

# Using Multistage Activation in an Ion Trap Mass Spectrometer

PSB 122

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Tandem mass spectrometry has become a valuable tool for identification and precise localization of post-translational modifications (PTMs). The preferred approach for analyzing samples using mass spectrometry is to produce structurally significant product ions using the process of ion dissociation. A method commonly known as Data Dependent<sup>TM</sup> Neutral Loss MS<sup>3</sup> (DDNLMS<sup>3</sup>) analysis enables selective fragmentation by isolating a neutral loss ion fragment from an MS/MS experiment and then subjecting it to further dissociation. The production of neutral loss ions in MS/MS, however, is almost always accompanied by partial fragmentation of the precursor ion and these diagnostic fragment ions are subsequently lost when the neutral loss ions are isolated for MS<sup>3</sup>. A new strategy, termed multistage activation (or pseudo MS<sup>3</sup>) produces a spectrum that is the combination of MS/MS and MS<sup>3</sup> fragmentation<sup>(1)</sup> and retains the informative fragments from the precursor ion.

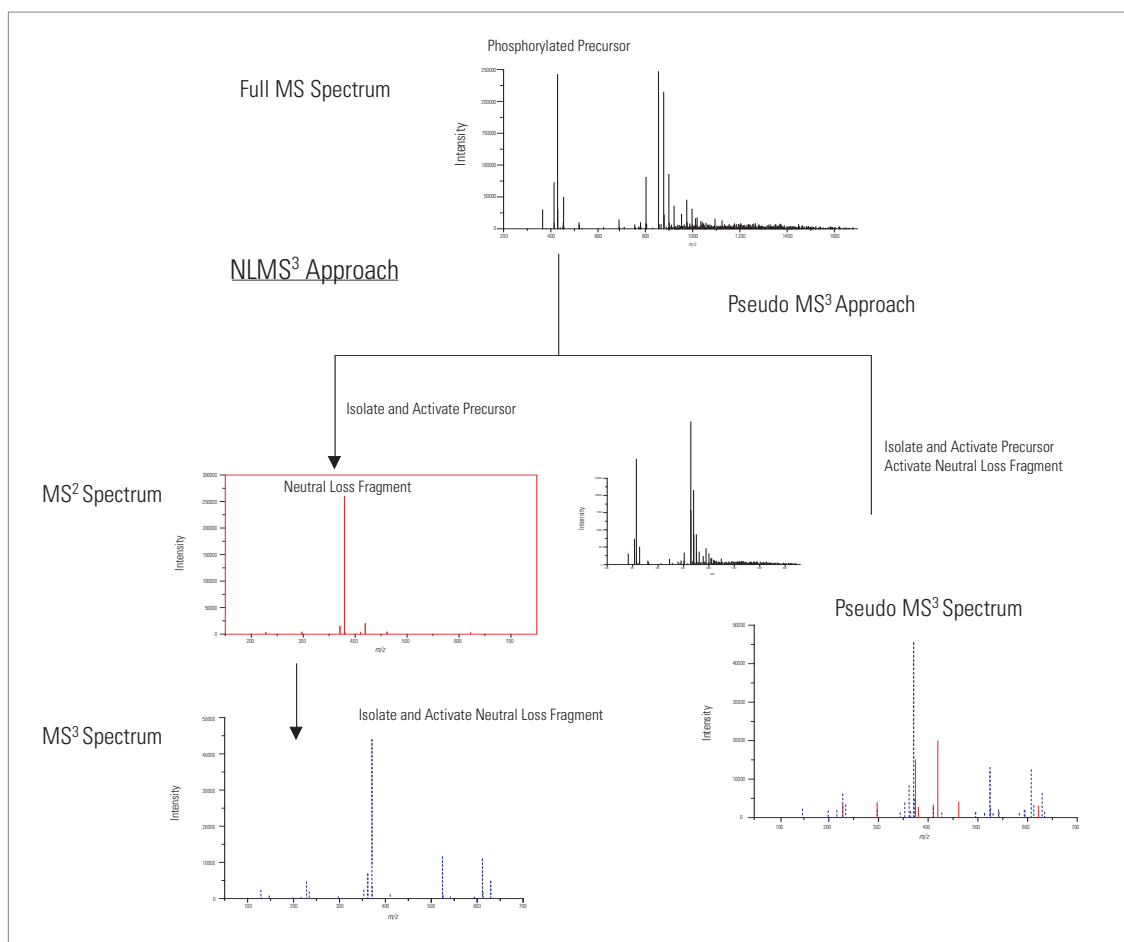


Figure 1: Schematic of the Multistage Activation experiment compared to a traditional MS<sup>3</sup> Neutral Loss experiment

