The use of software visualization tools for the identification of PTM patterns and artefacts in LC-MS experiments

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Introduction

Traditional analysis of LC-MS data by reviewing chromatograms and corresponding single or averaged mass spectra is evidently both tedious and difficult. When analyzing multiple proteomics samples, this part of the data analysis is therefore often omitted and only the final list of identified proteins is reviewed. This may lead to unnecessarily complex or even contradictory results. By representing LC-MS data as a 2D-image as demonstrated in the novel DeCyder™ MS software, it is possible to identify phenomena, which are very difficult to recognize using conventional LC-MS data representation. By discarding appropriate phenomena as artefacts the quality of the result will be significantly improved.

Methods

The samples were analyzed by 1D LC-MS using an Ettan™ MDLC system (GE Healthcare) in High Throughput configuration directly connected to a Finnigan LTQ™ system (Thermo Electron Corp.). After desalting on a trap column (Zorbax™ 300 SB C18 0.3 x 5 mm, 5 µm particles, Agilent) the bound peptides were separated through a RPC column (Zorbax 300 SB C18 0.075 x 150 mm, 3.5 µm particles, Agilent).
Full scan mass spectra were collected in profile mode and MS/MS spectra in centroid mode. Detection and intensity map comparison was done on the full scan precursor mass spectra using DeCyder™ MS. The peptides were identified using the information in the MS/MS spectra and TurboSEQUEST™. Peptides with Xcorr values 1.5, 2, 2.5 for charge states 1+, 2+, 3+, respectively were considered as positively identified.

Results

Assessment of LC-MS data

The visualization of an LC-MS analysis in form of a 2D intensity map gives a good overview of the sample complexity and allows for easy identification of possible contaminations like PEG (*Fig. 1*). The indication of embedded MS/MS experiments by small red stars supports a fast and easy judgment of the experimental conditions in respect to sample complexity and MS-method setup (*Fig 2*). This example shows clearly that MS/MS experiments were performed on all major m/z signals, but not on many of the minor ones. Based on this observation the experimental setup could be optimized for each sample in respect to the LC and MS methods.

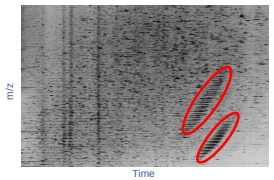

Fig1. *Visualization of an LC-MS run as a 2D intensity map using DeCyder MS. The circled areas show contamination of the sample with PEG*

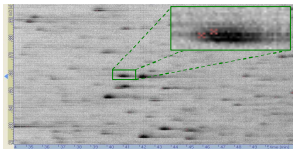

Fig 2. *Red crosses indicate the presence of MS/MS experiments, allowing for a judgment of the experimental setup*

Confirmation and identification of PTM`s using the 2D intensity map

We looked at PTM's like Methionine oxidation and deamidation and their behavior in an LC-MS analysis. The change in the m/z value depends on the charge (Met ox +16 for (M+H)$^+$, +8 for (M+2H)$^{2+}$; Deamidation +1 for (M+H)$^+$, +0.5 for (M+2H)$^{2+}$).

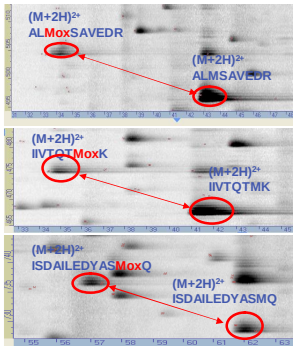

Fig 3. *Details of a 2D intensity map illustrating the chromatographic behavior in RPC of peptides with oxidized Methionine in contrast to the same peptides without oxidation*

In case the shift in retention time of the modified peptide in respect to the unmodified peptide is similar for peptides with different amino acid sequence the PTM's could be at least confirmed or even identified by the pattern in the 2D intensity map.
The examples presented for the oxidation of Methionine (*Fig 3*) show very clearly that the oxidized version was eluting several minutes before the unmodified peptide.
A deamidation of Asparagine containing peptides resulted in a change in elution time of around 1 min in the RPC analysis (*Fig .4*). A database search using TurboSEQUEST was not able to identify the deamidation and assigned both separated peptide spots, which differ clearly by 1 Da, to the same peptide sequence.

