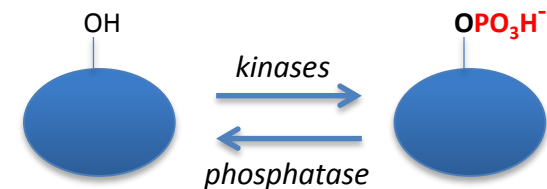
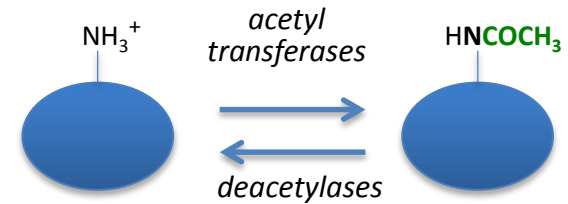


Skyline MS1 Filtering for label-free quantification targeting posttranslational modifications



Birgit Schilling

Buck Institute for Research on Aging



Webinar #1: Getting the Most Out of DDA Data with Skyline

GIBSON LAB 2014

Ellerby
Hughes
Brand
Mooney

Huntington
(CHDI)

Metabolic
Disorders
(R24)

E. Verdin (Gladstone)
R. Kahn (Joslin)
C. Newgard (Duke)
E. Goetzman (Pittsburg)

Alzheimer's

Biology of
Aging
(NIA)

Hughes, Lithgow
Kapahi, Campisi
Melov, Jasper,
S. Ghaemmaghami
(SUNY), others...

Bredesen
Melov
Brand
Ellerby/Gan

**Gibson
lab**

Tissue
Homeostasis
(‘Secretomics’)

Campisi,
Kapahi
C. Adams
(Iowa)

Clinical
Proteomics,
Biomarkers

Fisher (UCSF)
Benz
Mooney

**Chemistry
Core**

Stem
Cells
(AMD)

Lamda

Benz
Fisher

Cancer
Biology
(R01)

**Methods
Development**

MacCoss (UW)
Vitek (Northeastern U)
Tabb (Vanderbilt)
Held (WU)
BioMarin

Infection
Disease & Innate
Immunity
(R01, PPG)

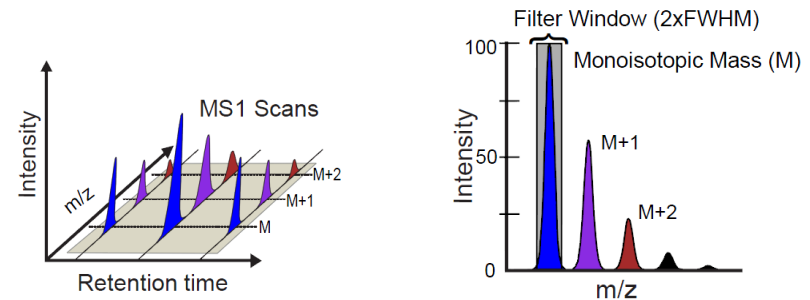
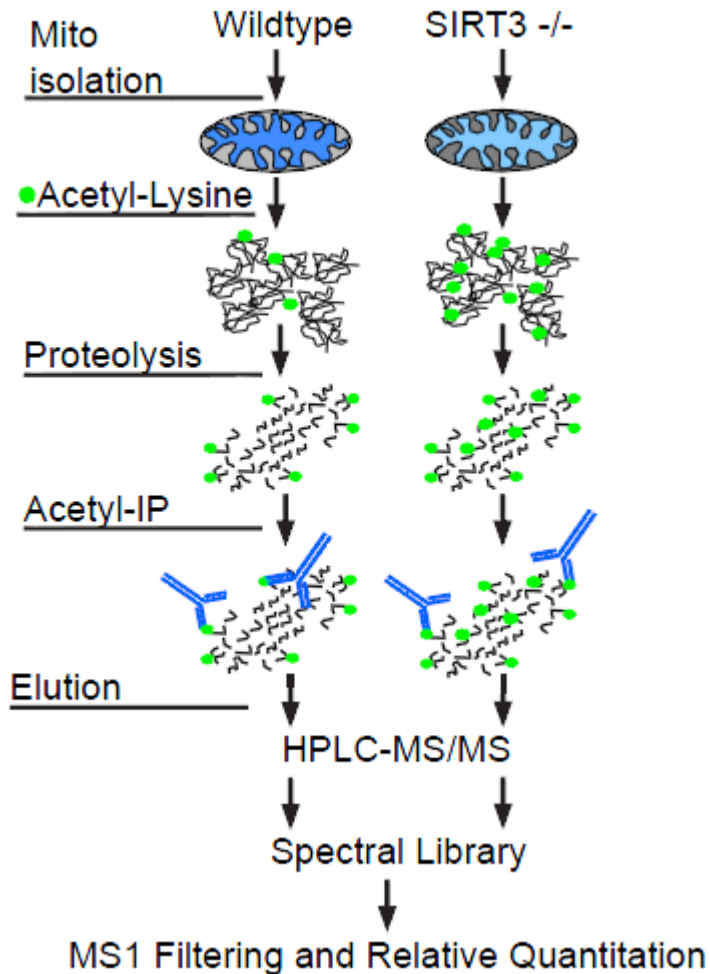
Prokaryotic
Metabolic
Regulation

Wolfe (Loyola)
Rao (Illinois)

Apicella,
Nauseef (Iowa),...

October, 2014

Development and Examples for MS1 Filtering Workflows



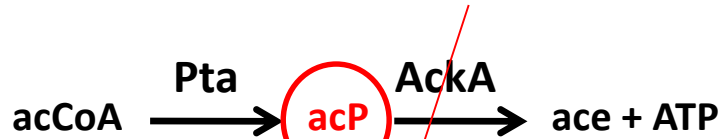
- Data-dependent acquisitions (discovery workflows) depend on dynamic sampling of MS/MS spectra.
- **MS1 scans** are truly **data-independent** and can be used for relative quantification.
- MS1 Filtering uses ion extracted chromatograms of peptide precursor ions for relative quantification
- Easy **interfacing** 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

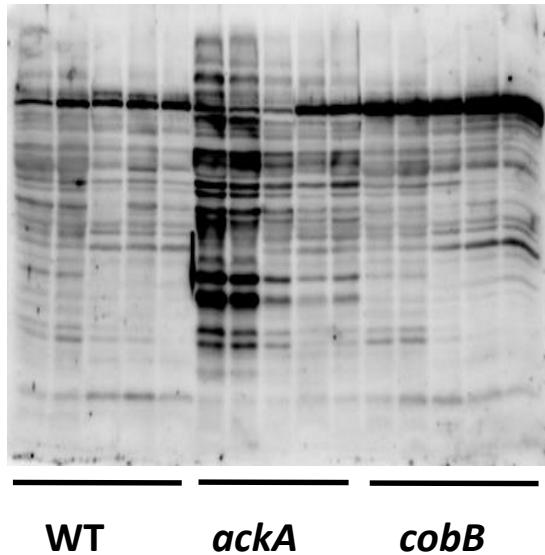
DATA examples

E. coli acetylation regulation - Background and Workflow



acP: acetyl phosphate (chemical acetylation)

anti-acetyl blots



MS Analysis

4x biological replicates of:



Protein lysates

↓ Trypsin Digestion

Proteolytic Peptides

↓ *Anti-acetyl-K- IP*

Acetyl-enriched Peptides

↓ Triple TOF 5600

3x technical LC MS/MS replicates

↓ Database Search

Skyline MS1 Filtering

PHASE 1: Biomarker Discovery Using Conditioned Media from Cancer Cell Lines to predict cancer-specific plasma glycosites candidates

