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A Data Independent Approach Towards the Qualitative and Quantitative Profiling of Complex Protein Mixtures

Johannes P.C. Vissers Waters

Corporation, MS Technologies Center, Manchester, UK

Mass spectrometry is widely accepted as an essential tool to better understand protein function, facilitating both the identification and quantification of proteins in complex samples. A mass spectrometry based protein identification strategy has previously been described that facilitates the simultaneous acquisition of qualitative and quantitative information in a data independent fashion [1]. This method alternates the energy applied to the collision cell of a mass spectrometer between a low and elevated energy state without applying precursor ion selection. The low energy portion of the data contains the intact molecular ions of the peptides and is typically used for quantification. The elevated energy portion comprises structurally informative, accurate mass fragment ion information and can be combinedly utilized with the low energy data for qualitative, identification purposes. More recently, this approach has been extended to generate estimated amounts for proteins contained in biological systems, allowing precise relative quantification to be performed [2].

The general challenges faced when analyzing complex protein mixtures and the validation of the associated search results will be discussed in detail [3]. The specificity of both qualitative and quantitative analysis is in general lost when chimeric peptides are considered and the effect that they have on the experiment design, and outcome, is not acknowledged during either the acquisition or searching of the data. It will be demonstrated through the use of experimental data and modeling studies that the highest peak capacity afforded to detect, separate and correlate all precursor and product ions by an analytical system is desired. The latter is however not only being offered by separation in time, mobility and mass but also through the use of theoretical models of (sub) proteomes, considering complexity, dynamic range and the inherent physiochemical properties of tryptic peptides in both the solution and gas phase [4].

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