

## A Novel Protein Quantitation Algorithm within Protein Prospector

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We have added a new feature to the SearchCompare program within the ProteinProspector package which facilitates accurate and automatic large scale protein identification and quantitation based on the use of stable isotopes. To achieve accurate quantitation we have developed an algorithm for carrying this out on the raw data sets generated by a set of experiments (eg LC-MS/MS runs) on a given sample at a given set of experimental conditions. Currently supported instruments are the ABI QSTAR and the Proteomics Platform 4700 TOFTOF instruments. The algorithm is general purpose and we have currently applied it to several methods such as ICAT, BEMAD, SILAC and chemical exchange of <sup>18</sup>O.

SearchCompare identifies proteins based on MS/MS peptide matches (Poster ThPA 004) and then selects peptide pairs containing the light and heavy isotope tags and uses the MS raw data to carry out the quantitation. Although this is designed for completely automated operation, it also provides links to both the MS and MS/MS data so that a manual validation can be carried out. This is useful to detect problems such as peak saturation, low intensity peaks, peak contamination by a second molecular ion, incomplete <sup>13</sup>C incorporation during reagent synthesis, incorrect peptide assignment, etc.

We evaluated this software on an ICAT analysis of proteins associated with Dent's disease that was previously carried out manually. Samples from patients and healthy controls were extracted from urine using precipitation and SCX chromatography, labeled with ICAT, mixed, digested and analyzed by LC-MS/MS. Quantitation was carried out on more than 50 identified proteins, many of which showed substantial differences in their isotope ratios, with several examples having ratios > 20. A comparison of ESI-QSTAR and MALDI-TOFTOF data indicated that both instruments identified the same proteins as up or down regulated. Fig.1 shows a report generated by the software showing the number of peptides found for three different patients and five different proteins from the experiment. The first three columns are for the QSTAR and the latter three for the 4700 TOFTOF. Fig. 2 is a report showing the heavy/light quantitation ratios for five peptides from Human CD59 glycoprotein for the three different patients. The report also has a time column that enables the peptide to be located in the data set and a column recording the intensities of the light and heavy peaks. The time column records the plate number, spot number, run number and MS/MS spectrum number when used with the TOFTOF and the LC run number and retention time when used with the QSTAR. There are links from the peptide report to subsequent reports showing the MS parent data and quantitation calculation (Fig. 3) and the MS/MS data (Fig 4). From the MS parent report it is possible for example to see that the ratio of 0.239 for Patient AB is incorrect because the light peak is saturated.

Patient AB QSTAR	Patient HSK QSTAR	Patient KD QSTAR	Patient AB TOFTOF	Patient HSK TOFTOF	Patient KD TOFTOF	Protein Name
14	12	11	14	12	12	Uromodulin
6	6	8	10	8	10	IGFBP-7
69	59	60	68	68	63	Albumin
4	1	3	5	1	5	Zn-alpha-2-glycoprotein
13	12	15	16	14	14	Alpha-1 microglycoprotein

**Fig. 1** Protein comparison report.

Peptide	Patient AB				Patient HSK				Patient KD			
	Time	Int 1	Int 2	H/L	Time	Int 1	Int 2	H/L	Time	Int 1	Int 2	H/L
FEHCNFNDVTTR	1-38-2-7	24329	1787	<b>0.066</b>	4-42-2-7	64887	6326	<b>0.088</b>	3-28-2-5	73375	4403	<b>0.054</b>
LRENELTYCCK	1-49-2-11	96262	28108	<b>0.239</b>	4-53-2-7	51324	3140	<b>0.050</b>	4-38-2-5	77294	8949	<b>0.095</b>
ENELTYCCK	1-42-2-2	6267	491	<b>0.064</b>	2-57-2-7	66303	3545	<b>0.044</b>	1-35-2-8	59316	1992	<b>0.027</b>
FEHC(IcatC13)NFNDVTTR	4-31-2-3	31823	6799	<b>0.193</b>								
LRENELTYCCKK									3-33-2-2	2640	204	<b>0.063</b>

Fig. 2 Peptide comparison report.

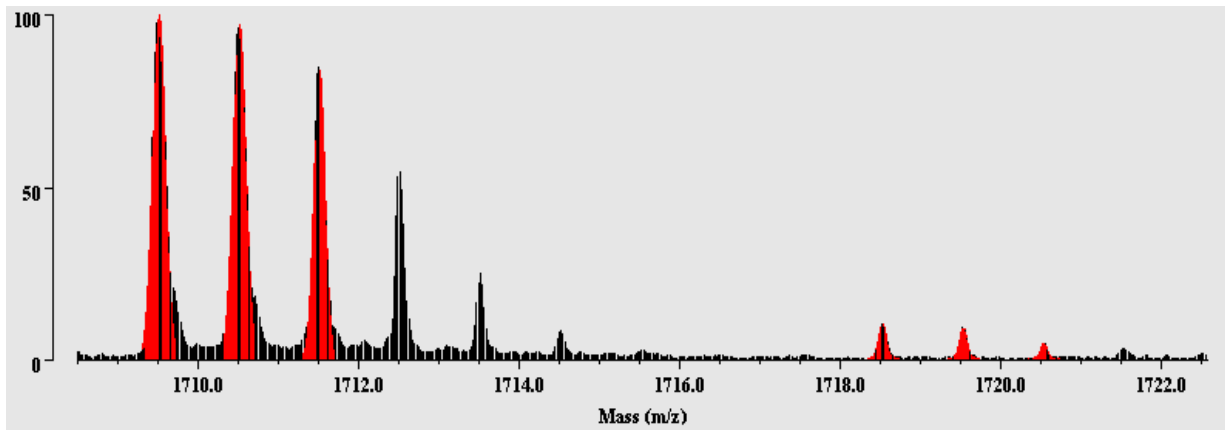


Fig. 3 Quantitation report

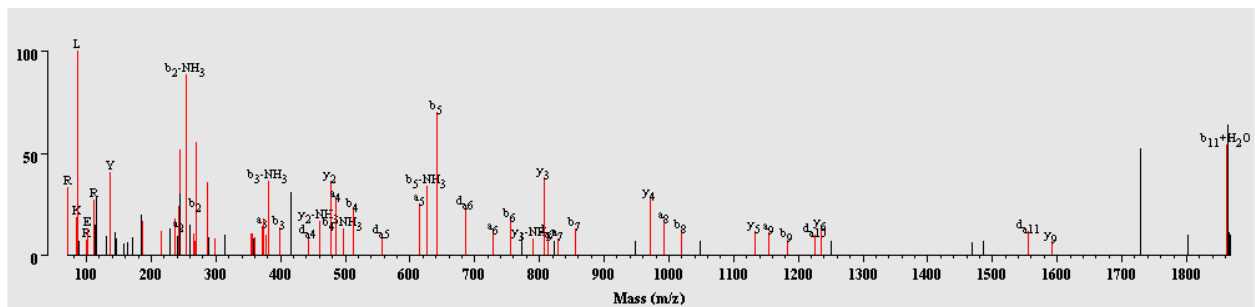


Fig. 4 MS/MS Peptide Report

This software is currently available for in-house use only but we anticipate that it will become a standard component within the ProteinProspector package. However, it is designed for the analysis of large datasets which may render it unsuitable for inclusion in our web-based package.

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