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Towards Turnkey Targeted Proteomics Solutions Using SureQuant Internal Standard Triggered Acquisition on Orbitrap MS

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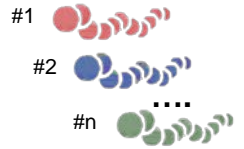
The world leader in serving science

Introducing SureQuant Targeted Protein Quantitation

Intelligent Data Acquisition Strategy for Large-scale, Sensitive, Precise and Reproducible Quantitation

Synthetic Heavy Peptides

Sample

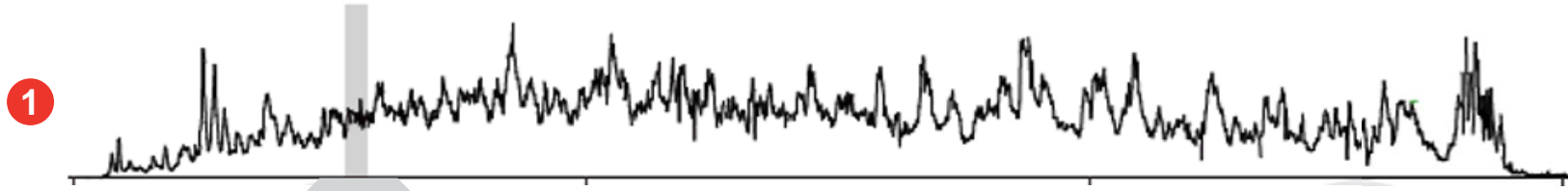
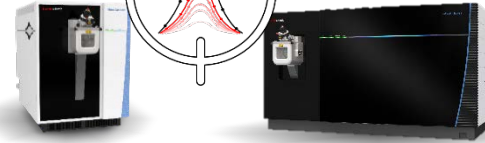


Mix

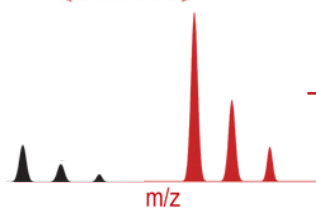


LC-MS Analysis

SureQuant



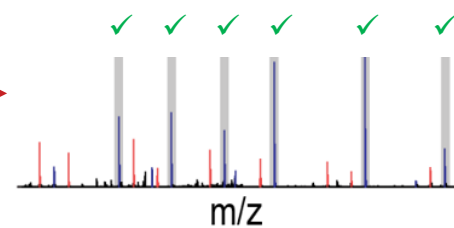
2 ENDO Peptides IS peptides (trigger)



MS1 Survey Scan
(Precursor m/z, intensity threshold)

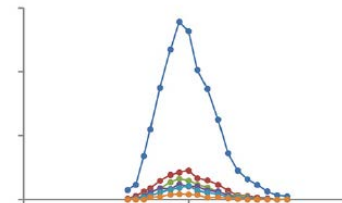
3 WATCH MODE
Fast, Low Res MS2
of Trigger

Pseudo Spectral Matching



4 MS/MS IS
(≥ Min. number fragment m/z)

5 QUANT MODE
High Quality MS2
of Target



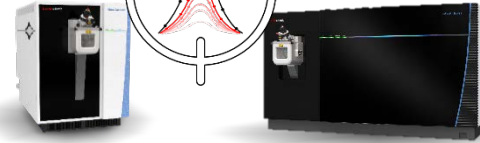
Introducing SureQuant Targeted Protein Quantitation

