

Comprehensive Proteome Analysis by Multi-Dimensional Separation Coupled to High Mass Accuracy MALDI-MS and MALDI-MS/MS

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Currently, shotgun strategy for proteome research involves a combination of chromatography and mass spectrometry for analysis of a complex enzymatic digest of protein samples. Continuous deposition was previously introduced to couple high resolution separation to MALDI-MS. In this work, a closely spaced external standard [1] was used to obtain 10 ppm mass accuracy in the LC-MALDI-MS with continuous deposition in order to improve database searching of MS/MS for peptide identification.

A tryptic digest of yeast protein mixture was fractionated by strong cation exchange chromatography into 20 fractions, and then analyzed by nanoLC-MALDI-MS and MS/MS. The eluent from LC was mixed with matrix solution and deposited via a continuous deposition interface onto a nitrocellulose coated target in the form of 250 μm -wide streaks. Five standard peptides, used as external calibrants, were deposited adjacent to the analyte streaks with a roughly 100 μm gap. The MS spectra were acquired by an AB 4700 TOF/TOF MS instrument. A new in-house developed algorithm, MEND [2], which minimizes both random and chemical noise in order to improve mass accuracy and to select low intensity peaks, was used to analyze MS spectra and generate lists of precursor ions for acquisition of MS/MS spectra. MS/MS data was then analyzed by Mascot for peptide identifications.

In Mascot MS/MS database searching, the threshold level for significant hits is determined by the number of potential peptide candidates, which depends on mass tolerance, the mass value of the precursor ion, and the size of the database. By improving of mass accuracy in the MS mode, mass tolerance used in database searching can be minimized to reduce the number of peptide candidates, which results in the decrease of significant score. Table 1 shows database search results for about seven thousand MS/MS spectra with 100, 50, 10 ppm mass tolerance. Reducing mass tolerance from 100 ppm to 50 ppm resulted in only 2% increase in the number of significant hits; however, the number of significant hits increased by 20% when mass tolerance was reduced from 50 ppm to 10 ppm. The new identifications, which had low ion scores, arose from MS/MS spectra with poor fragmentation and/or low intensity. The number of identified proteins also increased by 10%, due to the extra peptide identifications. These identifications are potentially useful in investigation of low abundant proteins and post-translational modifications.

A database containing yeast proteins with reversed amino acid sequences was used to the rate of examine false positives [3]. Since proteins in this reversed database are highly unlikely to be present in the sample, all significant matches were considered as false positives. No significant difference was observed in the rate of false positives while varying mass tolerances in MS/MS searching, which suggested the confidence the level of database search result was not affected by the mass tolerance.

Table 1 Mascot Search Result with Various Mass Tolerances¹

Mass tolerance	Threshold for significant hit ²	Significant hits	False positive rate
100 ppm	22	2219	2.5%
50 ppm	21	2312	2.3%
10 ppm	14	2708	2.7%

¹Number of total query: 13246

²Confidence level: 95%

References:

[1] Closely Spaced External Standard: A Universal Method of Achieving 5 ppm Mass Accuracy over the Entire MALDI plate in MALDI-TOF MS, E. Moskovets, H.-S. Chen, A. Pashkova, T. Rejtar, V. Andreev., and B. L. Karger, submitted.

[2] A Universal Denoising and Peak Picking Algorithm for LC-MS Based on Matched Filtration in the Chromatographic Time Domain, V Andreev, T. Rejtar, H.-S. Chen, E. Moscovets, A. Ivanov, and B. L. Karger, submitted.

[3] Qscore: An Algorithm for Evaluating SEQUEST Database Search Results, R. E. Moore, M. K. Young, and T. D. Lee, *J. Am. Soc. Mass Spectrom.* 2002, 13, 378-386.