RAPID MEASUREMENT OF PROTEIN MODIFICATIONS BY COMBINING EXACT MASS MEASUREMENT AND ADVANCED SPECTRAL DECONVOLUTION

Waters

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INTRODUCTION

Detecting and measuring modifications in protein structure that affect biological activity is required in protein chemistry research. For protein biopharmaceuticals, such changes must be monitored throughout process development, stability testing, and quality assurance because structural changes can affect efficacy and safety. While peptide mapping can be used, it is time-consuming and labor-intensive. It is attractive to consider using molecular weight measurement of the intact protein to determine the degree of modification. Such an approach would require measurement of exact mass in combination with spectral interpretation that preserves quantitative information. For this approach to be used, high quality, exact mass spectra are required. Of equal importance, the spectra must be analyzed using an algorithm that does not require prior knowledge of the sample composition and that preserves relative quantitative information about the protein components of the sample. A Tof mass spectrometer (LCT Premier) in conjunction with the MaxEnt1algorithm meets these basic criteria.

METHODS

Instruments
Waters 2796 Alliance Liquid Chromatography System

Waters Micromass LCT Premier ESI-orthogonal Tof mass spectrometer

Waters Micrmass Masslynx 4.0 software for control and data acquisition

Column:
MassPREP™ on-line desalting cartridge, 2.0 x 10 mm

Separation
Flow rate 250 µL/min
0.1% formic acid in water (Buffer A)
0.1% formic acid in acetonitrile (Buffer B)
Gradient 10-60% Buffer B, 2 minutes
Temperature 40 °C

Samples:
Standard proteins, bovine RNase B and yeast enolase, were purchased from Sigma Chemical Company, St. Louis, MO
Samples were dissolved and diluted to 10 pmol/µL each in aqueous 0.1% formic acid.
10µL injections = 100 pmol of protein on column

Tuning Tips

- Tune for maximum declustering. On the LCT Premier one adjusts the source pressure to 2.0—3.0 e0. At a set source pressure increase the voltage of Ion Tunnel 1 and Aperture 1 to decrease *m/z* peak widths caused by non-covalent clusters. Do not increase Ion Tunnel 1 and Aperture 1 voltages past the point when charge stripping begins. (Do not allow the charge-state envelope to distort to high m/z).
- Tune with a protein which has a MW as close a possible to the MW of the proteins to be analyzed.
- Acquire data over the widest possible *m/z* range to determine the charge-state distributions for the proteins of interest

DECONVOLUTION PROCEDURE

Step 1: Select ESI MS Spectral Data

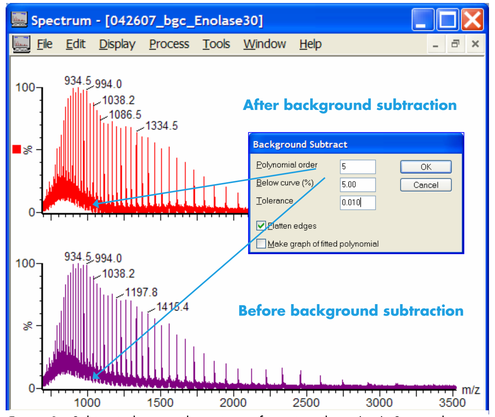


Figure 1. Subtracted summed spectrum of yeast enolase. (top) Summed spectrum of yeast enolase (bottom). Spectral subtraction should decrease the ambiguity derived from the raised baseline due to overlapping charge states. However, since MaxEnt1™ requires noise, therefore a modest polynomial order (≤ 5) and % if background subtraction (≤ 5) must be chosen.

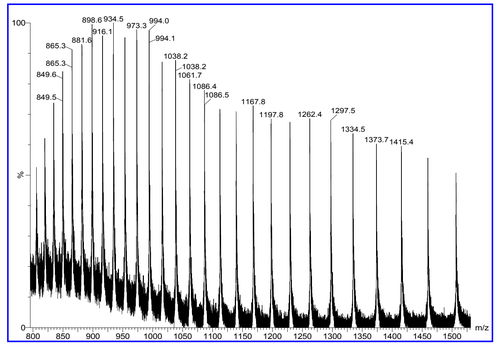


Figure 2. Choose the *m/z* range for deconvolution to include the most intense charge states. Deconvolution applies only to data in the spectrum view window.

Step 2: Set Deconvolution Parameters

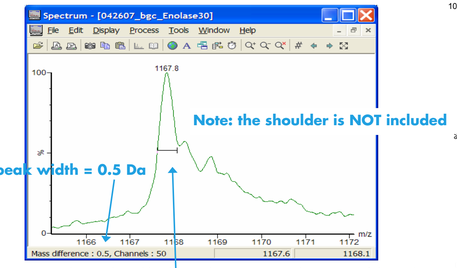


Figure 3: Measure the peak width of one charge state in the center of the m/z range chosen for deconvolution.

