Performance Characteristics of a New Hybrid Triple Quadrupole Time-of-Flight Tandem Mass Spectrometer

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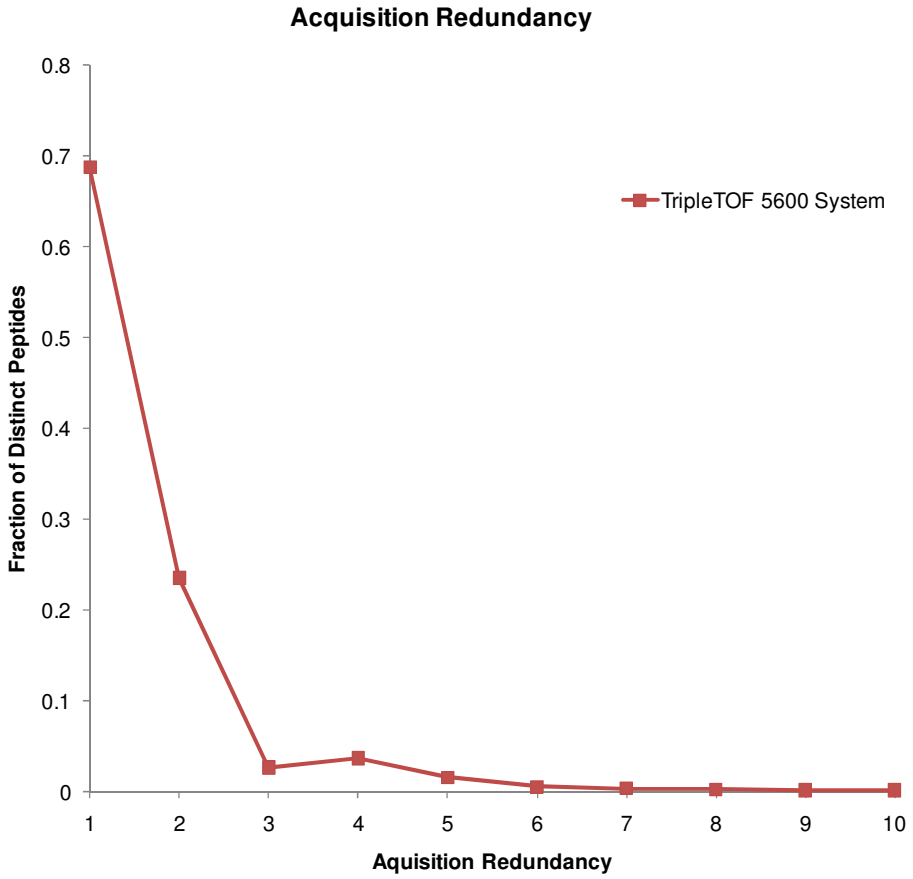

Figure S-1 - Assessing the acquisition redundancy of the TripleTOF which identified 9771 peptides at 1% FDR for a single injection. The TripleTOF is set to select ions approximately twice (see **Experimental**). However, this is not true for the majority of the time with this particular sample and parameters, and may be due to one of the following: (1) peak width could be shorter than the exclusion time of 8 s and then the precursor signal fell short of the threshold; (2) charge state could not be determined after the first round of MS/MS, thus would not be triggered again; or (3) there were 8, 20, or 50 more precursors that were of higher signal, thus those were prioritized over the precursor in question, and not picked up a second time.

