

LC MALDI with an Ion Trap - Orbitrap Hybrid Instrumentation: Decoupled Method Setup

Kerstin Strupat¹; Huy Bui²; Rosa Viner²; Justin Blethrow²; Yue Xuan¹; Viatcheslav V. Kovtoun²; George Stafford²

¹Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany; ²Thermo Fisher Scientific, San Jose, CA, USA



Overview

Purpose: Perform MS/MS data dependent decision on the apex of a given LC peak by de-coupling of LTQ Orbitrap survey full scans and MS/MS scan information in LC MALDI runs.

Methods: First, FT full scans are acquired from the LC MALDI plate. The raw file is used in an in-house software to create data dependent methods which include mass lists with monoisotopic m/z ratios, and locations (retention time, RT) where the individual peptide has shown a maximum output. The data are acquired to obtain a second, dependent raw files. This 2nd raw file contains MS/MS decisions only and is submitted to Proteome Discoverer. The results are compared with conventional data dependent, double play with data dependent decisions on top 50 peaks.

Results: More peptides are identified in a shorter amount of time. MS/MS quality is maximized by being performed only once on top of the LC peak.

Introduction

LC-MS/MS has become the method of choice for analysis of complex proteomic samples. In a typical protein sample digest, there may easily be thousands of different peptides and many of these peptides may co-elute to in an individual LC peak. When using LC directly coupled to ESI, not all peptides may be analyzed by the MS/MS experiments based on data dependent decisions when you have co-eluting peaks due to under sampling. By using the combined LC MALDI technique, this limitation can be overcome because the LC eluent is frozen in time on the MALDI plate and this provides the potential to perform MS/MS experiments on many more precursors.

Methods

Autosampler, LC and LC MALDI Spotter: A Thermo Scientific MicroAS Autosampler, a Thermo Scientific Surveyor MS Pump Plus and a SunChrom MALDI Spotter SunCollect (Friedrichsdorf, Germany; www.thermo.com) are used for providing the nano LC MALDI samples. The sample is injected via a pre-column into the 75 μ m nano LC column. LC pump flow is split; the final nano LC flow is 300 nl / min. The sample is mixed with matrix solution via a T-junction. Here, 2 parts of a diluted MALDI matrix solution is mixed with 1 part of the sample eluting from the column. Matrix: CHCA matrix is used for the experiments carried out. For this purpose a saturated matrix solution in acetone containing 3% water (containing 0.1% TFA) is prepared. The saturated stock solution is centrifuged and the supernatant is diluted using 1 part of the saturated matrix and 9 parts of a solvent mixture (6 parts ethanol, 3 parts acetone, 1 part water containing 0.1% TFA).⁽¹⁾
Spotting: The samples are fractionated into 20 sec or 30 sec "wide" spots; in particular, 100 nl or 150 nl of sample is mixed with 200 nl or 300 nl of matrix solution upon spotting onto the MALDI sample plate. Samples dry rapidly in a smooth stream of air in the MALDI spotter chassis. Samples are spotted onto disposable plates (see e.g. Figure 1)
Samples: An equimolar six bovine protein containing enzymatic digest (Michrom Bioresources, www.michrom.com) is used for the analyses. A stock solution – 200 fmol / μ l of each peptide is prepared using water 0.1% TFA. A 20 fmol / μ l solution is placed in the autosampler. A 3 μ l volume is injected onto the analytical column.