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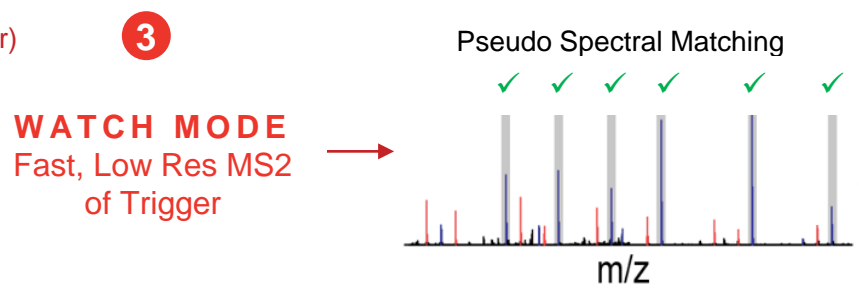
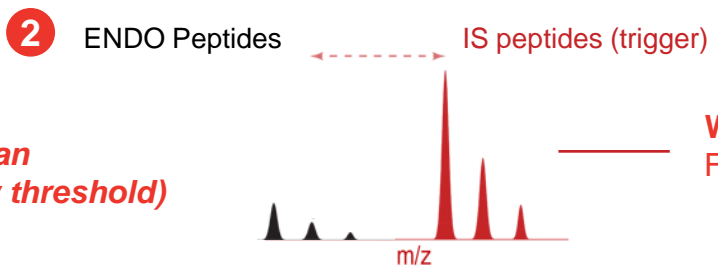
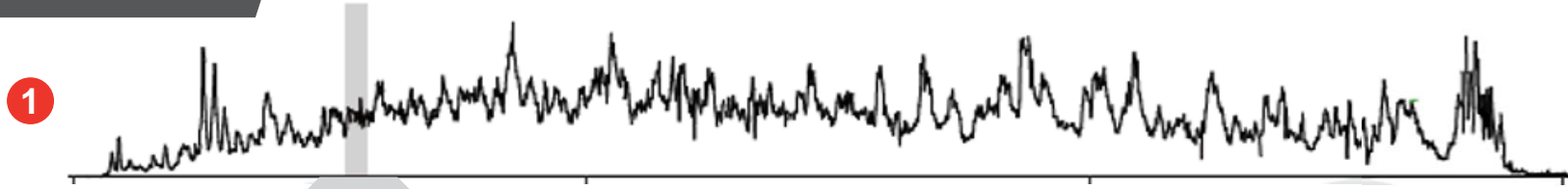
High Usability & Stability

Native implementation in ICSW
Generic scan events / filters
Optimized scan execution matrix

SureQuant



Orbitrap Exploris 480 MS
Orbitrap Eclipse Tribid MS
(& legacy Tribid w/ Tune 3.3 and higher)

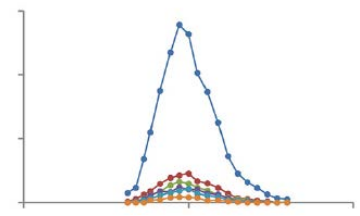


MS/MS IS
(≥ Min. number fragment m/z)

MS1 Survey Scan
(Precursor m/z, intensity threshold)

