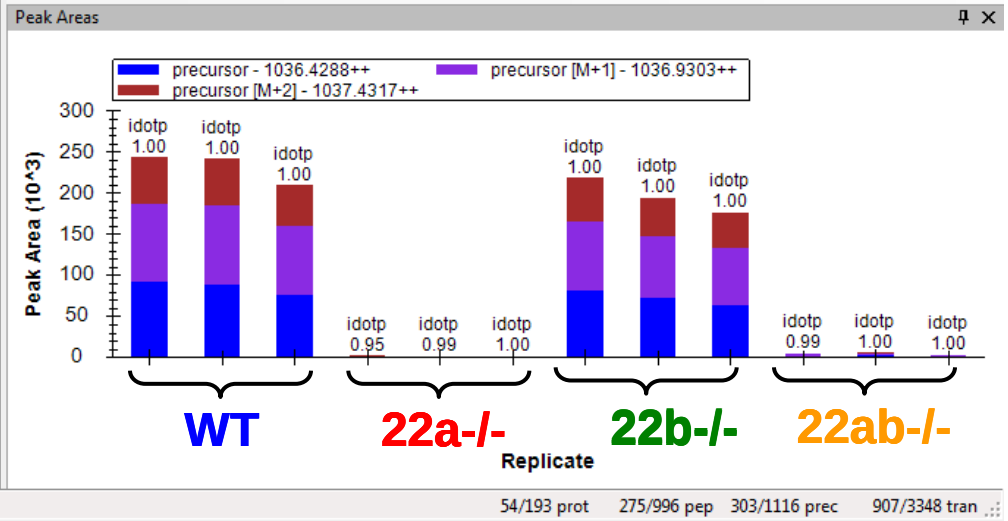
Spectral libraries are generated for 40S, 60S, and 80S yeast polysome fractions



WT **RL22a-/-** **RL22b-/-** **RL22ab-/-** **yeast strains**

MS1 Filtering to comprehensively quantitate ribosomal proteins, ‘RL’ and ‘RS’ proteins (including paralogs) in the polysome fractions.
Subunit changes ?
Complex composition changes ?

Protein RL22a quantitated
(in 4 different yeast strains)



Greatly improved Raw File Import Speed (64 bit) and Memory Allocation

Example: yeast polysomal proteins and interacting proteins
MS1 Filtering for 2309 peptides, each M, M+1, M=2; total of 7191 “transitions”

Import of 12x TripleTOF 5600 wiff files (90 minute gradient)

Comparison of Skyline file import speed with different computer systems	
32 bit, 2 core, 1 proc. 3.16 GHz, 3.3 GB RAM	276 min
64 bit, 4 cores, 1 proc. 3.30 GHz, 8.0 GB RAM	113 min
64 bit, 8 cores, 2 proc. 2.67 GHz, 12.0 GB RAM	99 min

