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

Sebastien Gallien, PhD

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Introducing **SureQuant** Targeted Protein Quantitation

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

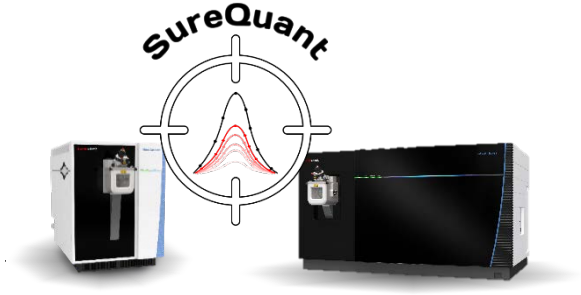
Synthetic Heavy Peptides

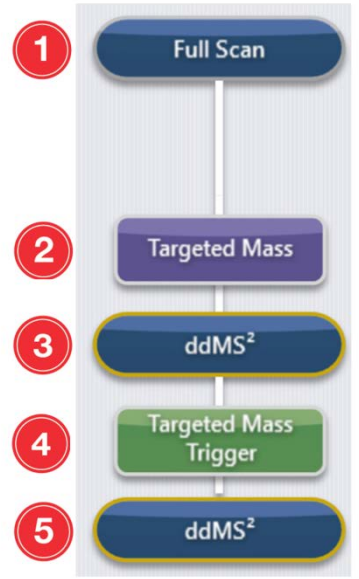
#1
#2
....
#n

Mix

Sample

LC-MS Analysis





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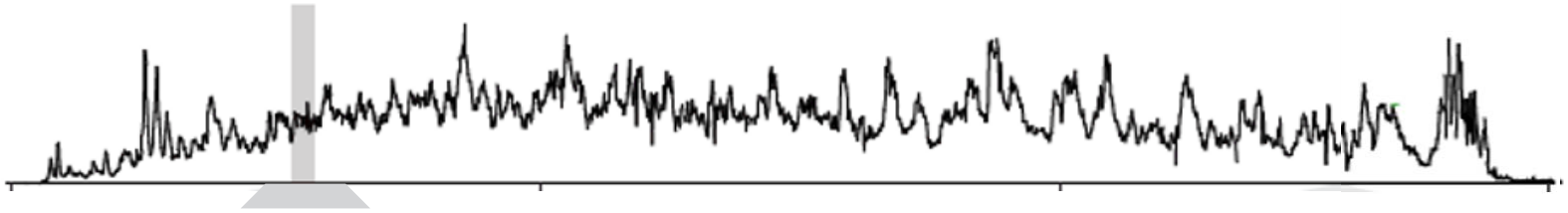
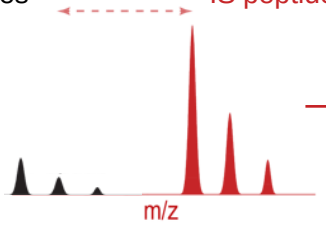


2 ENDO Peptides — IS peptides (trigger)

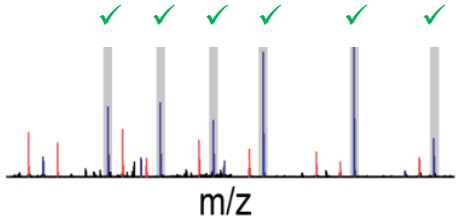


MS1 Survey Scan
(Precursor m/z, intensity threshold)

3

WATCH MODE
Fast, Low Res MS2
of Trigger

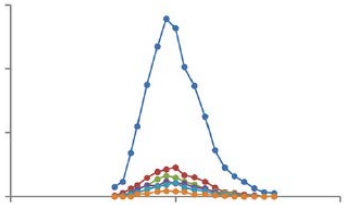
4 Pseudo Spectral Matching



MS/MS IS
(≥ Min. number fragment m/z)

5

QUANT MODE
High Quality MS2
of Target



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Introducing **SureQuant** Targeted Protein Quantitation

