Super-Resolution Analysis of Highly-Plexed Complement Reporter Ion Clusters Enables Quantitative Proteomics

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Introduction

The complement reporter ion approach was developed to address certain limitations of multiplexed quantitation using the TMT reporters. The recently released TMTproC reporters extend the heavy TMT reporter coverage to C-terminal peptides and allow the detection of peptides with C-terminal Met modifications. However, unlike the user-friendly, low-mass TMT reporters, the workflows with the heavy TMTproC reporters are challenging even for the state-of-the-art mass spectrometers. Previously, we introduced the lead-squares fitting (LSF) method for Fourier transform mass spectrometry (FTMS) super-resolution processing of apex processing and demonstrated its benefits in particular applications. Here, we extend the LSF for complement reporter ion clusters, the TMTproC, and TMR workflow, both with high-multiplexed channels and with sparse channels, and evaluate this extended LSF method for super-resolution LC-MS experiments.

Methods

FTMS Booster X2

Peak Separation

N-plex Suite

LSF

TMTproC & TMR processing pipeline

Spectroswiss

HPLC

LC-MS, MS2

Figure 1. Illustration of LSF signal processing in the LC-MS analysis of a 12-plex TMTpro TM labeled HeLa sample with aFT and mass error range of ±10 ppm. Each 12-plex TMTpro TM sample was processed with Spectroswiss software running on a 16-core desktop computer.

Conclusions

1. Complementary time-domain- and mass-domain instruments provide additional flexibility for post-acquisition signal processing and data analysis.

2. Unbiased-challenge (ULC) performance issues with Lumsen™ FTMS, to obtain one of the reference data sets in this work, was enabled by detection of extended-length transients followed by data processing with FT in absorption mode.

3. LC-MS method and data processing workflows have been extended to be applicable to LC-FTMS data with complement reporter ion clusters for the TMTproC or TMR tags.

4. The comparison of the complement reporters’ statistical data for the raw aFT and LSFT mass spectra (where applicable, viz., the signals from the 8-plex and 12-plex data, and the doublets from the 4-plex data) validate this implementation of the LSF method.

5. A fundamental possibility was observed, to reduce the required times to 105 ms (FT resolution scale) for the high-throughput workflows (IT values of ~100 μs) with the highly-plexed TMTproC channels (thirteen- and 950-da doublets), provided that ion interactions within the doublets are sufficiently below the cross-contamination threshold.

References