Mass Spectrometer: The ion trap – Orbitrap™ hybrid mass spectrometer equipped with a MALDI source is described in detail.⁽²⁾ Briefly, the basis of the instrumentation is an LTQ Orbitrap XL. The heated capillary, and the tube lens used in ESI mode are exchanged versus a quadrupole (q00) which is hold on a constant (low) pressure at 75 mtorr. For an analytical scan MALDI produced ions are collected and cooled in the quadrupole and sent as packages toward the ion trap or Orbitrap mass analyzer according to previously determined AGC conditions. Ions are produced with a nitrogen laser (337 nm wavelength, 3 ns pulse duration, 60 Hz repetition rate). The instrument is capable of acquiring 1 e6 charges per Orbitrap FTMS full scan using automatic gain control. To acquire this high number of charges, the laser is typically pulsed a few to some ten laser shots only.
Software: We have a developed software that creates precursor ion lists from previously acquired Orbitrap FT full scan raw data files. MS/MS decisions are made in a second raw file on top of the individual LC peaks using such a precursor ion mass list. Our approach decouples high mass accuracy, high mass resolution data (better than 3 ppm mass accuracy, mass resolution > 60k for a precursor @ m/z 1900 (for a typical enzymatic peptide choosing 100k @ m/z 400 settings) acquired by full scan Orbitrap detection from MS/MS data dependent scans acquired in a second raw file. Extracted ion chromatograms of various m/z ratios of the accurate mass full scan data are used to locate the sample spots (retention time) where the intensity of an individual precursor ion is maximized. The extracted ion chromatograms and retention times of all LC peaks are written into a subsequent method which is applied to the LC MALDI plate. Using data dependent decisions applied to the precursor ion list allow to acquire data from top of the LC peaks to give MS/MS data of the highest possible quality.

FIGURE 1. Workflow for LC MALDI applications. A raw file consisting of FTMS full survey scans (randomly taken over the sample spot, one survey scan per spot, some few microscans per survey scan per spot) is the basis for a second raw file with MS/MS scans only. The "LC MALDI Data Dependent Generator" creates extracted ion chromatograms and creates an output sequence file with a global MS/MS mass list and the retention time (spot position) at which the peak maximizes.

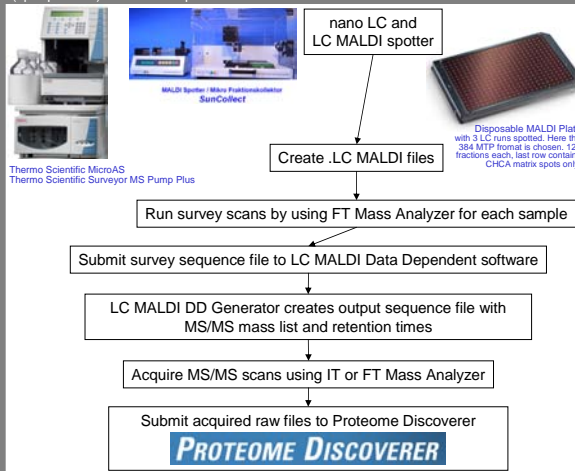


FIGURE 2. LC MALDI position files and Method Setup for a 1D LC or a 2D LC approach. A) Example for 4 LC MALDI runs on a 384 MTP plate. Regular, 1D, (horizontal) retention times or 2D, vertical retention times, can be considered by the software. B) Sequence Setup for FTMS full scan survey scans of the four LC MALDI runs in Xcalibur

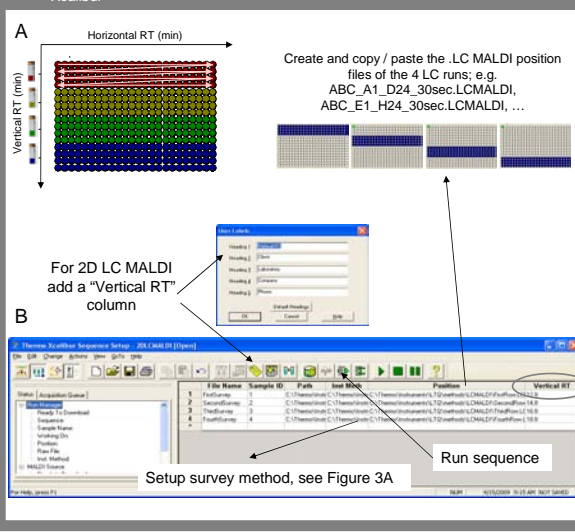


FIGURE 3. Method Setup for a 1D LC or 2D LC approach. A) Method Setup for the FTMS full survey scans. B) LC MALDI Data Dependent Generator User Interface. C) Sequence Setup for the corresponding data dependent 2nd raw files. Details are given on the right hand side of the figures

