Skyline MS1 Filtering for label-free quantification targeting posttranslational modifications

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

**DATA examples**
**E. coli** acetylation regulation - Background and Workflow

- **acCoA** → **acP** → **ace + ATP**
- **acP**: acetyl phosphate (chemical acetylation)

**MS Analysis**

4x biological replicates of:

- **WT**
- **ackA**
- **cobB**
- **others**

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**

(Kuhn, Schilling, Gibson, Wolfe et al., *PlosOne* 2014)
PHASE 1: Biomarker Discovery Using Conditioned Media from Cancer Cell Lines to predict cancer-specific plasma glycosites candidates

Luminal

Triple negative

4 claudin-low (basal B)

1 basal A

Centrifugation of CMs

3K MWCO; dialysis

Amino acid analysis (normalized)

Trypsin

Lectin Chromatography

(2x) SNA

Bound fractions (glycopeptides)

PNGaseF

10 SNA + 10 AAL deglycopeptide fractions

2x

80 HPLC MS/MS

(+ phosphatase inhibitors)

HILIC fractionation

TiO2 Phosphopeptide affinity chromatography

Bound fractions (P-peptides)

2x

HPLC MS/MS

Drake, Schilling et al., JPR 2012

Zawadzka, Schilling et al., MCP 2014