Optimization of wide window acquisition methods for improved proteome coverage

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ABSTRACT

Purpose: The CHIMERYS intelligent search algorithm provides a means to deconvolute complex spectra containing multiple PSMs in tandem mass spectrometry signals. To determine the effect of increasing window sizes in CHIMERYS, we used several gradient lengths and compared the results to peptide and protein identifications obtained using the SequestXR software.

Methods: We performed comparative studies using standard samples using various gradient lengths (# of minutes) and isolation widths for trap isolation methods. Data were acquired using a Thermo Fisher Scientific Orbitrap Exploris 480 mass spectrometer with or without a ChargeSwitch TRAP™ trapping column. Single gradient, pool injection methods were used for all samples. Data were processed using the Thermo Scientific Proteome Discoverer software and the CHIMERYS intelligent search algorithm. The CHIMERYS software and algorithm were compared with the SequestXR software using various isolation window sizes.

Results: Increasing the isolation window size increases the number of proteins identified for each time point. Compared to SequestXR, CHIMERYS provided a means to deconvolute complex spectra containing multiple PSMs. The CHIMERYS intelligent search algorithm rethinks the analysis of tandem mass spectra from the intelligent search algorithm provides a means to deconvolute complex spectra containing multiple PSMs.

Introduction

The identification of peptides in bottom-up proteomics relies on matching tandem mass spectra to proteins in sequence databases. Tandem mass spectra containing multiple PSMs require more complex computational approaches to ensure that the peptides identified are accurately assigned.

Materials and Methods

Sample Preparation

Thermo Scientific™ HisTag™ Tag Standard (10 ng) was reduced by adding 0.05 M of TCEP buffer (7:3 H2O:acetonitrile, pH 8.0) for 30 min at 25°C. The sample was then purified using a C18 Cartridge (1000 ng). The purified sample was then lyophilized and stored at -20°C. The purified sample was then lyophilized and stored at -20°C. The purified sample was then reconstituted in 50% acetonitrile (ACN) in 0.1% formic acid in water. It was further diluted with 0.1% formic acid to obtain a concentration of 200 ng/μl.

Comparison of different search strategies in various gradient lengths

To determine the impact of increasing window sizes in CHIMERYS, we performed data dependent acquisition experiments with isolation windows ranging from 0.5 to 4.0 Th. The same sample was processed using four different isolation window sizes (1, 2, 3, 4 Th) for each time point. The data were analyzed using three different search engines with SequestXR and CHIMERYS, with INFERYS software. Results show that increasing the number of protein groups with wider isolation widths for trap isolation methods show a significant improvement in protein identification rates.

Results

Figure 1. Average and standard deviation for identified unique peptides and proteins from 5 replicates of 200 ng HeLa lysate run with direct injection and a 2 Th isolation window. CHIMERYS identifies between 30 and 40% higher unique peptides and proteins than the SequestXR software.

Figure 2. Average unique peptides and proteins identified for 4 replicates of 200 ng of HeLa lysate run with a 2 minute gradient with varied isolation windows processed using SequestXR and CHIMERYS. CHIMERYS provides a 35% increase in unique peptides and 42% increase in proteins when compared to SequestXR.

Figure 3. Average and standard deviation for identified unique peptides and proteins from 5 replicates of 200 ng HeLa lysate run with direct injection and a 2 Th isolation window. CHIMERYS identifies between 30 and 40% higher unique peptides and proteins than the SequestXR software.

Figure 4. Average unique peptides and proteins identified for 4 replicates of 200 ng of HeLa lysate run with a 2 minute gradient with varied isolation windows processed using SequestXR and CHIMERYS. CHIMERYS provides a 35% increase in unique peptides and 42% increase in proteins when compared to SequestXR.

Figure 5. Average unique peptides and proteins identified for 4 replicates of 200 ng of HeLa lysate run with a 2 minute gradient with varied isolation windows processed using SequestXR and CHIMERYS. CHIMERYS provides a 35% increase in unique peptides and 42% increase in proteins when compared to SequestXR.

Figure 6. Average unique peptides and proteins identified for 4 replicates of 200 ng of HeLa lysate run with a 2 minute gradient with varied isolation windows processed using SequestXR and CHIMERYS. CHIMERYS provides a 35% increase in unique peptides and 42% increase in proteins when compared to SequestXR.

Figure 7. Average unique peptides and proteins identified for 4 replicates of 200 ng of HeLa lysate run with a 2 minute gradient with varied isolation windows processed using SequestXR and CHIMERYS. CHIMERYS provides a 35% increase in unique peptides and 42% increase in proteins when compared to SequestXR.

Figure 8. Average unique peptides and proteins identified for 4 replicates of 200 ng of HeLa lysate run with a 2 minute gradient with varied isolation windows processed using SequestXR and CHIMERYS. CHIMERYS provides a 35% increase in unique peptides and 42% increase in proteins when compared to SequestXR.

Figure 9. Average unique peptides and proteins identified for 4 replicates of 200 ng of HeLa lysate run with a 2 minute gradient with varied isolation windows processed using SequestXR and CHIMERYS. CHIMERYS provides a 35% increase in unique peptides and 42% increase in proteins when compared to SequestXR.

Figure 10. Average unique peptides and proteins for 3 replicates of 500 ng of HeLa lysate run with a 3 minute gradient with varied isolation windows. Increasing the isolation width between 2 and 4 Th improved performance, while wider windows decreased performance.

Figure 11. Average unique peptides and proteins for 3 replicates of 500 ng of HeLa lysate run with a 3 minute gradient with varied isolation windows. Increasing the isolation width between 2 and 4 Th improved performance, while wider windows decreased performance.

Figure 12. Average PSMs per MS scan for 200 ng of HeLa lysate run with various gradient lengths using CHIMERYS and SequestXR. CHIMERYS shows a pattern with improved performance between 2 and 4 Th isolation, while wider windows decreased performance.

Figure 13. Average unique peptides and proteins for 3 replicates of 500 ng of HeLa lysate run with a 3 minute gradient with varied isolation windows. Increasing the isolation width between 2 and 4 Th improved performance, while wider windows decreased performance.

RESULTS

Comparison of different search strategies in various gradient lengths

To determine the impact of increasing window sizes in CHIMERYS, we performed data dependent acquisition experiments with isolation windows ranging from 0.5 to 4.0 Th. The same sample was processed using four different isolation window sizes (1, 2, 3, 4 Th) for each time point. The data were analyzed using three different search engines with SequestXR and CHIMERYS, with INFERYS software. Results show that increasing the number of protein groups with wider isolation widths for trap isolation methods show a significant improvement in protein identification rates.

Comparison of different window sizes for trap isolation methods show a significant improvement in protein identification rates.

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