Introduction to the Thermo Scientific Q Exactive HF-X MS for Proteomics

October 2017
Josh Nicklay

Thermo Scientific Q Exactive HF-X Hybrid Quadrupole-Orbitrap MS

Pushing the leading edge in protein analysis

- Revolutionizing insights, from discovery to verification
- Enhance your productivity
- Achieve faster than ever scan speed
- Confirm with greater confidence
- Superior consistency in quantitative accuracy, sensitivity and reproducibility

Thermo Scientific Q Exactive HF-X Hybrid Quadrupole-Orbitrap MS

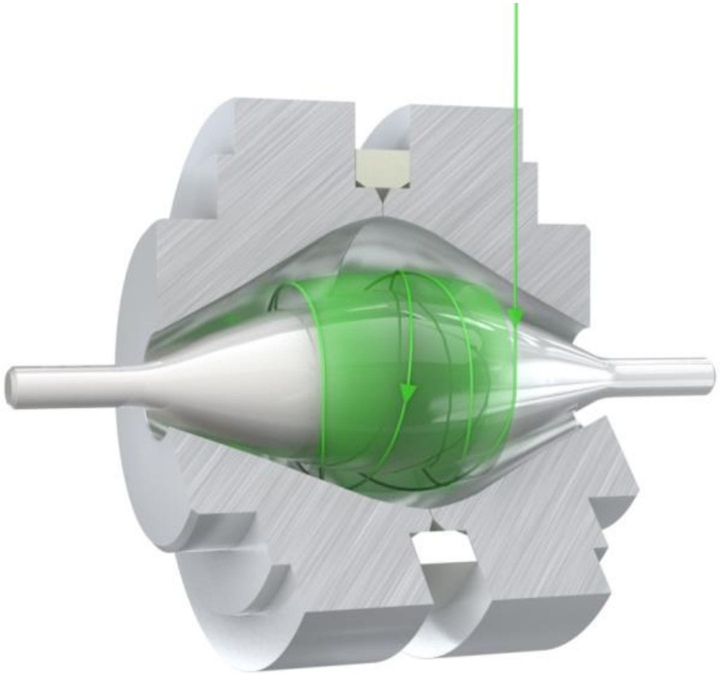
Novel architecture with a high capacity transfer tube and electrodynamic ion funnel

Ultra-high field Orbitrap analyzer

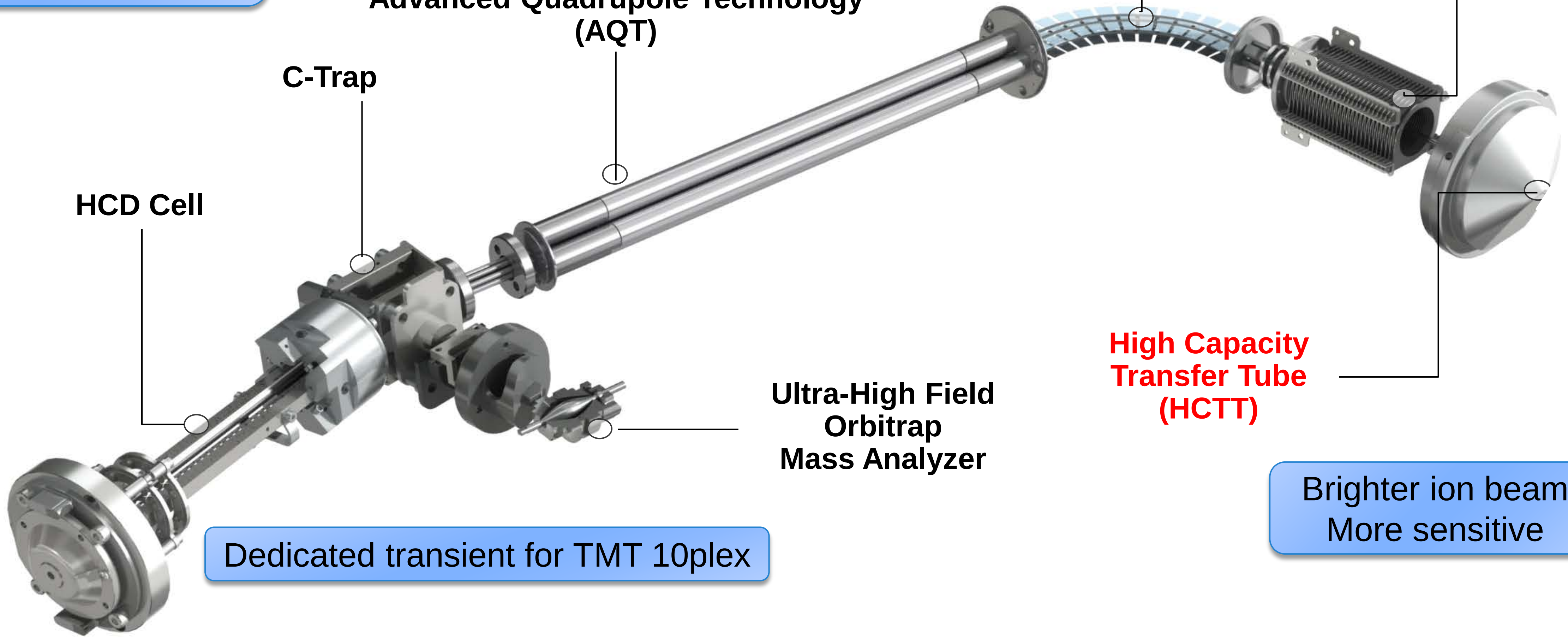
- 240,000 resolution at *m/z* 200
- 40 Hz data acquisition speed @ 7,500 resolution

Advanced Peak Determination (APD)

BioPharma Option for intact proteins



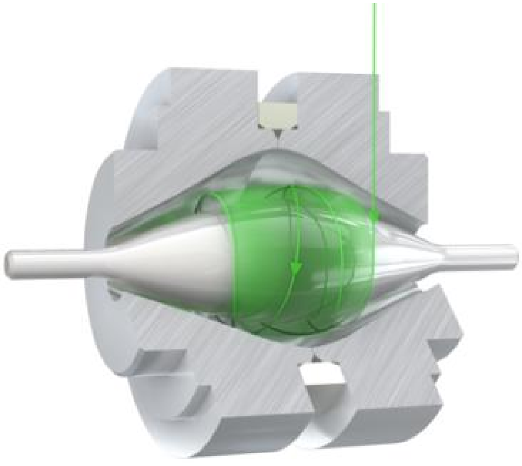
Thermo Scientific Q Exactive HF-X MS – New Architecture



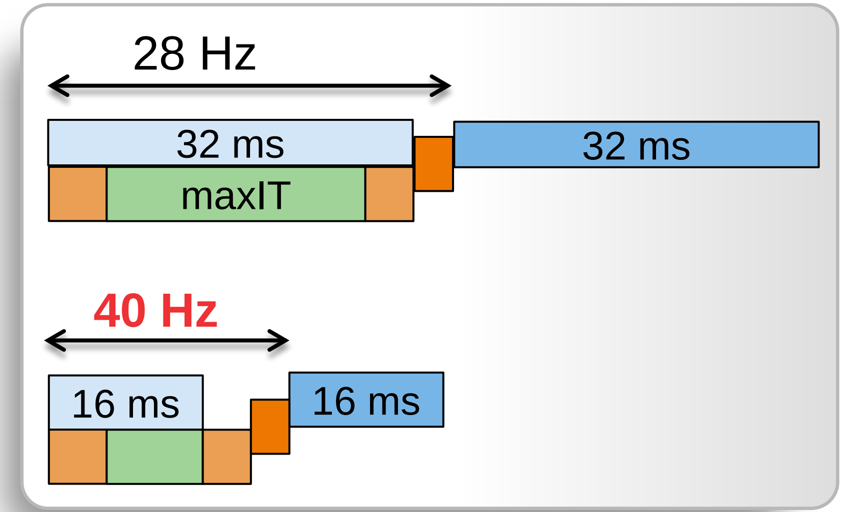
Key Technologies of Thermo Scientific Q Exactive HF-X MS

