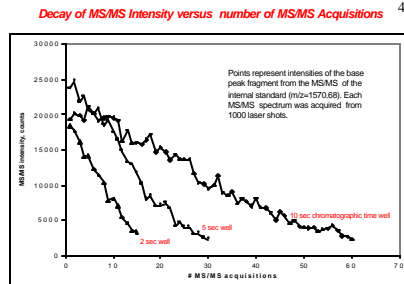


A New Algorithm for Selection of MS/MS Precursors in LC-MALDI TOF-TOF Analysis of Complex Proteomic Mixtures

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Introduction

The need to optimize selection of precursor ions for MS/MS analysis is a result of the complexity of biological samples. For LC-MS/MS of complex peptide mixtures, the number of coeluting peptides can be much higher than the maximum number of MS/MS spectra that can be acquired from a given section of the chromatogram. The fundamental advantage of the off-line LC-MALDI approach is the possibility to select precursor ions for MS/MS analysis based on an examination of the complete LC-MS data set. Typically the distribution of the peptide peak maxima along the chromatogram is not uniform. Our algorithm (PRESEL – precursor selector) enables a redistribution of positions for MS/MS acquisition on the MALDI plate in order to maximize the number of identified peptides.

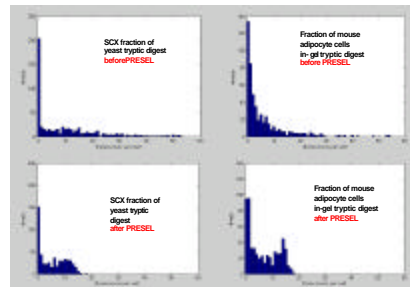


Maximum number of MS/MS acquisitions from one well is limited by the amount of material in the well.

Algorithm

PRESEL takes into account two types of peptide overlap: spatial (peptide chromatographic maxima occur at the same position on the MALDI plate) and spatial-spectral, where peaks overlap in both the chromatographic time and m/z domains. The problem with the spatial overlap is that the number of MS/MS acquisitions is limited by the amount of material in one well (spot). The spatial-spectral overlap is a result of a relatively broad MS/MS window for precursor ion selection, e.g. ± 5 Da or ± 3 Da for the present TOF-TOF instruments. MS/MS spectra for spatially-spectrally overlapping peaks (typically 10-15% of precursors for a complex mixture) can include fragments from several precursor ions and can consequently lead to false positive or negative identifications. While selecting precursors and redistributing positions for MS/MS acquisition, PRESEL minimizes the influence of both types of overlap.

Precursor Distribution on MALDI Plate Before and After PRESEL



After PRESEL: less empty wells, less highly populated wells, precursor distribution is more uniform.

Results

PRESEL was used for selection of precursors for MS/MS analysis (AB 4700 TOF-TOF) following denoising and peak picking by MEND (matched filtration with experimental noise determination) [1] of LC-MALDI/MS of 3 SCX fractions of a tryptic digest of yeast lysate and the fraction of the in-gel digest of mouse adipocyte cells. The LC system was coupled to MALDI MS using an off-line interface allowing both continuous and discrete droplet deposition. MEND typically picked about 3,000-4,000 candidates for MS/MS analysis out of which only about 1,500-2,000 could be acquired without redistribution, PRESEL increased the number of MS/MS spectra that could be acquired from the plate by 10-15%.

The likelihood of peptide identification from MS/MS analysis was shown to be correlated with a number of parameters: precursor intensity, S/N and extent of spectral-spatial overlap. It was found that, for the densely populated wells, the order of precursor submission is important (precursors submitted first have higher chances to produce identifiable MS/MS spectra). Thus, intensity corrected by the order of submission I_{cor} (high intensity first) was introduced.

The dependence of the total number of peptide identifications on the number of points (MS/MS acquisition wells) per chromatographic peak was also studied. The same fraction of a yeast digest was analyzed repeatedly by depositing droplets corresponding to 2, 5, 10 and 20 seconds of chromatographic time (15, 6, 3 and 1.5 points per peak). The ability to redistribute precursors was found to be higher for the greater number of points per peak. The number of identified peptides was about two times higher for 5 sec droplets relative to 20 sec droplets.

