

## Boosting FTMS performance via advanced data acquisition electronics and signal processing

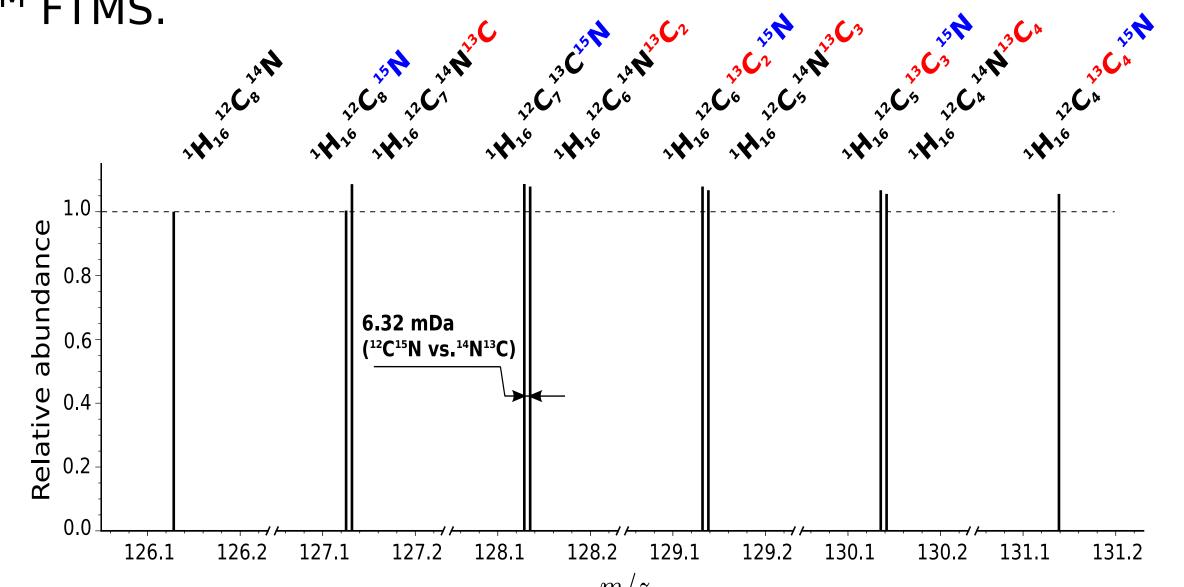
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### 1. Introduction

Data acquisition and signal processing capabilities of current FTMS instruments do not always match the performance advances in their mass analyzers and ion optics devices, thus inhibiting the progress in FTMS applications.

Here, we demonstrate improvements in FTMS performance and applications, enabled by the implementation and development of new high-performance data acquisition electronics and allied advanced signal processing methods.

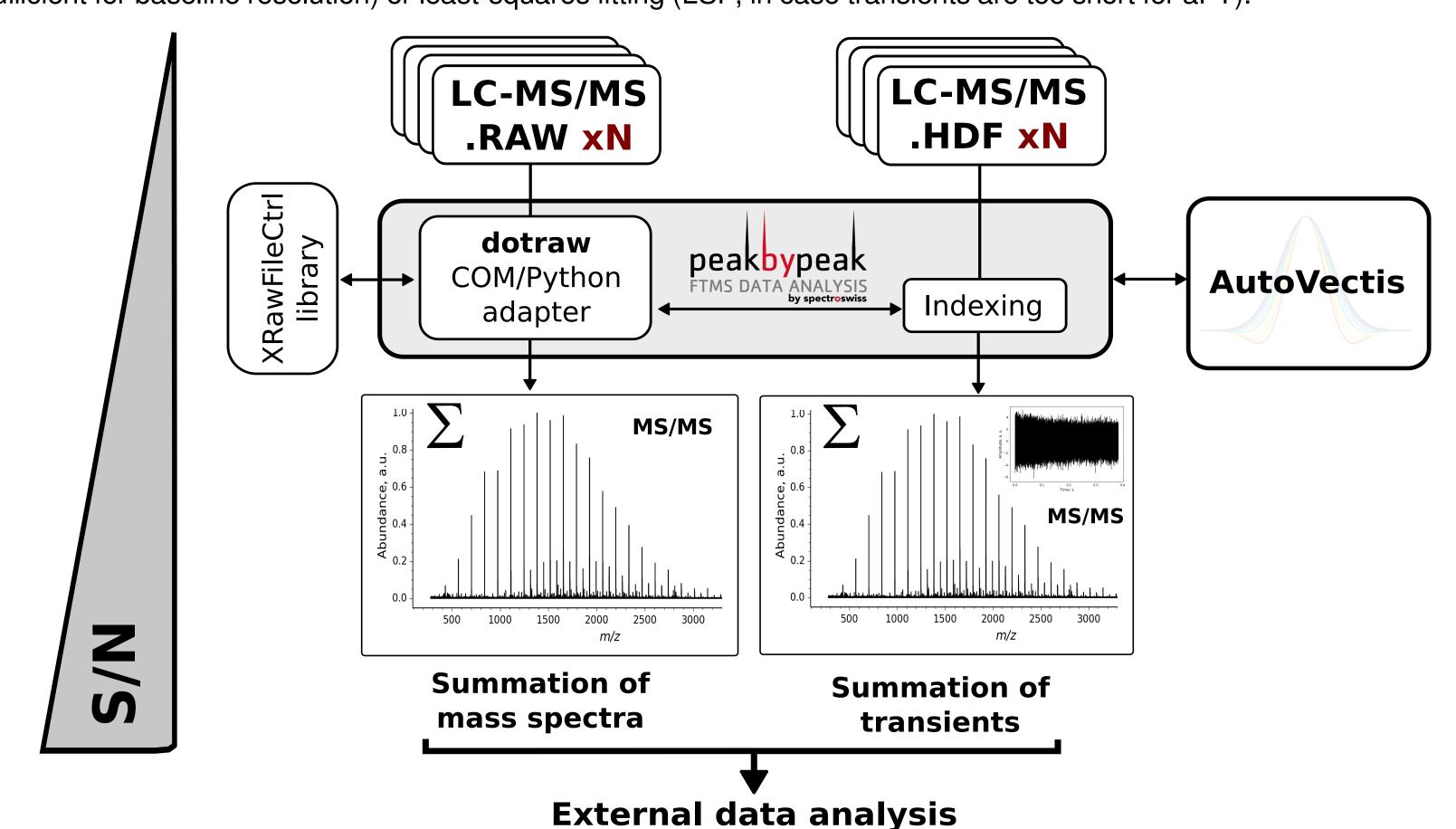
We focus on applications in multiplexed protein quantitation via bottomup proteomics, see Figure 1, and protein structural analysis following topdown protein analysis approach. Both of the applications are shown for Orbitrap<sup>TM</sup> FTMS.



