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**Integrating “Top-Down” and “Bottom-Up” Mass Spectrometry
Approaches for Comprehensive Eukaryotic Proteomic Analysis***

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With the current rapid expansion of the field of proteomics, there is much interest in the development of methods for rapid, large-scale mass spectrometry (MS) analyses of proteins from complex biological samples. Two major approaches for MS-based proteome analysis have been employed to date. In the most common “bottom-up” approach, proteins are separated, proteolytically digested, and subsequently identified via MS analysis of the resultant peptide mixture. An alternative approach, termed the “top-down” method, involves the characterization of *intact* proteins by high-resolution mass measurements combined with fragmentation information obtained from tandem MS experiments (MS/MS). Since an intact protein mass is measured, this method is advantageous for the detection of post-translational modifications, which may be easily missed in analyses by the bottom-up approach. Here we present a novel method for proteome analysis that integrates both the top-down and bottom up approaches, capitalizing on the unique capabilities of each method. This approach would provide the most comprehensive characterization of a proteome by yielding information not only on protein identities but also their intact molecular nature in the samples. Such information is vital for eukaryotic organisms, such as humans, because of the large amount of post-translational processing that occurs for the expressed proteins.

The experimental approach for this integrated “top-down, bottom-up” MS technique relies on the capabilities of LC-MS and high resolution Fourier transform ion cyclotron resonance MS (FTICR-MS). Cellular lysates or body fluids are initially fractionated via anion-exchange fast protein liquid chromatography (FPLC). No attempt is made to purify and collect individual proteins in a given fraction; rather this method simplifies the complex protein mixture into several separate fractions, each of which may contain between 75-200 *intact* proteins. Each FPLC fraction is divided equally into two samples. The first portion of each fraction is analyzed by proteolytic digestion with reversed phase-LC-MS/MS or LC/LC-MS/MS. The second portion of each fraction is analyzed on an electrospray FTICR-MS for high-resolution identification of the intact proteins with the top-down approach. The results from each of the separate methods are compared to correlate the protein information obtained from individual FPLC fractions. The use of the two methods in concert enables the facile detection of such common post-translational modifications as N-terminal methionine elimination, phosphorylation, glycosylation, and signal peptide cleavages. In addition, this information can also be used to identify translation frame shift errors from the gene prediction by bioinformatic techniques. We feel that this level of information is a unique capability of our combination “top-down, bottom-up” MS proteomic method. Results obtained by this integrated approach will be illustrated for yeast (used as a model eukaryotic system) and pooled human serum.

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