

## Nine-Step Strategy for the Interpretation of a Peptide Tandem Mass Spectrum

Adapted from Kinter and Sherman, Protein Sequencing and Identification by Tandem Mass Spectrometry, (2000)

- 1) **Inspect the low-mass region for immonium ions.** Immonium ions indicate the presence of amino acids within the peptide.
- 2) **Inspect the low-mass region for the  $b_2$ -ion.** Generally recognized by the  $b_2$ -ion/ $a_2$ -ion pair separated by 28 m/z. The m/z of the  $b_2$ -ion is then used to calculate the mass of the corresponding  $y_{n-2}$ -ion.
- 3) **Inspect the low-mass region for the  $y_1$ -ion.** This will assign the C-terminal amino acid. An ion at m/z 147 indicates C-terminal lysine, whereas, an ion at m/z 175 indicates C-terminal arginine. The m/z of the  $y_1$ -ion is then used to calculate  $b_{n-1}$ -ion
- 4) **Inspect the high-mass region for the  $y_{n-1}$  ion.** Based on the combinations indicated by the  $b_2$ -ion assign the N-terminal amino acids.
- 5) **Extend the y-ion series toward the lower m/z.** As a y-ion is identified, calculate the m/z of the corresponding b-ion and identify that ion in the spectrum.
- 6) **Extend the b-ion series toward higher m/z.** As a b-ion is identified, calculate the m/z of the corresponding y-ion and identify that ion in the spectrum.
- 7) **Calculate the mass of the proposed peptide sequence.** Compare this with the measured mass.
- 8) **Reconcile the amino acid content with the spectrum data.** Check that the amino acid content agrees with the immonium ions observed. Also consider the charge state of the peptide in terms of the presence of histidine, and internal lysine and arginine residues. Consider the intensity of proline peaks.
- 9) **Attempt to identify all ions in the spectrum.** Work to identify the other ions in the spectrum based on the proposed peptide sequence and pay particular attention to the ions from the loss of  $H_2O$ ,  $NH_3$ , and  $HSOCH_3$ ; any doubly charged ions; and any ions due to internal cleavages.