**Figure 1.** Isotopic labeling with N-plex TMT<sup>TM</sup> reagents, whose reporter groups represent N multiplexed channels for protein quantitation. Shown are mass-to-charge ratios, ion abundances, and isotopic compositions of the reporter ions for N=10. The ion abundances illustrate a particular case of equal concentrations of analytes from the 10 channels.

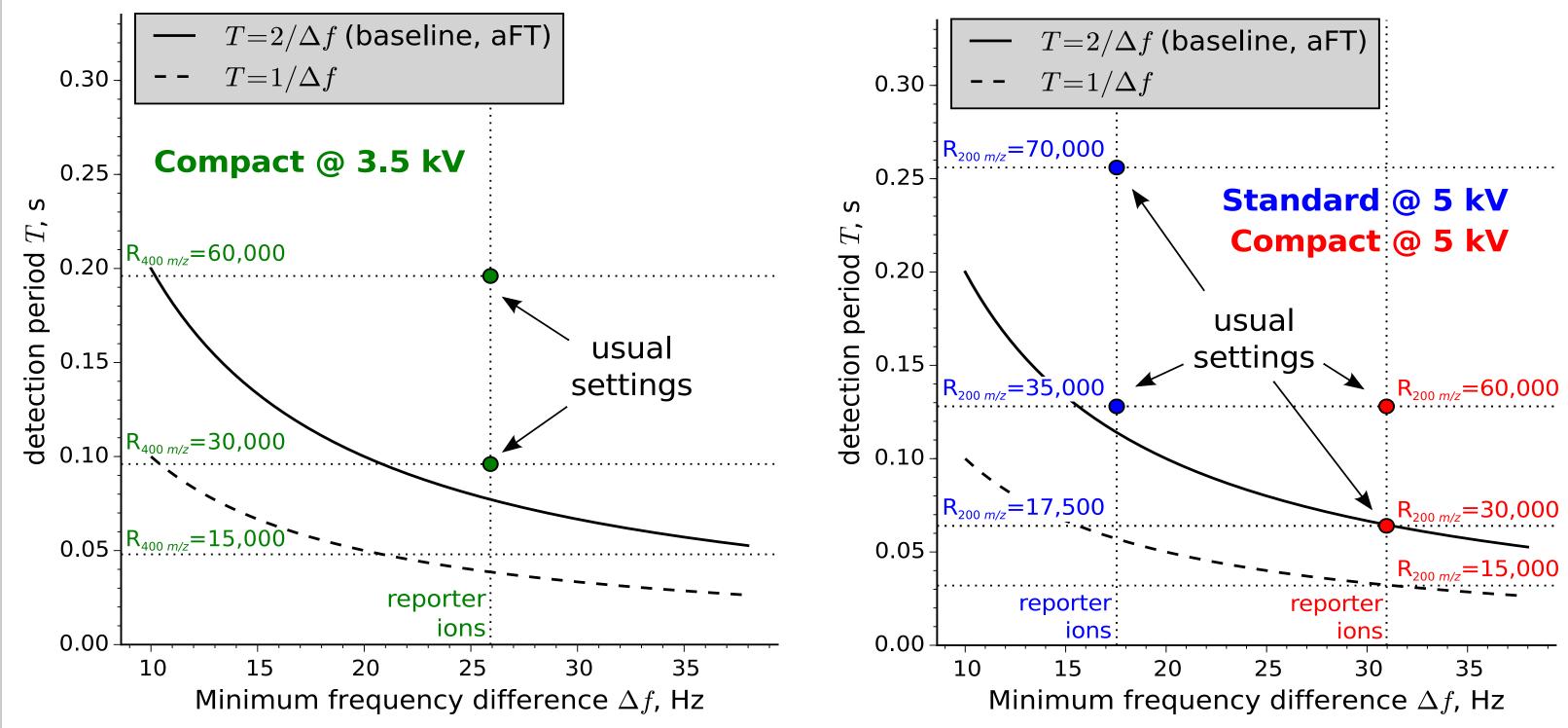
# Orbitrap™ FTMS N-Plex Suite SUPER-RESOLUTION SIGNAL PROCESSING Peakbypeak FTMS DATA ANALYSIS by spectrowns .CSV .mgf .ibrary .mzXML

**Figure 2.** Workflow for improved multiplexed protein quantitation using parallel data acquisition via both in-built electronics of FTMS instruments (for example, mass spectra in .RAW format acquired using Q Exactive<sup>™</sup> Plus Orbitrap<sup>™</sup> FTMS or Fusion<sup>™</sup> Lumos<sup>™</sup> Orbitrap<sup>™</sup> FTMS instruments from Thermo Scientific) and an external data acquisition system (FTMS Booster X1, Spectroswiss). Transient data analysis for the latter depends on the acquired transient duration and can be made using either *in-line* absorption mode FT (aFT, if provided transient length is sufficient for baseline resolution) or least-squares fitting (LSF, in case transients are too short for aFT).