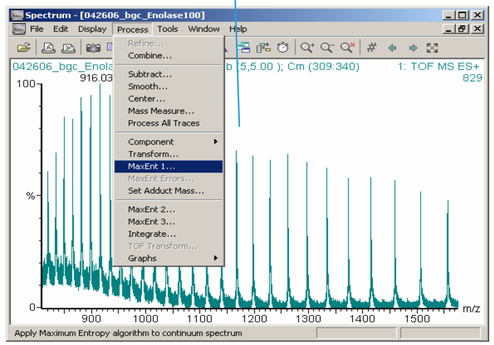


Figure 4. Open MaxEnt 1™ window.

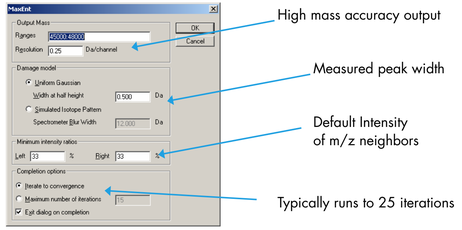


Figure 5. MaxEnt 1™ parameter window.

Step 3: Begin Deconvolution Calculation

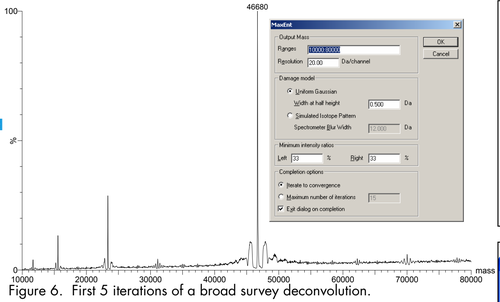


Figure 6. First 5 iterations of a broad survey deconvolution.

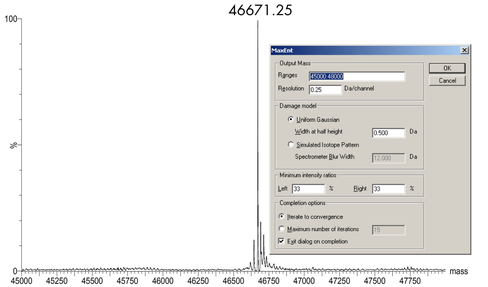
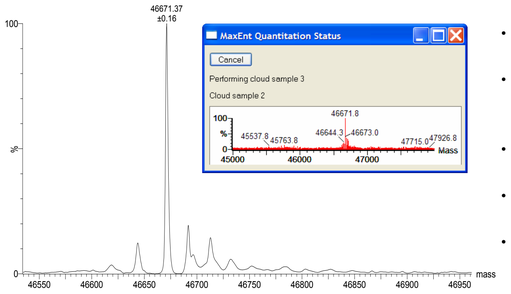
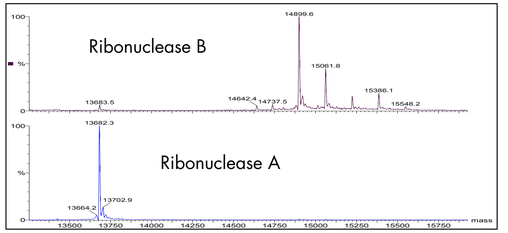
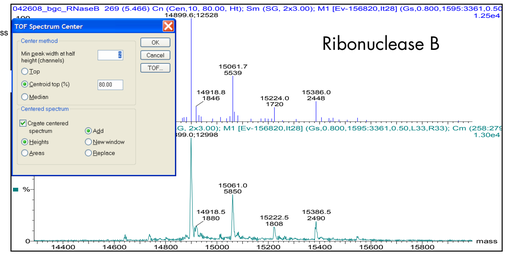


Figure 7. Final iteration of a accurate, narrow MaxEnt 1 ™ deconvolution. After centering proceed to calculate MaxEnt Errors



Step 4: Centering for Quantitation





56% 5-Mannose
25% 6-Mannose
8% 7-Mannose
11% 8-Mannose
1% 9-Mannose

= Mannose = N-Acetyl glucosamine

CONCLUSIONS

- MaxEnt 1™ Deconvolution of m/z data produces a quantitative representation of the protein MWs.
- The peak width measured in the m/z data is required for deconvolution accuracy, but no other prior knowledge of the protein MW is required.
- After deconvolution the MaxEnt 1 ™ output must be centered without sticks prior to MaxEnt 1 error calculation.
- MaxEnt 1 ™ errors measure the precision of the deconvolution.
- After deconvolution the MaxEnt 1 ™ output must be centered by heights to calculate the relative abundance of the forms of a protein.

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