Top Down Proteomics Using Online Polymer Reversed Phase (PLRP) Nanocapillary-LC Coupled Fourier Transform Mass Spectrometry

Adakilam Vellaichamy1; John F. Kellie1; John C. Tran1; Ji Eun Lee1; Adam Catherman; Jaeyun Sung1; Nathan D. Price1; Gary Valaskovic2; Neil L. Kelleher1

1University of Illinois, Urbana, IL; 2New Objective, Inc., Woburn, MA

OVERVIEW
In comparison to Bottom Up proteomics, the growing field of Top Down proteomics is impeded for high throughput due partly to the lack of comparable studies in sample preparation and online separation methods.

Here, we evaluate the performance of Polymer Reversed Phase (PLRP) for online nano-LC coupled Top Down analysis in combination with pre-fractionation by gel-eluted liquid fraction entrapment electrophoresis (GELFEE).

INTRODUCTION
Due to its uniformity hydrophobic nature, enhanced chemical, mechanical, and pH stability, the polymeric (polystyrene divinylbenzene) reversed phase resin is well suited for biopolymer separation. The silica based reversed phase hydrophobic separation media such as C4, C5, and C18 have been widely used for nano-LC based separation of peptides, and proteins. Suitability of capillary packed PLRP for online nano-LC separation of intact proteins in combination with ultra high resolution Fourier Transform mass spectrometry (FTMS) has yet to be studied in detail.

For reducing the complexity of eukaryotic proteomes as yeast and human, we have recently adopted a separation device known as gel-eluted liquid fraction entrapment electrophoresis (GELFEE). GELFEE separates intact proteins according to mass into well resolved liquid fractions. The advantage of PLRP nano columns over the conventional C4 nano columns, and its application to high throughput online identification and characterization of high mass proteins using 12T LTQ-FTMS is discussed.

METHODS

NanoLC-MS/MS work flow

GELFEE Separation

RESULTS
Recovery and Resolution

PLRPS Pore Size and Retention

Separation of six protein standards ranging from low mass to medium high mass were conducted on nano-LC using varying pore size. Chromatograms show inverse relationship between pore size and retention factor. These figures show that 1000 Å is the optimal pore size for both medium (30-49kDa) and high MW proteins (>50kDa) in terms of resolution. In addition, recovery was observed to be inversely proportional to pore size. Protein standards used were: Cytochrome C; 2. α-lactalbumin; 3. Myoglobin; 4. Carbonic anhydrase; 7. Ovalbumin; 6. Bovine serum albumin.

PLRPS and High Mass Detection

Ion trap charge state distribution profiles for BSA fractionated online using PLRPS (300 Å) from six protein standard mixture with increase in number of micro scans. The S/N intensity increases with micro scans.

PLRPS and High Mass Identification

ShOWN here is a fragmentation spectrum from single FT scan obtained during the online nano-LC MS/MS of six protein standards mixture. Monoisotopic fragment masses were obtained from this scan using Xlink and searched against a standard protein database consisting of 7965 uniprot protein forms. The search result showed 23 fragments matching to BSA with mass error of less than 5 ppm.

ACKNOWLEDGEMENTS
Thanks to Kelleher Lab informatics team members: Paul M. Thomas, Leonid Zamdborg, Dorothy Ahn, Haylee Thomas, Kenneth R. Durbin, and Shannee Babai. Thanks also to other Kelleher Lab members including Minhui Lee, Steve Sweet, and Joanna Nita. We thank Jeremiah Tipton and Alan Marshall from National High Magnetic Field Laboratory, Tallahassee, FL. AV thanks Neil and IGB for the Institute for Genomic Biology fellowship position.