

## **A New Strategy for Comprehensive Proteome Analysis Using Parallel Replicate Separation with Multiplexed LC-MALDI MS**

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Complex proteome analysis requires multiple stage separation processes in order to reduce the sample complexity prior to MS analysis. However, only a partial number of peptides in a complex sample can be identified in a single LC-MS/MS analysis due to the limitations of instrumentation. Analyzing the same sample multiple times is preferable in order to obtain comprehensive information. The LC-MALDI MS permits simultaneous deposition of eluents from multiple separation columns on MALDI plates, allowing rapid multiple LC-MS analyses [1,2]. Moreover, decoupling the separation from MS analysis leads to a strategy for precursor ion selection in replicate analyses, thus reducing redundant MS/MS acquisition and increasing the number of peptide identifications. We have developed a new strategy for comprehensive proteome analysis by using a new multiplexed LC-MALDI MS system with a high deposition rate, which preserves the resolution of separation, and apply a new MS/MS analysis strategy in replicate LC-MS analyses.

Multiplexed 4-channel HPLC system was off-line coupled to MALDI-MS using a lab-built deposition interface. Each separation channel consisted of an individual injection valve with a sample loop and a separation column. The eluents from individual LC columns were mixed in micro-Tees with MALDI matrix solution and deposited simultaneously on MALDI plates in the form of spots. Each spot represented 5 seconds of chromatographic time. The deposited samples were analyzed by AB 4700 TOF/TOF MS instrument in both the MS and MS/MS modes. The MS spectra were processed by an in-house algorithm (MEND), which generated a list of precursor ions for subsequent MS/MS analysis. MS/MS spectra were then analyzed by Mascot for peptide identifications.

The new developed strategy of comprehensive proteome analysis involved analyzing the same sample multiple times and adjusting the list of precursor ions for MS/MS acquisition according to previous analyses. A tryptic in-gel digest of 50-60 kDa gel band from the SDS-PAGE of a yeast cell lysate was used as a model sample. The sample was injected into all 4 channels of the multiplexed LC and then analyzed simultaneously. The extracted ion chromatograms revealed that the difference in elution time was less than 1 minute from column to column even at the end of one-hour of separation, demonstrating the high reproducibility of separation among channels. MS/MS spectra were acquired in all 4 channels in order to test the reproducibility of LC-MS/MS analyses. The database searching results showed that the overlap of peptide identifications between two channels was about 75-80%. The result suggested that replicated analyses were necessary in order to obtain more comprehensive information from a single sample. Figure 1 shows the scheme of this new strategy for MS/MS analysis. In order to avoid redundant MS/MS acquisition and increase the possibility to identify low abundant peptides, the MS/MS data obtained from the previous analysis was used to generate an exclusion list. Figure 2 shows the result of the MS/MS analysis of the model sample after applying the new strategy. In the first LC-MS/MS analysis, 248 unique peptides were identified. With the new strategy, 79 additional unique peptides were identified in the second LC-MS/MS analysis, representing 32% increase in the number of peptide identifications. With all four LC-MS/MS analyses, a 60% increase in the number of peptide identifications was observed, compared to a single LC-MS/MS analysis.

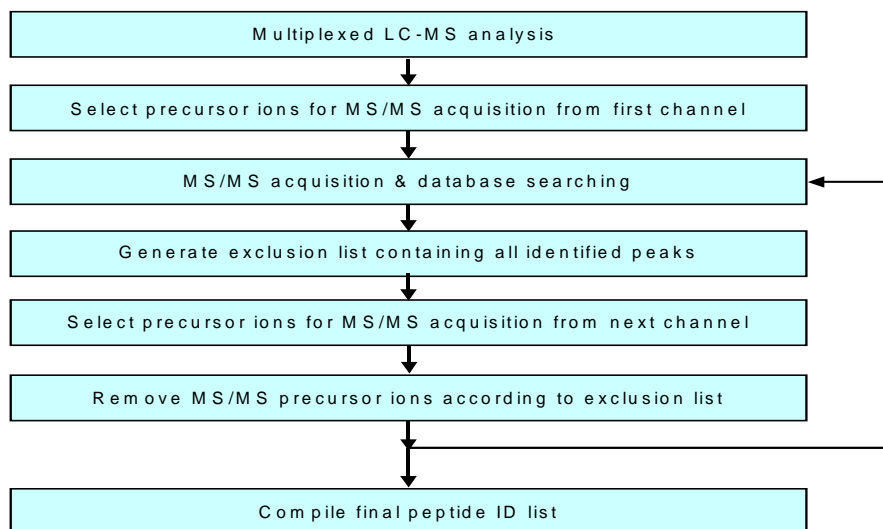


Figure 1 Flowchart of new strategy of LC-MS/MS analysis

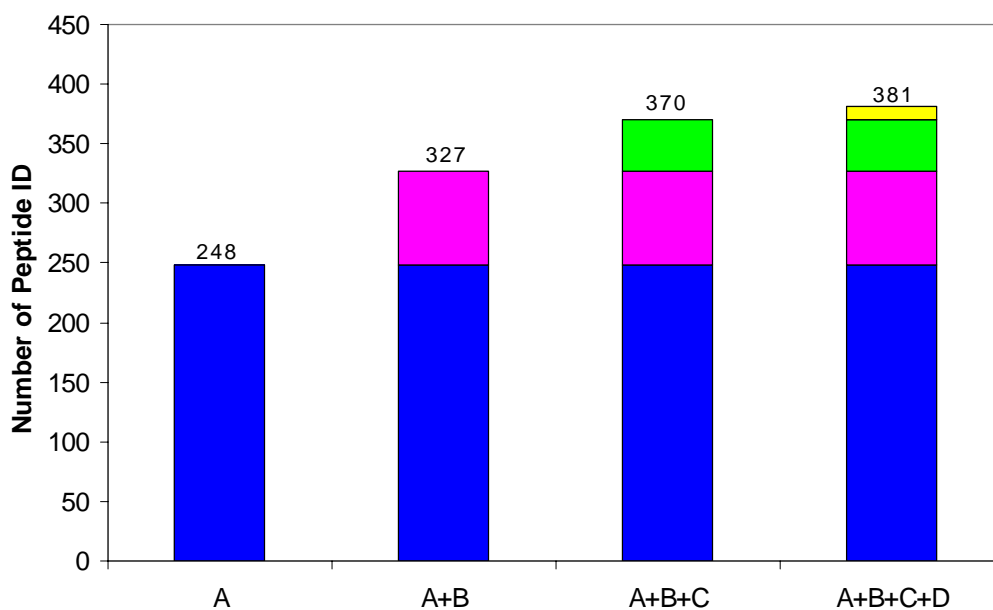


Figure 2 Peptide identifications in replicate LC-MS/MS analysis

Reference:

[1] Development of a Multiplexed Microcapillary Liquid Chromatography System for High-Throughput Proteome Analysis, H. Lee, T. J. Griffin, S. P. Gygi, B. Rist, and R. Aebersold, *Anal. Chem.* 2002, 74, 4353-4360.

[2] An Automated Noncontact Deposition Interface for Liquid Chromatography Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry, C. Ericson, Q. T. Phung, D. M. Horn, E. C. Peters, J. R. Fitchett, S. B. Ficarro, A. R. Salomon, L. M. Brill, and A. Brock, *Anal. Chem.* 2003, 75, 2309-2315.