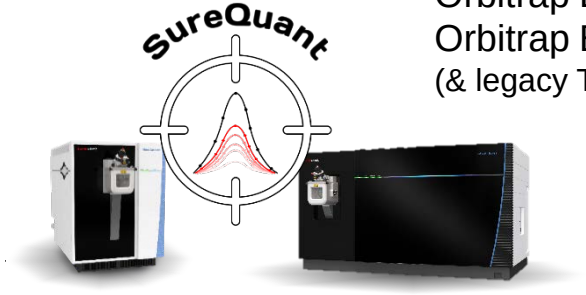
Intelligent Data Acquisition Strategy for Large-scale, Sensitive, Precise and Reproducible Quantitation

High Usability & Stability

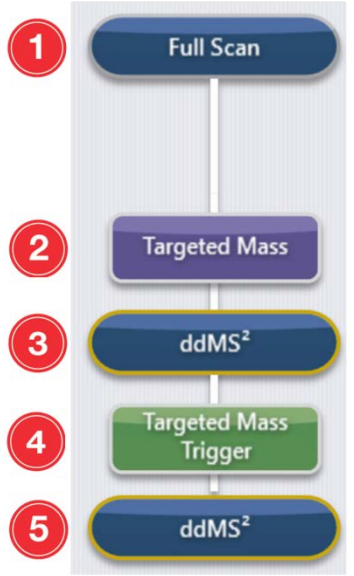
Native implementation in ICSW

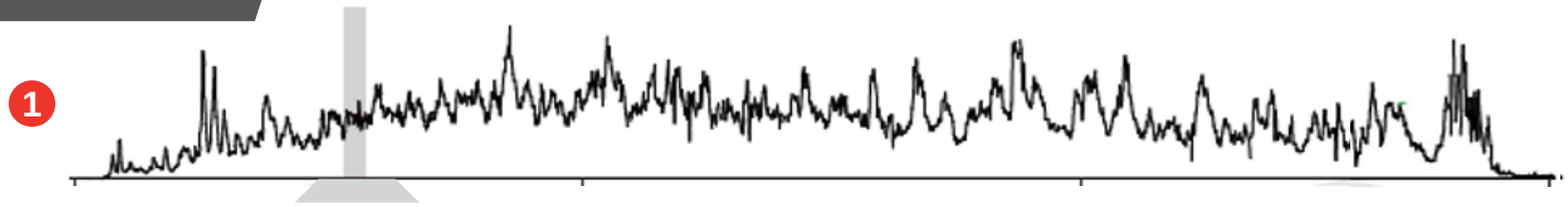
Generic scan events / filters

Optimized scan execution matrix



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





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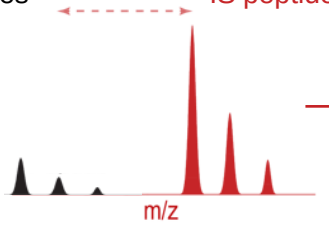
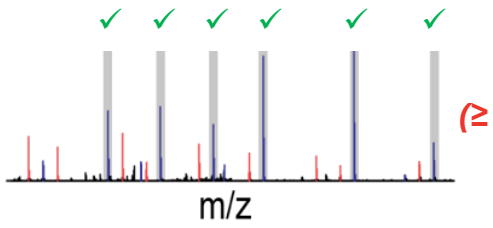
Pseudo Spectral Matching 4



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

WATCH MODE
Fast, Low Res MS2
of Trigger



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High Quality MS2
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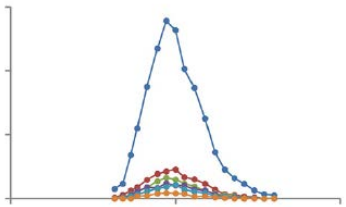

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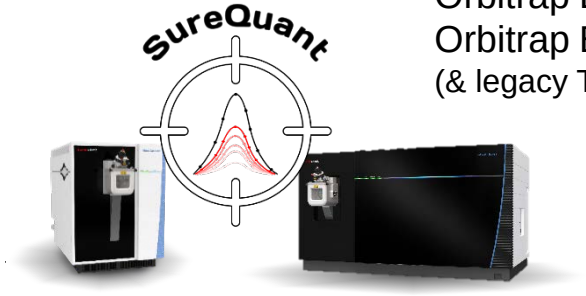