WATCH MODE
Fast, Low Res MS2
of Trigger

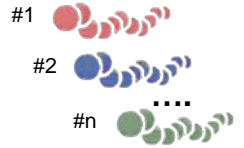
QUANT MODE
High Quality MS2
of Target



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Synthetic Heavy Peptides

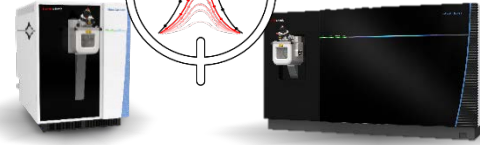


Sample

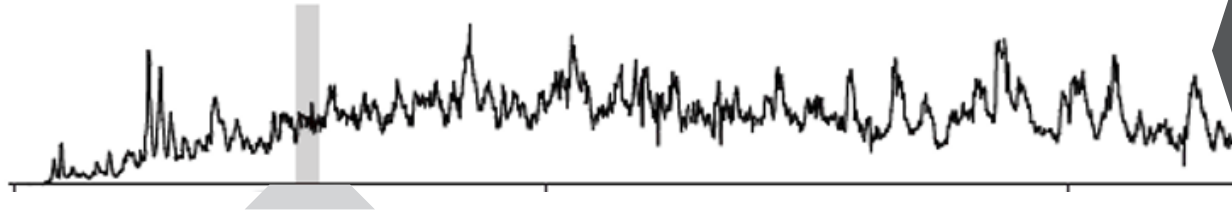


LC-MS Analysis

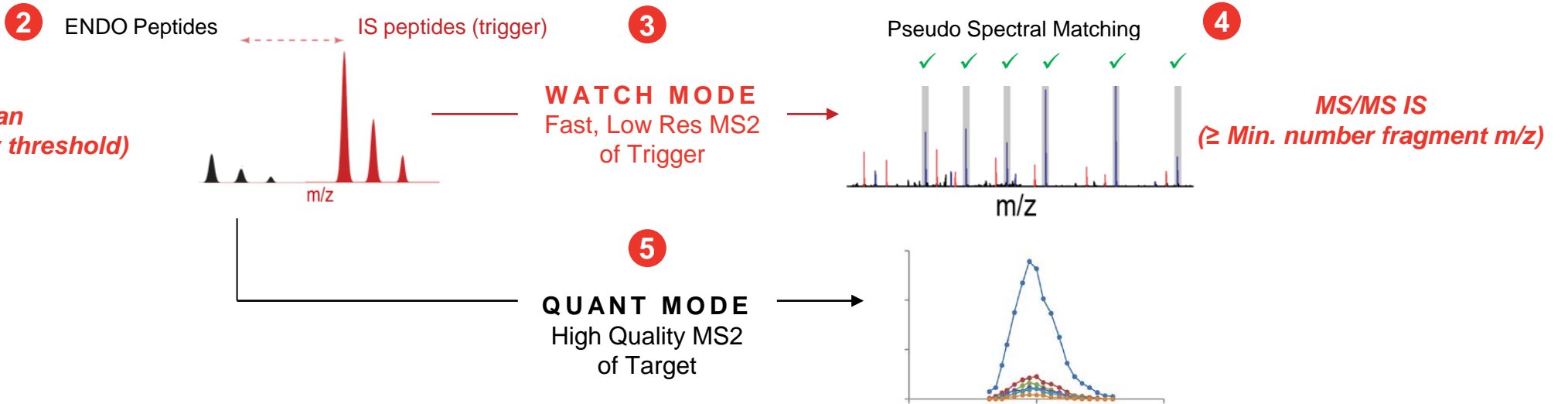
SureQuant



Orbitrap Exploris 480 MS
Orbitrap Eclipse Tribrid MS
(& legacy Tribrid w/ Tune 3.3 and higher)





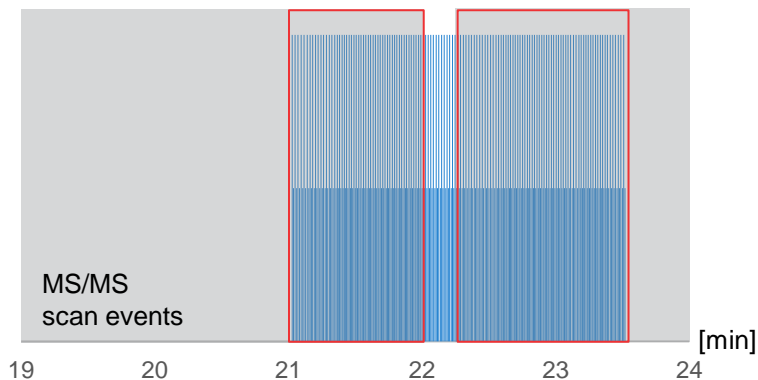
Robust Acquisition
No retention time scheduling
Insensitive to chromatographic fluctuations
No acquisition-driven missing value



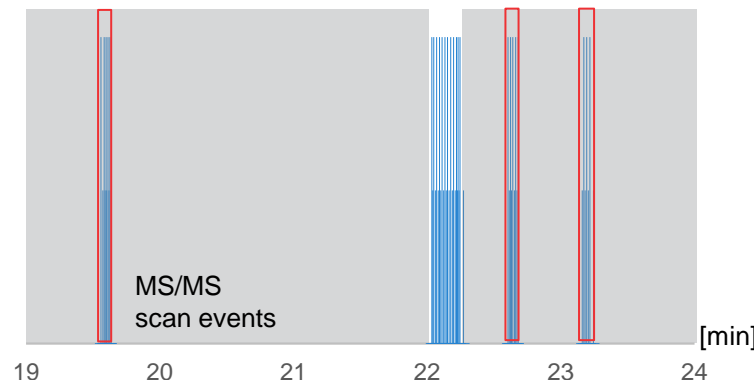
SureQuant Acquisition Delivers Intelligent Detection of Targets, Enhancing Productivity

