

Quantitative Proteomics at US HUPO

DDA to Targeted: Differential Statistics with Skyline

*PROTEOMICS COURSE,
US HUPO 2018*

**Buck Institute for Research on Aging
Novato, CA, USA**

Thank you
to Brendan MacLean !!



Birgit Schilling
March 10th, 2018

Live better longer

Schilling Lab



Proteomics Research and MS Capabilities - Buck Institute



Protein expression changes

- Precise Quantification
- High throughput MS quantifying ~ 5000 proteins per acquisition
- Technology Development
- State of the art label-free workflows (data-independent acquisitions)

Biomarkers Aging & Disease

- Processing of Bio-fluids, plasma, exosomes, synovial fluid
- Protein secretion into bio-fluids from diseased tissues
- Clinical sample sets

Post-translational Modifications

- Dynamic regulation of PTM : Phosphorylation, acetylation, succinylation, malonylation, ubiquitination, AGES etc.
- Affinity Enrichments
- Optimized for low sample material (from tissues)
- PTM site stoichiometry

Protein Networks

- Protein-Protein Interactions
- Immunoprecipitations

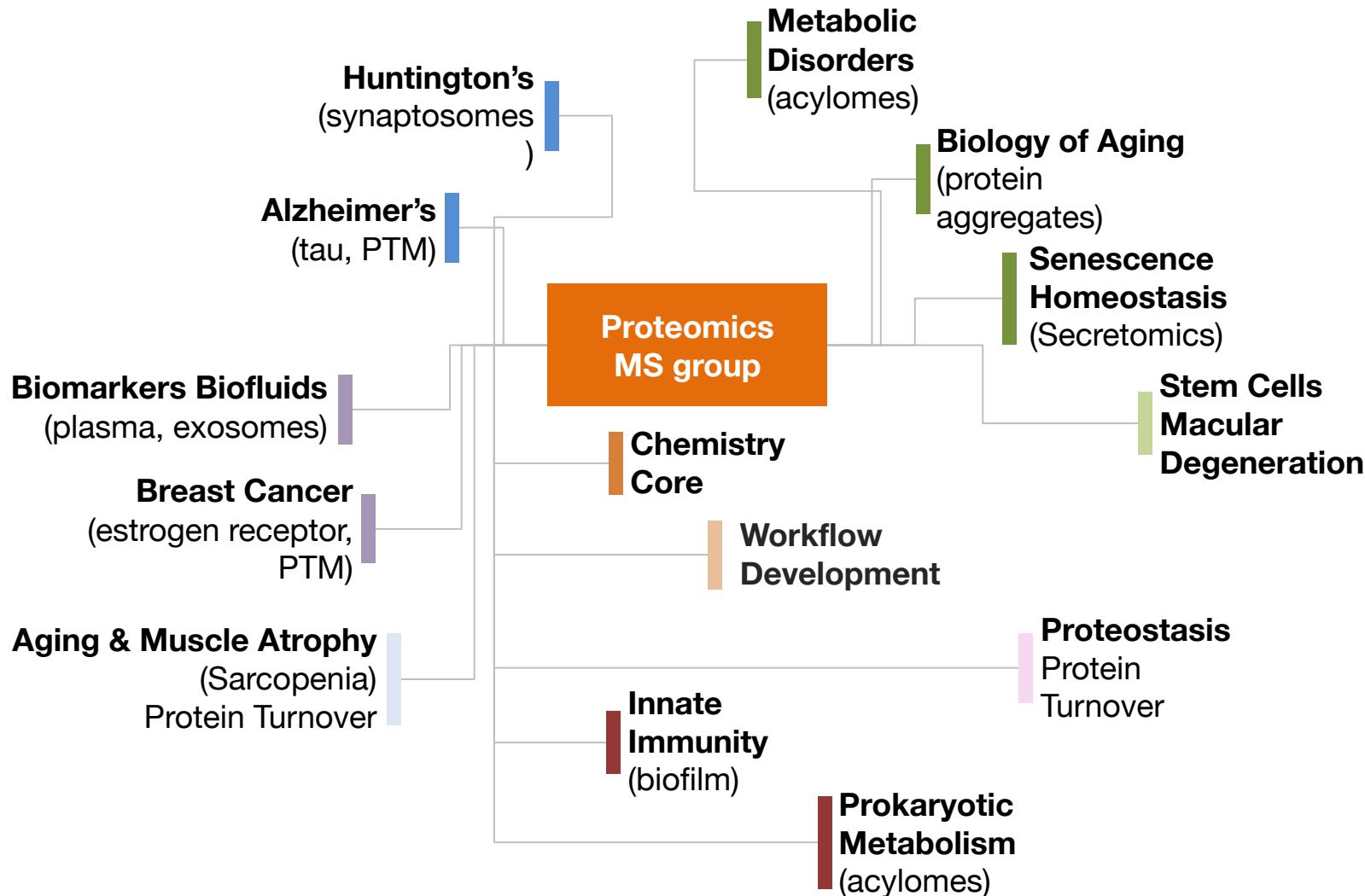
Protein Turnover

- Changes in protein synthesis & degradation
- In-depth analysis of protein half-life and protein flux