Orbitrap Exploris 480 MS
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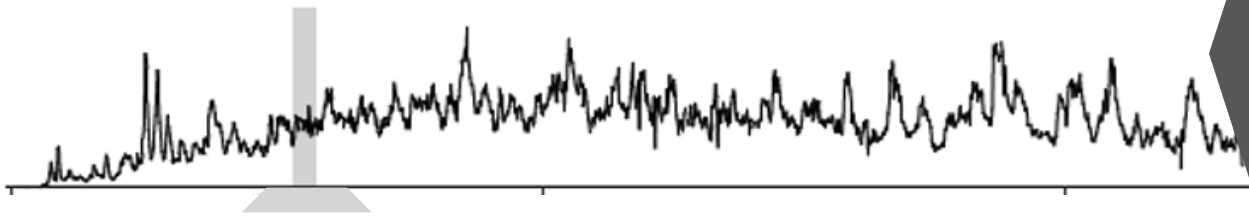
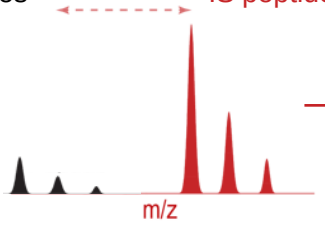


Robust Acquisition

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

2 ENDO Peptides — IS peptides (trigger)

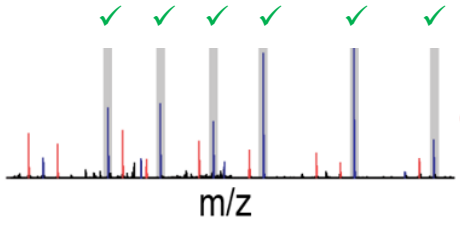
MS1 Survey Scan
(Precursor m/z, intensity threshold)



3

WATCH MODE
Fast, Low Res MS2
of Trigger

Pseudo Spectral Matching **4**



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(≥ Min. number fragment m/z)

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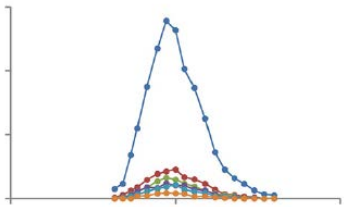
QUANT MODE
High Quality MS2
of Target



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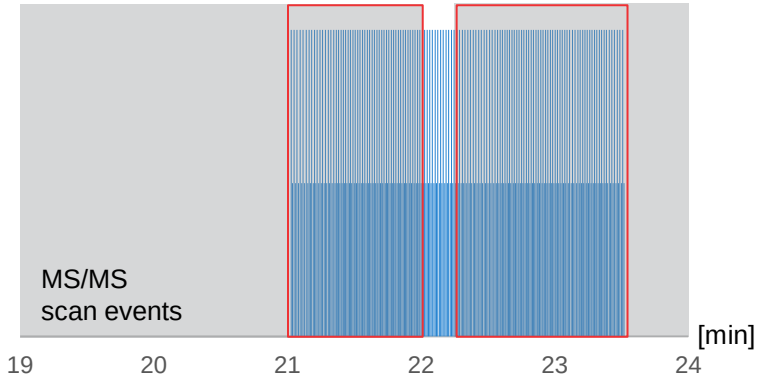
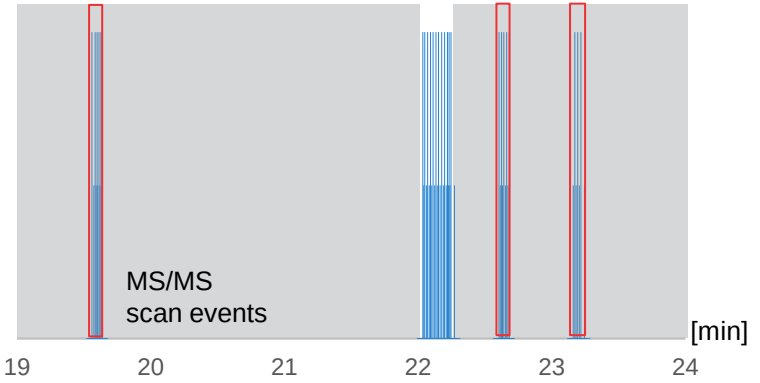
SureQuant Acquisition Delivers Intelligent Detection of Targets, Enhancing Productivity