High Capacity Transfer Tube

Electrodynamic Ion Funnel

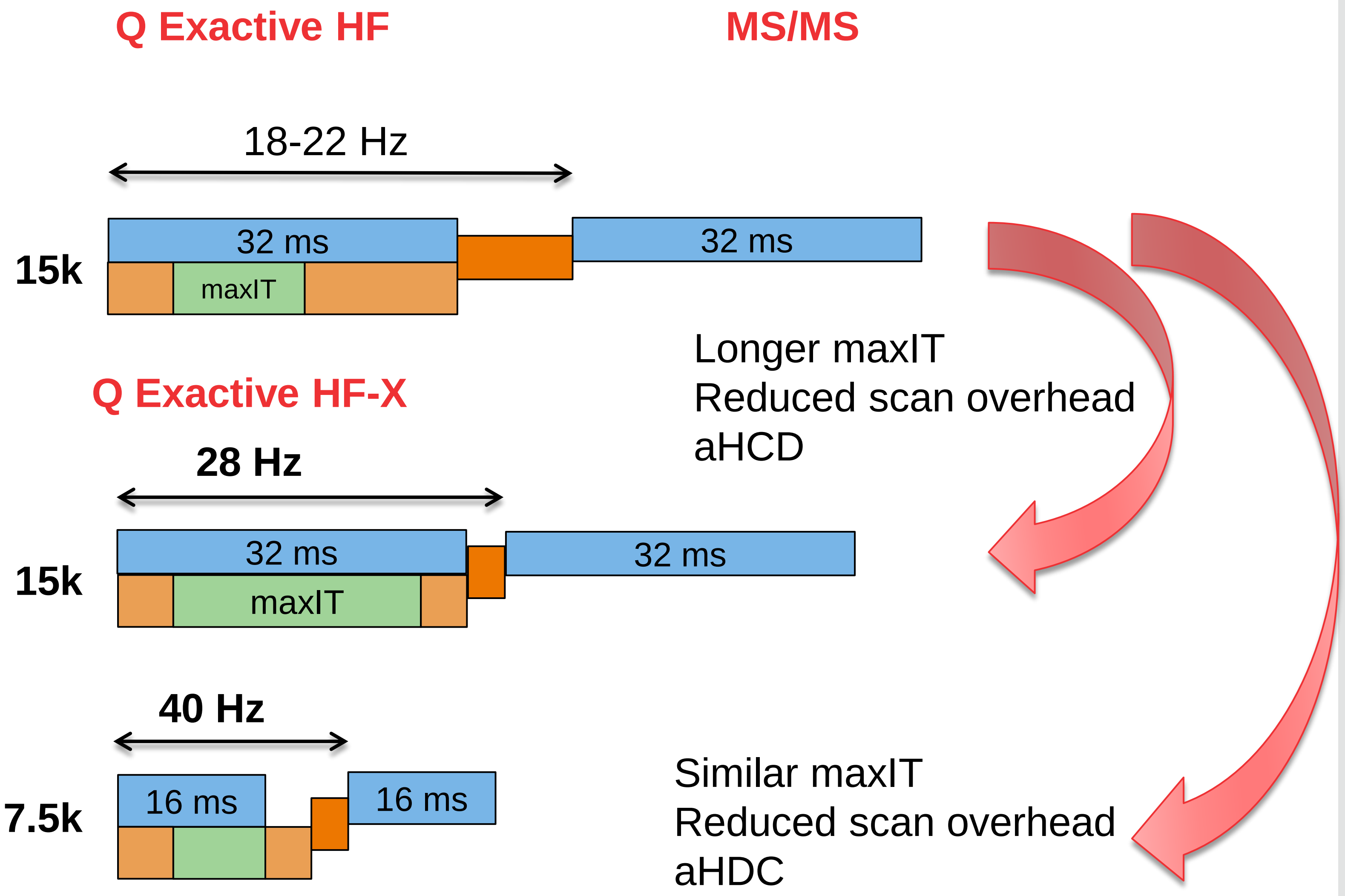


Ultra-High Field Orbitrap



Optimized Scan Matrix and new Transient lengths

Optimized Scan Matrix



Longer maxIT
Reduced scan overhead
aHCD

Similar maxIT
Reduced scan overhead
aHDC

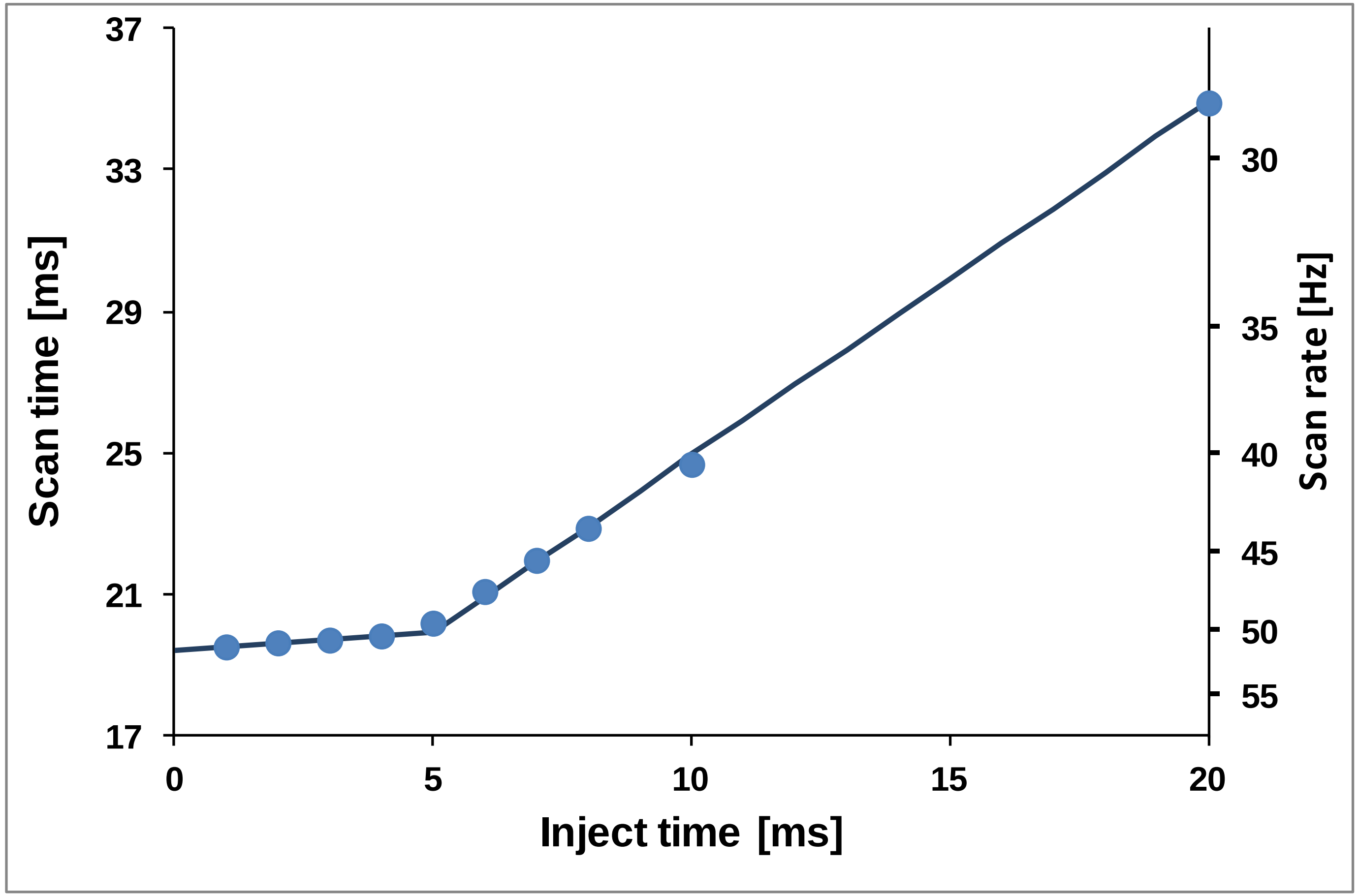
- Brighter ion beam, reduced scan overhead, and accelerated HCD (aHCD) is boosting acquisition speed
- Advantage for both MS and MS/MS mode
- Fast and high quality MS/MS acquisition up the 40 Hz with new 16 msec transient (7,500 resolution setting)

🟦 Orbitrap detection

🟩 Maximum fill time with precursor ions

🟧 Inter and intra scan overheads

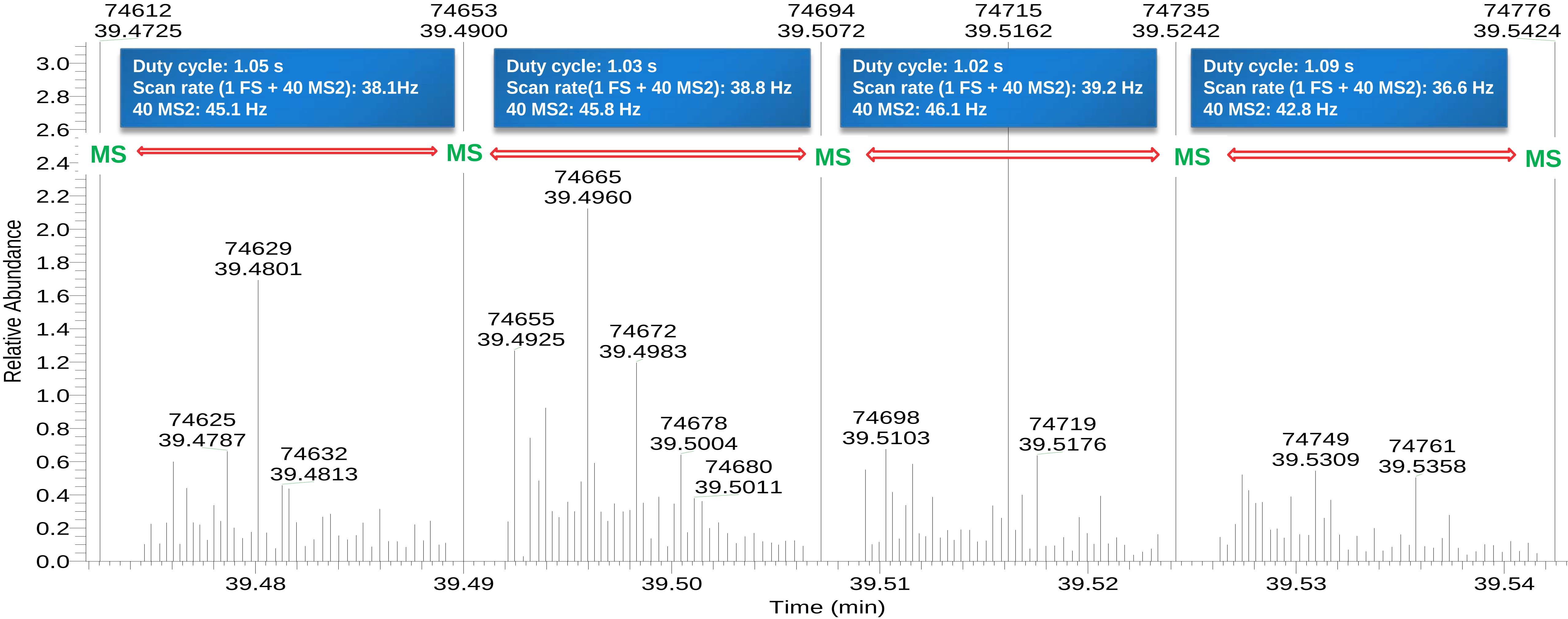
MS/MS Scan Rate



- MS/MS scan rate at resolution setting 7,500 @ m/z 200 for Q Exactive HF-X with respect to the ion injection time is displayed.
- Sample: Calmix
- 40 Hz MS/MS scan rate is reached with a maximum injection time (maxIT) of 10 ms

ASMS’17: TP 389, T.N. Arrey et al. New innovations implemented on the Thermo Scientific™ Q Exactive™ HF MS.