250 ng cell line digest, 50 fmol internal standards
60 peptides
30-min gradient

 Non-productive MS2 scans  Productive MS2 scans



SIL



Limited false positive triggering MS/MS of Internal Standard peptides

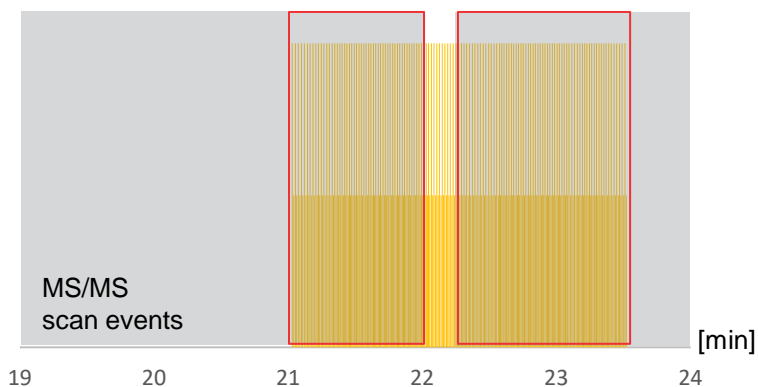
'No' false positive triggering MS/MS of Endogenous peptides

PRM

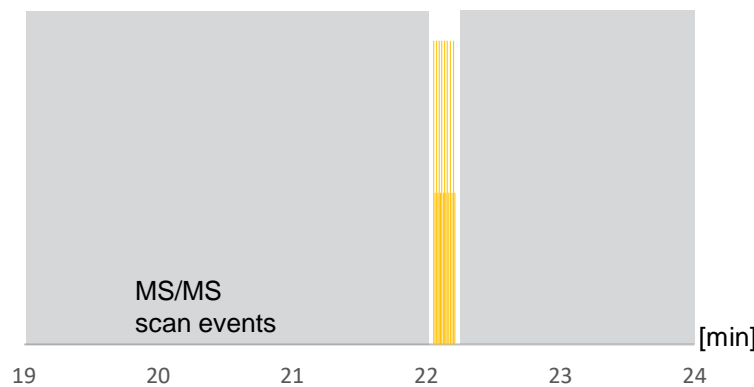
2.5-min monitoring window

LCDSGELVAIK

SureQuant



ENDO



Enhanced Productivity

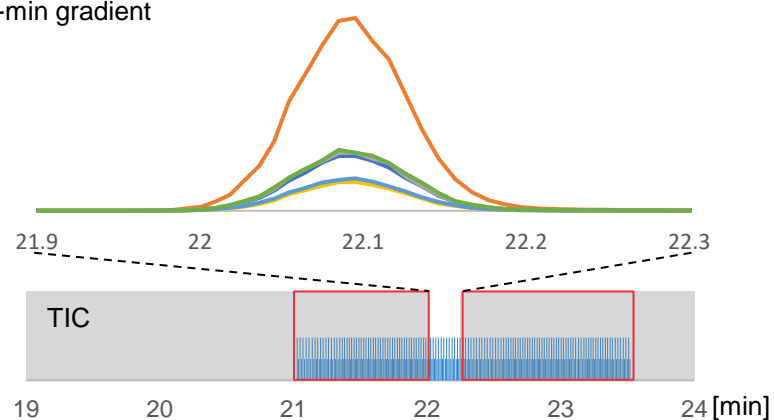
Proportion productive MS2 scans
(% MS2 acquisition time)

PRM ≈ 10-15 %

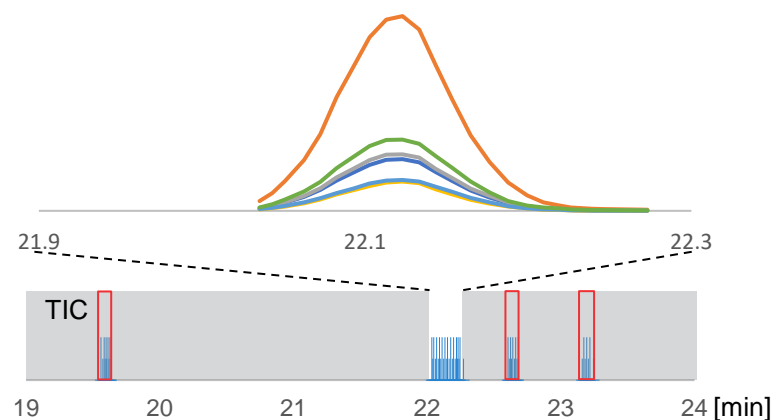
SureQuant = 80-90 %

SureQuant Acquisition Delivers Intelligent Detection of Targets, Enhancing Productivity