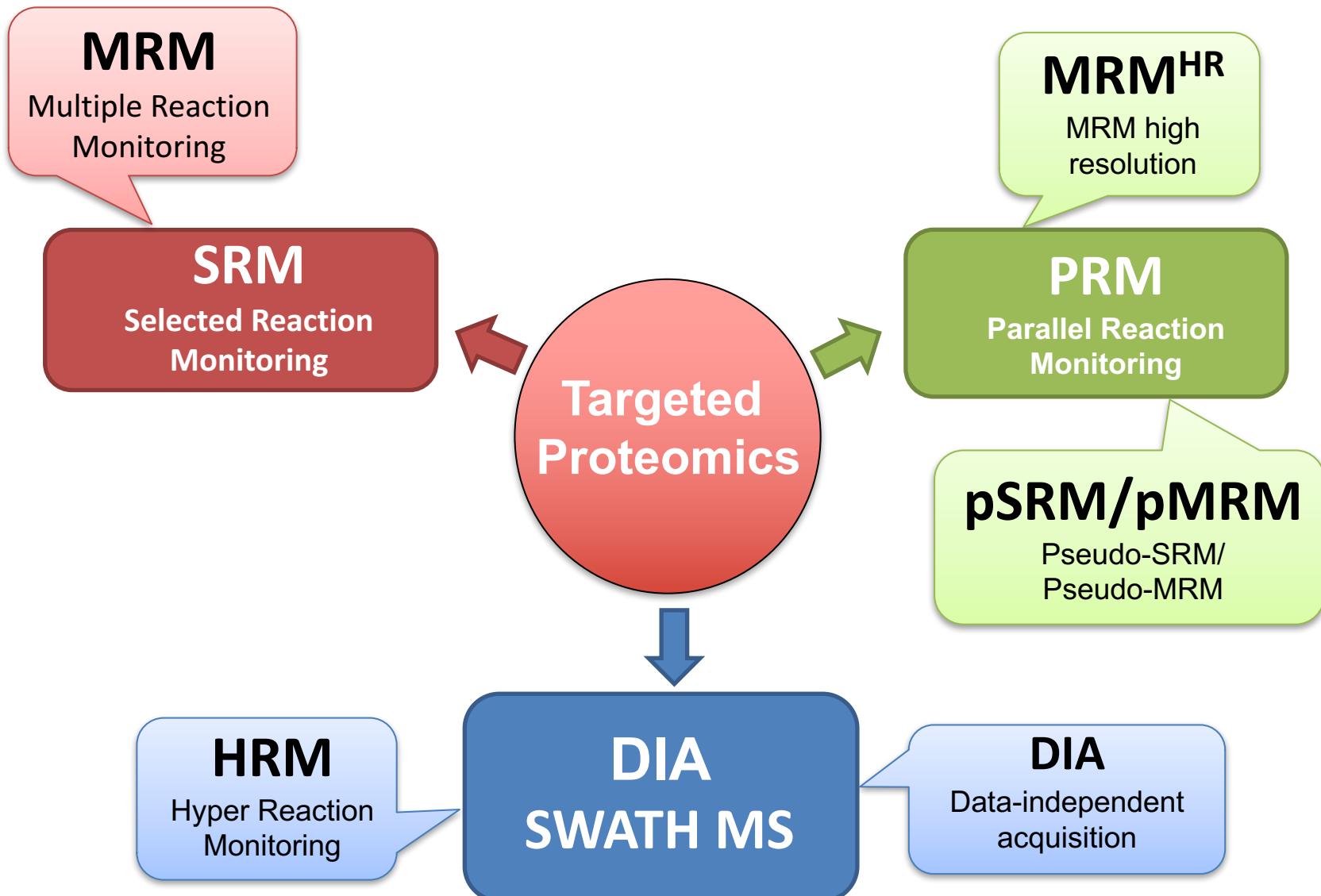
Software Development

- Custom software for data processing and reports
- Skyline (MacCoss, U. Washington) –
- Algorithm, QC - Vitek (NEU)

