

PSB 116 Analysis of Post-Translation Modifications Using a Data Dependent Neutral Loss Scan Function

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Post-translational modifications, such as phosphorylation and glycosylation, are associated with regulation, location and function of proteins. Phosphoproteins and glycoproteins are generally found in low quantities within cells. This low abundance, coupled with the higher acidity of phosphopeptides and low hydrophobicity of glycoproteins, complicates their analysis by mass spectrometry in positive ESI mode.

In tandem mass spectrometry, phosphopeptide and glycopeptide precursor ions typically exhibit a prominent neutral loss during fragmentation, i.e., of a phosphate (98 Da) or mannose (162 Da) moiety. Consequently, identification of the

phospho/glycoproteins is often limited by inadequate peptide fragmentation and diagnostic sequence ion information. To overcome the characteristic lack of peptide fragmentation information which is observed in the analysis of phospho/glycopeptides, a new Data Dependent™ scan function selectively triggers MS³ scans on MS/MS fragment ions when a specific, prominent neutral loss ion is detected. This new instrumental method was designed, in part, to take advantage of the very high MSⁿ sensitivity on the Thermo Scientific LTQ series of linear ion traps, enabling detailed analysis of low abundance ions in MS³ and higher order MSⁿ spectra. This capability is key to unambiguous identification of post-translational modifications.

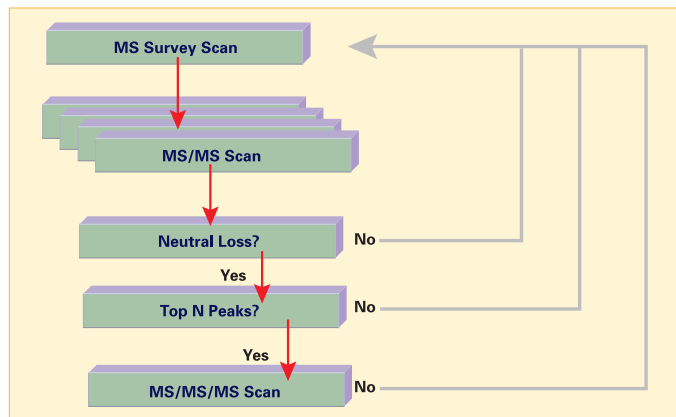


Figure 1: Flow chart of scan events for the Data Dependent Neutral Loss experiment

The flow chart of scan events for the Neutral Loss Data Dependent experiment is shown in Figure 1. In this Top 3 experiment, an MS survey scan is performed first, followed by three MS/MS scan events. When an MS/MS scan event detects a neutral loss ion and this neutral ion intensity is one of the 3 most intense ions (or 5, depending on the user defined settings), it will trigger the MS³ scan event. If a neutral loss is not detected, it will repeat with another series of MS and MS/MS scans and continue the process. Figure 2 shows the base peak chromatogram generated by capillary LC-MS/MS analysis of a mixture of alpha and beta casein digest using the LTQ series linear ion trap mass spectrometer. The full-scan survey detects a peptide at *m/z* of 1031.6 which, upon MS/MS, shows a neutral loss of 49 (doubly charged loss of phosphate) resulting in a peak at *m/z* of 982.6. The Data Dependent neutral loss algorithm then triggers an MS³ event on the ion at *m/z* 982.6 in the consecutive scan, resulting in robust peptide fragmentation and the ability to identify the peptide with high confidence.

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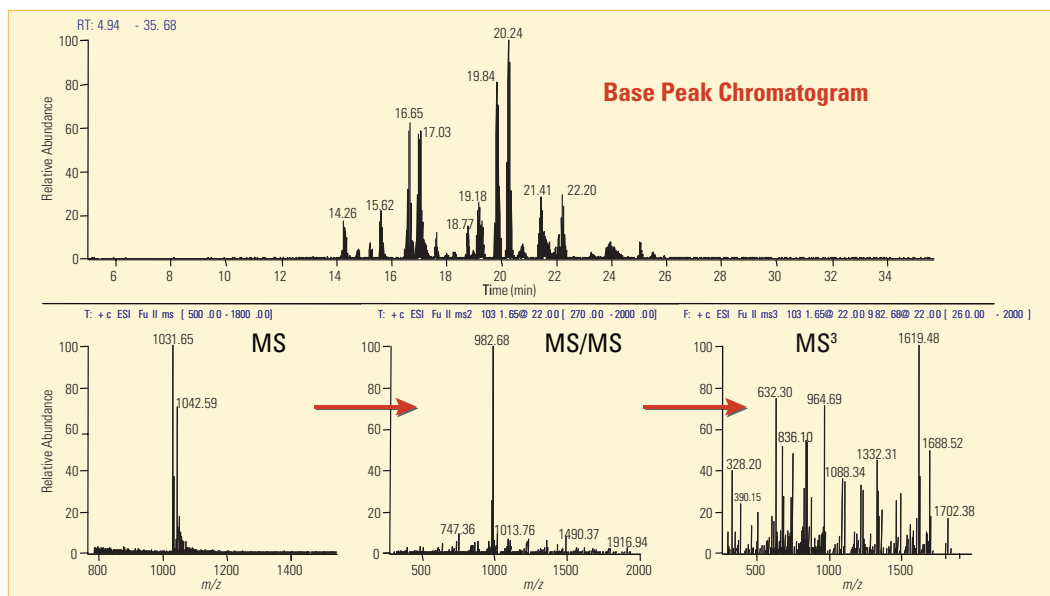


Figure 2: LC-MS/MS analysis with Data Dependent Neutral Loss MS³ scanning of a mixture of alpha and beta casein digest using the LTQ.

The Data Dependent Neutral Loss functionality is available on all LTQ series and Thermo Scientific LCQ Deca XP Series ion trap mass spectrometers with Thermo Scientific Xcalibur 1.4 and above software versions.