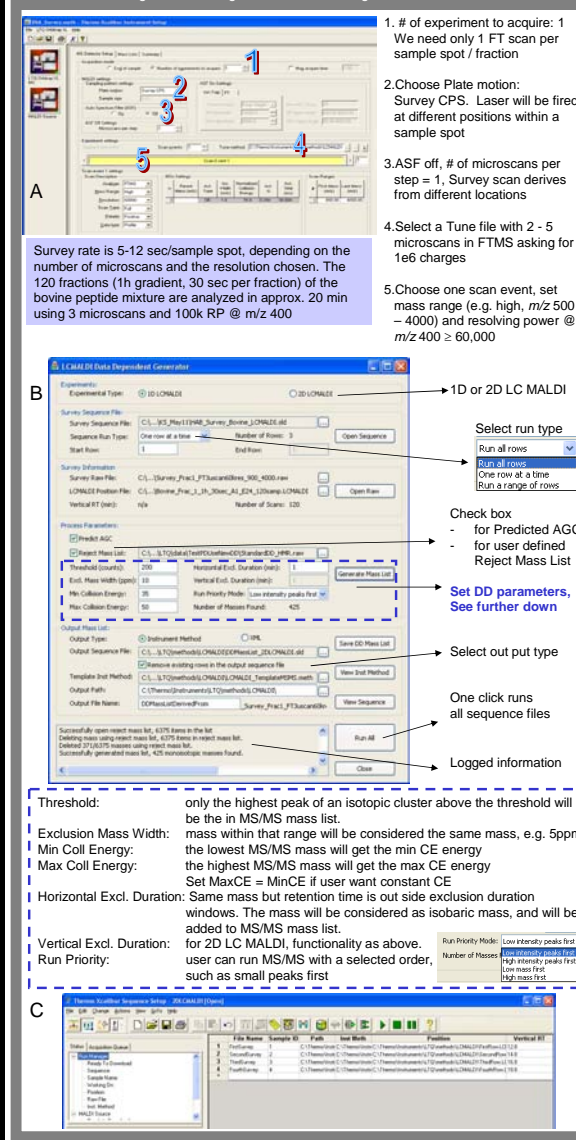


FIGURE 4. Comparison of new Data Dependent Mode versus conventional DD mode. FTMS full scan information, single scan, from which the data dependent decision for ITMS/MS on m/z 1983 is made, see also Figure 5.

A) FTMS survey raw file serves as decision maker for two subsequent raw files of the same LC MALDI run, acquired for the reason of proofing reproducibility. B, C) FTMS master scans from a convention set up in which an FT full scan is directly followed by the DD decision. This is done for the top 50 peak of each LC MALDI fraction. Right hand of each figure displays an inset into the precursor ion of interest @ m/z 1983. Note the difference in absolute intensities.