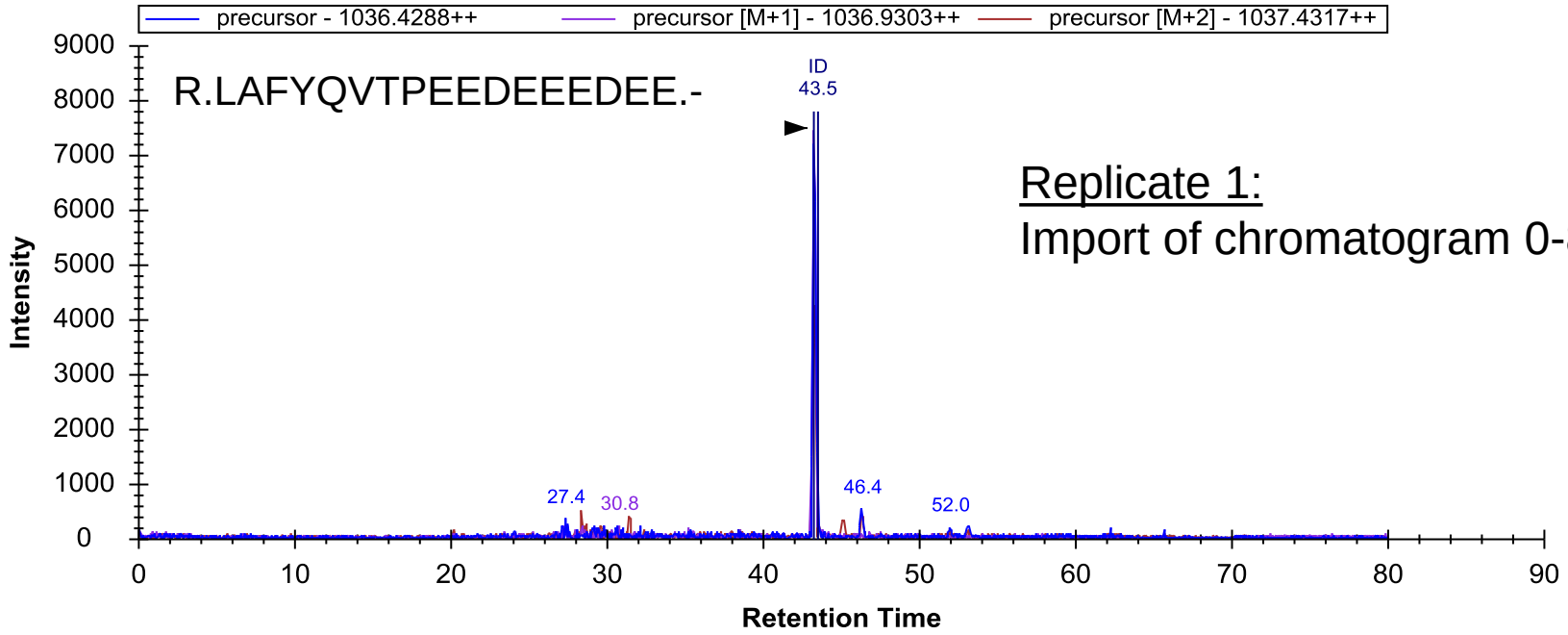
Advantage of file import with scheduled time window (Δ 2 min) around peaks	
32 bit, 2 core	78 min
64 bit, 4 cores	29 min
64 bit, 8 cores	20 min

Scheduled File Import Advantages:

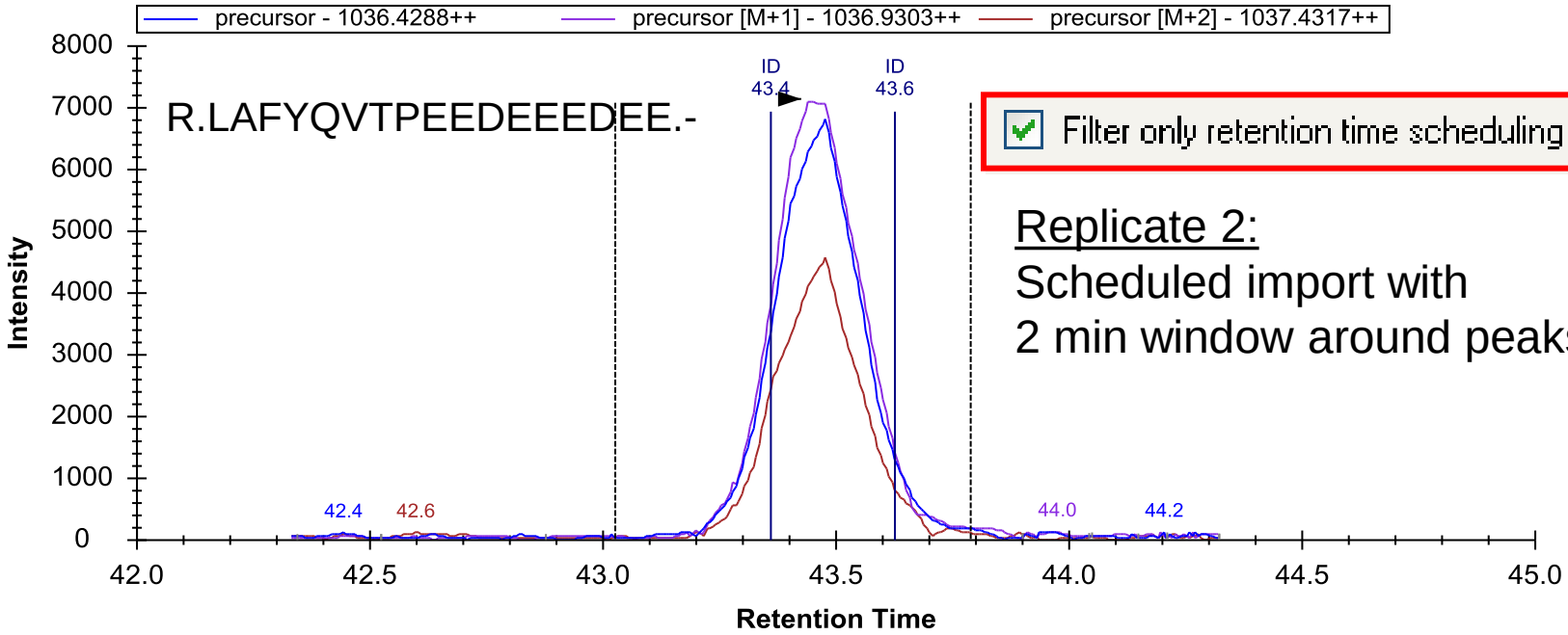
- reduced import time
- greatly reduced Skyline file size **(8.6 GB vs 0.08 GB)**
- easier follow-up peak processing