Selected Proteomics Projects - Buck Institute 2018

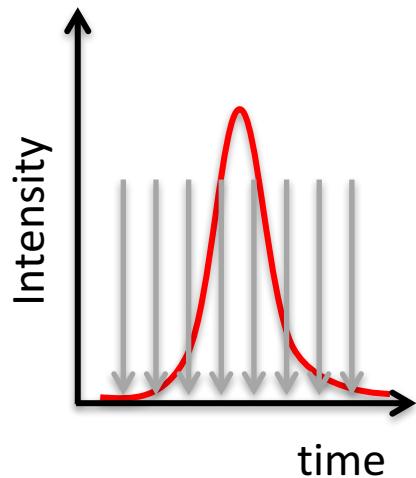


Targeted proteomics – various approaches



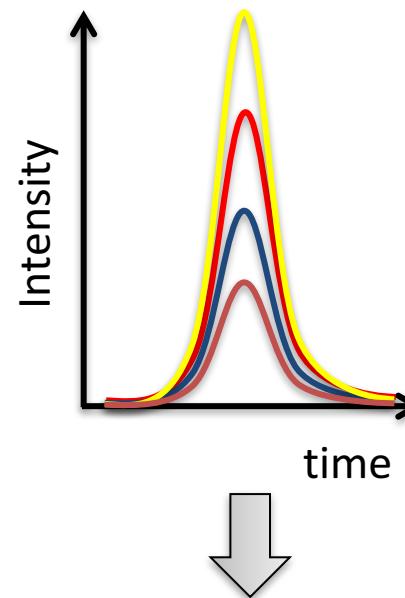
Identification and Quantification – Targeted

PRECURSOR
chromatographic peak



FRAGMENTATION

FRAGMENT ION
chromatographic peak

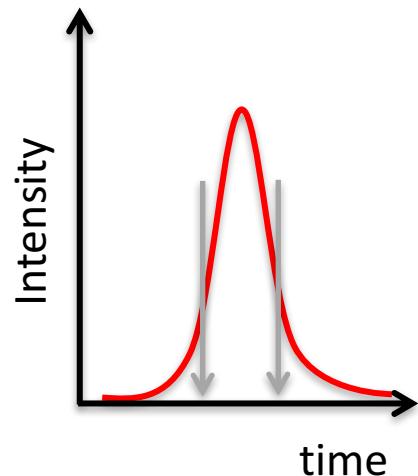


ideal technique for
• **Reproducible peptide
identification**
• **quantitative accuracy**

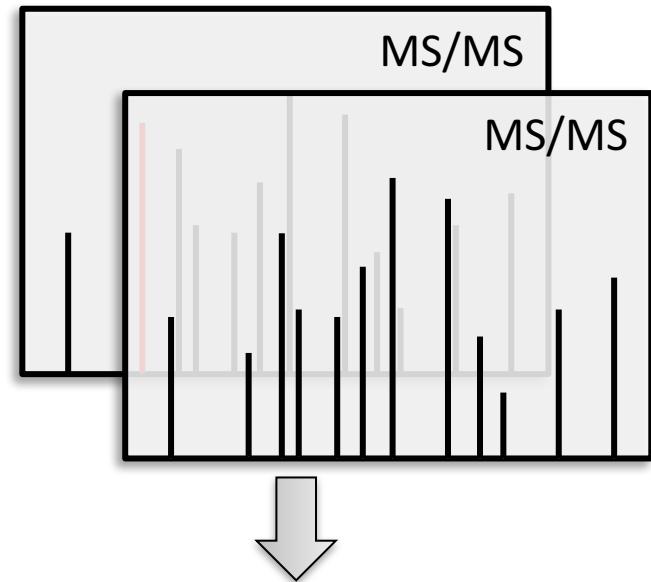
IDENTIFICATION:
Hypothesis test
QUANTIFICATION:
Extracting fragment
ion chromatograms

Identification and Quantification – DDA

PRECURSOR
chromatographic peak



FRAGMENTATION



QUANTIFICATION:
Extracting precursor ion
chromatograms

IDENTIFICATION:
peptide to spectrum
match

ideal technique for **global identification of peptides**.

Agenda

- Welcome
- **DDA to Targeted: Differential Statistics**
- Introduction with Birgit Schilling
 - Workflow and data set overview
 - Hands on with Birgit Schilling
 - DDA data processing review
 - Hypothesis generation from DDA data

