SHIMADZU

MP 608

Peptide Mapping of a Monoclonal Antibody Using an Integrated Protein Digestion LCMS Platform (Perfinity-QTOF LCMS-9030)

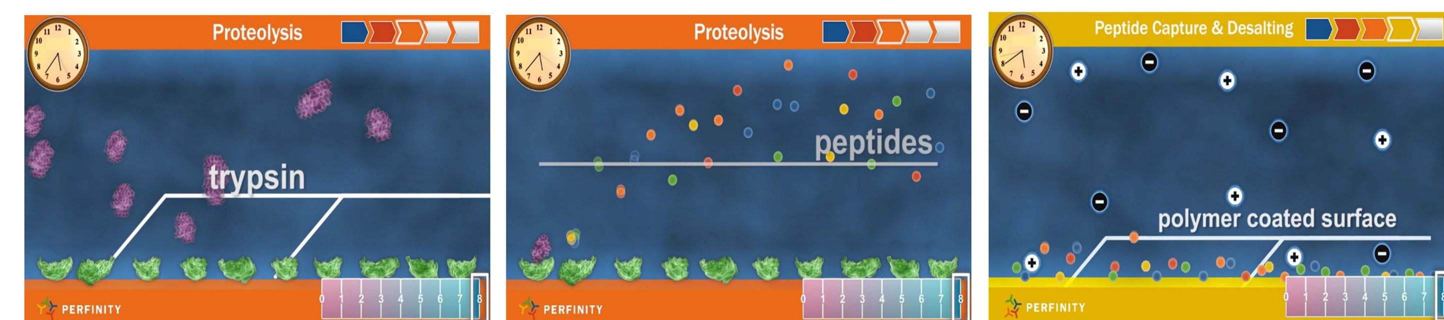
Xiaomeng (Kate) Xia[1], Evelyn Wang[1], Stephen Kurzyniec[1], Tairo Ogura[1], Yoshiyuki Okamura[1], M. Nazim Boutaghou[1]
1 Shimadzu Scientific Instruments, Columbia, MD 21046 U.S.A.

1. Overview

The combination of the Perfinity, an integrated online protein digestion platform, and a Q-TOF provides an efficient and reproducible protein digestion protocol for peptide mapping analysis.

2. Introduction

Typical peptide-mapping procedures, especially for protein digestion, consist of many laborious steps, which can vary due to the differences among protocols or operators. As such they are susceptible to changes that affect reproducibility, reduce assay sensitivity, and significantly increase analysis time. An automated, new method is developed to overcome these drawbacks and provide a simple, efficient, and reliable alternative. We utilized Perfinity workstation – an automated on-column protein digestion platform – directly coupled with a LCMS-QTOF mass spectrometer for high throughput and high sequence coverage peptide mapping.



3. Methods

Peptide mapping was performed using the NISTmAb standard (reference material 8671). Three types of enzymatic columns – trypsin column, non-reduction/alkylation (NoRA) trypsin column, and Lys-C column – were used for protein digestion on Perfinity. A C18 desalt column was used to clean up the samples after digestion and a Restek Ultra C18 (2.7µm, 150mm x 2.1mm) column was used for reserved phase separation.

All data was obtained on a Q-TOF mass spectrometer, LCMS-9030. Data dependent acquisition (DDA) with three dependent events was used for data acquisition. For one set of experiments, NISTmAb was diluted to 1 mg/mL with water then directly injected into Perfinity for a 6-minute on-column protein digestion. For another set of experiments, NISTmAb was first reduced by adding 50 mM dithiothreitol (DTT), then alkylated with 500 mM iodoacetamide prior to the injection into Perfinity.

Protein Metrics software was used for comprehensive peptide identification of the digested protein.

4. Results

Sequence Coverage. The sequence coverage maps of NISTmAb digestion on different types of enzymatic columns are shown in Figure 1 and the results are summarized in Table 1.

Figure 1: NISTmAb peptide coverage. Black: trypsin column without reduction/alkylation (RA). Blue: NoRA trypsin column without RA. Red: trypsin column with RA. Green: NoRA trypsin column with RA. Magenta: Lys-C column with RA.

Table 1. Summary of sequence coverage

NISTmAb	Sequence coverage (%)				
	Trypsin column w/o RA, 50 °C	NoRA trypsin column w/o RA, 70 °C	Trypsin column with RA, 50 °C	NoRA trypsin column with RA, 70 °C	Lys-C column with RA, 50 °C
Heavy chain	30.21	37.33	98.67	100	94.42
Light chain	44.13	50.23	100	100	95.31

Critical PTMs Identification and Quantification. A set of quality attributes post translational modifications (PTMs), such as oxidation, glycosylation, and deamidation, was identified and quantified by relative abundance.

Figure 2 illustrates the MS/MS spectra comparison of the native (precursor at m/z = 418.2207, +2) and Met-oxidized peptides (precursor at m/z = 426.2182, +2) in the heavy chain 252-258. The differences in m/z (+15.99 Da in the oxidized form) for the y4–y5 fragment ions (green boxes) clearly distinguish the modified forms from the native species, and the m/z of y1-y3 ions remain constant, indicating that the Met-4 in the peptide is the location of oxidation.

Table 2 summarizes the quantification results of the critical PTMs identified with different digestion conditions.