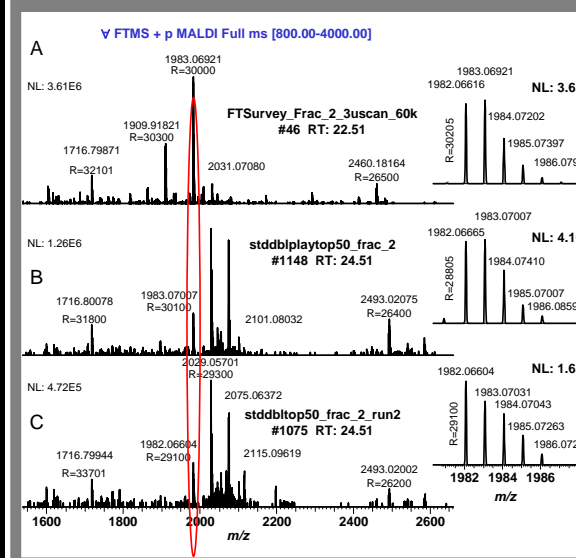


FIGURE 5. Comparison of MS/MS quality of the precursor ion shown in Figure 4. A, B) MS/MS decision on top of the peak elution, new workflow. Less laser shots are needed and spectra quality is better than in C, D) MS/MS decision according to a top 50 method using appropriate LC dynamic exclusion conditions. MS/MS spectra require more laser shots and spectra quality is decreased.

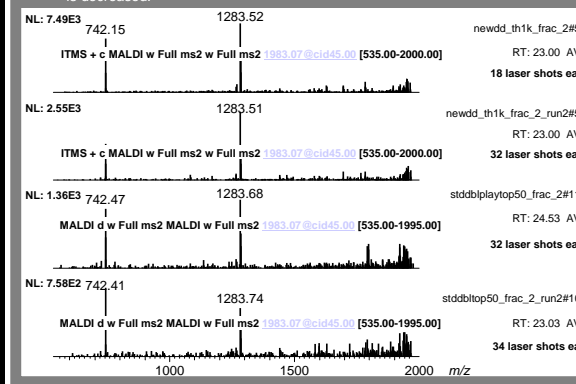
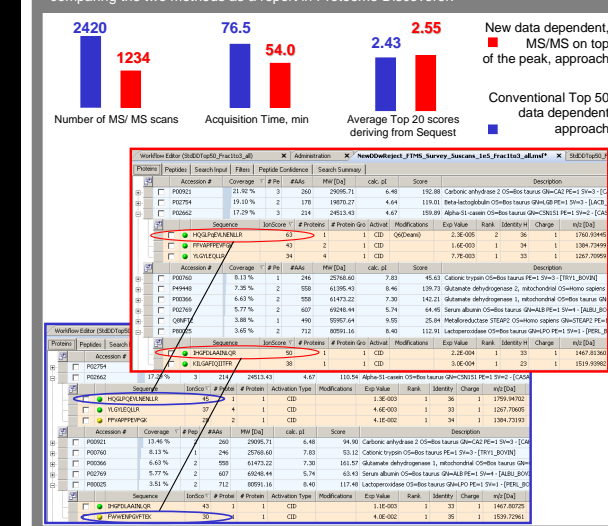


FIGURE 6. Comparison of the number of data dependent MS/MS scans, acquisition time, and average scores of the top 20 identified peptides between conventional DD mode and New DD mode. Also displayed is the Mascot™ Score for various peptides comparing the two methods as a report in Proteome Discoverer.



Conclusions

- Results show that more peptides are identified in a shorter time by using the new workflow for LC MALDI with MALDI LTQ Orbitrap XL instrumentation. The new approach
- Maximizes MS/MS spectral quality by performing the MS/MS scan on the top of the LC peak
 - Increases the number of unique peptide identified
 - Reduces the number of redundant MS/MS by up to 50%
 - Reduces the total acquisition time by 30% (FTMS survey scan + IT MS/MS)
 - Can utilize rejection mass lists directly from ESI-LC MS/MS raw file or user defined lists (e.g. to exclude matrix cluster peaks for the entire run)
 - Peak list obtained by FTMS survey scans can be used in other standalone ion trap based instruments
 - MS/MS only raw files are searchable by Proteome Discoverer

References

- (1) Gorman J., Dave, K. personal communication
- (2) Strupat K., Kovtoun, V., Bui, H., Viner, R.; Stafford, G.; Horning, S.; Journal of the American Society for Mass Spectrometry, available online: 3-MAY-2009, <http://dx.doi.org/10.1016/j.jasms.2009.04.013>

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