Proteolytic Digestion

Trypsin is the most commonly used protease for digesting proteins, but other endoproteinases, such as lysylendopeptidase, Asp-N and *S. aureus* V8, are frequently used. Each protein is digested into smaller peptide components, parts of the overall primary sequence. The m/z values of the resulting peptides can be used to identify the protein, if it has been previously identified and its sequence is available in a database (e.g., NCBI or Swiss-Prot/TrEMBL). The measured m/z values observed in spectra obtained from digested proteins are then matched to *in silico* digestion of all proteins in the database using Protein Prospector, Mascot or Sonar. Some post-translational or chemical modifications can be selected as well.

Accurate Mass Measurement with MALDI MS

Accurate mass measurements can be made with MALDI MS to within 50 ppm with external calibration and to within 10 ppm if internal calibrants are used. The measured m/z values observed in spectra obtained from digested proteins can be matched to *in silico* digestion of all proteins in the database using Prospector at UCSF, Mascot or Prowl at Rockefeller University.

Accurate Mass Measurement with ESI MS

In PMF using ESI MS, mass measurements can be made more accurately because orthogonal-TOFMS is used. Even with external calibration, mass measurement accuracy is often below 20 ppm. Unfortunately, a few ion signals may be observed for each peptide, corresponding to different charge states, denoted $[M + nH]^{n+}$ where n is usually 1-4 depending on the peptide size, peptide sequence and ESI instrument used for analysis (See Intact Mass Measurement using ESI MS for further detail). In ESI MS, the m/z values and the charge state (if known) must be entered for database searching to be successful.

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

proteomicfacility@mdanderson.org