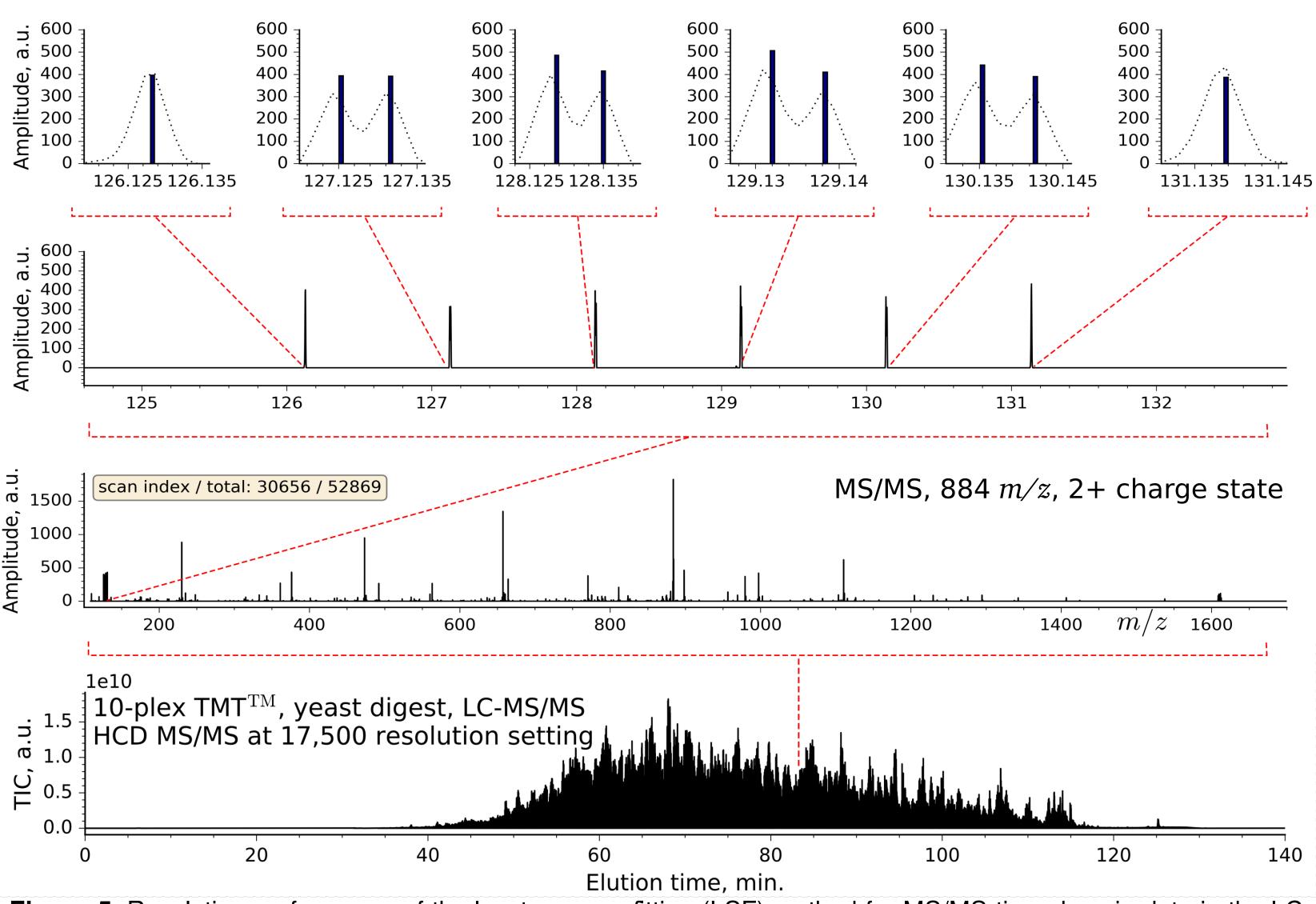


**Figure 3.** Workflow for bottom-up or top-down protein analysis using parallel data acquisition via both in-built electronics of FTMS instruments (for example, mass spectra in .RAW format acquired using Q Exactive™ Plus Orbitrap™ FTMS or Fusion™ Lumos™ Orbitrap™ FTMS instruments from Thermo Scientific) and an external data acquisition system (FTMS Booster X1, Spectroswiss). Data analysis performed by Peak by Peak software (Spectroswiss) enables indexing of .RAW files and advanced transient processing, including absorption-mode FT post-processing with the AutoVectis software (in collaboration with Dr. David Kilgour).