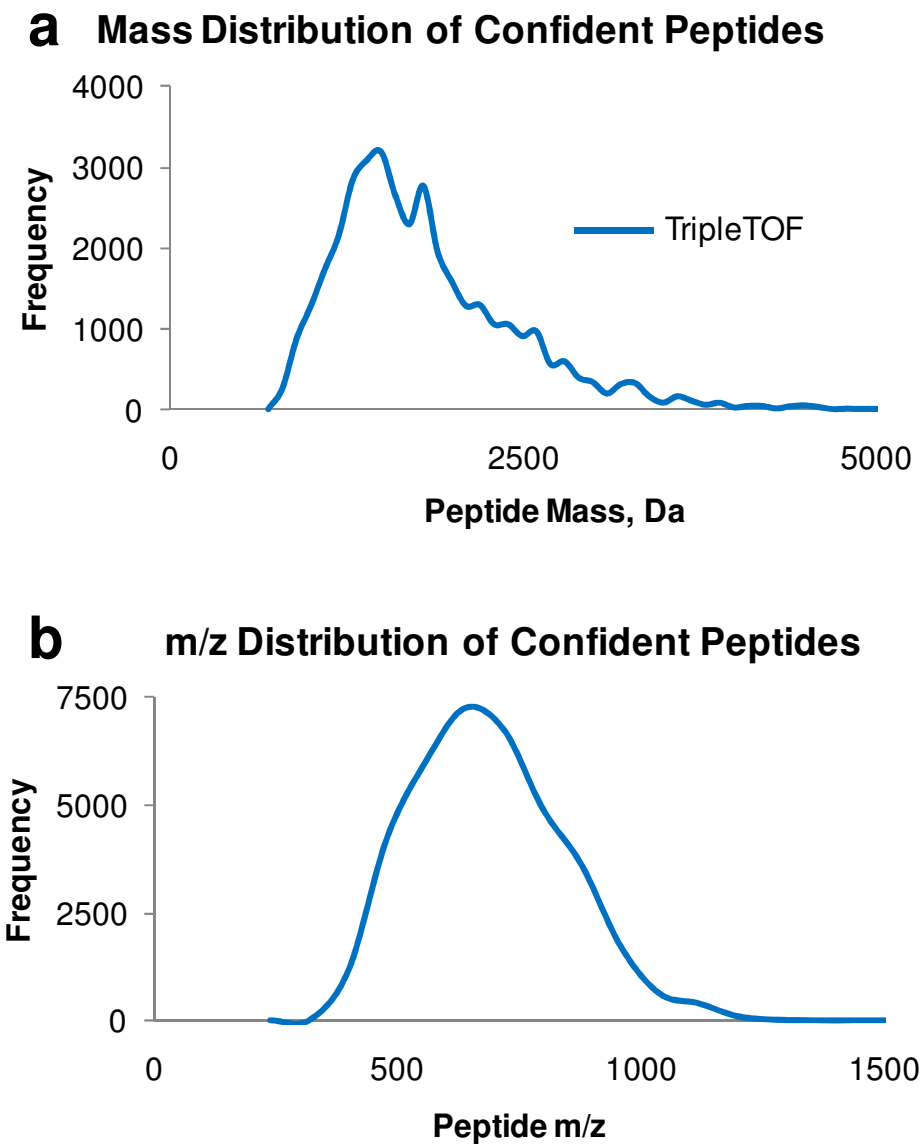

Figure S-2 - Assessing the dynamic range of mass and *m/z* of triplicate analysis. The mass distribution (**a**) and *m/z* distribution (**b**) are evaluated by the frequency of the measurement.

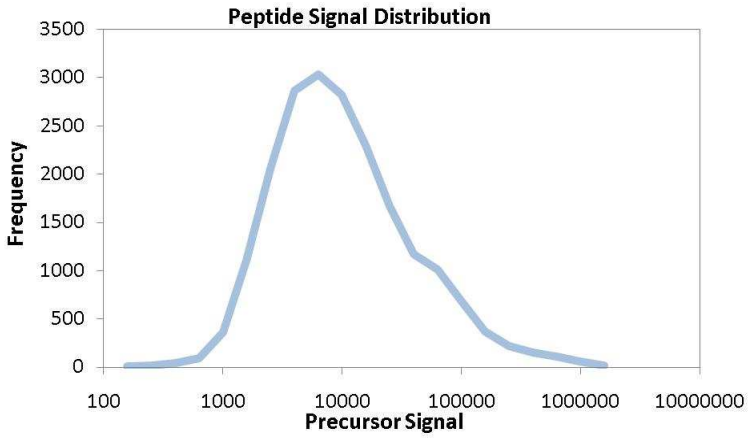

Figure S-3 – Demonstration of the dynamic range of the precursor ion. Frequency is plotted as a function of the dynamic range (signal intensity) of the precursor signal. Here, precursor ions afforded signal intensity from approximately 500 to over 1,000,000 which is almost 4 orders of magnitude.

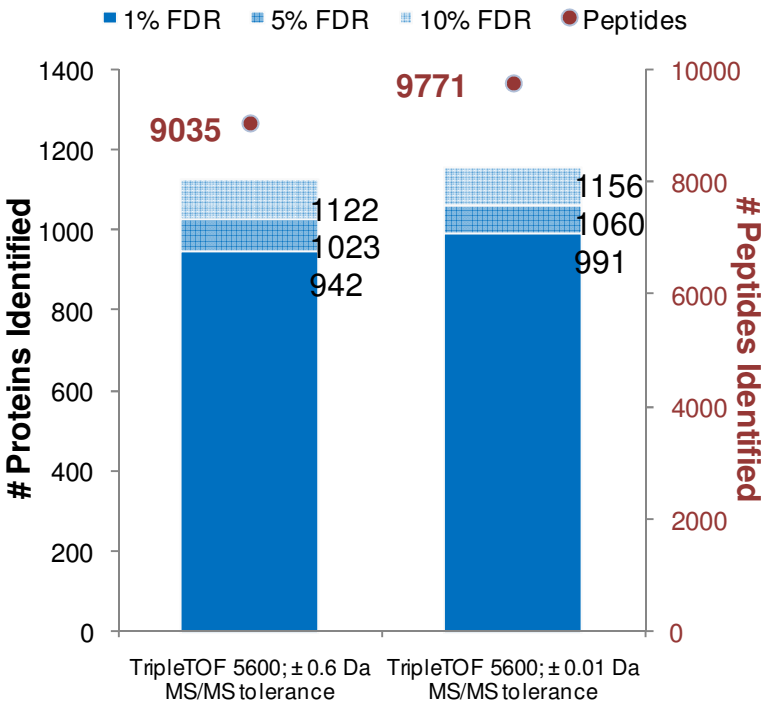

Figure S-4 - The influence of the MS/MS tolerance window on the TripleTOF platform, indication of number of proteins identified at three different FDR maximums, and the number of peptides identified at 1% FDR for each search condition of a single nLC-MS run. The conservative improvement in identified proteins as a function of decreasing the MS/MS tolerance window demonstrates that the high RP of the fragment ions is not the key figure of merit in protein identification by the TripleTOF; the speed on the instrument platform key Concerning FDR, similar trends are exhibited at either tolerance window suggesting that a similar numbers of decoy species relative to the number of proteins at 1% FDR are identified and the fragment ion high-mass accuracy does not exclusively contribute to greater proteome coverage.

Top Modifications
Carboxamidomethyl (C)
Deamidation
Oxidation (M)
PyroGlutamic Acid (Q)
Peptide N-terminal Formyl
Methyl Ester (D,E)
O-Phosphoryl (S,T,Y)
Dehydration (D,E)
Kynurenin (W)
Formylkynurenin (W)
Protein N-Terminal Acetyl
Peptide N-Terminal Acetyl
Nitro (Y)
Methyl (K)
Carbamyl (K)

Table S-1 - The Paragon protein searching algorithm searches common peptide and protein modifications described here as well as others detailed in Shilov *et al.*