Ultra Fast MS/MS Scan Speed > 40 Hz



Duty cycle: 1.05 s
Scan rate (1 FS + 40 MS2): 38.1Hz
40 MS2: 45.1 Hz

Duty cycle: 1.03 s
Scan rate(1 FS + 40 MS2): 38.8 Hz
40 MS2: 45.8 Hz

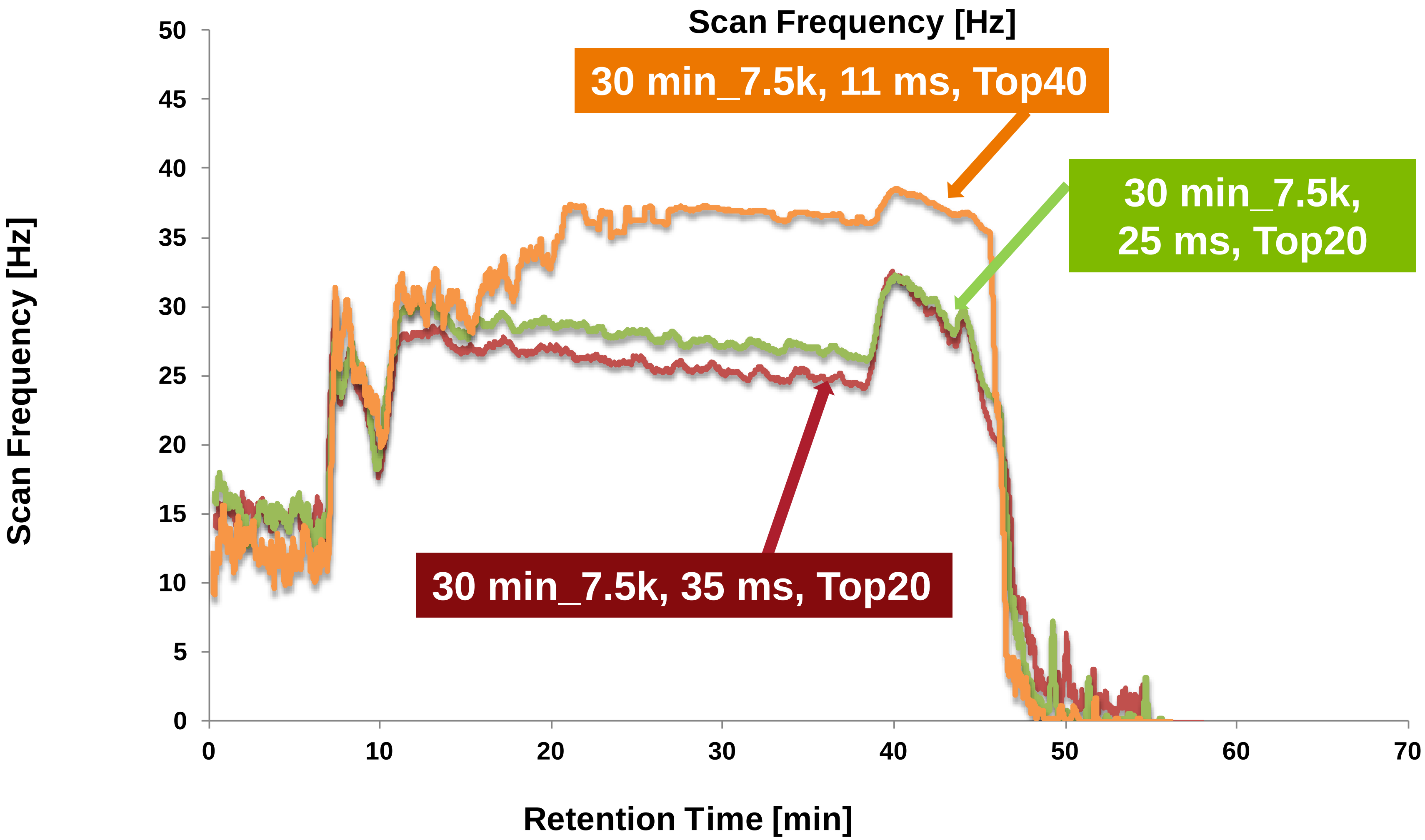
Duty cycle: 1.02 s
Scan rate (1 FS + 40 MS2): 39.2 Hz
40 MS2: 46.1 Hz

Duty cycle: 1.09 s
Scan rate (1 FS + 40 MS2): 36.6 Hz
40 MS2: 42.8 Hz

1 full scan (60,000 @ m/z 200) and 40 MS^2 scans 7,500@ m/z 200) at LC time scale in 1 second.
30 min gradient, MS2 max IT: 11 ms

ASMS’17: TP 389, T.N. Arrey et al. New innovations implemented on the Thermo Scientific™ Q Exactive™ HF MS.

Calculated Scan Rate Across the LC Gradient for 1 µg HeLa Digest

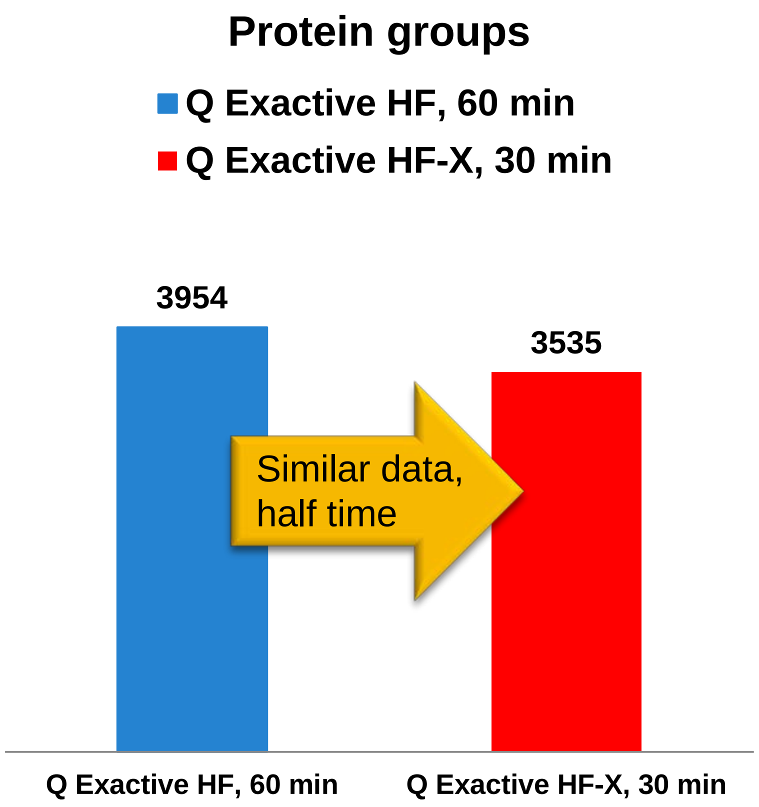


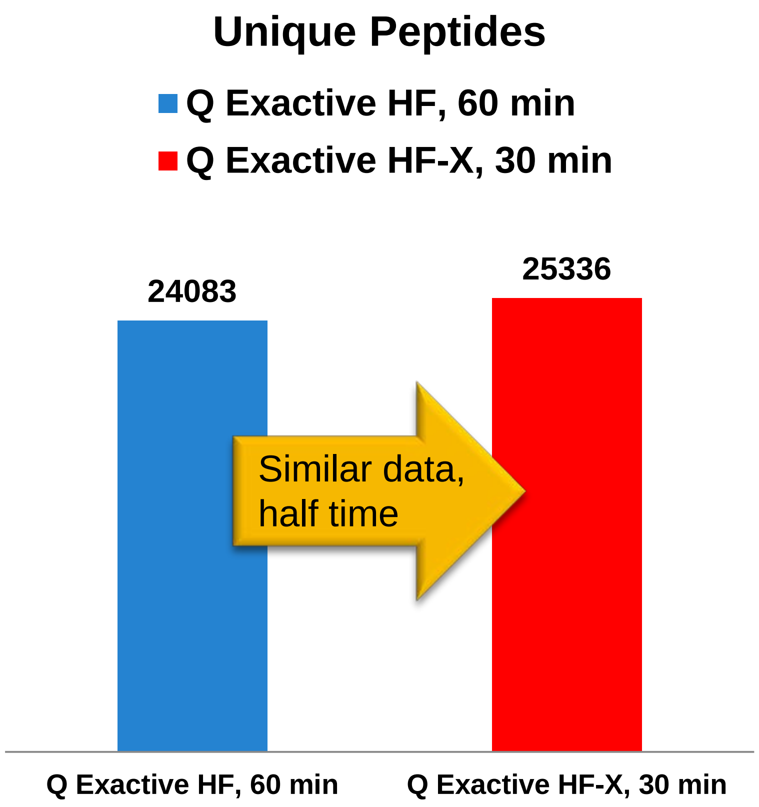
30 min gradients:

- Top40 method:
  Scan rates of 38 Hz are obtainable at high ion flux with 11 ms max. inj. times applied
- Top20 method:
  Scan rates of > 25 Hz are obtained with longer max. inj. times (25 ms and 35 ms, resp.)

ASMS'17: TP 389, T.N. Arrey et al. New innovations implemented on the Thermo Scientific™ Q Exactive™ HF MS.