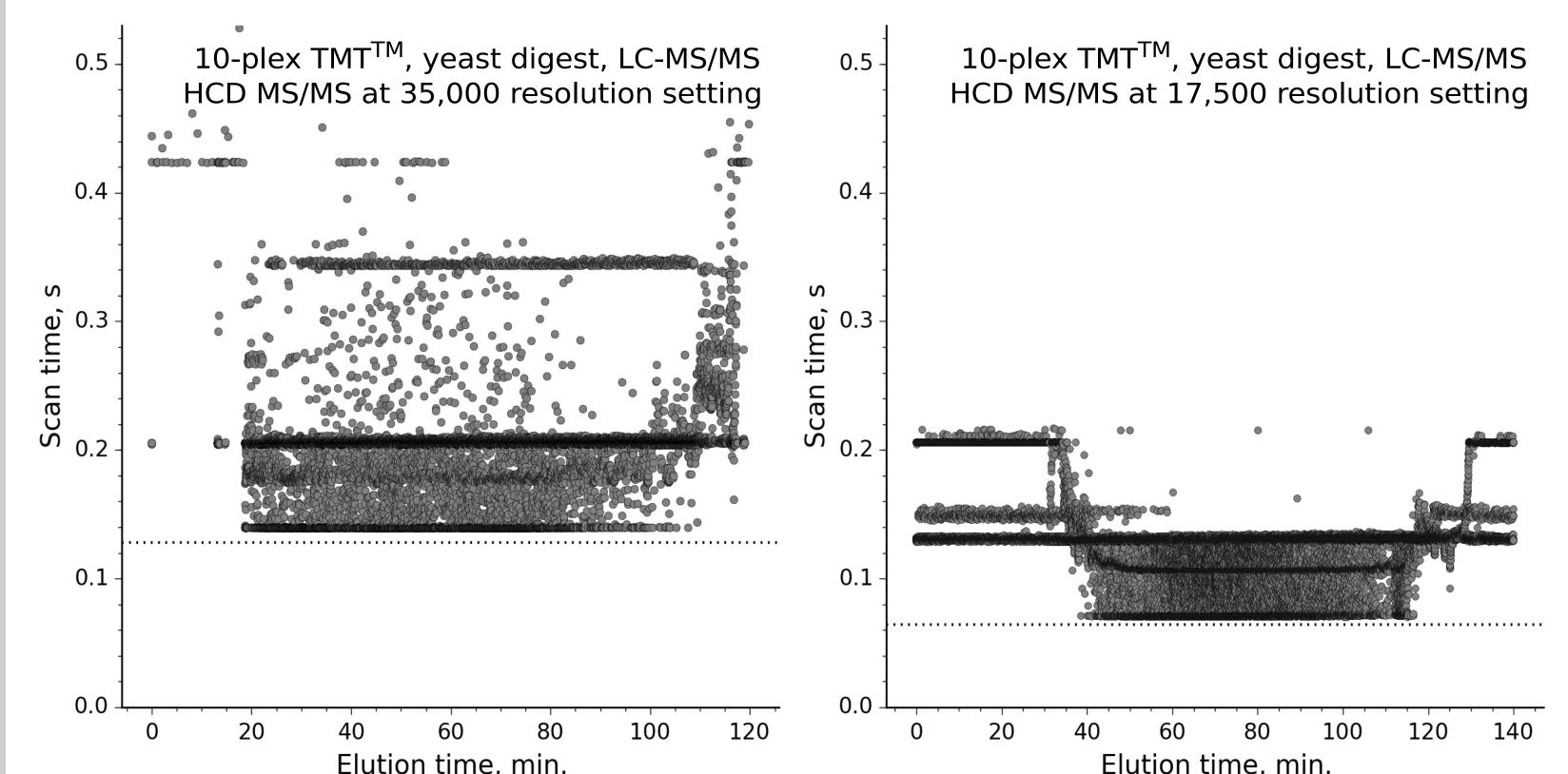
### 3. Super-resolution signal processing



**Figure 4.** Resolution settings of MS/MS scans in 10-plex TMT<sup>™</sup> experiments as usually selected for different orbitrap mass analyzers, illustrated on top of the FT uncertainty principle plot. The baseline peak resolution limit (full line) is estimated for the case of absorption-mode FT with moderate signal apodization. The FT resolution limit is shown for reference (dashed line). The reporter ions' minimum frequency differences and available resolution settings (detection periods) are plotted for Orbitrap<sup>™</sup> Elite<sup>™</sup> FTMS (left panel), Q Exactive<sup>™</sup> (right panel, in blue), and Fusion<sup>™</sup> Lumos<sup>™</sup> / Q Exactive<sup>™</sup> HF FTMS (right panel, in red).

