

Multidimensional Chromatographic Separation of Intact Proteins Coupled with Atmospheric Pressure Matrix Assisted Laser Desorption Mass Spectrometry for Rapid, Comprehensive Proteomic Analysis

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The goal of comprehensive analysis of complex proteomic samples such as cell lysates or biological fluids has focused primarily either the use of 2D Gel Electrophoresis couple with Mass Spectrometry or, increasingly, Multidimensional HPLC approaches to Peptide Identification from a whole proteome proteolytic digests. The coverage of the 2DGE approach is limited by the loadability, imaging detection limits and linearity. The Mudpit[1] approach on the other hand is limited by a combination of MS/MS acquisition speed and chromatographic resolution of the extremely complex mixtures resulting from digestion of complex samples since a given protein generates approximately 50 peptide fragments. Furthermore, in the case of MDLC approaches, the relationships between specific proteolytic fragments and the parent protein are lost. Thus characterization of samples which may contain multiple post-translational modifications only reflect an average distribution of those PTMs.

As an alternative, we are investigating the use of Multidimensional HPLC of intact proteins followed by fraction collection, proteolytic digestion and mass spectral evaluation with Atmospheric Pressure MALDI or Nanospray LC-MS. This approach is comprehensive in the sense that all eluting proteins are collected and analyzed and thus limits of coverage will be due to MS sensitivity rather than the ability to visualize and excise specific protein spots. Additionally, this approach offers the advantage of maintaining the association of related fragment peptides. Although limitations might be anticipated concerning the capability of chromatographic separation modes and equipment to adequately separate and yield accurate profiles of complex sample containing proteins of extremes in pI, molecular weight and hydrophobicity, careful selection of separation modes and conditions appear to yield equivalent results to 2D Gel Electrophoresis.

In the current research, protein extracts from *S. cerevisiae* were reduced and alkylated and separated by Offline 2D HPLC. A number of chromatographic modes were evaluated for the first dimension including Size Exclusion, Anion Exchange and Chromatofocusing as well as preparative isoelectrofocusing (Rotofor). Fractions were collected and then each fraction was separated using a high-speed reversed phase separation on Poroshell 300SB-C18 packing material. The use of the Poroshell material allowed total RP separation cycles of 5-10minutes, resulting in a total 2D fractionation Time of approximately 4 hours. Fractions were collected from the RP separation in 0.5-1min intervals containing approximately 5-10 proteins per fraction. These fractions were digested with Trypsin and then analyzed by AP-MALDI with Ion Trap MS/MS characterization of each fraction for comprehensive MS/MS of all parent ions above a preset threshold and sub-femtomole identification of collected proteions. Alternatively, the collected fractions were analyzed by Nanospray LC/MS using fast (5-10min) gradients on 75um i.d. capillary columns. It should be noted that the RP separation of digest peptides is orthogonal to the prior protein separation and thus constitutes a 3rd dimension of chromatographic separation.

1. Wolters, D.A., M.P. Washburn, and J.R. Yates, *An automated multidimensional protein identification technology for shotgun proteomics*. Analytical Chemistry, 2001. **73**(23): p. 5683-5690.