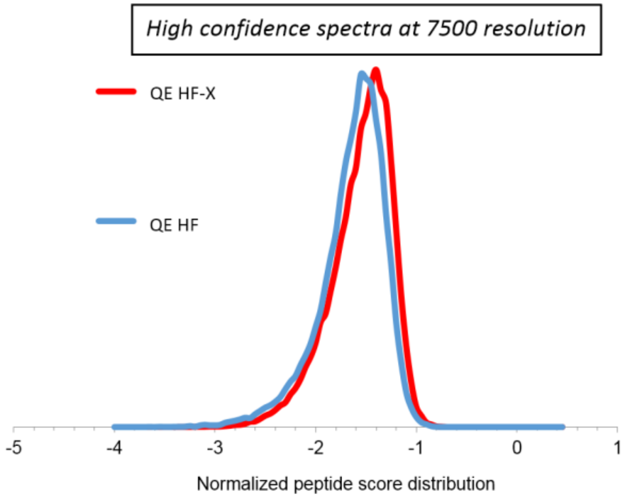
Protein Identification Faster than Ever





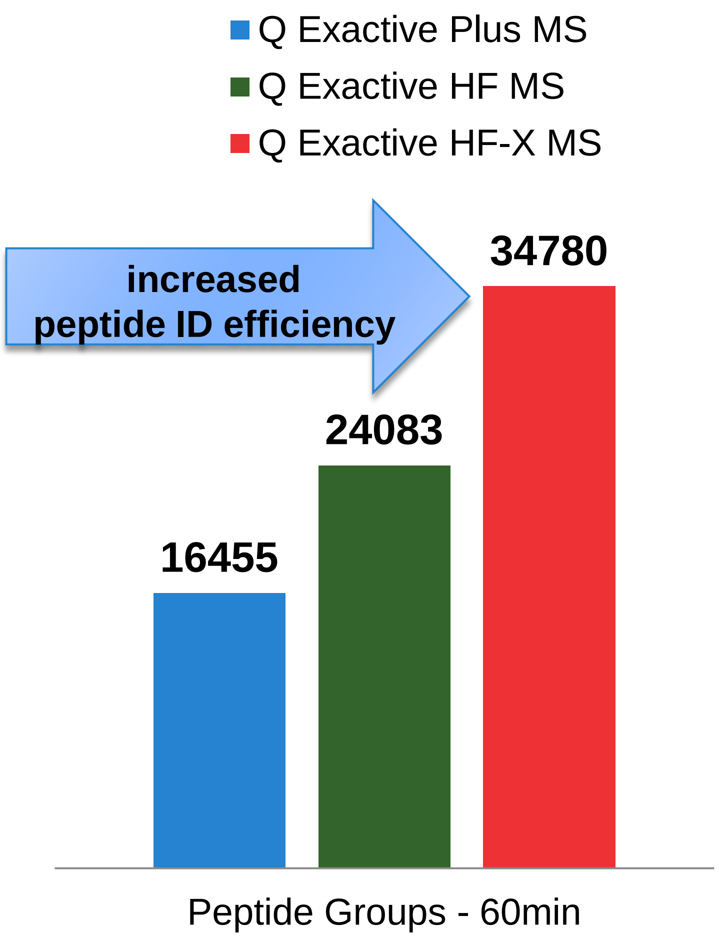
1 µg HeLa, nano LC, 120k res MS1, DDA Top 20/40, minimum injection time 45/25 msec

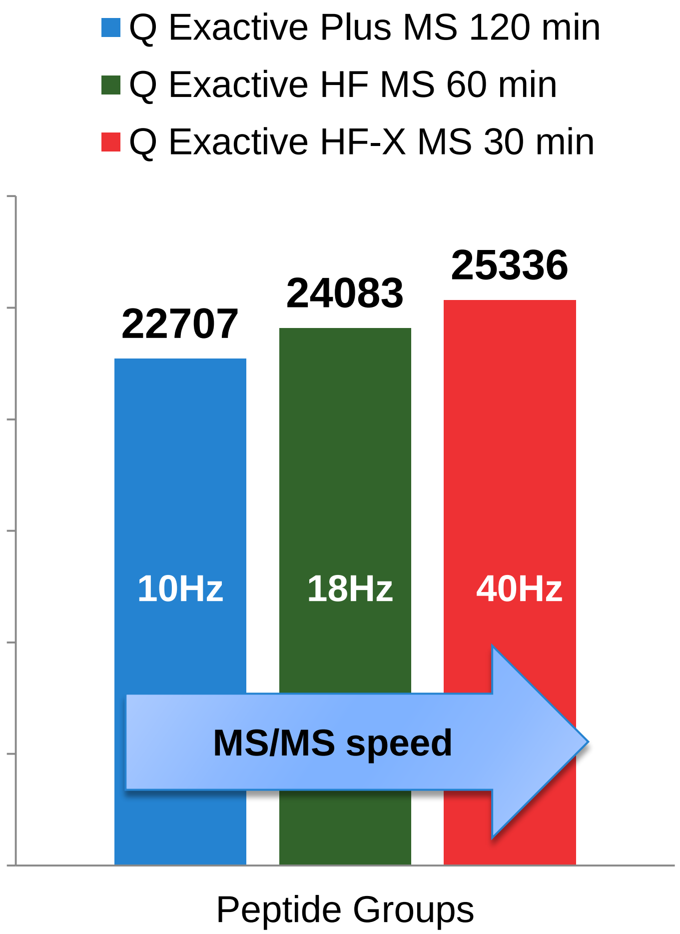
- Maximizing protein identifications
- Same protein identifications in half the analysis time
- Faster, with same high quality results



ASMS’17: TP 389, T.N. Arrey et al. New innovations implemented on the Thermo Scientific™ Q Exactive™ HF MS.

Deeper Dive into Proteome - More Productivity with Thermo Scientific Q Exactive HF-X MS





Maximizing peptide identifications

- Highest peptide coverage
- Deep proteome analysis
- Spectral library building

- **2x** productivity increase vs. Q Exactive HF

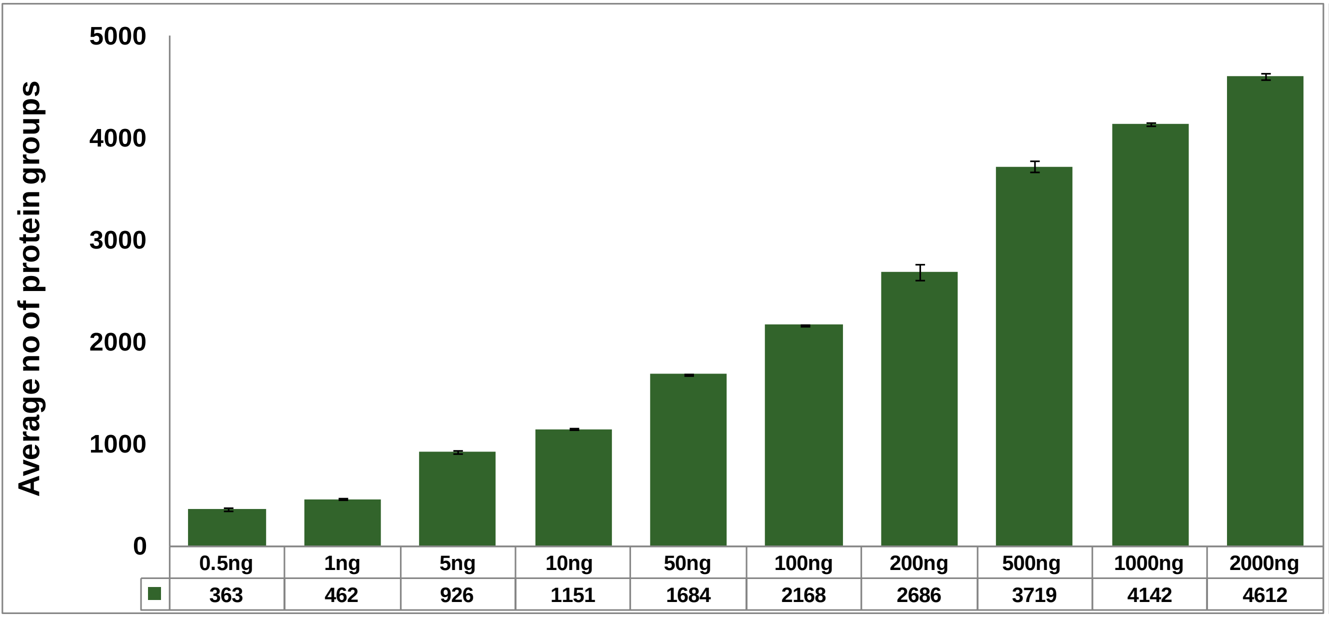
- **4x** productivity increase vs. Q Exactive Plus

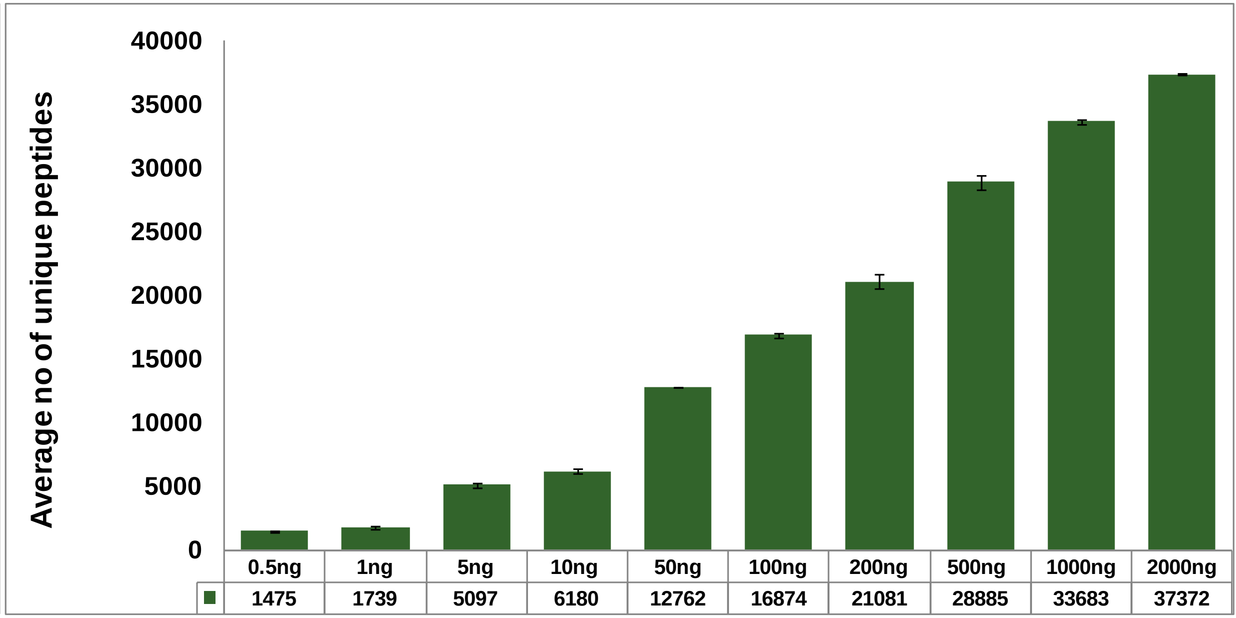
- Sample: 1 ug Pierce HeLa digest

ASMS’17: TP 389, T.N. Arrey et al. New innovations implemented on the Thermo Scientific™ Q Exactive™ HF MS.