Chromatography-based Quantification

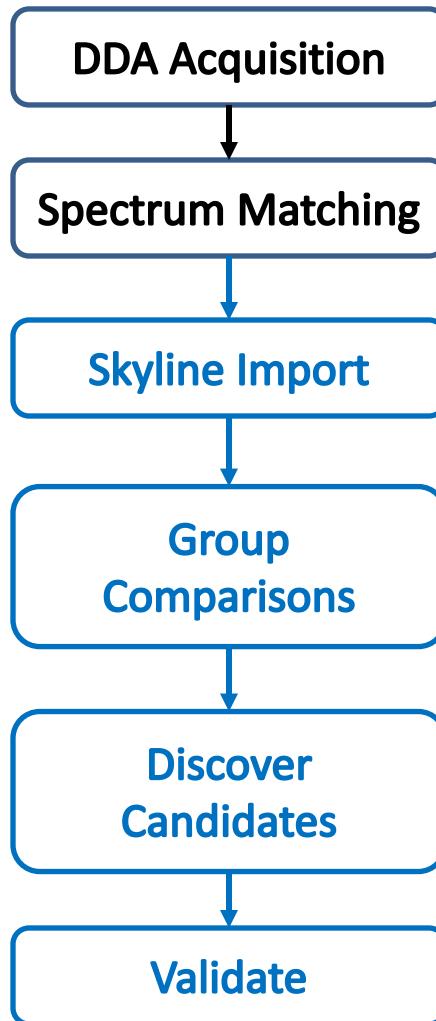
- Hypothesis testing (Verification)
- SRM
- **DDA - MS1 chromatogram extraction**
- Targeted MS/MS (PRM)
- Data independent acquisition (DIA/SWATH)



Acquisition	Targeted	Survey
More Selective	PRM	DIA
Less Selective	SRM	DDA-MS1

HYPOTHESIS??

Discovery to Targeted with Skyline



Got HYPOTHESIS!!

Case Study: ABRF iPRG 2014

	Fake Accession	Name	Origin	Molecular Weight
A	P44015	Ovalbumin	Chicken Egg White	45KD
B	P55752	Myoglobin	Equine Heart	17KD
C	P44374	Phosphorylase b	Rabbit Muscle	97KD
D	P44983	Beta-Galactosidase	Escherichia coli	116KD
E	P44683	Bovine Serum Albumin	Bovine Serum	66KD
F	P55249	Carbonic Anhydrase	Bovine Erythrocytes	29KD

Sample Preparation

	A	B	C	D	E	F (fmol)	
Sample 1	65	55	15	2	11	10	+ 200 ng yeast digest
Sample 2	55	15	2	65	0.6	500	+ 200 ng yeast digest
Sample 3	15	2	65	55	10	11	+ 200 ng yeast digest

Group Comparisons

	A	B	C	D	E	F	(fold change)	
Sample 1-2	0.85	0.27	0.13	32.5	0.055	50		+ 200 ng yeast digest
Sample 1-3	0.23	0.036	4.33	27.5	0.91	1.1		+ 200 ng yeast digest
Sample 2-3	0.27	0.13	32.5	0.85	16.7	0.022		+ 200 ng yeast digest

Group Comparison Maxima

	A	B	C	D	E	F	(abs log2 fold change)
Sample 1-2	0.2	1.9	2.9	5.0	4.2	5.6	+ 200 ng yeast digest
Sample 1-3	2.1	4.8	2.1	4.8	0.1	0.1	+ 200 ng yeast digest
Sample 2-3	1.9	2.9	5.0	0.2	4.1	5.5	+ 200 ng yeast digest
Maximum	2.1	4.8	5.0	5.0	4.2	5.6	

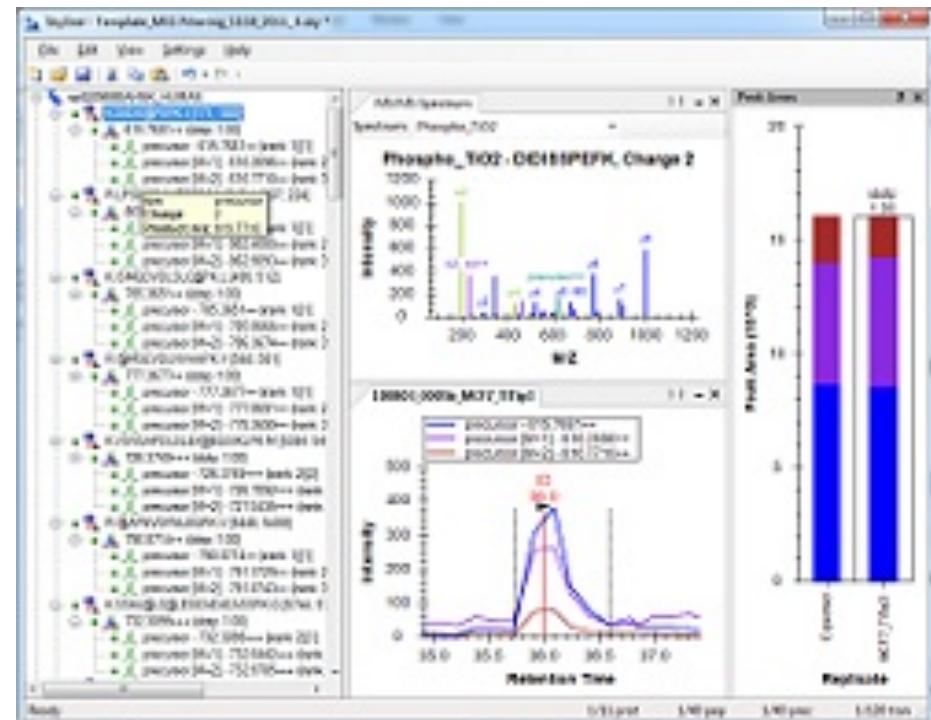
DDA Acquisitions Searched

	Identified yeast proteins
sample 1-a	3016
sample 1-b	3073
sample 1-c	2905
sample 2-a	2916
sample 2-b	2984
sample 2-c	2907
sample 3-a	2883
sample 3-b	2972
sample 3-c	2913