250 ng cell line digest, 50 fmol internal standards
60 peptides
30-min gradient

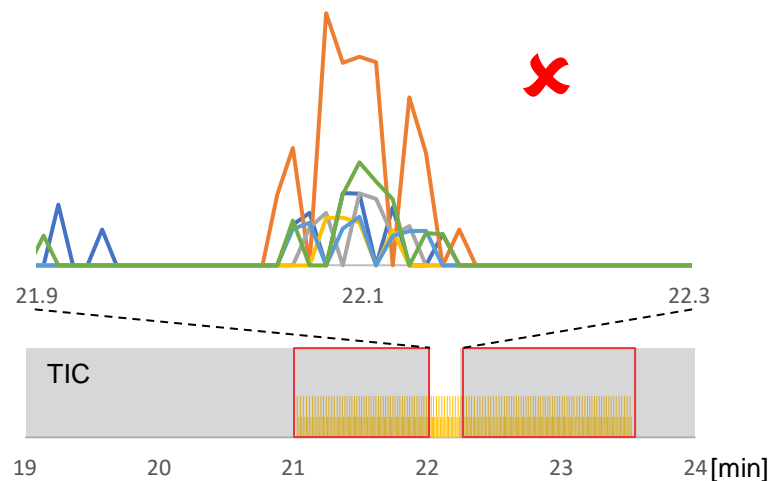


SIL



PRM

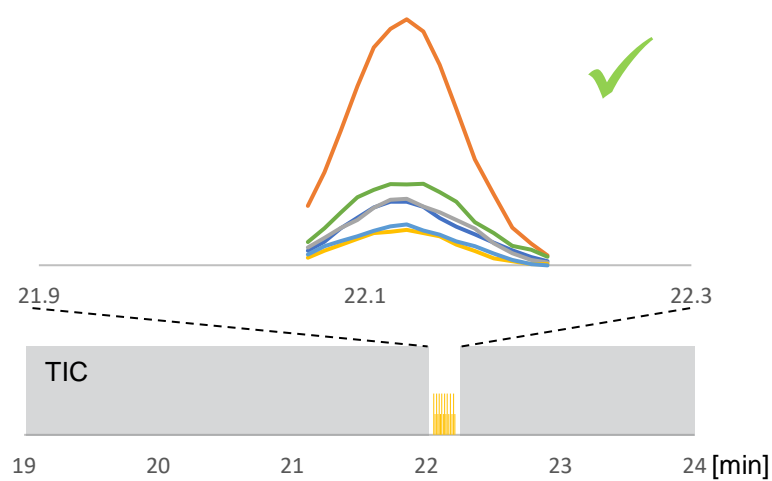
7.5k / 10 ms ; 2.5-min monitoring window



LCDSGELVAIK

SureQuant

Watch mode: 7.5k / 10 ms ; Quant mode: 60k / 116 ms



Enhanced Productivity
Proportion productive MS2 scans
(% MS2 acquisition time)
PRM \approx 10-15 %
SureQuant \approx 80-90 %

Higher Performance
(From extra acquisition time)

Enhanced data quality
Enhanced experiment scale
(number of peptides monitored)
Enhanced throughput

SureQuant Workflow Support in Current Version of Skyline-Daily

MacCoss Lab Software

Home

Start Page / Tips

Triggered Acquisition in Skyline 20.2

Triggered Acquisition in Skyline 20.2

As of Skyline-Daily 20.1.1.83, Skyline-Daily has support for "Triggered Acquisition" methods.

A Triggered Acquisition method is one where the mass spectrometer has been told to begin collecting MS2 scans for one analyte when the mass spectrometer sees particular transitions for a different precursor.

This will enable Skyline to work better with assays such as Thermo's SureQuant Targeted Mass Spec Assay Kits

The Transition Settings > Instrument tab will have a "Triggered Acquisition" checkbox which tells Skyline that there may be large gaps between the points in an analyte's chromatograms.

When Triggered Acquisition is selected, Skyline will detect these gaps and make sure that integration boundaries do not cross these gaps. Also, Skyline will perform no background subtraction when Triggered Acquisition is enabled.

