High Throughput Proteomic Analysis of Proteins Separated by Gel Electrophoresis Using MALDI TOF/TOF MS and Automated Data Processing

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Development of high throughput technology for global identification of every protein expressed in any given cultured cell lines or organism have become essential for our understanding of protein functions under different biological conditions. In order to achieve such large scale protein identification, a mass spectrometry-based high throughput system in conjunction with automated sample handling and automated data processing needs to be developed.

Although electrophoretic separation, proteolytic digestion, mass spectrometric analysis of unseparated digests and database searching have become standard methods in widespread use, peptide sequence information obtained by collision induced dissociation and tandem mass spectrometry is required to establish the most comprehensive and reliable results. Currently, we are developing methodology for high throughput proteomic analysis of gel-separated proteins, using robotic sample preparation techniques for in-gel digestion, desalting and MALDI target spotting, a novel matrix assisted laser desorption/ionization-time of flight/time of flight (MALDI-TOF/TOF) tandem mass spectrometer with automated data collection, and automated data transfer and interpretation by newly developed software packages of Protein Prospector.

This high throughput approach has been employed for comprehensive identification of *S. cerevisiae* nucleoporin interacting proteins at large scale. The *S. cerevisiae* nuclear pore complex (NPC) is a supramolecular assembly of thirty nucleoporins that cooperatively facilitate nucleocytopalsmic transport. Thirteen nucleoporins that contain FG peptide repeats (FG Nups) are proposed to function as stepping stones in karyopherin-mediated transport pathways, but the actual mechanism of protein transport across the NPC is unknown. In order to understand the nucleocytoplasmic transport processes, we are studying the nucleoporin interacting protein network using mass spectrometry. Here, protein interactions that occur at individual Nups were sampled with immobilized GST-nucleoporins and yeast extracts under different growth conditions[1]. In addition, nucleoporin interacting proteins were also captured in the presence of Ran-GTP mixed with yeast extracts, and the effect of Ran-GTP on the binding patterns was investigated. Bound proteins were eluted with NaCl and subsequently with SDS. All eluted proteins were separated by 1-D SDS-PAGE, and small portions of each gel band were cut and robotically digested by trypsin in multiple 96-well plates. The unseparated tryptic digests were robotically desalted by C18 Ziptips and spotted onto MALDI target. Hundreds of MS and MSMS spectra were acquired automatically in high throughput fashion using MALDI TOFTOF MS. The raw mass spectrometric data were automatically extracted, transferred and submitted for database searching using Protein Prospector software (http://prospector.ucsf.edu)[2]. The comprehensive search results were summarized based on the search results from both MS-Fit and MS-Tag searches, which provides protein identities for hundreds of samples simultaneously. As a result, hundreds of nucleoporin interacting protein gel bands were identified by such integrated and automated system for high throughput proteomic analysis. Figures 1and 2 illustrate an example of MALDI-TOF MS and MSMS spectra from an unseparated tryptic digest of one selected band. Peptide sequencing using MALDI-TOFTOF tandem mass spectrometry can not only provide unambiguous protein identification, but also generate comprehensive fragmentation information to distinguish I and L and to elucidate detailed structures for chemically modified peptides[2]. Most of the proteins identified were nucleoporins and kyropherins, which were unique for various nucleoporins used as baits. And the binding patterns changed in the presence of Ran-GTP, which supports the notion that Ran-GTP modulates the association of karyopherins and nucleoporins to facilitate karyopherin-mediated transport of macromolecules across nuclear pore complex. The results have not only provided a basis for our great understanding of the transport mechanism, but also provide a general guideline to use MALDI-TOFTOF MS-based high throughput system for study protein-protein interactions at large scale.