Dilution Series Pierce HeLa Digest

Reproducible and consistent identifications across a large range of sample load on column





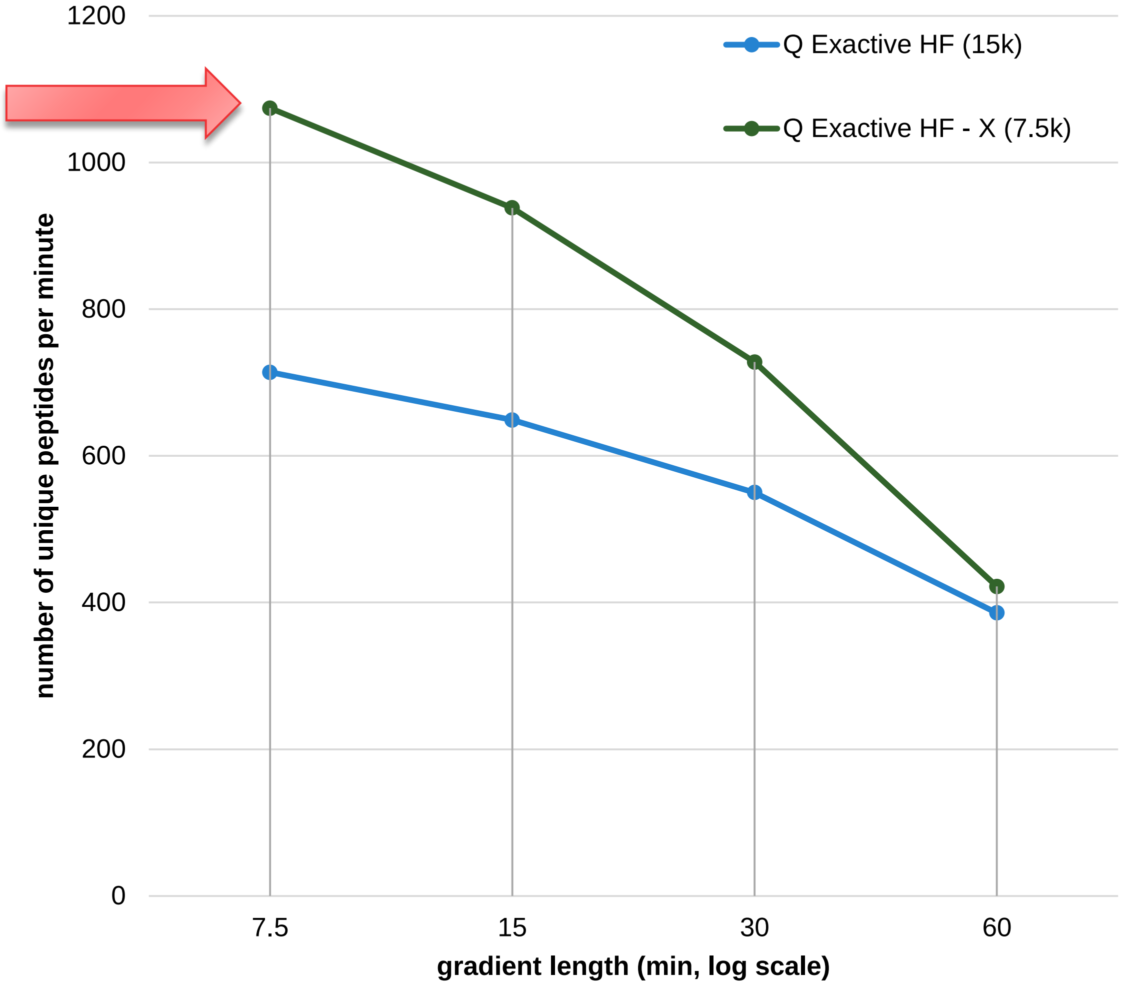
60 min gradients each. Method set-up adapted to according to different sample load on column from 0.5 ng … 2000 ng

Rapid Proteomics - More than 1,000 Peptides Identified per Minute

50% increased rate of peptide identifications when ion flux is max

Up to ~ 1100 peptides identified per minute

Sample: 1 µg HeLa

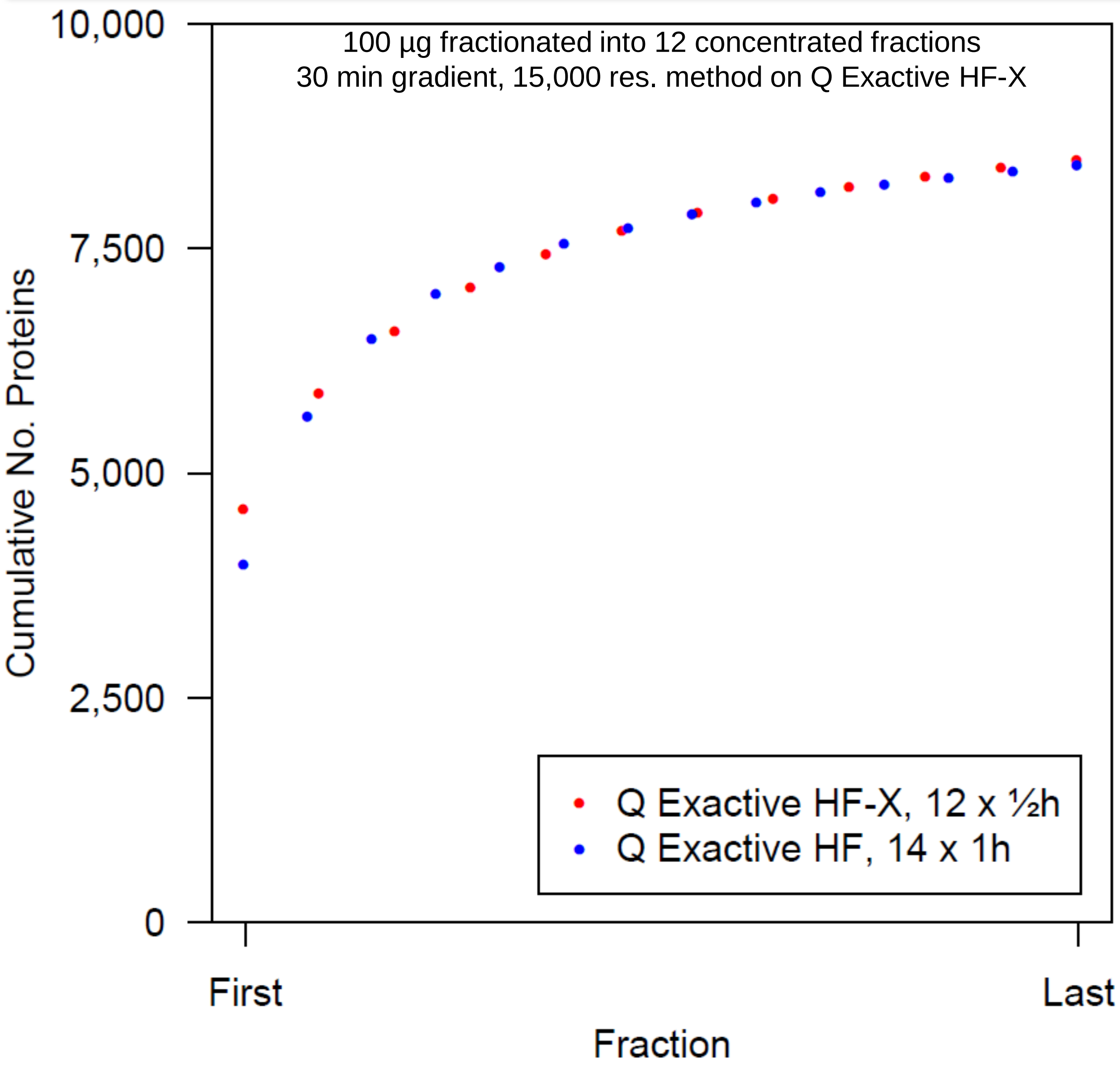


- Short transient time of 16 ms combined with accelerated HCD allows for scan rates up to 40 Hz in data dependent and targeted analyses
- Benefit is increased productivity, expressed by more peptide IDs per time.