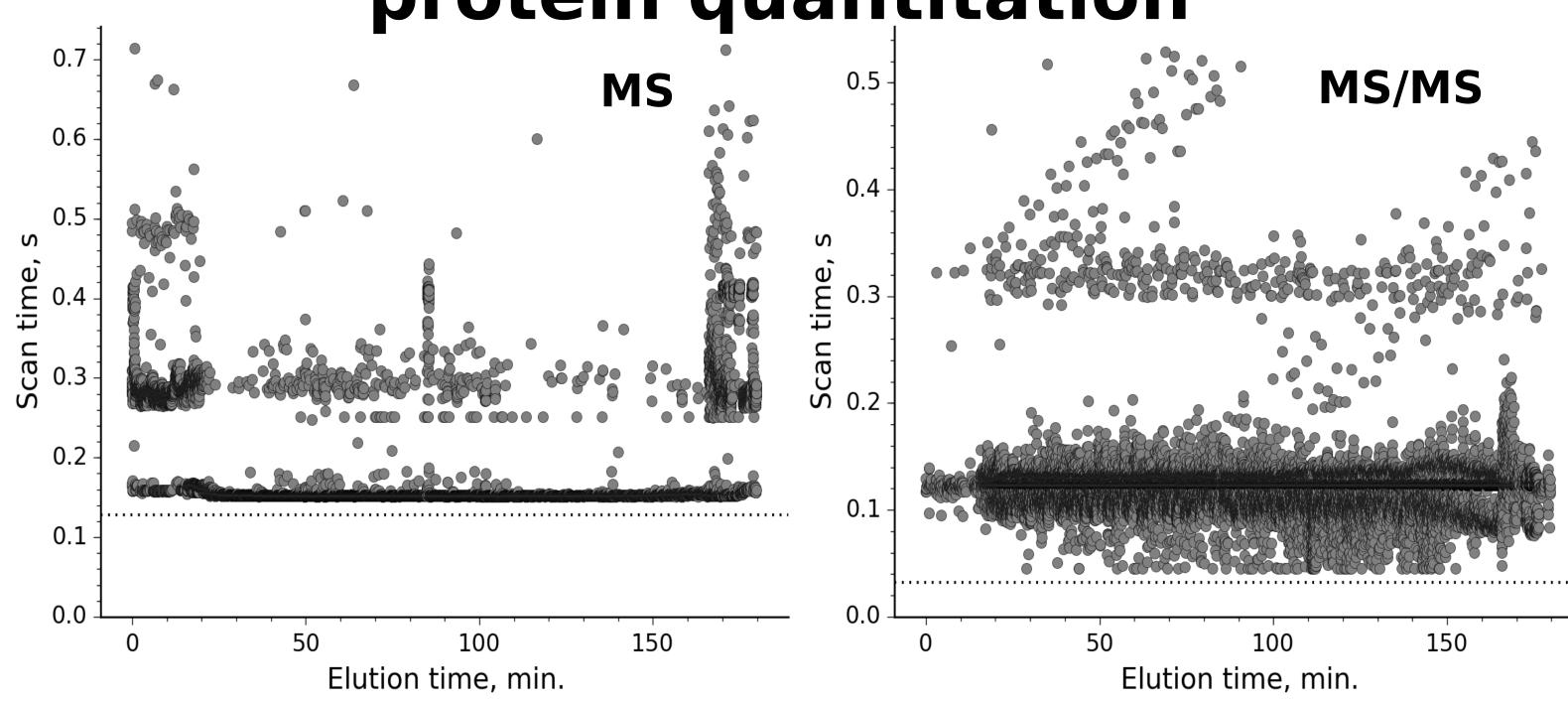


**Figure 5.** Resolution performance of the least squares fitting (LSF) method for MS/MS time-domain data in the LC-MS analysis of 10-plex TMT<sup>™</sup>-labeled yeast digest on Q Exactive<sup>™</sup> Plus Orbitrap<sup>™</sup> FTMS, with MS/MS scans acquired at the resolution setting of 17,500. From bottom to top panels: total ion current (TIC) chromatogram; MS/MS mass spectrum of a peptide example; a magnified view of the MS/MS mass spectrum into the reporter ions' *m/z* range; magnified views of the MS/MS spectrum into individual *m/z* regions of the reporter ions, with experimental ion abundances shown with vertical bars (LSF data). All data in black relates to original data acquisition electronics and software. MS/MS transient signals were digitized using an external data acquisition system. An initial length of 1 ms of each transient signal was skipped prior to LSF calculations.

