Application Note: PEAKS de novo performance on LTQ Orbitrap data

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High resolution, high mass accuracy instruments like Thermo's LTQ Orbitrap, promise to significantly enhance proteomics analysis. De novo sequencing is one of the applications of peptide mass spectrometry that will be most affected by the increase in data quality. Here the authors present the improvement in results obtainable by PEAKS peptide de novo sequencing when using an LTQ Orbitrap mass spectrometer.

Using Orbitrap MS/MS data, PEAKS de novo correctly assigned 97% of the residues in the sample peptides, leading to 8/10 completely correct sequences (the only errors being single transpositions on two peptides). Using LTQ MS/MS data, PEAKS de novo performed admirably, correctly assigning 85% of residues, leading to 3/10 completely correct sequences. Allowing for K being indistinguishable from Q in LTQ MS/MS data, this becomes 5/10 completely correct sequences. Table 1 shows the sequences obtained by each. The results show a significant improvement in the confidence in and quality of de novo sequences obtainable by peptide de novo sequencing using an LTQ Orbitrap mass spectrometer.

It should be noted that while transposition errors are still possible, the high accuracy of the LTQ Orbitrap data eliminates de novo sequencing errors that result from the masses of certain combinations of amino acid residues being very similar in mass. For instance, using an LTQ Orbitrap, it is easy to resolve the difference between MM (262.081 Da) and YV (262.131 Da).

The sample peptides were taken from a bovine lactoglobulin beta. MS2 scans were generated using both an LTQ Orbitrap, and an LTQ. The same 10 peptides were selected from each run, obtaining the correct sequences by PEAKS Protein ID and manual verification. Low quality data were discarded. PEAKS de novo sequencing algorithm (Ma et Al, 2002) was re-tuned to take advantage of the high resolution, mass accuracy and other characteristics of Orbitrap data.

Table 1: Correctness of sequences derived by PEAKS de novo using data from two instruments (Grey letters indicate incorrect residues)

m/z	Correct sequence	-LTQ	-Orbi
458.73856 2	IDALNENK	LDALNENK	LDALNENK
467.2729 2	LIVTQTMK	LLVTKTMK	LLVTQTMK
533.2925 2	VLVLDTDYK	VLVLDESYK	VLVLDTDYK
545.92804 3	TPEVDDEALEKFDK	TPEVDDEALEQVYK	TPEVDDEALEKFDK
597.3386 2	VLVLDTDYKK	VLVLDTDYKK	VLVLDTDYKK
623.2947 2	TPEVDDEALEK	TPEVDDEALEK	TPEVDDEALEK
673.38513 1	GLDIQK	GLDLKK	LGDLQK
771.75635 3	VYVEELKPTPEGDLEILLQK	VYVEELGAVVPEDGLELLLKK	VYVEELKTPPEGDLELLLQK
818.3876 2	TPEVDDEALEKFDK	TPEVDDEALEKVYK	TPEVDDEALEKFDK
1157.1289 2	VYVEELKPTPEGDLEILLQK	VMMEELKPTLLGDLELLLKK	VYVEELKPTPEGDLELLLQK
	Accuracy	85% of residues	97% of residues
	Correct Seq.	3/10	8/10
	Accuracy (K=Q)	89% of residues	97% of residues
	Correct Seq. (K=Q)	5/10	8/10

References

Bin Ma, Kaizhong Zhang, Christopher Hendrie, Chengzhi Liang, Ming Li, Amanda Doherty-Kirby, Gilles Lajoie. **PEAKS: Powerful Software for Peptide De Novo Sequencing by MS/MS.** Rapid Communications in Mass Spectrometry, 17(20):2337-2342. 2003. Early version appeared in 50th ASMS Conference 2002.