For more information, see the attached PowerPoint.

Attached Files

TriggeredAcquisitionTip.pptx

Settings Tools Help

Default

Save Current...

Edit List...

Share...

Import...

Peptide Settings...

Transition Settings...

Document Settings...

Integrate All

Transition Settings

Prediction Filter Library Instrument Full-Scan

Min m/z: 180 m/z Max m/z: 1700 m/z

Dynamic min product m/z

Method match tolerance m/z: 0.055 m/z

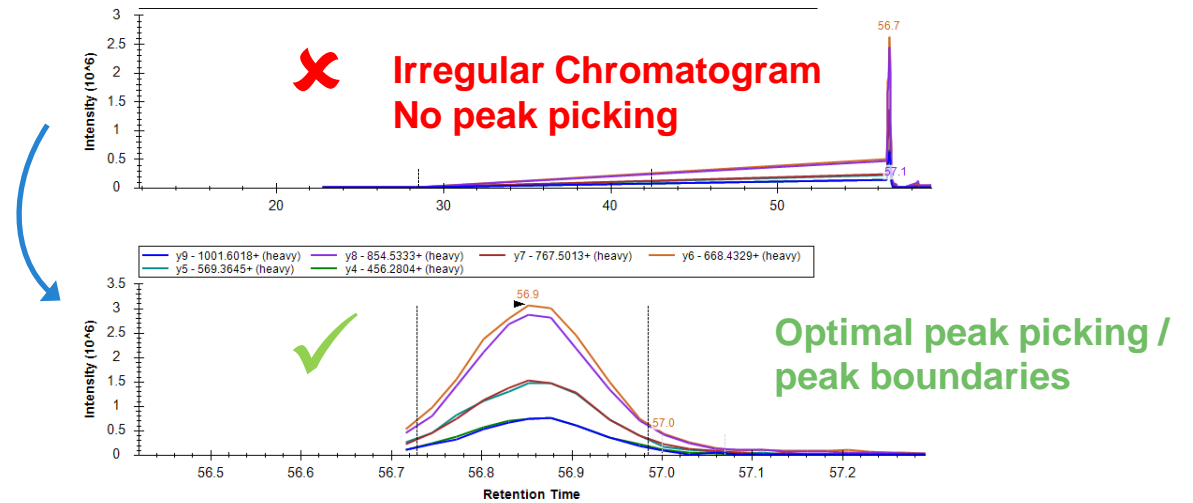
Firmware transition limit: min Max time: min

