

Repeats versus Mass-windows – optimization strategies for Mudpit type experiments to increase proteome coverage

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Introduction

For the separation and visualization of complex protein mixtures two dimensional gel electrophoresis combined with MALDI MS is a well established technique. However, some of the limitations associated with the 2D/MS approach (e.g. difficulties in the handling of membrane proteins) have prompted the development of gelfree strategies. In the so-called shot gun approaches, the proteins are usually enzymatically digested. The analysis of the resulting digested protein mixture has high demands on the separation system (SCX, RP HPLC) and the data acquiring system (MS and MS/MS) [1], [2]. In this poster we discuss the use of mass windows in repeated injections for the MS system which influence the performance of a MUDPIT type analysis.

Methods

A tryptic yeast digest separated manually on a SCX column into 9 fractions, was used for analysis. All the MS measurement were done on a Q-ToF 2 Instrument equipped with a Cap LC HPLC system. The SCX fractions were trapped onto a 0,3 mm i.d. x 0,5 mm precolumn and eluted into a 75 µm i.d. RP18 column. To enable a long time stable electrospray analysis a nanospray needle (New Objective) is used. The Q-ToF 2 settings were optimized with regard to scan times and precursor selection. All MS/MS data were processed with SEQUEST and analyzed according to well established criteria (Xcorr , delta Cn).

Results

The impact of different MS/MS analysis strategies on the total number of identified proteins is discussed and demonstrated: Repeated sample injection within a MUDPIT experiment: - There is a high reproducibility for the number of identified proteins of each run (245 to 266 proteins out of the same SCX fraction). - In each run of a series of repeats new proteins can be identified. - The number of overall identified proteins behaves like a saturation curve, 438 proteins were identified.

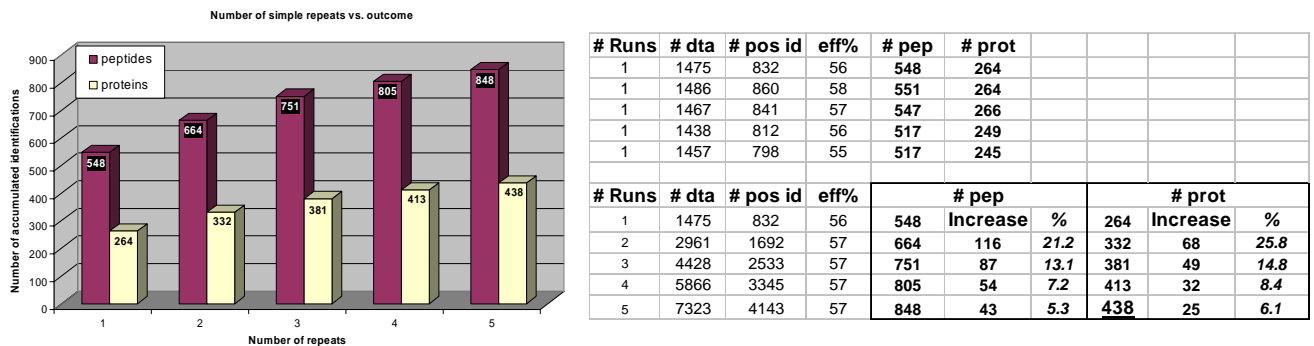


Figure 1: Chart and table for the result of repeated injections of the sample.

Repeated sample injection analyzing fixed and slightly overlapping mass windows within the MUDPIT experiment: - In comparison to simple repeats the number of identified proteins by using mass windows is higher. - There is a sample and protease dependent inhomogeneous distribution of the peptid ions over the whole m/z range with a main focus in the m/z region 550 to 1000. - Adapting the mass windows to the peptide distribution results in a high overall number of 572 identified proteins for 5 replicate injections out of one SCX fraction.

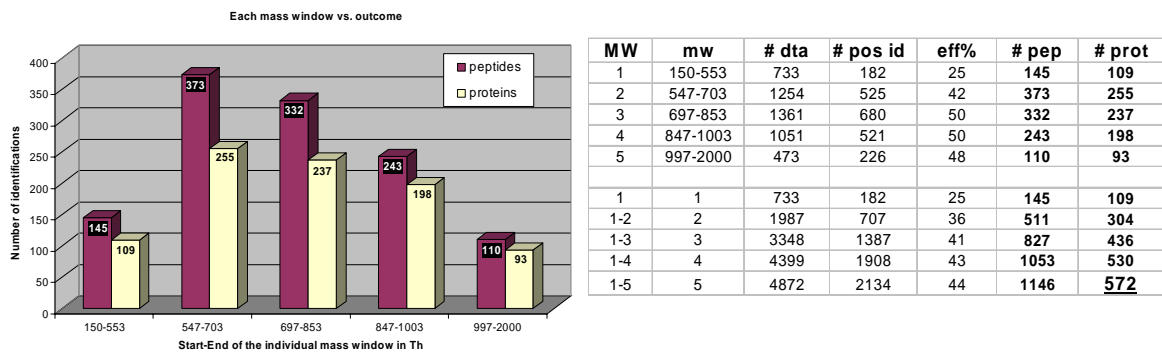


Figure 2: Chart and table for the result of the usage of mass windows for the analysis.

Repeats of a selected mass window show a similar saturation behavior for the identification number. The usage of mass windows reduces the frequency a peptide ion is identified without the use of exclude lists.

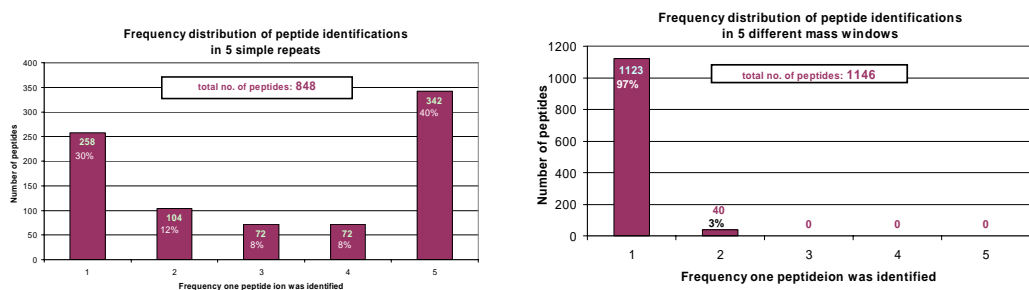


Figure 3: Identification frequency distribution chart for simple repeats and the use of 5 mass windows as described.

Conclusions & Perspectives

- Repeats using different mass windows results in a higher number of identified proteins than simple repeats.
- Repeats of one mass window show a saturation behavior for the number of identified proteins like simple repeats.
- The use of mass windows reduces the redundancy.
- Mass windows allow to fragment more precursors than in simple repeats and enables a deeper look into a proteome.
- Combination of mass windows and repeats can be used together for high identification numbers.

References

- [1] Washburn, M.P., D. Wolters, et al. "Large-scale analysis of the yeast proteome by multidimensional protein identification technology" *Nature Biotechnology* **2001**, 19 (3): 242-247
- [2] Washburn, M.P., D. Wolters, et al. "An automated multidimensional protein identification technology for shotgun proteomics" *Anal. Chem.* **2001**, 73 (23): 5683-90