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

Non-productive MS2 scans Productive MS2 scans



SIL

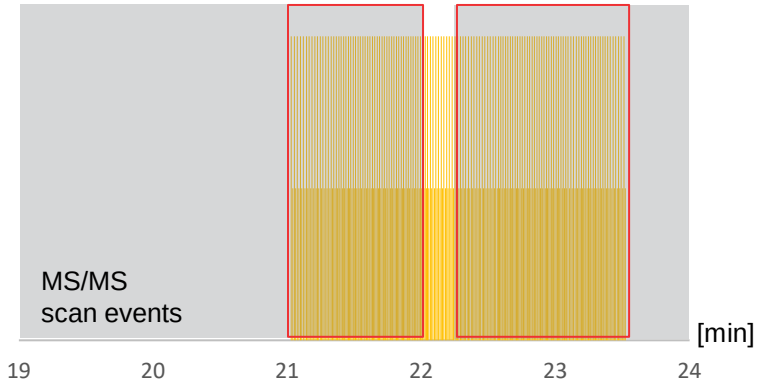


PRM

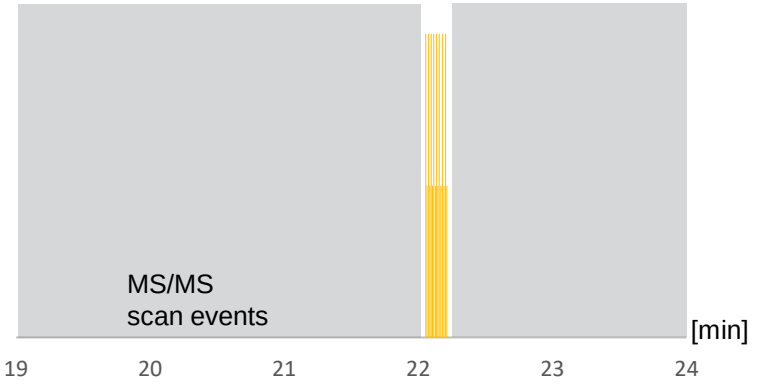
LCDSGELVAIK

SureQuant

2.5-min monitoring window



ENDO



Limited false positive triggering MS/MS of Internal Standard peptides

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

Enhanced Productivity

Proportion productive MS2 scans
(% MS2 acquisition time)

PRM ≈ 10-15 %

SureQuant ≈ 80-90 %

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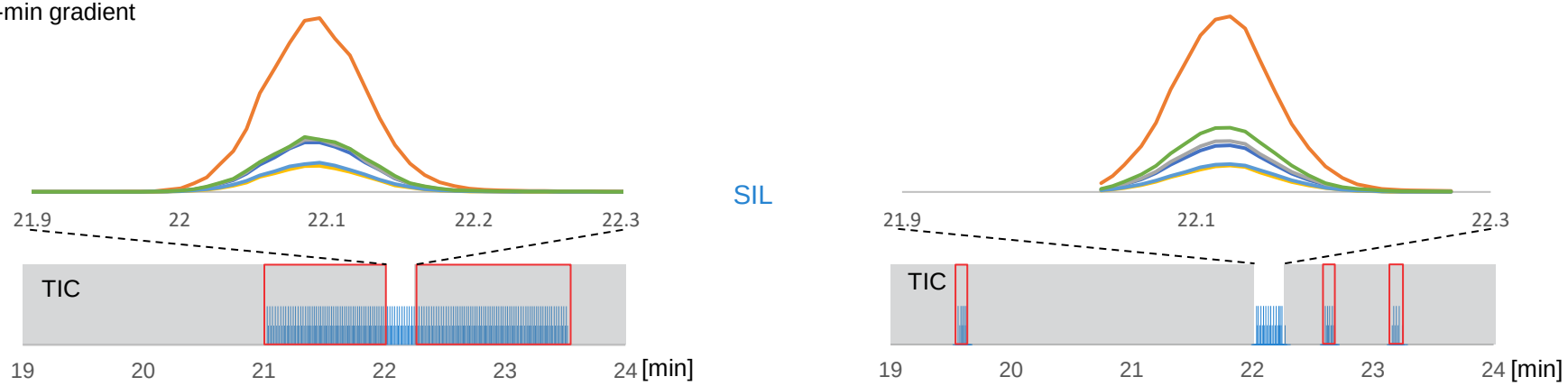
SureQuant Acquisition Delivers Intelligent Detection of Targets, Enhancing Productivity