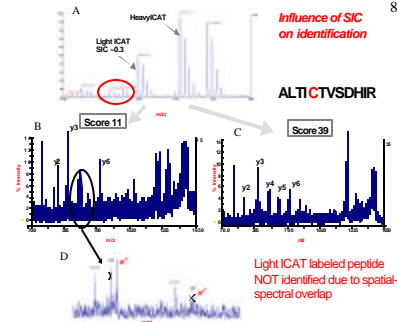
Spatial-Spectral Overlap Presence of Several Peaks within MS/MS Window

$$SIC = \sum_{n=1}^N I_n / I_0$$

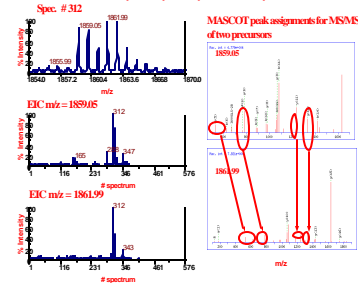
I_0 - intensity of precursor ion peak
 I_n - intensities of other MS peaks within MS/MS precursor window
 N - number of MS peaks within precursor window (not including precursor and its isotopes)

Spectral Interference Coefficient (SIC) estimated and used as a criterion for precursor selection

MS/MS window :	± 5 Da	± 3 Da	± 1 Da
3 SCX fract. avg. (SIC>1)	10.5%	6.7%	1.4%
3 SCX fract. avg. (SIC>0.3)	29.3%	24.5%	7.7%

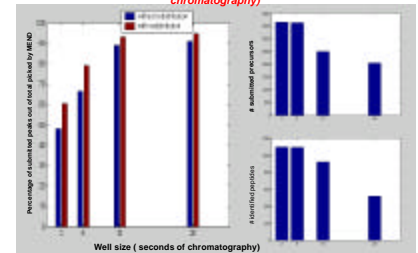


Example of Spatial-Spectral Overlap

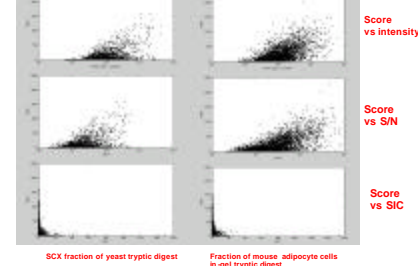


Fragments from one peptide (circled) prevent identification of another peptide

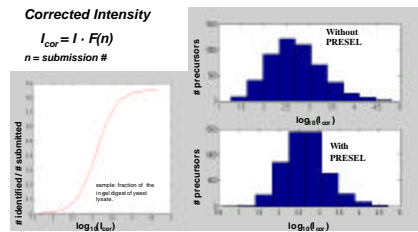
Increase (due to PRESEL) in the Percentage and Number of Precursors Submitted and Identified by MS/MS versus the Size of the Well (sec of chromatography)



Factors Influencing the Likelihood of Identification



Submission Order and Redistribution Are Important



PRESEL increases the number of precursors with intermediate I_{cor} values and thus increases # of IDs. Number of IDs with PRESEL is 25% higher than without PRESEL (preliminary results).

Conclusions

- Main factors influencing the likelihood of identification are: corrected intensity, S/N, SIC (spectral interference coefficient).
- PRESEL maximizes the likelihood of identification by redistributing positions for MS/MS acquisition in order to maximize corrected intensity and minimize SIC.
- PRESEL can be used with different strategies: e.g. preferential selection of high or low abundance peptides, exclusion of precursors identified during previous runs, combining identifications from several runs (see poster # 442 on multiplexing).

Reference
1. Andreev V. P.; Rejtar T.; Chen H.-S.; Moskovets E. V.; Ivanov A. R.; Karger B. L. Anal. Chem. 2003; 75: 6314-6326