Comet, OMSA, MSGF+ - iProphet

Keys to Success with MS1 in Skyline

- Use Import DDA Peptide Search wizard
- Make sure you have ID annotations
 - Diagnose with Spectral Library Explorer
 - <http://tinyurl.com/Skyline-missing-ids>
- Review RT alignment in alignment viewer
- Got HYPOTHESIS??
- Review and manually adjust <5% of peaks
- MS1 Filtering Tutorial



Discovery versus Validation

- **Discovery**

- asking your data what changed

- **Validation**

- asking if there is evidence your candidates changed

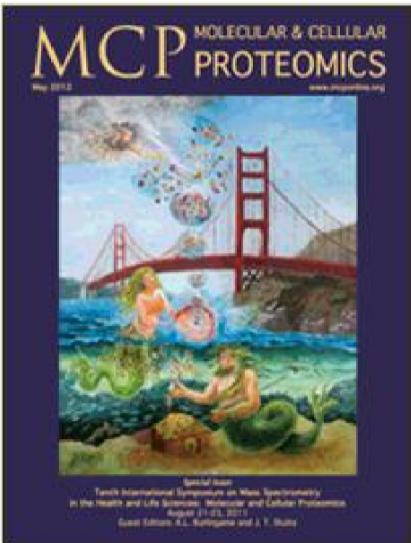
HYPOTHESIS??

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



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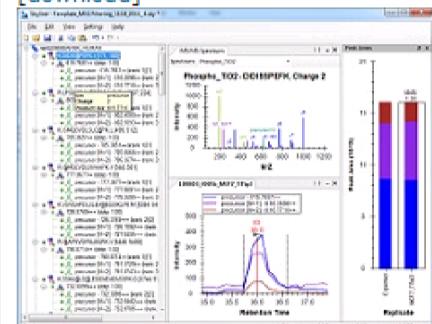
Skyline > Start Page > Tutorials >

MS1 Full-Scan Filtering Tutorial

PRINT

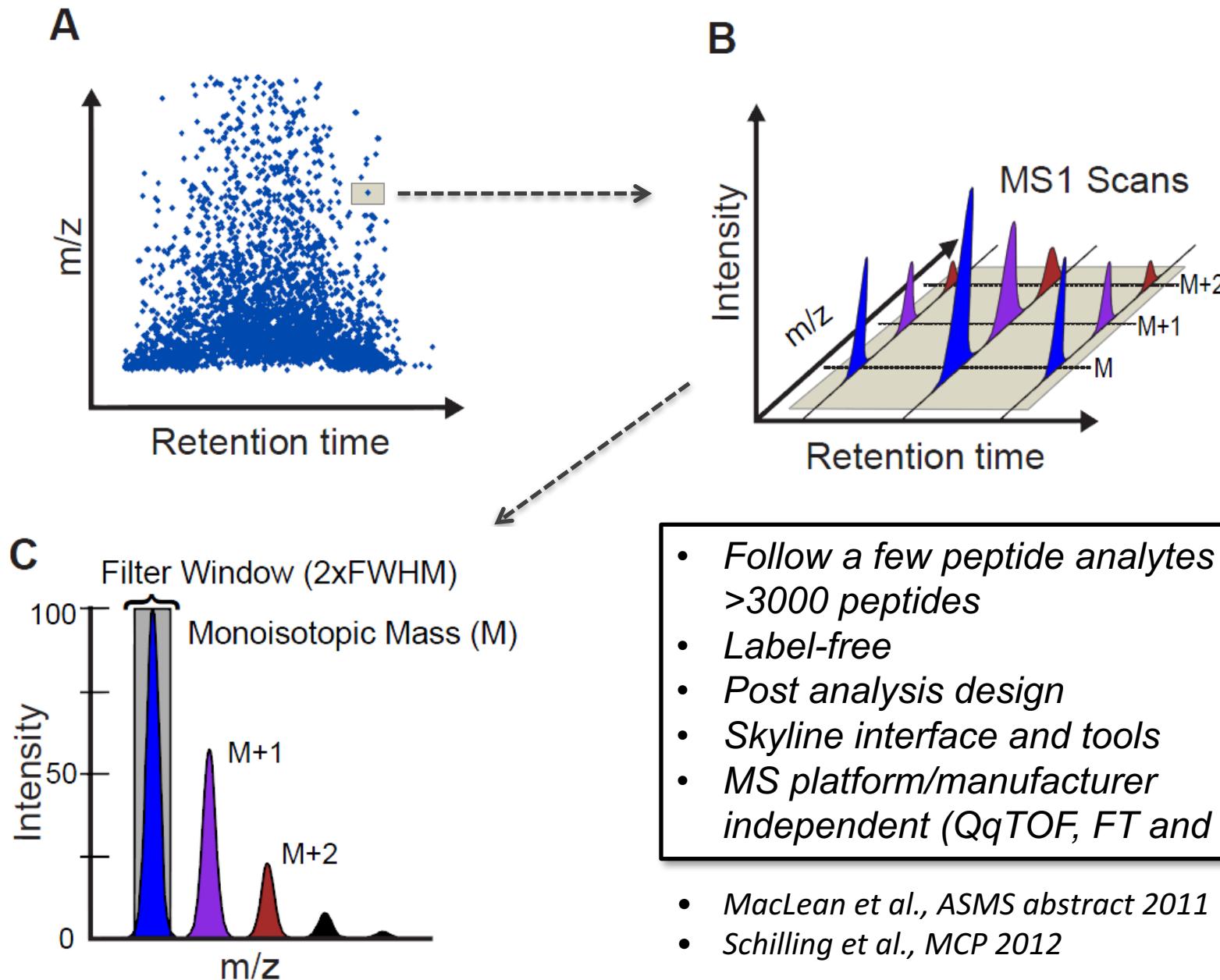
Get hands-on experience creating a Skyline document to measure quantitative differences in peptide expression using the MS1 scans from your data dependent acquisition (DDA) experiments. In this tutorial, you will generate a spectral library from a discovery data set, set up a Skyline document for MS1 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)