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



Enhanced Productivity

Proportion productive MS2 scans
(% MS2 acquisition time)

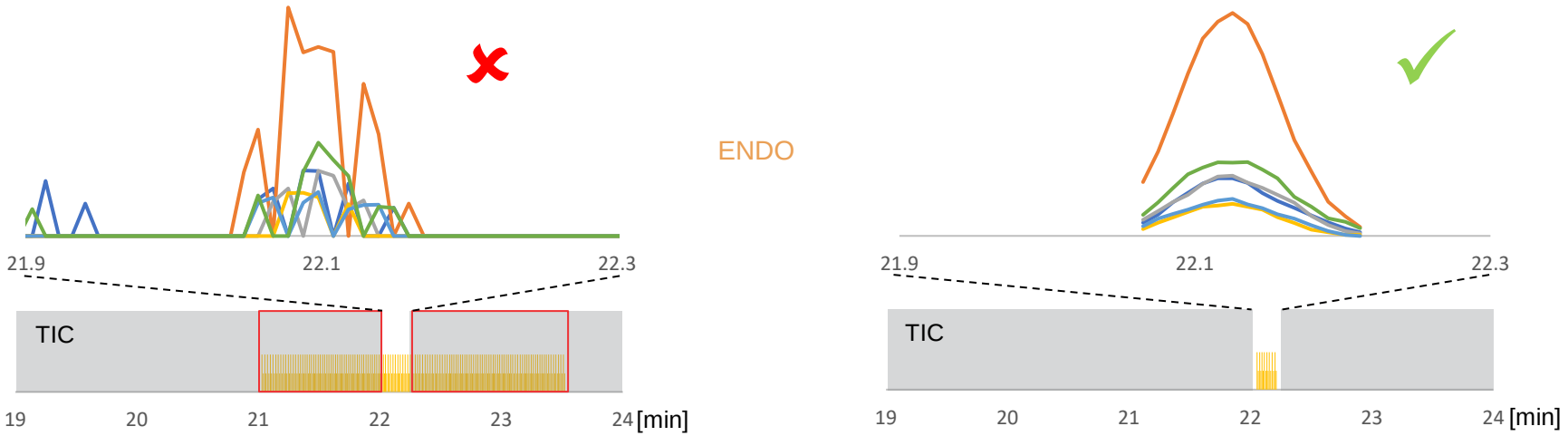
PRM ≈ 10-15 %

SureQuant ≈ 80-90 %

PRM **LCDSGELVAIK** ***SureQuant***

7.5k / 10 ms ; 2.5-min monitoring window

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



Higher Performance

(From extra acquisition time)

Enhanced data quality

Enhanced experiment scale (number of peptides monitored)

