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Top Down Proteomics

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The field of proteomics has reached a level of maturity where significant biological discoveries are being made with current technology. By far the largest numbers of proteomics experiments employ a “bottom-up” protein analysis. In this strategy proteins or protein mixtures are digested with a protease and then analyzed *en masse* in a “shotgun” format. Tandem mass spectrometry is then used to sequence peptides, and informatics tools are used to identify the sequences and assign them to proteins or genes. This same strategy can be used to identify modifications to peptides. This strategy works very well to identify the proteins present and their modifications. A challenge for bottom-up proteomics is the proper assignment of protein isoforms and patterns of modifications that create the many proteoforms present in cells. Why is this important? The functional sophistication of complex organisms, like humans, is driven by the ability to use a limited number of gene sequences in many different ways. Determining the precise proteoform involved in a specific function will be key for dissecting out the molecular mechanisms used by eukaryotic organisms. A key technology to determine this information is “top down” mass spectrometry. In this method, intact proteins are analyzed to both identify the protein and to determine the identity and location of modifications within the protein using a tandem mass spectrometer. The analysis of intact proteins by mass spectrometry is a major challenge. The first problem for top down analysis is fractionation of proteins. Traditional methods such as gel electrophoresis have limitations on the recovery of proteins in a form suitable for analysis by mass spectrometry. Extracting gel-separated proteins from the polyacrylamide matrix requires electroelution or electroblotting, frequently resulting in sample losses. Chromatographic methods to separate intact proteins can work well, but the resolving power for intact proteins with more than ~500 amino acids is poor. After introduction into the mass spectrometer, amide bonds in the protein backbone must be fragmented to create ladders of sequence information. Because proteins have many chemical bonds that can “soak up” energy put into ions, vigorous research into excitation methods to more efficiently and completely fragment intact protein ions is bearing fruit. The nonergodic methods for protein

ion excitation are increasingly able to fragment larger and larger protein ions, which when coupled with improvements in mass resolving power are aiding in robust protein identification. A key technological change underway is the development of lower cost mass spectrometers that are able to analyze intact proteins. This change will put the technology into more hands, which will speed up innovations and developments. A recent American Society for Mass Spectrometry Sanibel Conference meeting for top down mass spectrometry was very well attended and the lectures demonstrated a vibrant and growing subfield of mass spectrometry-based proteomics and biopharmaceutical analysis. To complement this very successful meeting and highlight a growth area, the *Journal of Proteome Research* and *Analytical Chemistry* have assembled and published a joint thematic virtual issue of the papers published in these journals on top down proteomics. This thematic issue emphasizes the challenges and successes in the field from preparation and fractionation of intact proteins to methods for direct fragmentation of whole proteins and the informatics for their identification by database retrieval. Recent developments clearly show that top down proteomics is a vigorous and energized area of research as described in a recent *Chemical and Engineering News* article by Celia Arnaud.