Skyline daily version 1.2.1.3628

Scheduled File Import during MS1 Filtering using RT from prepicked peaks



Replicate 1:
Import of chromatogram 0-80 min



Filter only retention time scheduling windows

Replicate 2:
Scheduled import with
2 min window around peaks

Integration of Skyline MS1 Filtering into Laboratory Workflows / Pipelines

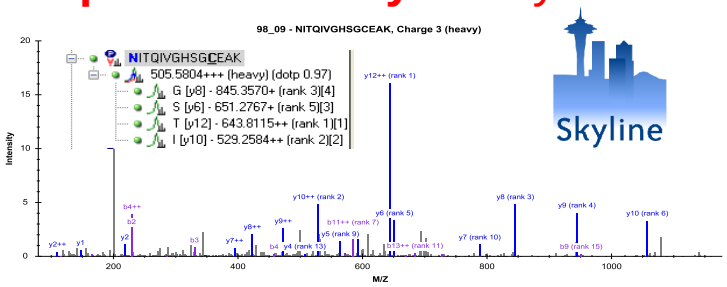
Discovery Mass Spectrometry
data dependent data set

MS1 spectra

MS/MS spectra
(data dependent)

Peptide
Identifications

Generation of a
Spectral Library in Skyline



MS/MS-directed
MS1 peak-picking

MS1 Filtering in Skyline

quantitative information
from Discovery Experiment

Candidate Lists for Follow-up

MRM transition selection

LC-MRM-MS assays
(Verification)

MS1 full scan Filtering - Conclusion and Future Outlook

- Platform and Vendor Independent
- Open Source, continuous development and improvements (Skyline Team)
- Easy label-free quantitation, particularly good for PTM peptide quantitation
- Taking advantage of existing Skyline graphical displays and QC features

- High throughput quantitative screening of discovery workflow experiments
- Easy integration of MS1 Filtering results with follow-up MRM experiments

- Combination of MS1 Filtering with Skyline iRT features
- Retention Time (RT) alignment, also when no MS/MS was sampled