Enhanced throughput

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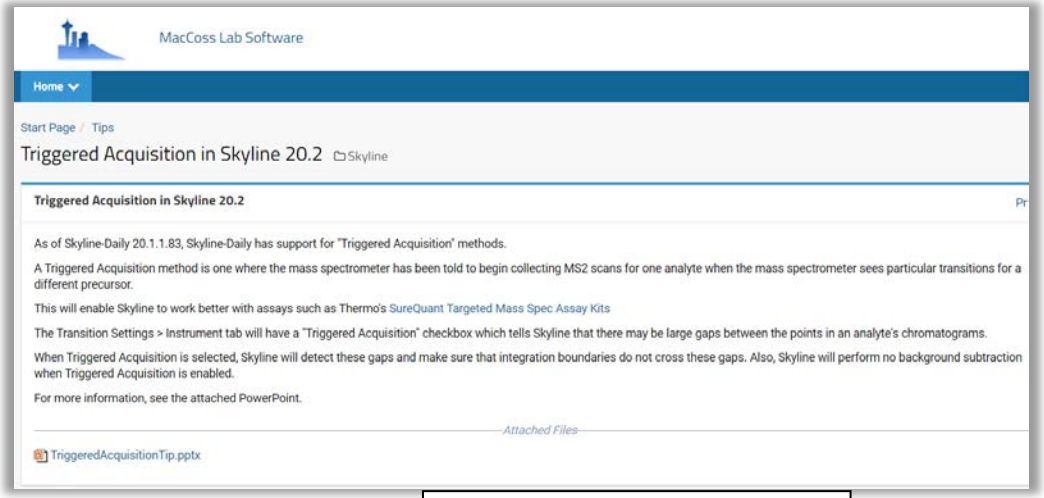
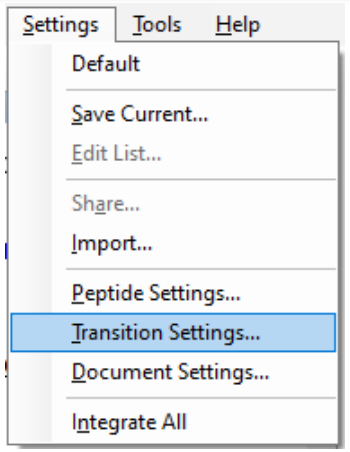
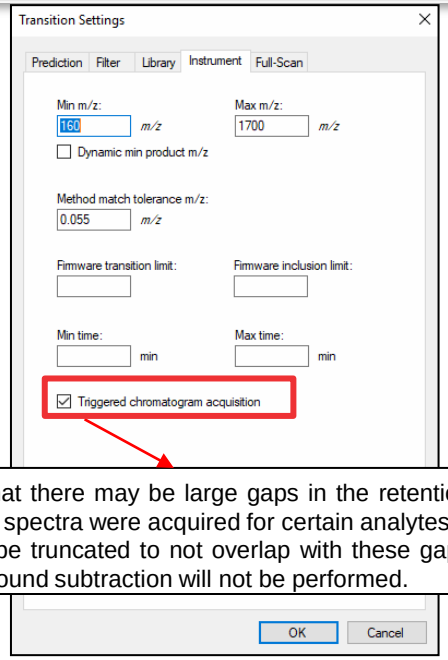
SureQuant Workflow Support in Current Version of Skyline-Daily