[\[download\]](#)

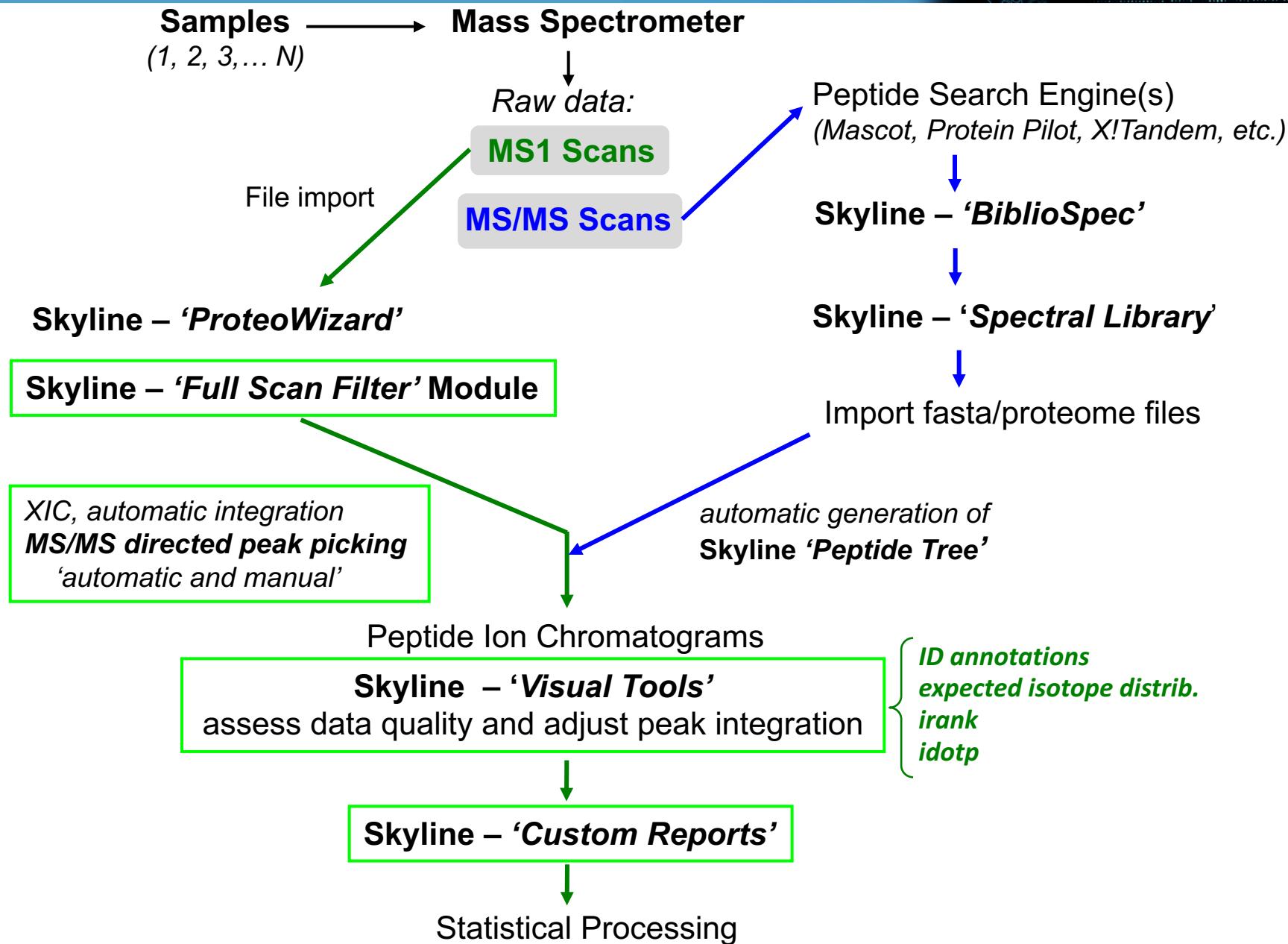


<http://proteome.gs.washington.edu/software/Skyline/tutorials/ms1filtering.html>

MS1 Filtering - a quantitative, label-free tool



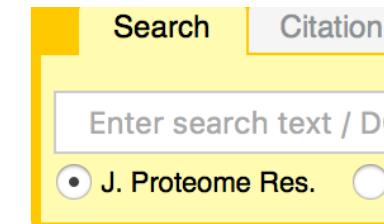
Proteomic Data Flow in Skyline MS1 Filtering



Skyline data also on Panorama Webbrowser

<https://panoramaweb.org/project/Panorama%20Public/2016/iPRG%202015/begin.view?>

(but all course participants can use the data from the flash – drive)



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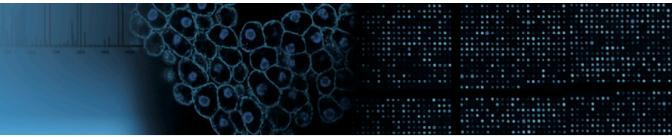
Article

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ABRF Proteome Informatics Research Group (iPRG) 2015 Study: Detection of Differentially Abundant Proteins in Label-Free Quantitative LC–MS/MS Experiments

Meena Choi^{#†} , Zeynep F. Eren-Dogu^{#‡}, Christopher Colangelo[§], John Cottrell[¶], Michael R. Hoopmann[⊥], Eugene A. Kapp[¶], Sangtae Kim[®], Henry Lam[□], Thomas A. Neubert[■], Magnus Palmblad[○], Brett S. Phinney[●], Susan T. Weintraub[△], Brendan MacLean[▲], and Olga Vitek^{*†} 

Hands-on Skyline !!



MS1 Filtering Transition Settings

Transition Settings

Prediction Filter Library Instrument Full-Scan

Precursor charges: 2, 3, 4, 5 Ion charges: 1, 2 Ion types: p

Productions

From: ion 3 To: last ion

Special ions:

- N-terminal to Proline
- C-terminal to Glu or Asp
- N-terminal to Proline (legacy)
- iTRAQ-114
- iTRAQ-115
- iTRAQ-116
- iTRAQ-117
- TMT-126

Precursor m/z exclusion window: m/z

Auto-select all matching transitions

OK Cancel

Prediction Filter Library Instrument Full-Scan

Ion match tolerance: 0.5 m/z

If a library spectrum is available, pick its most abundant peak

Pick: 10 productions

From filtered ion charges and types

From filtered ion charges and types plus filtered

From filtered productions

Transition Settings

Prediction Filter Library Instrument Full-Scan

MS1 filtering

Isotope peaks included: Count Precursor mass analyzer: TOF

Peaks: 3 Resolving power: 30.000

Isotope labeling enrichment: Default

MS/MS filtering

Acquisition method: None Product mass analyzer:

Isolation scheme: Resolution: m/z

Retention time filtering

Use only scans within 5 minutes of MS/MS IDs

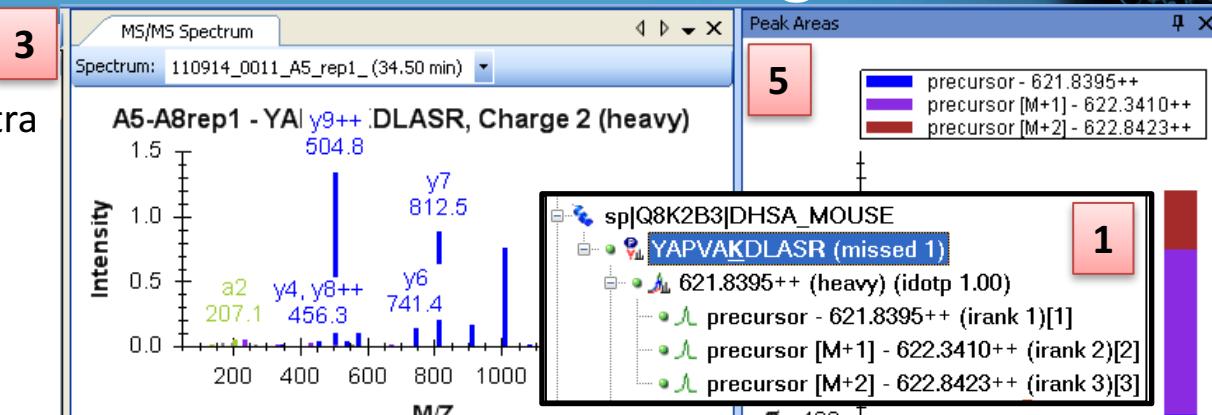
Use only scans within 5 minutes of predicted RT

Include all matching scans

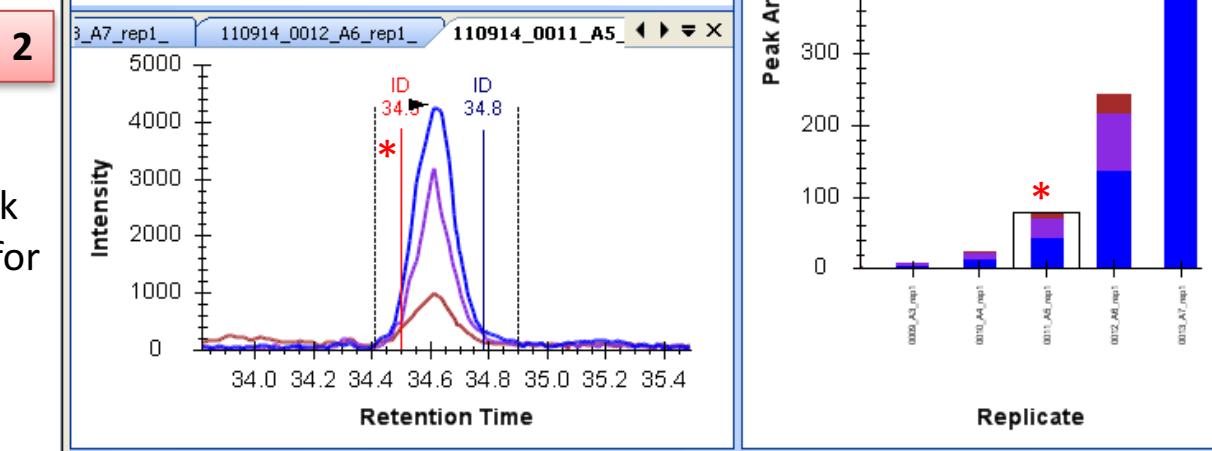
OK Cancel

Skyline interface for MS1 Filtering data

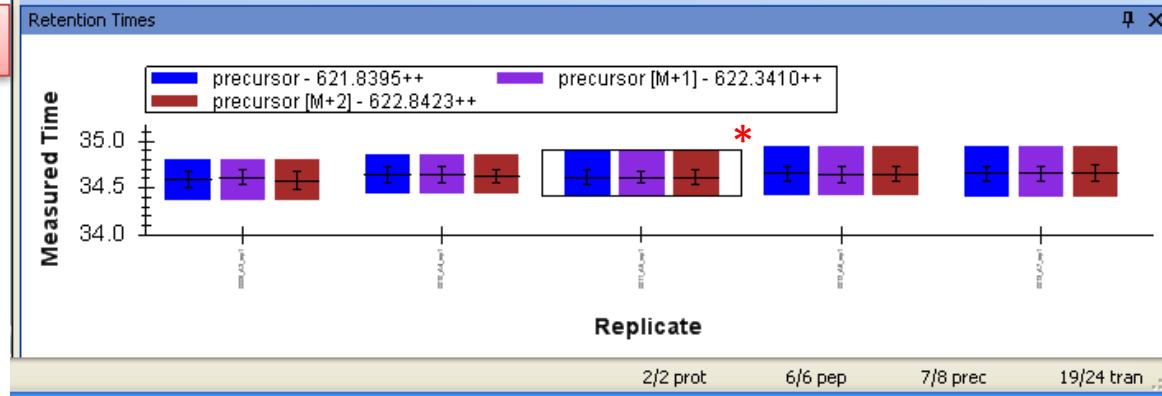
3) MS/MS spectra and ID



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

MS1 Filtering Standard Concentration Curves for Lys-Ac

TripleTOF 5600

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