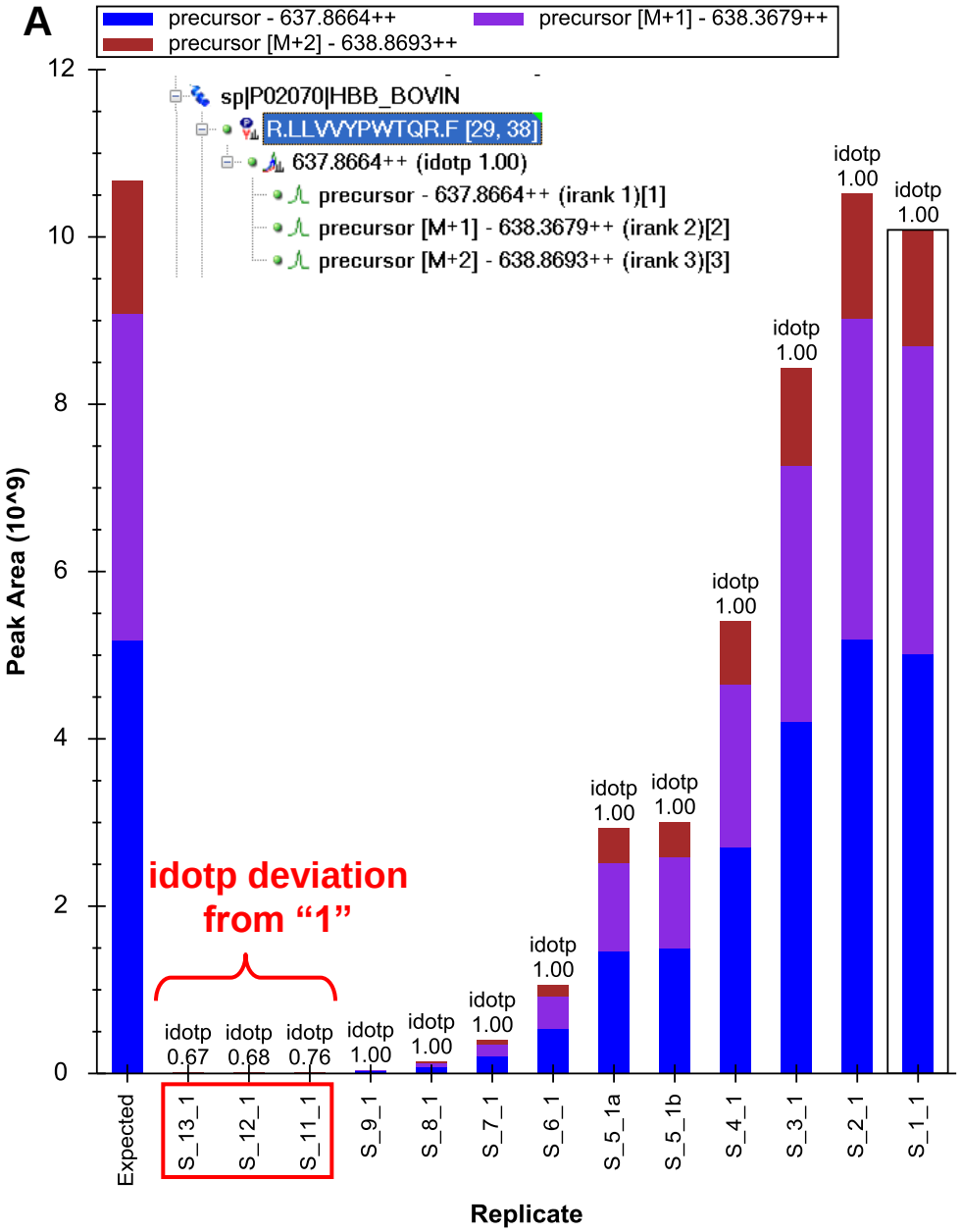
Acknowledgments

- **B.W. Gibson, M.J. Rardin**, M.P. Cusack, A. Zawadzka, D. Sorensen, S. Danielson, Monique O'Leary, Brian Kennedy – **Buck Institute**
- **B. MacLean**, M.S. Bereman, C. Wu, B. Frewen, M.J. MacCoss – **Univ. Washington**
- E. Jing, R. C. Kahn – **Harvard**
- E. Verdin – **Gladstone**
- P. Drake, S. Fisher – **UCSF**
- C. Hunter, S. Seymour – **AB SCIEX**
- J. Cottrell – **Matrix Science**

NIH, NCI (CPTAC)

Skyline MS1 Filtering specific parameters and Utilizing Grid View

A



B Skyline Results Grid View

Results Grid

Replicate Name	Identified	Isotope Dot Product	Best Retention Time	Total Area
S_13_1	False	0.6727	45.65	14147540
S_12_1	False	0.6758	45.77	10551740
S_11_1	False	0.7591	45.81	12591120
S_9_1	True	0.9978	45.62	41318950
S_8_1	True	0.9999	46.04	141312200
S_7_1	True	0.9999	46.29	401702100
S_6_1	True	0.9998	46.23	1060402000
S_5_1a	True	0.9999	46.42	2932124000
S_5_1b	True	0.9999	46.37	3006426000
S_4_1	True	0.9999	46.03	5404440000
S_3_1	True	0.9999	45.85	8435309000
S_2_1	True	0.9999	45.87	10517210000
S_1_1	True	0.9998	45.61	10083990000

Record: 13 of 39 Filter:

(LTQ FT-ICR-MS platform)

All parameters such as
- irank, observed rank
- idotp, isotope distribution %
- underlying MS/MS signal for MS1 peak?

can be exported to Skyline custom reports