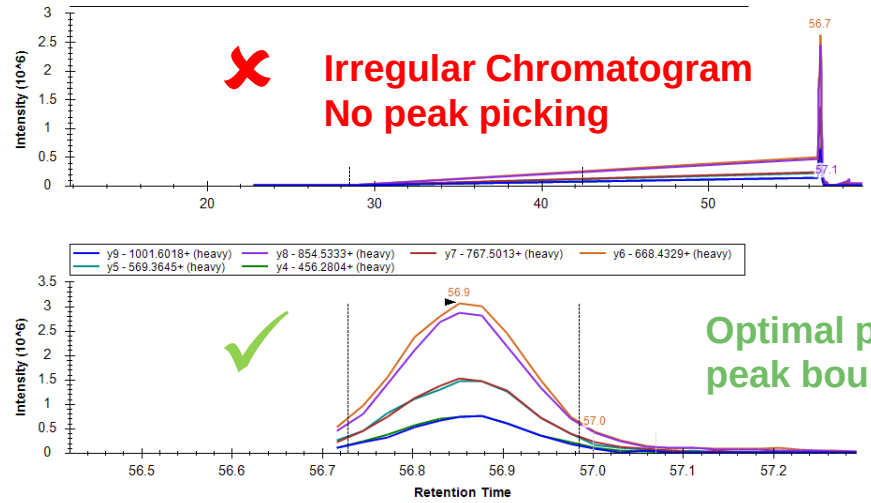




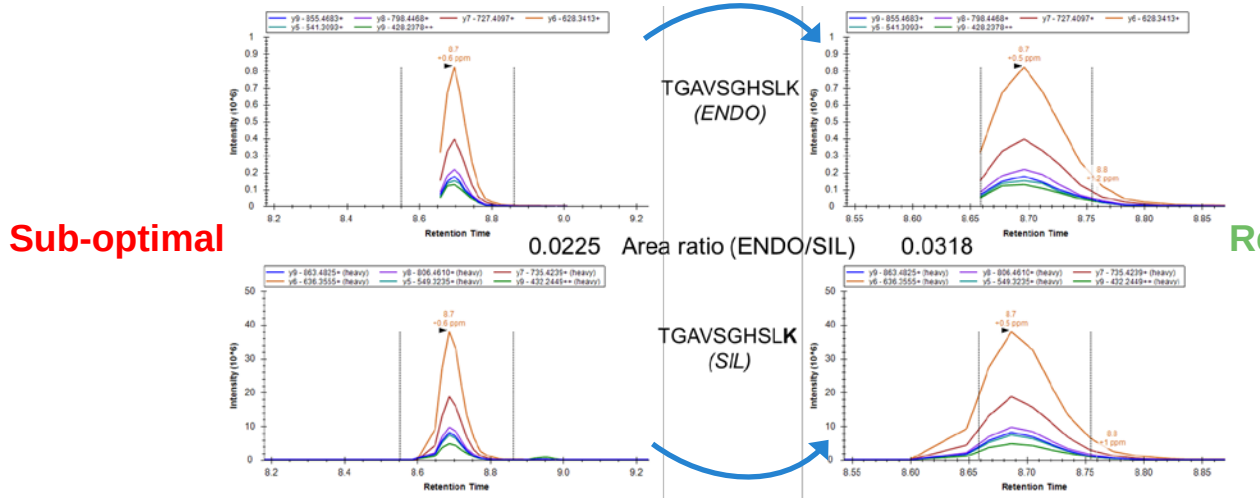


Indicates that there may be large gaps in the retention times when spectra were acquired for certain analytes. Peaks will be truncated to not overlap with these gaps and background subtraction will not be performed.

Benefit #1: Improved peak picking



Benefit #2: Improved peak boundaries definition



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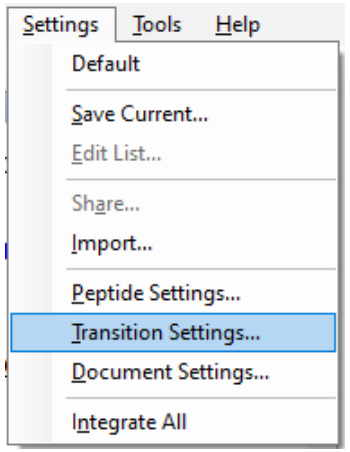
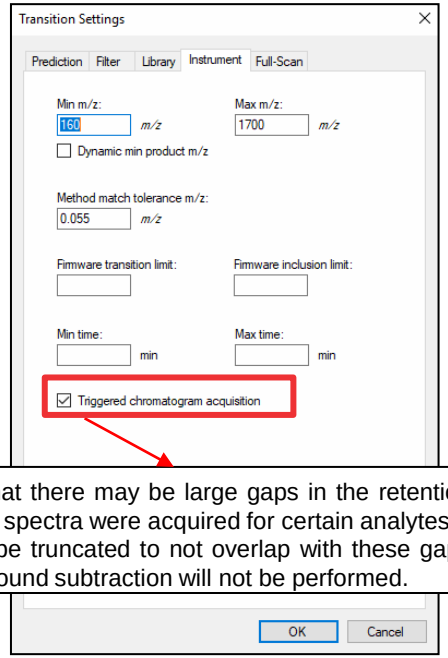
SureQuant Workflow Support in Current Version of Skyline-Daily

Improved execution of SureQuant workflow

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





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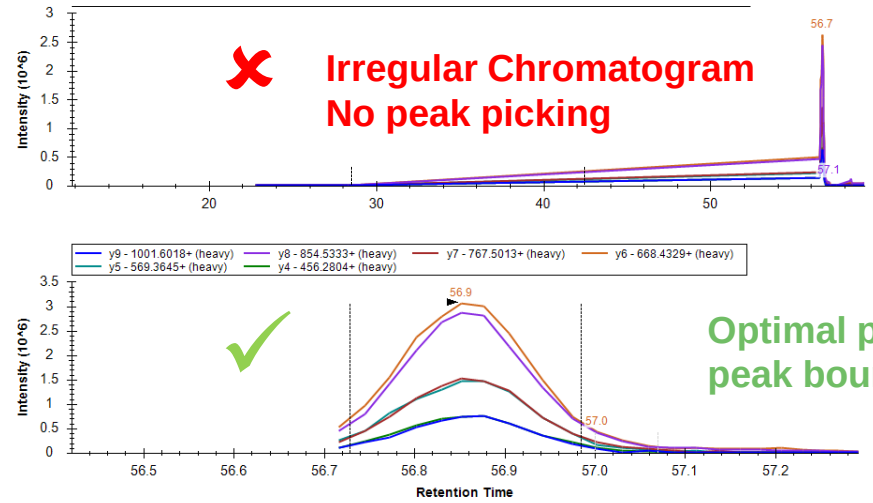