- Complete conversion of N-terminal glutamine (Gln) to pyroglutamate (Pyro-Glu) was observed under all conditions.
- The loss of lysine from the C-terminus of the heavy chain, in the range of 87-90%, was comparable at different digestion conditions.
- Methionine oxidation at heavy chain M255, and asparagine deamidation at heavy chain N387 and N392 are usually monitored for NISTmAb. Similar levels for the two modifications were detected for all digested samples.
- High abundance of glycosylation at the heavy chain N300 was observed for the reduced and alkylated samples where the main glycans were complex biantennary oligosaccharides containing from 0 or 1 non-reducing galactose with fucose attached to the reducing end of N-acetylglucosamine (A2G0F, A2G1F).

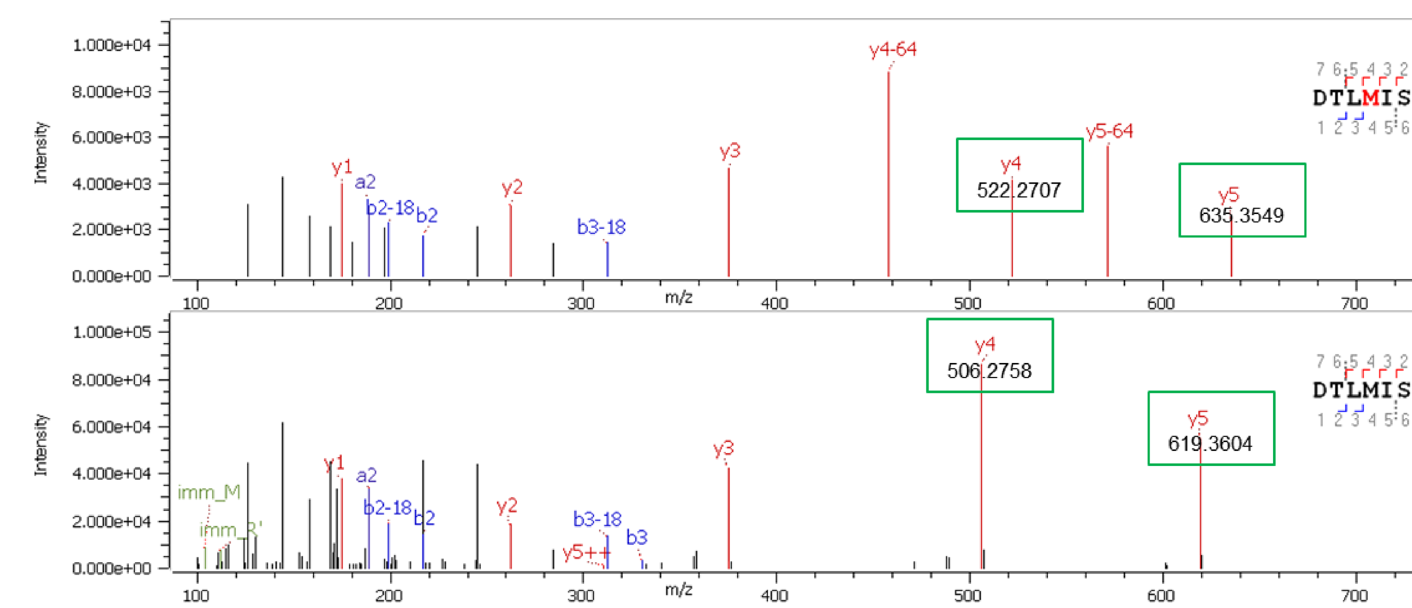


Figure 2: PTM (methionine oxidation) analysis. MS/MS spectra of Met-oxidized and native peptides (heavy chain peptide 252-258). Top: oxidation at Met 255 in heavy chain. Bottom: native peptide. Green boxes show confirmed fragment ions.

- The sequence was not covered for the non-reduced/alkylated samples, so the glycosylation modification was not detected.
- Overall, the reported PTM values are very close among experiments and show low amounts of sample preparation-induced modifications.

Table 2: Summary of critical PTMs identified and quantified for NISTmAb with different digestion conditions

Modification	Relative abundance (%)				
	Trypsin column w/o RA, 50 °C	NoRA trypsin column w/o RA, 70 °C	Trypsin column with RA, 50 °C	NoRA trypsin column with RA, 70 °C	Lys-C column with RA, 50 °C
HC Q1+Gln -> PyroGlu	100	100	100	100	100
HC M255 + Oxid	2.39	3.01	3.63	4.97	2.53
HC ~N392/N387 + Deam	1.24	2.89	1.21	1.95	2.54
HC K450 Lys loss	90.55	89.75	87.73	88.79	90.23
HC N300+A2G0F	NA	NA	40.14	38.36	37.52
HC N300+A2G1F	NA	NA	36.65	34.11	33.56
HC N300 unglycosylated	NA	NA	1.53	1.28	1.28

5. Conclusions

- We demonstrate here a fast, reproducible peptide mapping workflow using Shimadzu's unique solution: Perfinity-LCMS-9030 system.
- The combination of automated sample preparation, reversed-phase separation, and accurate detection upgraded the process of peptide mapping from time-consuming and tedious to an easy, reproducible and automated.
- The analysis of NISTmAb RM 8671 protein produced outstanding sequence coverage with high confidence due to the QTOF mass accuracy. Reduction and alkylation steps were necessary in order to improve sequence coverage. Even with the added steps, the total analysis time was still less than two hours.
- The most targeted modifications were successfully identified and quantified relatively. We confirmed that PTM induced by sample preparation were low and insignificant.

Disclaimer: All content contained herein resulted solely from Shimadzu, and no conflict of interest exists. The products and applications are intended for Research Use Only (RUO). Not for use in diagnostic procedures.