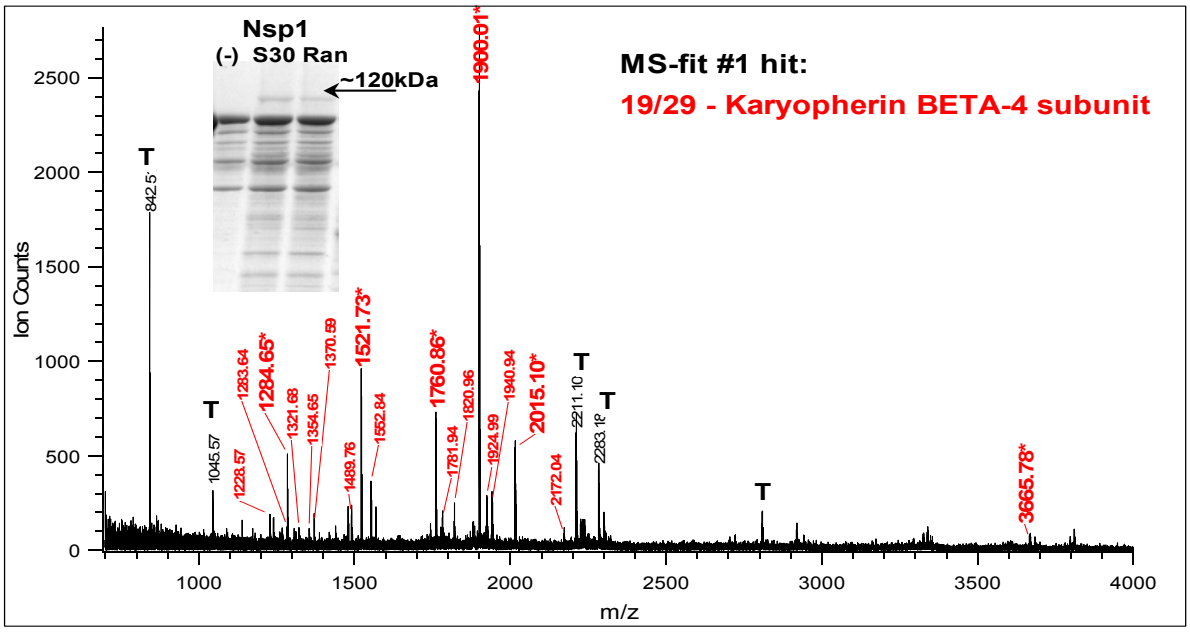

Figure 1. MALDI-TOF MS of unseparated tryptic digest from one band at ~120kDa using GST-Nsp1 as bait. T represents trypsin autolysis product. (-)control;S30:yeast extract; Ran:yeast extract with Ran-GTP.

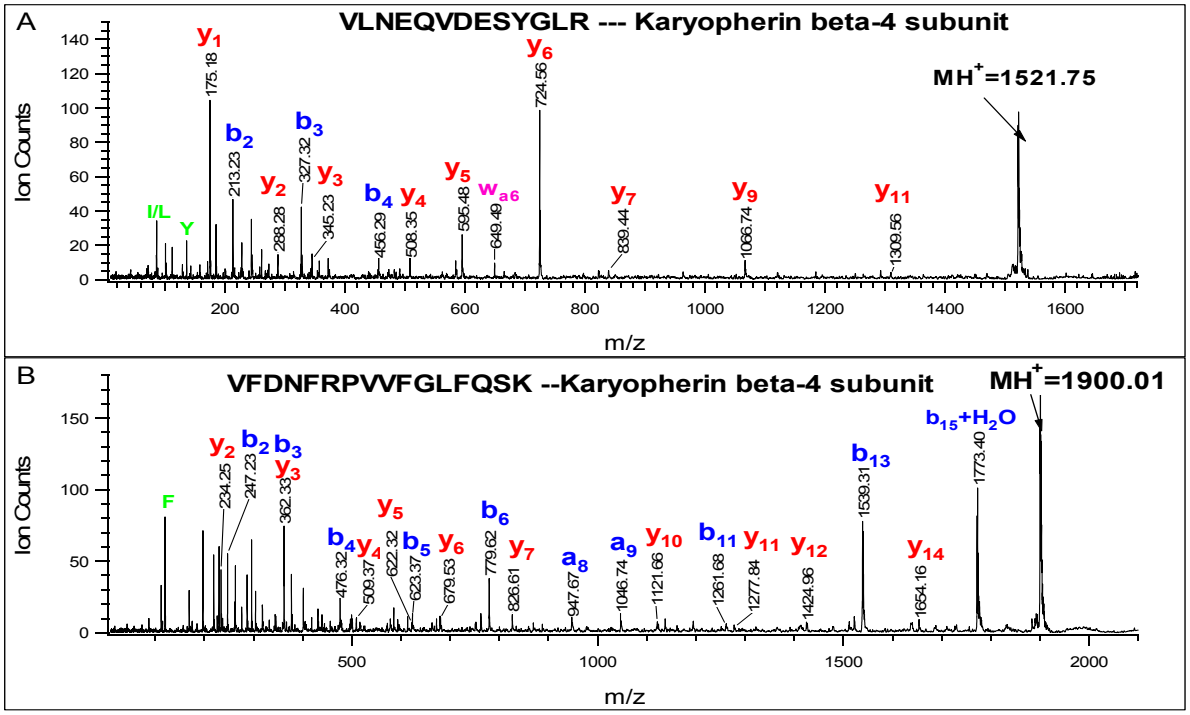

Figure 2. MALDI TOFTOF CID spectra of two tryptic peptides from tryptic digest as shown In Figure 1. (A) MH^+ 1521.7; (B) MH^+ 1900.1. Both peptides matched to karyopherin beta-4 subunit by MS-tag searching.

References:
1.Allen NP, Huang L, Burlingame A, Rexach M. *J Biol Chem*. **2001**, 276(31):29268-74.
2.Huang L, Baldwin M., D. Maltby, P.R. Baker, et al. Molecular and Cellular Proteomics, 2002, May 29. (Http://www.mcponline.org).
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