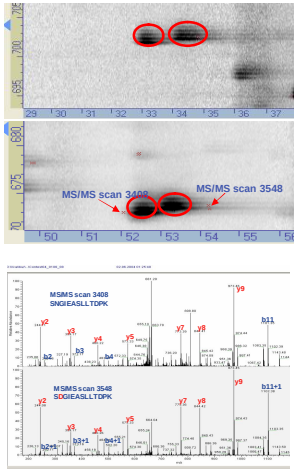

Fig 4. *Details of a 2D intensity map illustrating the chromatographic behavior in RPC of peptides showing deamidation. Below are the MS/MS spectra for scans 3408 and 3548. The b-fragment ions in scan 3548 show a 1 Da shift from the b2-ion compared to scan 3408 indicating a deamidation of Asn.*

Identification of artefacts with DeCyder MS

Peptides eluting from the RPC can during the ionization process undergo artificial modifications like for example non-covalent adduct formation, non-covalent dimer formation and source fragmentation. These artefacts can be identified, because they co-elute with the parent peptide and the co- elution can be very easily observed using the 2D intensity map.

A typical example is shown in *Fig 5*. The m/z signal marked with 1 was assigned by TurboSEQUEST to be a Methionine containing peptide from Glucose oxidase (see *Table 1*) and the m/z signal 2 to be the oxidized version of the same peptide. With the help of the 2D visualization it is very easy to see that m/z signals 3, 4 and 5 are co-eluting with the m/z signal 1 suggesting that they may be artefacts created during the ionization process. The result of the TurboSEQUEST search for these peaks is shown in table 1. Peak no. 3 was assigned as the oxidized version of peak no. 1. Peaks no. 4 and 5 were assigned as unrelated peptides from different proteins.
The manual interpretation of the MS/MS spectra for the different peaks (Fig 6.) revealed clearly that peak no. 1 is the parent peptide with the amino acid sequence ISDAILEDYASMQ and peaks 3, 4 and 5 are artefacts created during the ionization process. The assignments done by TurboSEQUEST were obviously wrong. The addition of 16 amu to the parent ion could be due to an oxidation, and replacement of H+ by Na+ and K+ could account for the mass shift of 22 and 38 respectively.

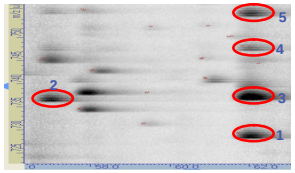

Fig 5. *Details of a 2D intensity map visualizing the co-elution of artificially modified peptides during the ionization process.*

Peak	Protein	Sequence	(M+H)+	Xcorr
1	GOX	ISDAILEDYASMQ	1456.6	4.2
2	GOX	ISDAILEDYASMoxQ	1472.6	3
3	GOX	ISDAILEDYASMoxQ	1472.6	2.7
4	RecA protein	TTLALHVVANAQKK	1494.7	2.7
5	GMP Synthase	FFAALSGVTDPEQK	1510.7	2.8

Table 1. *TurboSEQUEST results for the peaks shown and marked in Fig 5*

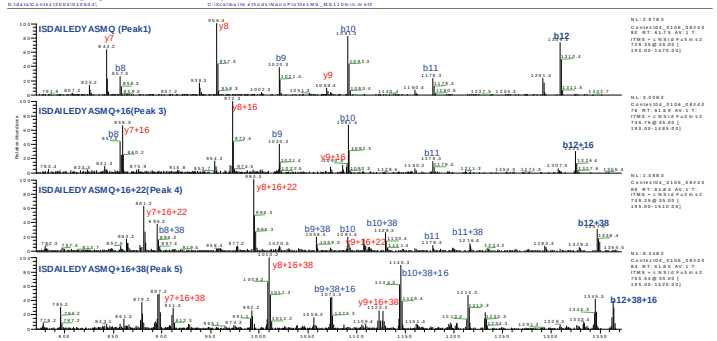

Fig 6. *MS/MS spectra with y and b ion assignments of peaks marked in Fig 5. as peak 1, 3, 4 and 5*

Conclusions

- 2D visualisation of LC-MS raw data as intensity maps with integrated MS/MS data points provides an excellent means for the assessment of LC-MS runs.
- Sample contamination, for example by PEG can easily be spotted.
- MS/MS data markers help to judge the MS settings for each sample.
- The confirmation and identification of PTM`s is supported by the recognition of corresponding 2D patterns.
- Artefacts like non-covalent adducts produced during ionisation can be easily spotted as a pattern and this information can be used to confirm or reject unclear assignments made by TurboSEQUEST.

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