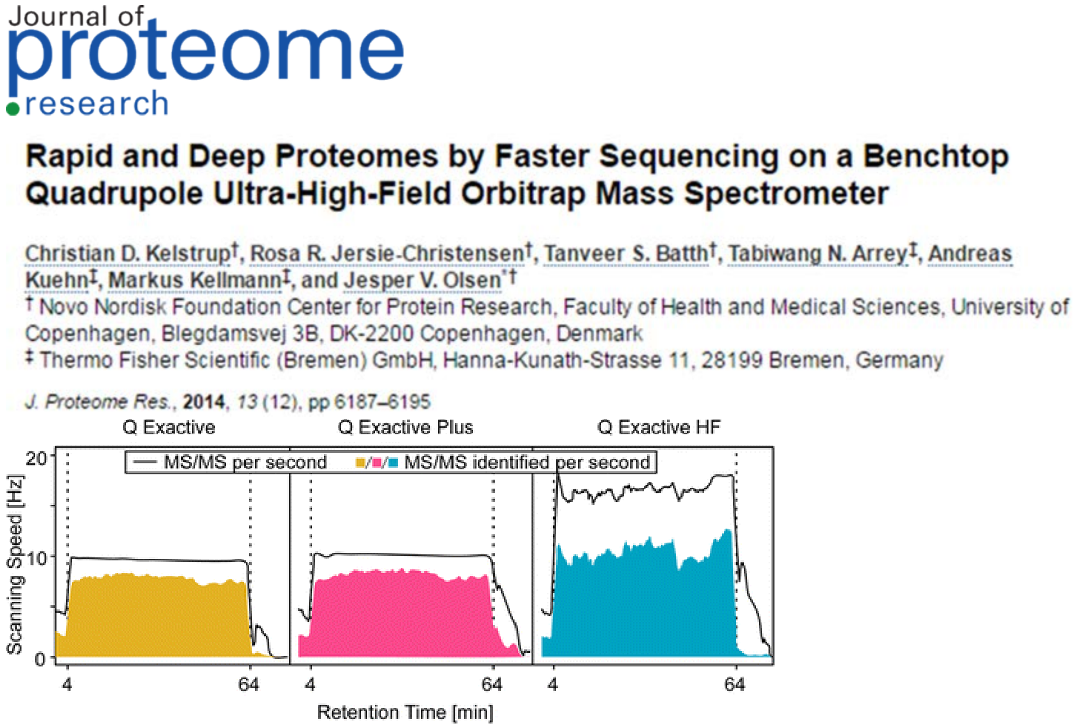
Data with courtesy from J. Olsen, Novo Nordisk Foundation, Center for Protein Research, University of Copenhagen.

Proteome Perspectives: Rapid and Deep Proteome Sequencing in Less than Half the Acquisition Time

New data from Thermo Scientific™ Q Exactive™ HF-X MS overlaid with published data



J. Proteome Res. 2014, 13(12), pp6187-6195



Journal of proteome research

Rapid and Deep Proteomes by Faster Sequencing on a Benchtop Quadrupole Ultra-High-Field Orbitrap Mass Spectrometer

Christian D. Kelstrup[†], Rosa R. Jersie-Christensen[†], Tanveer S. Batth[†], Tabiwang N. Arrey[‡], Andreas Kuehn[‡], Markus Kellmann[‡], and Jesper V. Olsen[*†]

[†] Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen, Denmark

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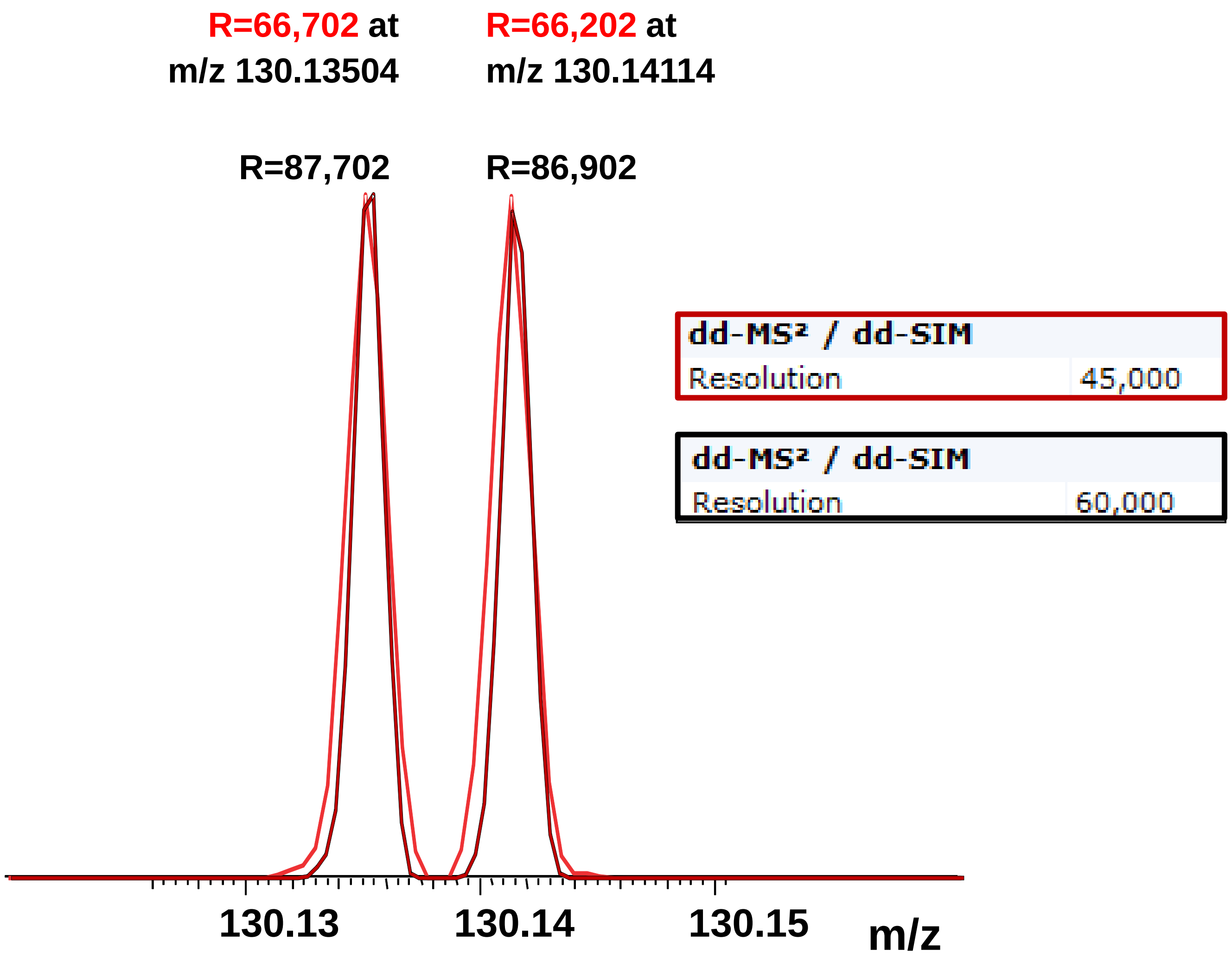
J. Proteome Res., **2014**, *13* (12), pp 6187–6195

Data with courtesy from J. Olsen, Novo Nordisk Foundation, Center for Protein Research, University of Copenhagen

New 96 msec Transient – Ideal for TMT 10plex



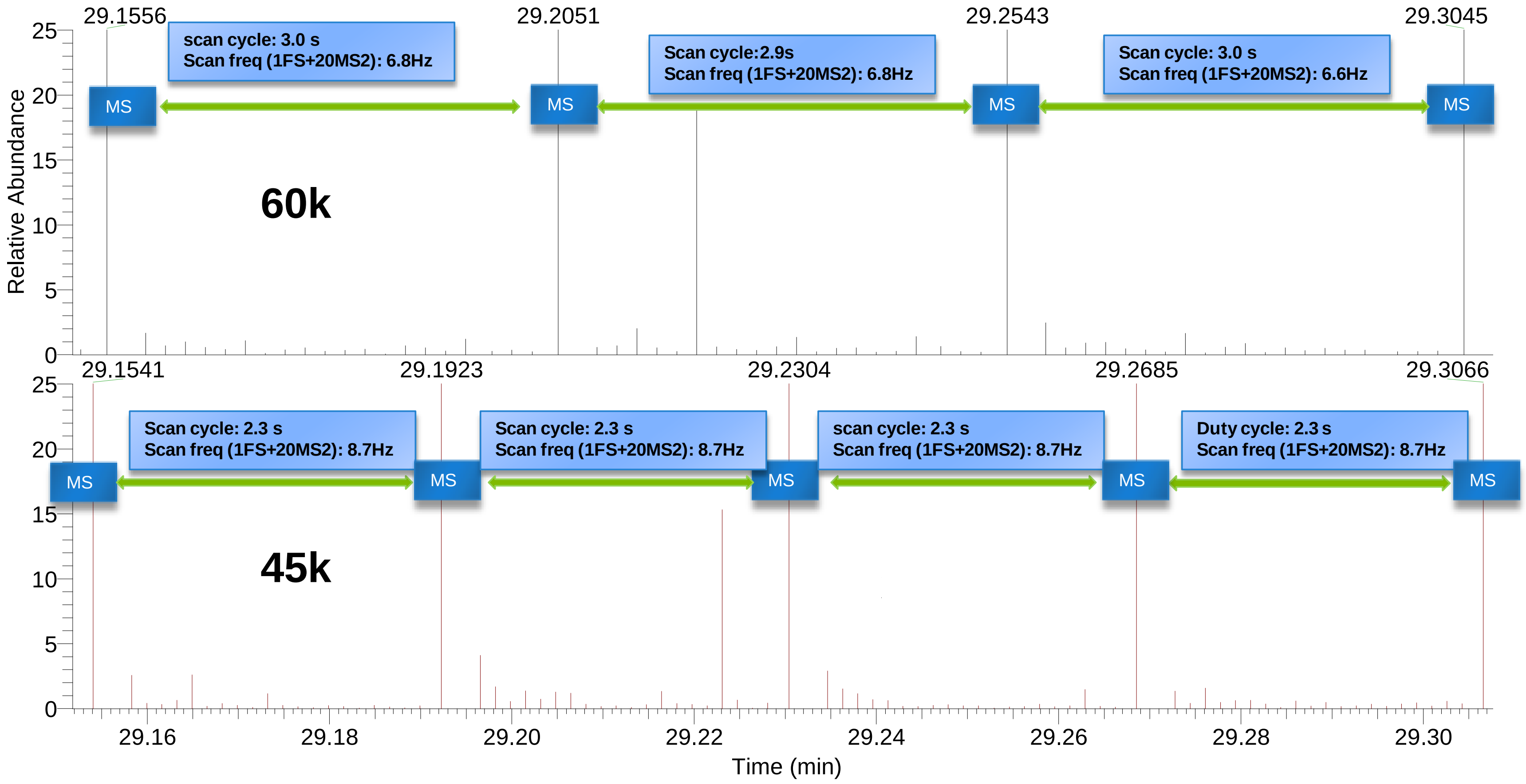
dd-MS² / dd-SIM
Resolution 45,000

dd-MS² / dd-SIM
Resolution 60,000

Optimal separation of TMT reporter ions

- A dedicated resolution setting of 45,000 at *m/z* 200 (FWHM)
- Optimally resolves reporter ions of the Thermo Scientific™ TMT10plex™ Isobaric Mass Tag Labeling Kit
- Frees up scan time to quantify 10–20% more peptides in discovery experiments