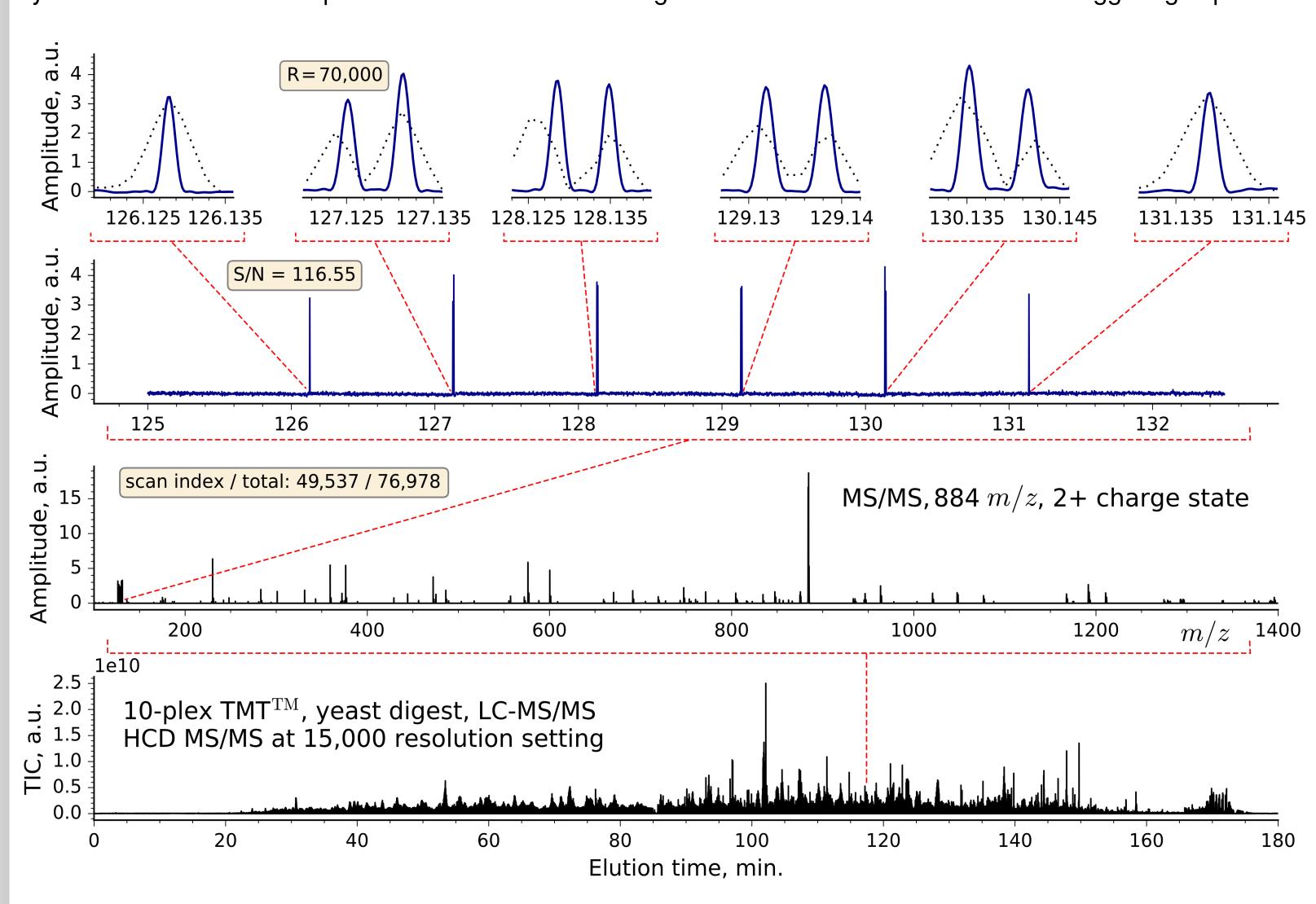


**Figure 6.** Distributions of MS/MS total scan times in two 10-plex TMT<sup>TM</sup> LC-MS measurements carried out on Orbitrap<sup>TM</sup> FTMS instruments of the Q Exactive<sup>TM</sup> series: with MS/MS scans acquired at the resolution setting of 35,000 (left panel, Q Exactive<sup>TM</sup>) and 17,500 (right panel, Q Exactive<sup>TM</sup> Plus). Detection periods, 0.128 s and 0.064 s, corresponding to these settings are shown with dotted lines. The time difference between the distributions on the left and right panels is consistent with the detection period change, 0.064 s. This shows time efficiency of Q Exactive<sup>TM</sup> instruments, allowing efficiently using faster MS/MS data acquisition strategies, *viz*, super-resolution signal processing, on these platforms, relative to the standard approaches (Figure 4).

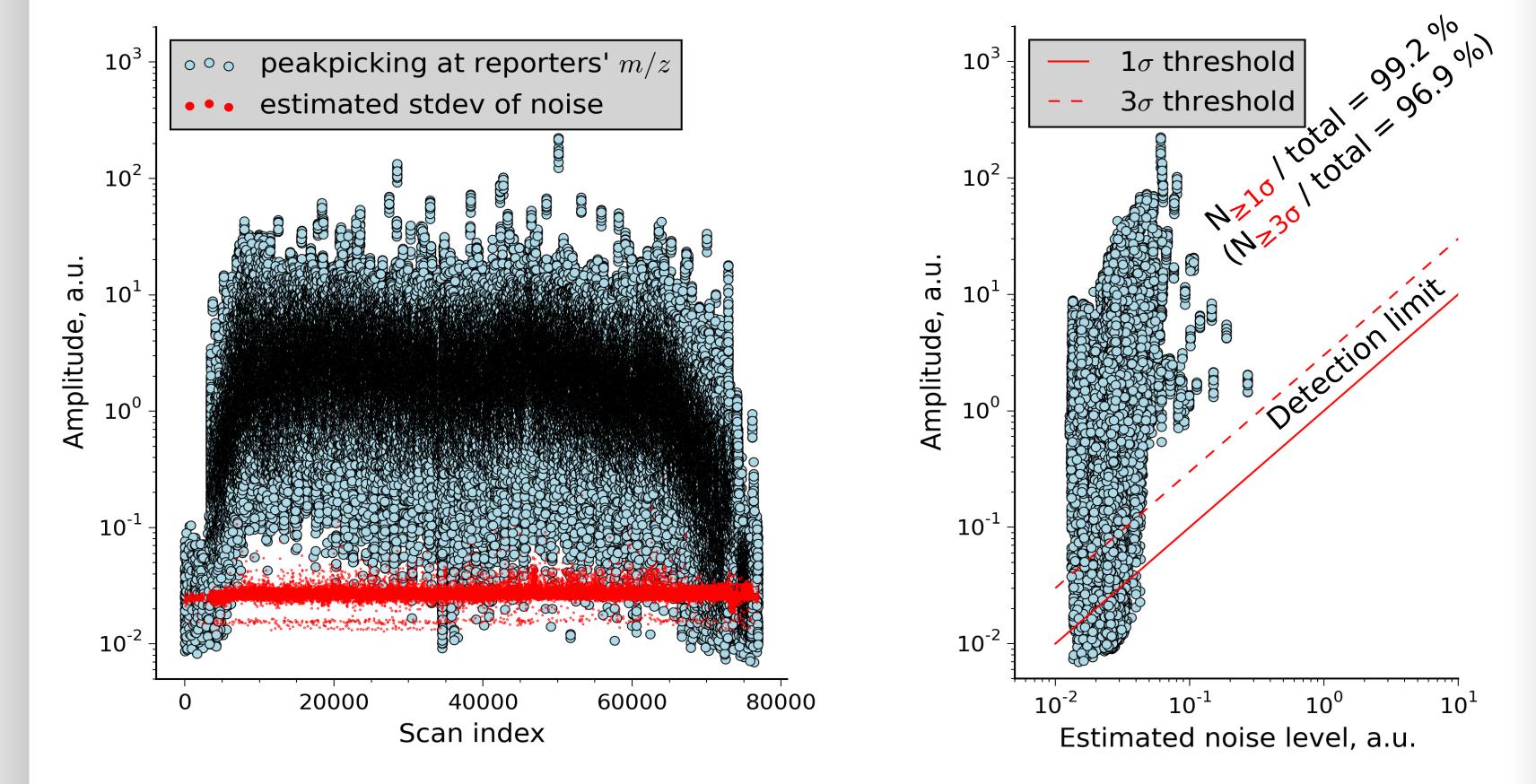
# 4. Advanced electronics: protein quantitation



**Figure 7**. Distributions of MS (left panel) and MS/MS (right panel) total scan times in the LC-MS analysis of 10-plex TMT<sup>™</sup>-labeled yeast digest on Orbitrap<sup>™</sup> Fusion<sup>™</sup> Lumos<sup>™</sup> FTMS, with MS scans acquired at the resolution setting of 60,000 and MS/MS scans at 15,000. Dotted lines show detection periods, 0.128 s and 0.032 s, respectively, corresponding to these settings. While the MS scan times are distributed close to the selected MS detection period, the MS/MS scans distribution is located at a longer time than the MS/MS detection period. Given that an FTMS instrument is designed such that ions are trapped in its mass analyzer during almost the full scan time, increased duty cycles of transient data acquisition can be achieved using external electronics with advanced triggering capabilities.