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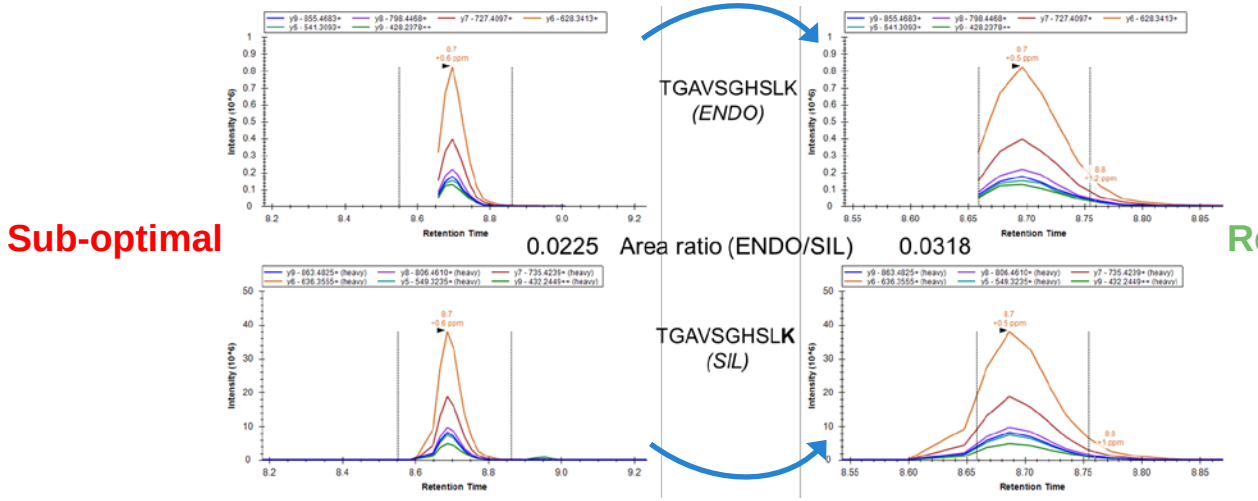

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Acknowledgements – SureQuant Related Work in the Spotlight

ThermoFisher SCIENTIFIC

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

Skyline

- Nick Shulman
- Brendan MacLean

janssen PHARMACEUTICAL COMPANIES OF Johnson&Johnson

- Michael Poltash
- Andy Mahan

Massachusetts Institute of Technology

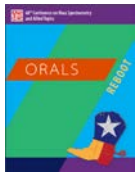
- Lauren Stopfer
- Forest White
- Cam Flower

BIOGNOSYS

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

- Multi-Pathway Phosphopeptide SureQuant Profiling (MP538)
- SureQuant Analysis of Ras and AKT Signaling Pathways (TP476)

ASMS 2020 Reboot



- SureQuant HCP Analysis for BioPharma Production (MOC pm 03:10)

janssen PHARMACEUTICAL COMPANIES OF Johnson&Johnson

- "Plasma Proteome in a Kit: Targeted Proteomics on a Discovery Scale"

May 26-28

vLC-MS.com

BIOGNOSYS

- "High-density Monitoring of Tyrosine Phosphorylation Signaling Targets in Human Tumors using IS triggered targeted quantitation"

Massachusetts Institute of Technology

bioRxiv THE PREPRINT SERVER FOR BIOLOGY

- SureQuant Workflow Support in Proteome Discoverer - Specific Nodes used in Combination with Skyline (version 2.5 and higher)

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