Scan Cycle Comparison



scan cycle: 3.0 s
Scan freq (1FS+20MS2): 6.8Hz

Scan cycle:2.9s
Scan freq (1FS+20MS2): 6.8Hz

Scan cycle: 3.0 s
Scan freq (1FS+20MS2): 6.6Hz

Scan cycle: 2.3 s
Scan freq (1FS+20MS2): 8.7Hz

Scan cycle: 2.3 s
Scan freq (1FS+20MS2): 8.7Hz

scan cycle: 2.3 s
Scan freq (1FS+20MS2): 8.7Hz

Duty cycle: 2.3 s
Scan freq (1FS+20MS2): 8.7Hz

Acquisition speed increase by ca. 25% allowing quantitation of more peptides per unit time with TMT 10plex.

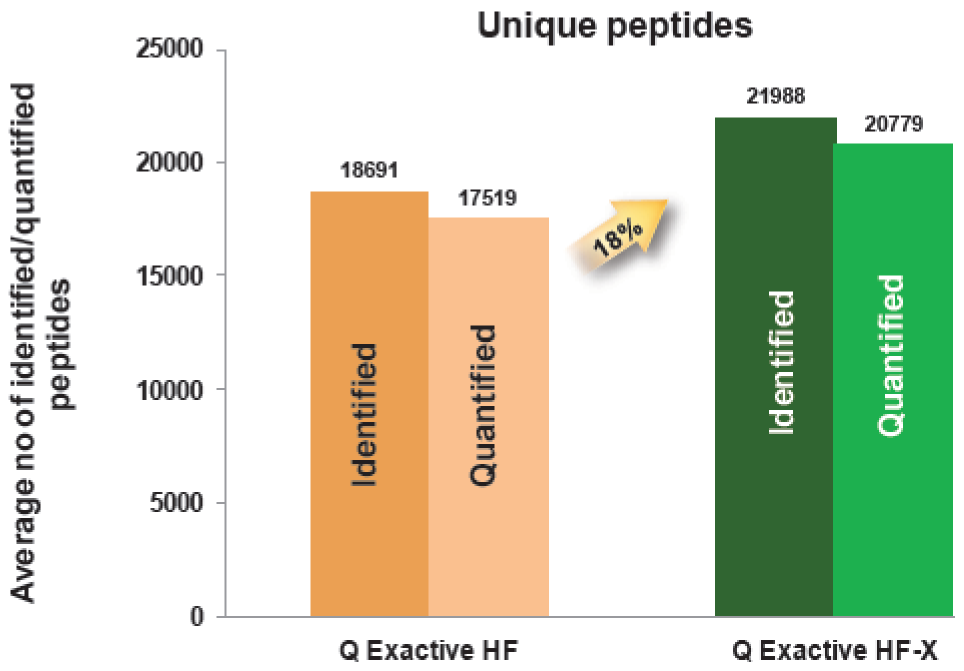
Scan cycle comparison between

- 60k resolution setting, Thermo Scientific™ Q Exactive™ HF (top)
- Novel 45k resolution setting Thermo Scientific™ Q Exactive™ HF-X (bottom)

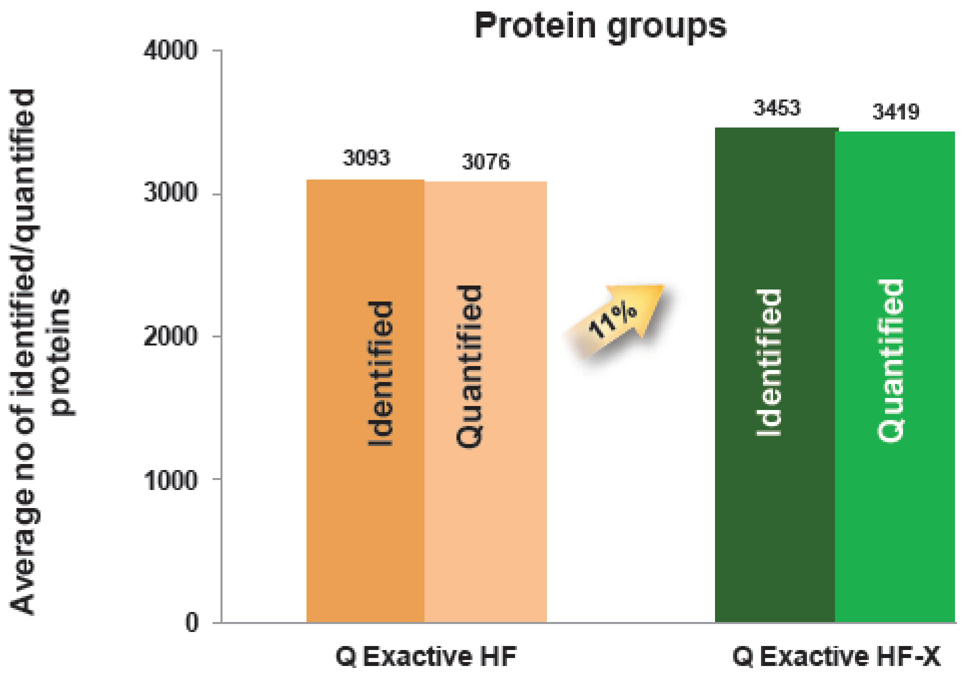
Method:
FullMS (120k res),
dd Top 20 MS/MS
(60k or 45k) scans

60k on HF vs. 45k on HF-X

TMT11-plex labeled HeLa
1 µg on column, ~90 min gradient
120/86 ms max inject on Thermo Scientific™ Q Exactive™ HF/HF-X MS

