PSB 126

Merging CID and ETD Database Search Results to Increase Confidence in Protein ID

Electron transfer dissociation (ETD) in the Thermo Scientific LTQ XL™ linear ion trap mass spectrometer takes less than 350 ms per experiment, which is fast enough to allow alternating CID and ETD scans for every selected precursor ion in a chromatographic time scale. ETD is an activation method that is complementary to CID. It not only favors multiply charged peptides, but also preserves labile post translational modifications (PTMs). Recent studies indicate that increased identification confidence is obtained using a combined CID and ETD approach for peptide/protein identification.

A typical Data Dependent™ MS/MS experiment in the LTQ XL with ETD can now be set to perform alternating CID and ETD scans. Approximately 2.5 seconds is needed for an experimental cycle of seven scans that includes a survey scan and Data Dependent CID and ETD scans for each of the top three selected ions (Figure 1).

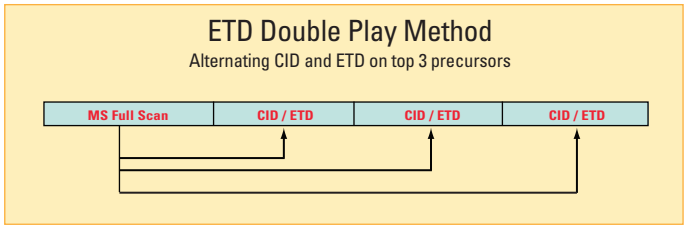

Figure 1. Data Dependent alternating CID and ETD instrument method of LTQ XL.

Raw data files obtained using the Data Dependent alternating CID and ETD instrument method contain adjacent pairs of CID and ETD spectra for each of the precursor ions selected by the routine of the instrument. Usually, a peptide precursor ion is fragmented well by either CID or ETD, or by both of the activation methods, as illustrated in Figure 2.

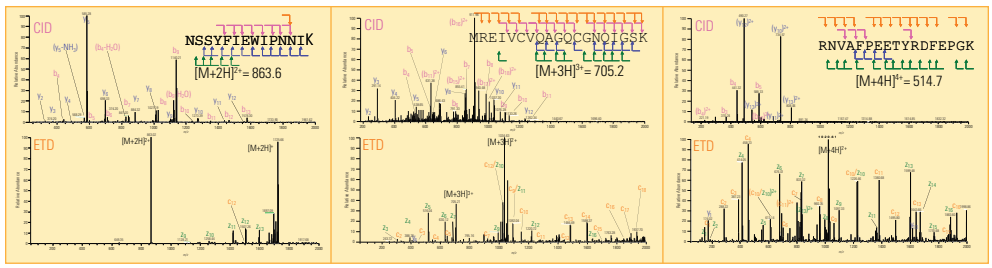

Figure 2. Examples showing that ETD and CID are complementary activation methods: three pairs of CID and ETD spectra from Data Dependent alternating CID and ETD scans. (A): Spectra of a peptide that fragments well with CID, but not with ETD. (B): Spectra of a peptide that fragments well with both CID and ETD. (C): Spectra of a peptide which fragments well with ETD, but not with CID.

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A database search of CID and ETD scans in a single raw file may be done separately using BioWorks™ 3.3.1 by selecting 'Activation Type' on the SEQUEST® search set up view (Figure 3). The results from the separate CID and ETD searches can be merged together in a BioWorks multi-consensus view after the searches are completed (Figure 4).

The merged CID and ETD result displays beneath each identified protein the peptides that are detected by ETD and CID. A peptide can be identified by both methods, such that the result of one dissociation experiment confirms the other. Thus, ETD and CID together increase the confidence of peptide identification by cross-validating peptide identifications. Protein sequence coverage is also increased by this approach, in particular, when some of the peptides can only be confidently identified by one of the activation methods (Figure 4). Search results of ETD and CID scans from a single raw file, as well as from different raw files can be merged using the multi-consensus feature of BioWorks.

After CID and ETD results are loaded, they are merged together and the combined result is displayed in the results table. In the result display, the peptides highlighted in blue are those confidently identified only by CID. The peptides highlighted in orange are those confidently identified only by ETD. The rest of the peptides are identified by both methods. Inside the red circles are examples of the adjacent CID and ETD scans of the same precursor ion. The identification of the same peptide from spectral pairs provides independent validation for peptide identification.

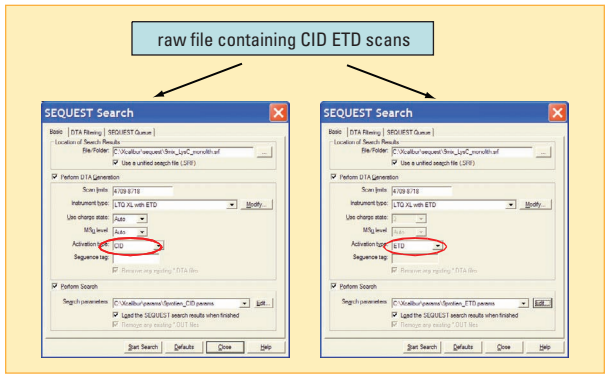

Figure 3. Search CID and ETD scans separately using BioWorks 3.3.1.

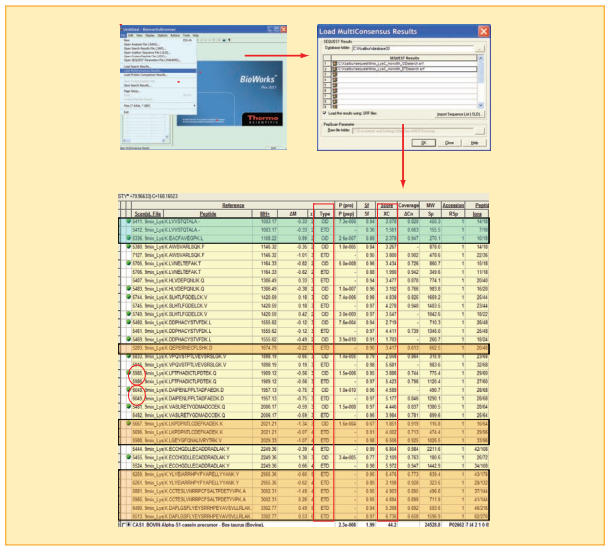

Figure 4. Merge CID and ETD results together using multi-consensus view and evaluate the increased confidence in identification.