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Multistage activation produces more structurally informative ions by eliminating the ion isolation step between MS/MS and MS<sup>3</sup>. A schematic of the multistage activation process is shown in Figure 1 with comparison to the traditional DDNLMS<sup>3</sup> process. Both processes start with a survey MS scan. The trap is then re-filled for an MS/MS scan of a particular precursor ion. In DDNLMS<sup>3</sup>, the resultant MS/MS spectrum is used to identify the neutral loss fragment. The trap is then refilled and the extra steps of isolating and fragmenting the neutral loss ion are performed to produce the MS<sup>3</sup> spectrum. In multistage activation, the neutral loss ion is collisionally-activated while the fragments from the precursor ion are still present in the trap; there is no second isolation step. The result is a composite spectrum that contains product ions from both the precursor and the neutral loss product. In addition, multistage activation is a faster route to a more information rich spectrum since the trap does not require refilling for the MS<sup>3</sup> scan, as with the traditional neutral loss experiment (DDNLMS<sup>3</sup>).

When compared with DDNLMS<sup>3</sup>, multistage activation generates spectra with increased signal intensity and a greater number of structurally diagnostic ions. An example is shown in Figure 2 where spectra were obtained for a phosphorylated peptide using a) DDNLMS<sup>3</sup> and b) multistage activation. A comparison of the two spectra demonstrates an increase in the signal intensity of the fragment ions from multistage activation compared with the DDNLMS<sup>3</sup> experiment. Further benefits of using multistage activation are demonstrated in a study of synthetic phosphopeptides as shown in Table 1. The information-rich spectra generated using multistage activation are especially important for these compounds because there is often significant loss of sequence informative fragment ions generated in MS/MS. In this study, each phosphopeptide was analyzed at different charge states using both DDNLMS<sup>3</sup> and multistage activation methods. For ten of the eleven cases studied, more ions were identified with multistage activation than with MS/MS or MS<sup>3</sup> in the DDNLMS<sup>3</sup> method. In addition, the signal intensities were generally higher with multistage activation compared to MS/MS or MS<sup>3</sup> of DDNLMS<sup>3</sup> method. Overall, multistage activation resulted in more information for the suite of phosphopeptides studied.

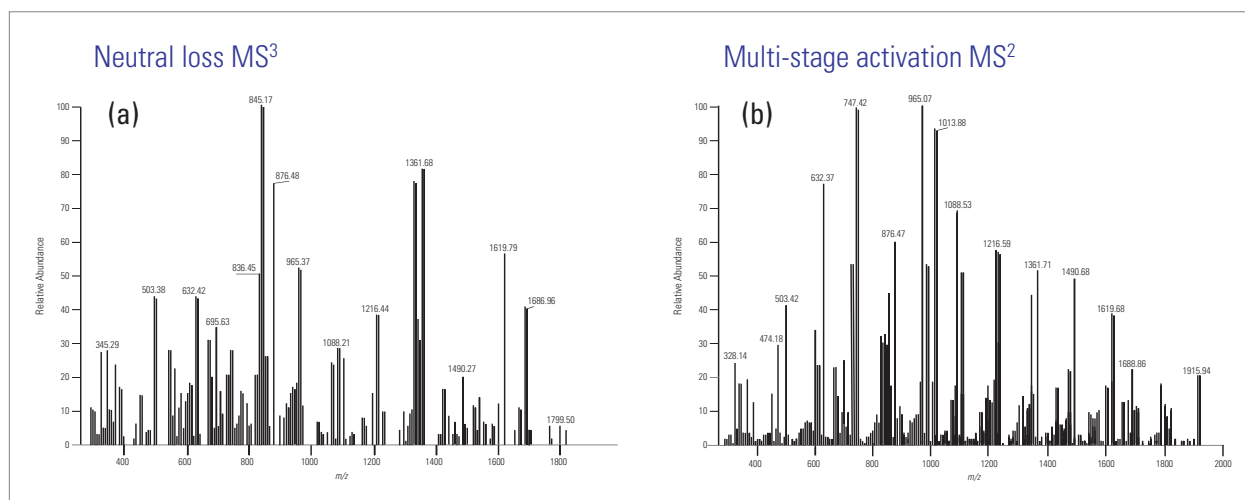


Figure 2a: MS<sup>3</sup> spectrum of a phosphopeptide using DDNLMS<sup>3</sup> NL = 2.2 e<sup>3</sup>  
Figure 2b: Multistage activation spectrum of the same phosphopeptide; NL = 1.4 e<sup>4</sup>