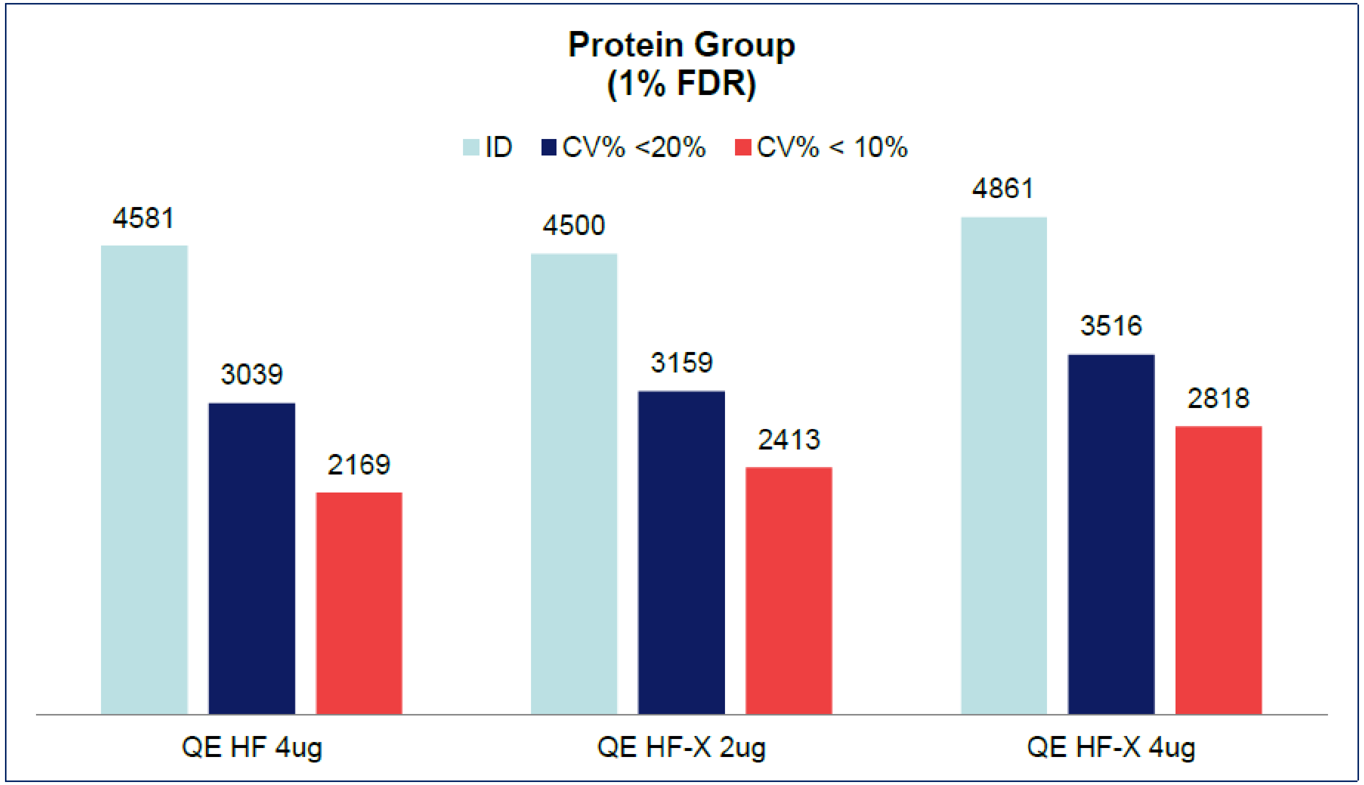


>90% quantified >90% quantified



Robust DIA Proteome Profiling using CapLC with Thermo Scientific QE HF-X MS

VALUE: Highest depth of coverage and robust quantitative analysis



HR-DIA data processed with Spectronaut software, Biognosys AG, Switzerland

Similar data on HF-X with 2 µg as HF with 4 µg

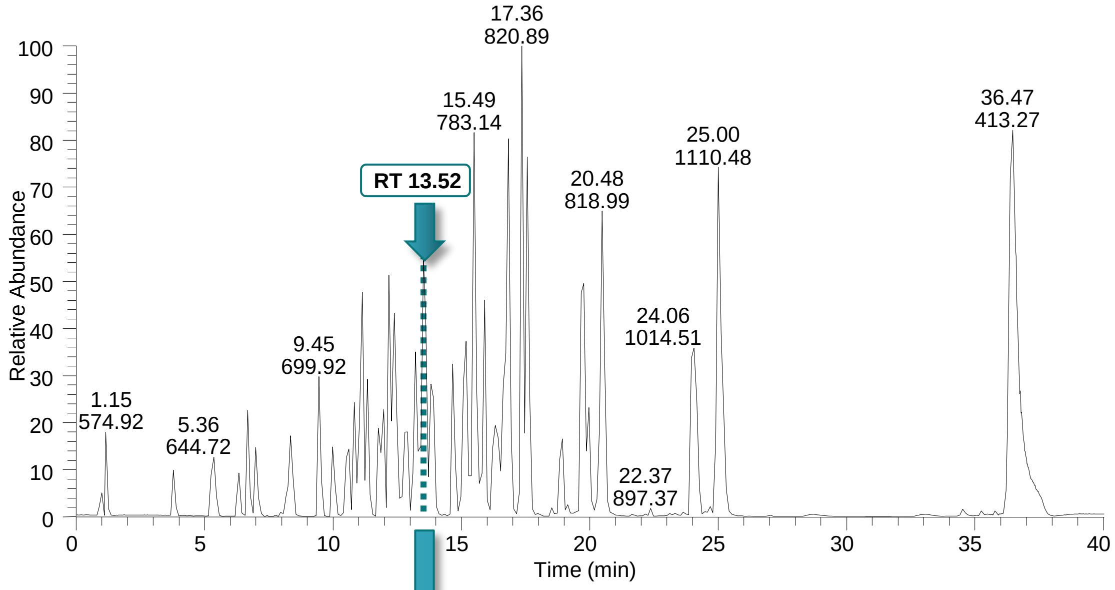
Greater proportion of peptides with CVs < 10% on HF-X

HeLa digest, 3 technical replicates each, 60 mins total run time

ASMS'17: ThP 237 Y. Xuan et al. Revolutionary Proteome Profiling and Quantitation without Compromising Speed, Sensitivity, and Selectivity.

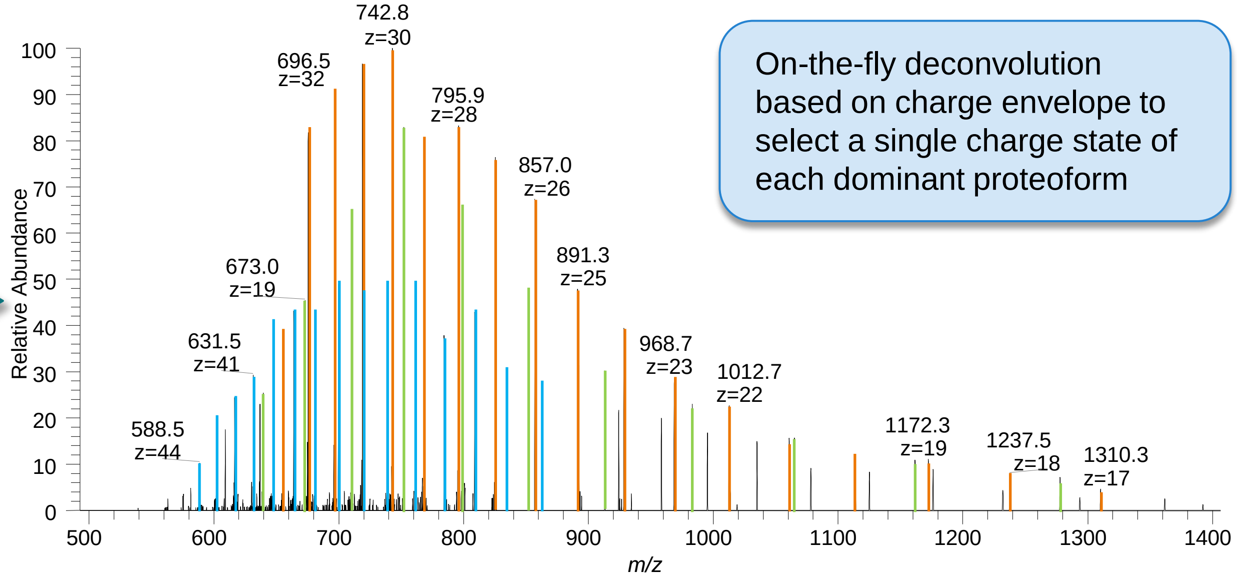
Top Down Proteomics - A Novel Workflow Brings Intact Proteins Into Focus

Base peak chromatogram of *E. coli* ribosomal proteins separated in a 30 min gradient.



Full MS @ RT 13.52 min

Thermo Scientific™ Q Exactive™ HF-X MS Full scan acquired in Protein Mode at a resolution setting of 7,500, detecting multiple charge envelopes.

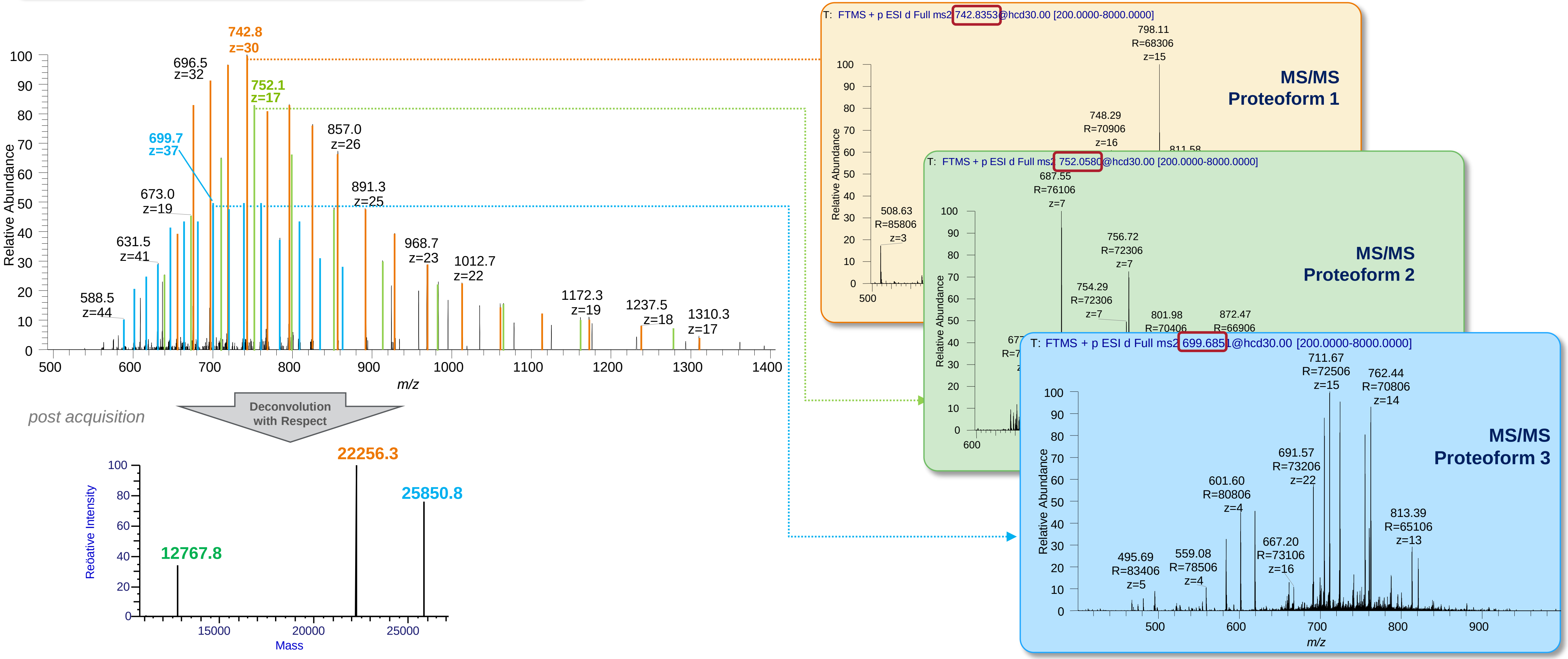


On-the-fly deconvolution based on charge envelope to select a single charge state of each dominant proteoform

Top Down Proteomics - A Novel Workflow Brings Intact Proteins Into Focus

On-the-fly deconvolution based on charge envelope to select a single charge state of each dominant proteoform.

MS/MS analysis of each proteoform fragmented with optimal collision energy and detected at a resolution setting of 120,000.



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