Triggered chromatogram acquisition

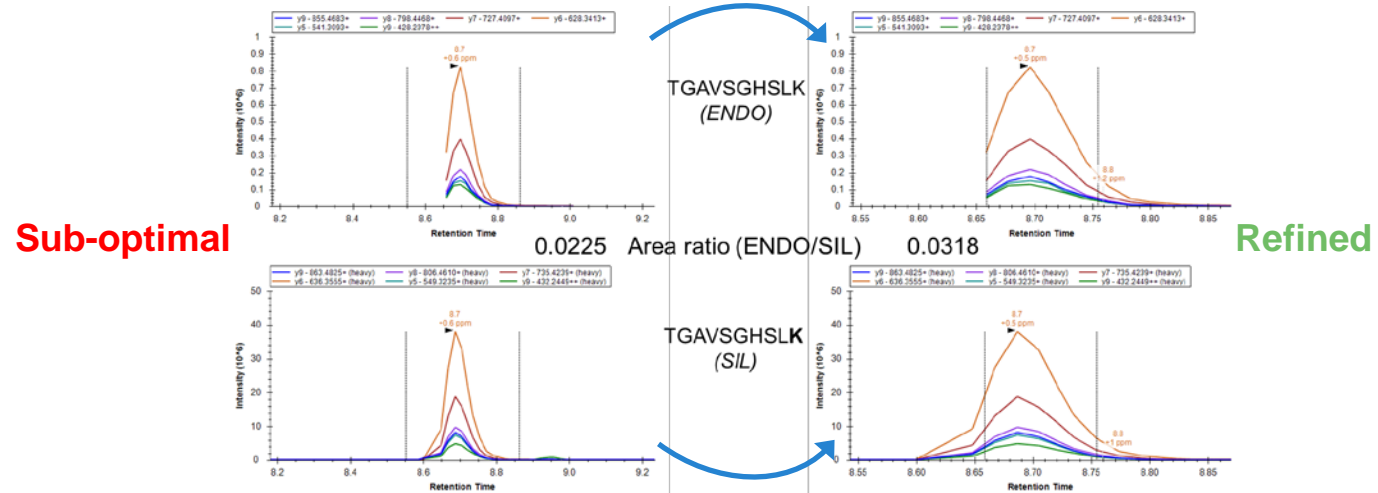
OK Cancel

Indicates that there may be large gaps in the retention times when spectra were acquired for certain analytes. Peaks will be truncated to not overlap with these gaps and background subtraction will not be performed.

Benefit #1: Improved peak picking



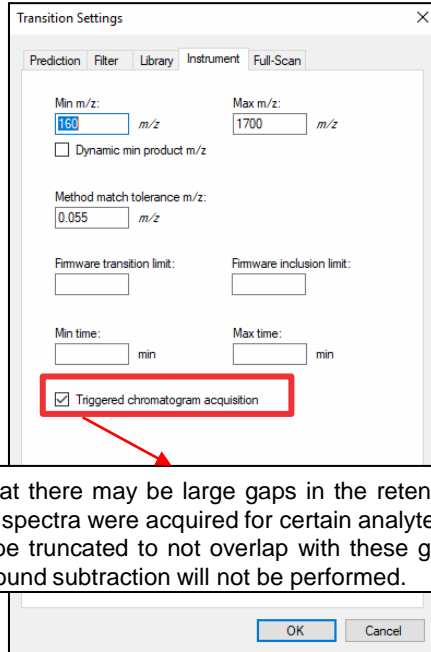
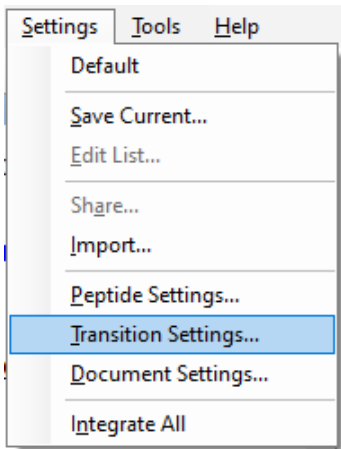
Benefit #2: Improved peak boundaries definition



SureQuant Workflow Support in Current Version of Skyline-Daily

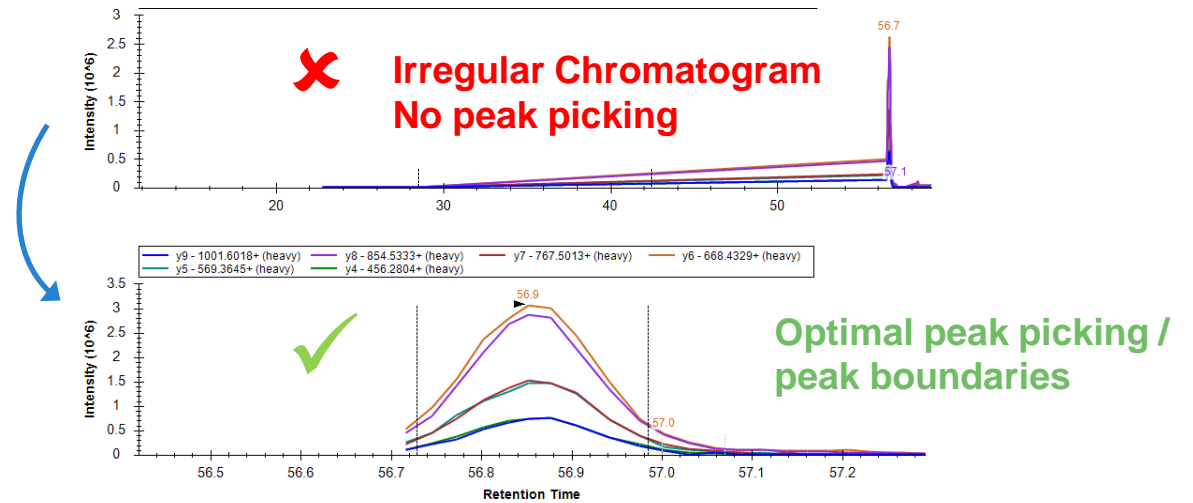
Improved execution of SureQuant workflow

