

On the Advantage of Denoising and Peak Picking by MEND in LC-MALDI-QqTOF Analysis

Werner Ens¹; Victor Andreev²; Oleg Krokhin¹; Tomas Rejtar²; Hsuan-shen Chen²; Eugene Moskovets²; Kenneth G. Standing¹; Barry L. Karger²

¹ University of Manitoba, Winnipeg, Canada; ² Northeastern University, Boston, MA 02115

The MEND (matched filtration with experimental noise determination) algorithm for denoising of LC-MS data and selecting precursors for MS/MS analysis has been recently demonstrated for LC-MALDI-TOF-TOF analysis [1]. The main advantage of MEND is the ability to minimize chemical noise in LC-MS by exploiting the difference between the patterns of noise and true signal in the chromatographic time domain. In this work, MEND was used in LC-MALDI-QqTOF analysis for minimization of chemical noise and selection of MS/MS precursors, leading to a significant increase in the number of identifications of low abundance peptides.

A single SCX fraction of a tryptic digest of a yeast lysate was separated by RPLC and deposited on the MALDI plate as a series of 400 spots, each corresponding to 5 seconds of LC separation. MS and MS/MS analyses were performed with the Manitoba/Sciex prototype QqTOF mass spectrometer [2]. Denoising of LC-MS data set and selection of precursors for MS/MS analysis were performed with MEND software. An example of efficient denoising of LC-MALDI-QqTOF data is presented in Fig. 1. The acquired MS/MS spectra were submitted for database searching and identification to SONAR (www.proteome.ca).

The goal of the experiment was to determine if MEND could aid in the identification of low abundance peptides in the presence of high abundance peptides. From a total of 2262 precursors selected by MEND, 90 were chosen with the lowest values of S/N (below S/N=10) and submitted to MS/MS analysis. About 80% of these low S/N peaks provided good MS/MS spectra and 23 peptides (26% of those submitted to MS/MS) were identified by database searching with SONAR. Table 1 presents sequences of the above low abundance peptides. In order to evaluate the peak picking ability of MEND, the same LC-MS data was analyzed with the "M/Z" software that is typically used with the LC-MALDI-QqTOF Manitoba/Sciex instrument. The S/N threshold was set to the value 2.5. Nevertheless "M/Z" picked only 25 out of the 90 low S/N peaks. Out of these 25, only 5 were identified by SONAR. Thus, application of denoising and peak picking MEND software enabled a significant (23 versus 5) increase in the number of identified low abundance peptides.

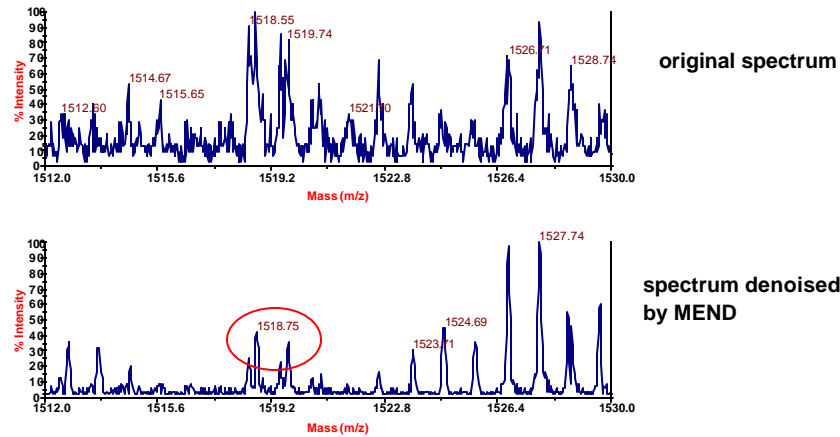
Conclusions:

- MEND minimized both chemical and random noise in LC-MALDI-QqTOF data
- MEND significantly (by a factor of 4.5) increased the number of identified low abundance peptides relative to standard software.

References

1. Andreev V.P., Rejtar T., Chen H.S., Moskovets E. V., Ivanov A.R., Karger B.L. Anal. Chem. 2003, 75: 6314-6326.
2. Loboda A.V., Krutchinsky A.N., Bromirski M., Ens W., Standing K.G. Rapid Commun. Mass Spectrom. 2000, 14:1047-1057.

Low S/N Peak Selected and Identified due to MEND



Chemical noise reduced. Overlapping peaks distinctly separated.
Mass accuracy improved.

As a result :

precursor m/z=1518.75 identified by SONAR with significant score.

Low Abundance Peptides Selected by MEND and Identified by SONAR

m/z	Peptide sequence, identified protein	S/N	"M/Z" software
1684.85	NI VWEYESVVQYR_gi2780955	3.2	-
1518.81	EL SASLNL PAAASEFK_gi6323056	3.7	-
1041.59	NLIQLISLK_gi1586982	4.7	-
1646.79	EYDSNYPEFPI R_gi29420847	5.8	-
1742.76	SGFESEFCIYQVNAK_gi6322126	6.8	+
1156.66	QILDNREI R_gi6319290	7.3	-
1103.62	VOPDAAVYL K_gi171545	7.3	-
1840.91	QTNDYYIL YNPAL PR_gi136482	7.5	+
1351.64	FWGLDPIDGTK_gi6324508	7.8	-
1020.48	SDDNIESIK_gi999493	7.9	-
1152.55	QVYFELESR_gi6321650	8.0	-
1032.56	VAPLOYWR_gi6322761	8.1	-
1674.78	TAODDSQPEEFYVK_gi6320079	8.1	-
1422.71	SFEVTDVNSSI K_gi6323570	8.3	-
1746.74	INEGLEIFNSYER_gi3495	8.6	-
2289.05	DYSEELSELSSNGEISODLR_gi6323768	8.6	+
1027.54	QVLI RPSK_gi6322271	8.9	-
1126.64	IIGANPTALEK_gi6319925	8.9	-
1701.84	SAYVTTEFLDAVEK_gi6320137	8.9	-
2806.39	LQEQQLQEQQLQQQQQQQQQLR_gi433624	9.0	-
1393.66	TYEQYAL TNYK_gi6325097	9.6	+
1969.07	VNNLIELSGQIPVTPDNK_gi6320899	9.6	+
1366.77	VLDTGGPISVPVGR_gi114575	9.7	-