**Figure 8.** Resolution performance of Fourier transform with increased duty cycles of time-domain data acquisition in the LC-MS analysis of 10-plex TMT<sup>TM</sup>-labeled yeast digest on Orbitrap<sup>TM</sup> Fusion<sup>TM</sup> Lumos<sup>TM</sup> FTMS, with MS/MS scans acquired at the resolution setting of 15,000. From bottom to top panels: total ion current (TIC) chromatogram; MS/MS mass spectrum of a peptide example (see also Figure 5); a magnified view of the MS/MS mass spectrum into the reporter ions' *m/z* range; magnified views of the MS/MS spectrum into individual *m/z* regions of the reporter ions. MS/MS transient signals were digitized in a form suitable for straightforward use of absorption-mode FT and Fourier transformed followed by frequency-to-*m/z* conversion (data in blue), including for transients from the lower edge of the MS/MS distribution in Figure 7. All data in black relates to original data acquisition electronics and software.



**Figure 9.** Entire range of signal amplitudes for all 10 channels of reporter ions in the LC-MS analysis of Figure 8. Left panel shows signal amplitudes and noise standard deviations as a function of scan index, obtained from corresponding MS/MS transient signals. Right panel shows a correlation plot of the signal amplitude vs. the noise level for each of the data points on the left panel, thus allowing one to distinguish between the reporter ions and the noise level (false positives) based on a selected noise threshold value for absorption mode FT data, e.g. 3 standard deviations.

### 5. Advanced electronics: top-down MS

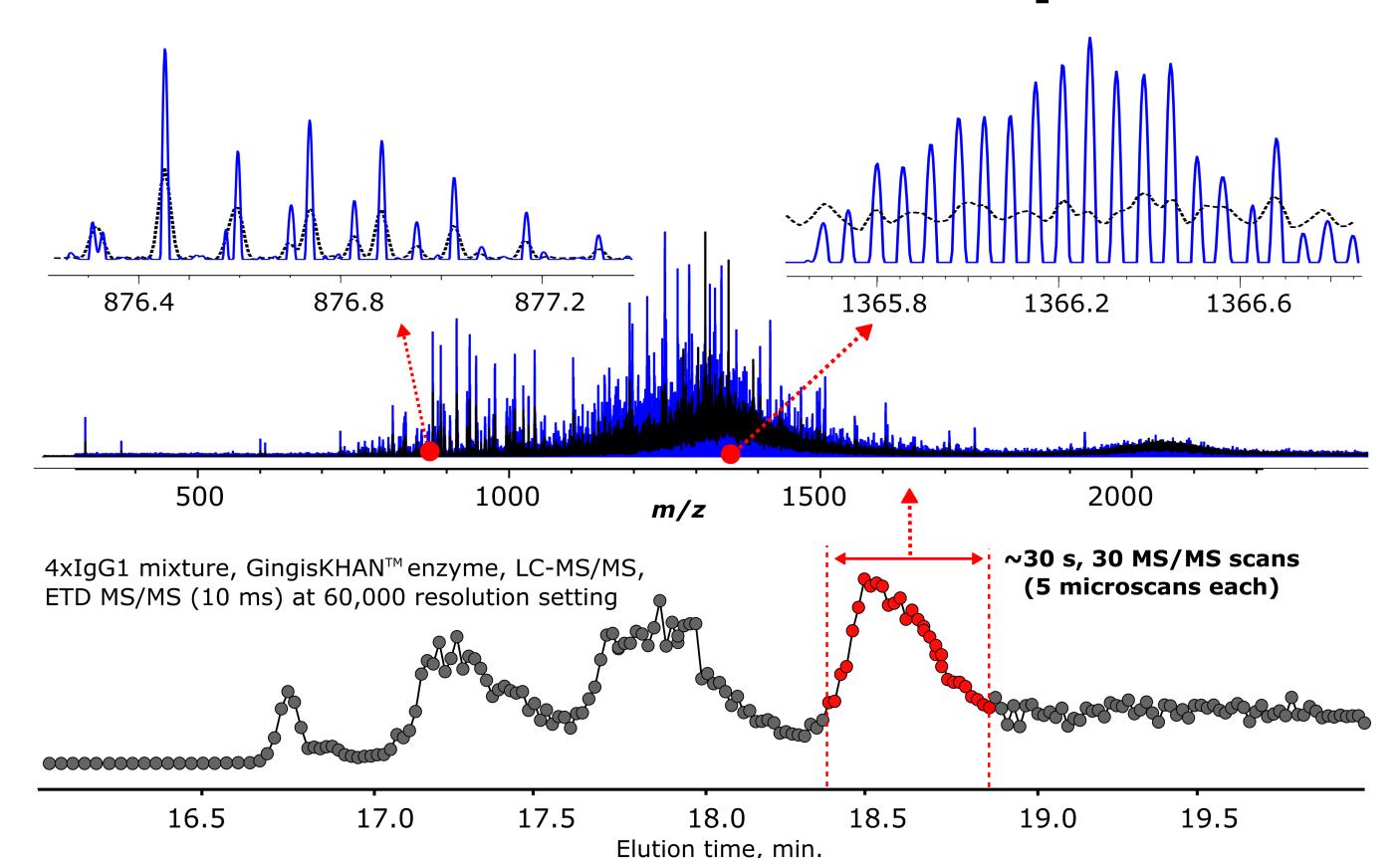
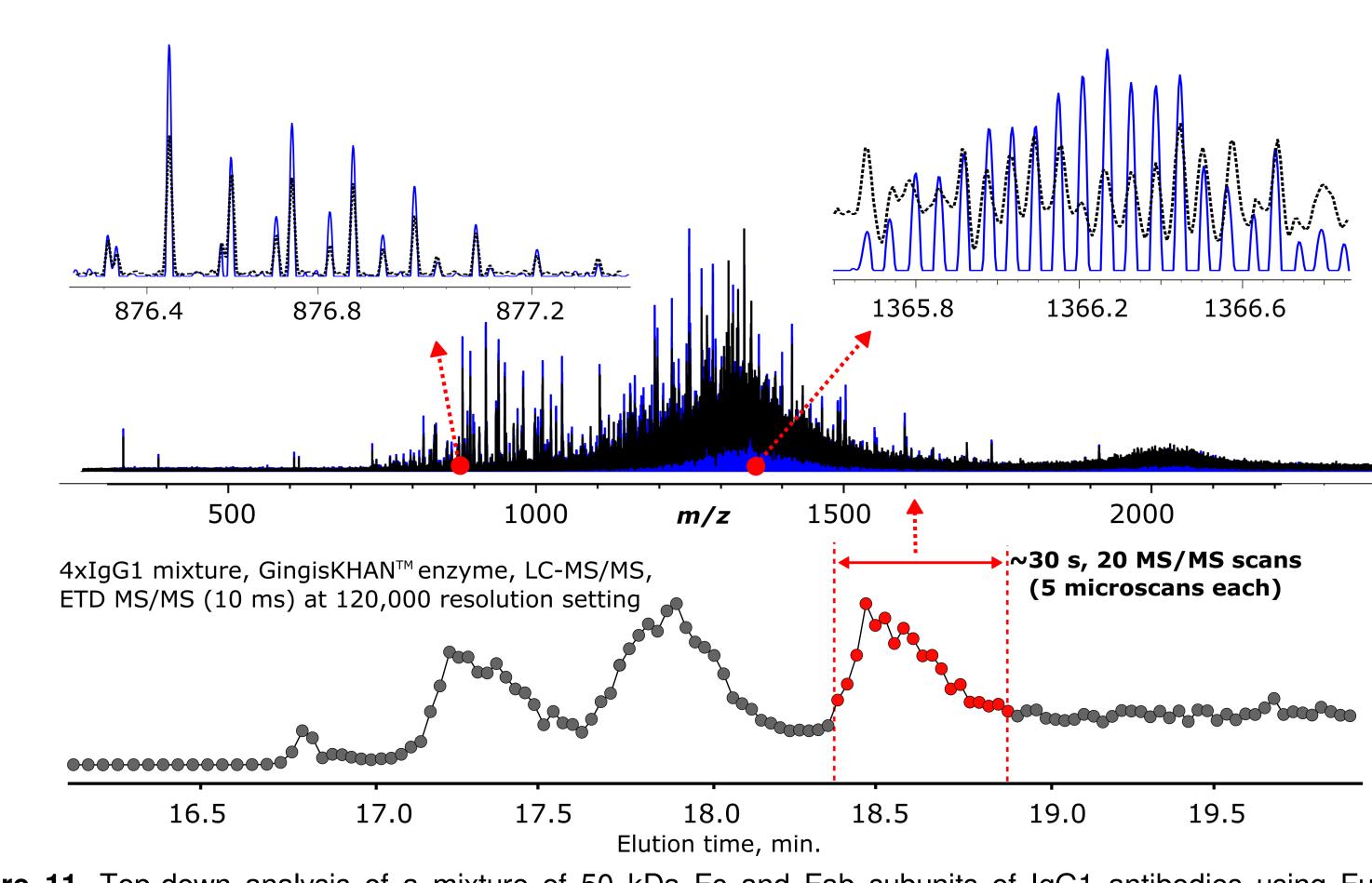