- Improved peak picking
- Improved peak boundaries definition
- Improved signal extraction (better consistency)
- Limited manual input

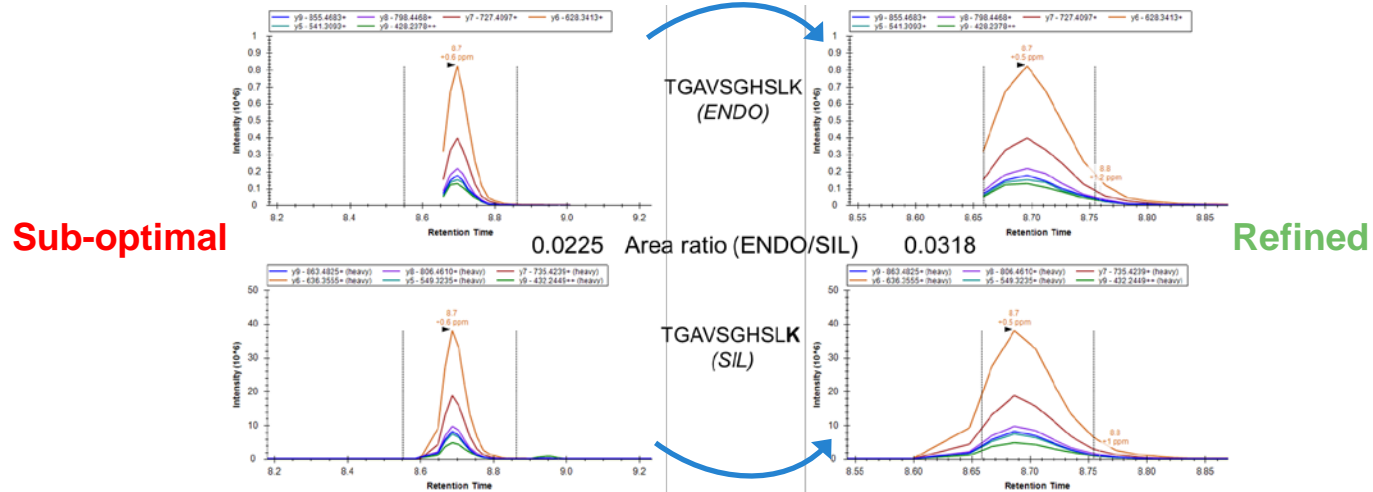


Indicates that there may be large gaps in the retention times when spectra were acquired for certain analytes. Peaks will be truncated to not overlap with these gaps and background subtraction will not be performed.

Benefit #1: Improved peak picking



Benefit #2: Improved peak boundaries definition



Acknowledgements – SureQuant Related Work in the Spotlight

ThermoFisher
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- Aaron Gajadhar
- Bhavin Patel
- Penny Jensen
- Christian Thoeing
- Dave Horn
- Carmen Paschke
- Bernard Delanghe
- Shannon Eliuk
- Emily Chen
- Daniel Lopez-Ferrer
- Andreas Hühmer



- Nick Shulman
- Brendan MacLean

- Multi-Pathway Phosphopeptide SureQuant Profiling (MP538)
- SureQuant Analysis of Ras and AKT Signaling Pathways (TP476)
- SureQuant HCP Analysis for BioPharma Production (MOC pm 03:10)
- "Plasma Proteome in a Kit: Targeted Proteomics on a Discovery Scale"
- "High-density Monitoring of Tyrosine Phosphorylation Signaling Targets in Human Tumors using IS triggered targeted quantitation"
- SureQuant Workflow Support in Proteome Discoverer - Specific Nodes used in Combination with Skyline (version 2.5 and higher)



- Michael Poltash
- Andy Mahan



- Lauren Stopfer
- Forest White
- Cam Flower

ASMS 2020 Reboot



May 26-28



BIOGNOSYS



Massachusetts
Institute of
Technology

bioRxiv
THE PREPRINT SERVER FOR BIOLOGY



BIOGNOSYS

- Sebastian Müller
- Tejas Gandhi
- Jan Muntel
- Lukas Reiter