Figure 10. Top-down analysis of a mixture of 50 kDa Fc and Fab subunits of IgG1 antibodies using Fusion™ Lumos™ Orbitrap™ FTMS with ETD MS/MS. Resolution setting of 60k is not sufficient for baseline resolution of ETD product ions with built-in electronics and eFT spectra representation (black color). Parallel transient data acquisition with an external data acquisition system, followed by absorption-mode FT post-processing (AutoVectis software package), enabled baseline resolution, demonstrating performance of 120k resolution and corresponding SNR increase (blue color). Palivizumab analysis is performed during 30 s elution period within which 30 scans of 5 micro-scans each could be recorded. That is 50% increase in the number of scans acquired in the same 30 s elution period when 120k resolution setting is employed, see Figure 11.



**Figure 11**. Top-down analysis of a mixture of 50 kDa Fc and Fab subunits of IgG1 antibodies using Fusion™ Lumos™ Orbitrap™ FTMS with ETD MS/MS. Resolution setting of 120k allowed baseline resolution of ETD product ions with built-in electronics and eFT spectra representation (black lines). Transient data acquisition with an external data acquisition system, followed by absorption-mode FT post-processing (AutoVectis software package), demonstrated above for 60k resolution setting (blue color), correlates with the eFT data quality.

### 6. Conclusions

- Complementary transient data acquisition from commercial FTMS instruments provides additional flexibility for post-acquisition signal processing and data analysis.
- Advanced electronics and firmware enable acquisition of full transients, increasing the duty cycle of data acquisition.
- For applications where full transient duration is sufficient for baseline resolution in absorption mode FT mass spectra, proportional, up to two fold, increase in throughput can be obtained. Examples include multiplexed protein quantitation and ETD-based top-down protein analysis on Fusion™ Lumos™ Orbitrap™ FTMS.
- For applications where full transient duration is comparable with the transient duration required to achieve the pre-set resolution, super-resolution signal processing, e.g., least-squares fitting (LSF) provides the desired increase in throughput. An example here is a multiplexed protein quantitation on Q Exactive™ Plus FTMS.
- Further benefits of high-performance acquisition and processing of transients in FTMS is in improved sensitivity, limit of detection and limit of quantitation.

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