イオンモビリティ-飛行時間型質量分析計を用いた Top / Middle-Downフラグメンテーション解析によるタンパク質配列解析および酸化部位の同定

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OVERVIEW

- Fragmentation of large therapeutic proteins (top /mid down analysis) tends to generate many different types (and charge states) of fragment ions, making the spectra interpretation and sequence deduction very challenging.
- The biopharmaceutical Platform Solution with UNIFI provides a workflow for protein top/middle down analysis including targeted MSMS acquisition, data processing and automatic report generating.
- In this work, we demonstrated the top/middle down analysis using the developed UNIFI workflow to characterize Trastuzumab (Tmab) sub - units on a Xevo G2-XS quadrupole time-of-flight mass spectrometer.
- In addition, Waters SYNAPT G2-Si HDMS provides a unique instrument platform for top down protein analysis to simplify the terminal sequence confirmation using ion mobility separation (IMS) of product ions.

UNIFI Top Down Analysis Workflow

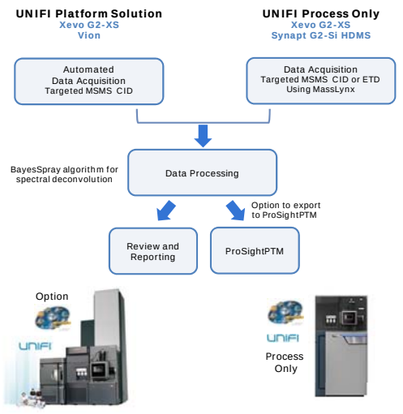


Figure 1: The Biopharmaceutical Platform Solution with UNIFI provides a workflow for protein top/middle down analysis including targeted MSMS acquisition, data processing and automatic report generating. UNIFI process only option supports MassLynx data with complementary CID and ETD fragmentation.

METHODS

Sample Preparation:

FaBricator (IdeS) was used to cleave Transtuzumab at the hinge region, followed by limited reduction and alkylation using DTT and iodoacetamide (IAA) to reduce the inter-chain disulfide bonds and generate the light chain and two heavy chain fragments. Neat acetic acid was added to terminate all digestions. The digests were diluted to 0.5 µg/µL prior to LC-MS analysis.

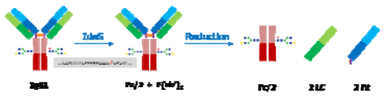


Figure 2: Schematic of Ides digestion followed by reduction to generate Fc/2, Light Chain (LC), and Fd subunits from monoclonal antibodies.

LC-MS and LC-MS/MS Analysis:

LC: ACQUITY UPLC H-Class Bio System
Column: ACQUITY UPLC Protein BEH C4 Column (300Å, 1.7 µm, 2.1 mm X 50 mm) (P/N=186004495)
Mobile Phases:
A: 0.1% TFA in Waters; **D:** 0.1% TFA in Acetonitrile

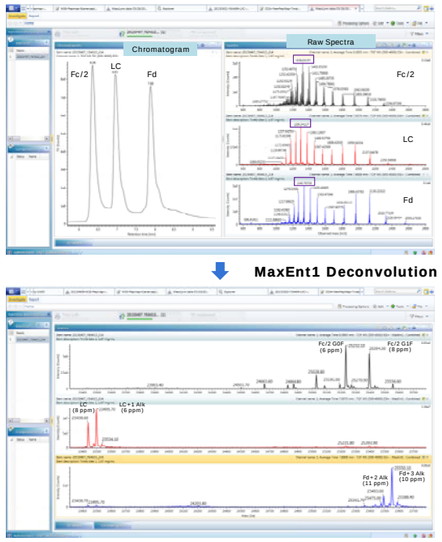
Time(min)	Flow Rate(mL/min)	%A	%B	%C	%D	Curve
Initial	0.400	95.0	0.0	0.0	5.0	Initial
1.00	0.400	95.0	0.0	0.0	5.0	6
1.10	0.200	95.0	0.0	0.0	5.0	6
2.50	0.200	70.0	0.0	0.0	30.0	6
12.50	0.200	50.0	0.0	0.0	50.0	6
13.00	0.200	5.0	0.0	0.0	95.0	6
13.10	0.400	5.0	0.0	0.0	95.0	6
14.90	0.400	5.0	0.0	0.0	95.0	6
15.00	0.400	95.0	0.0	0.0	5.0	6
20.00	0.400	95.0	0.0	0.0	5.0	6

MS Conditions
Capillary: 3kV;
Sample Cone voltage: 150 v;
Source Temp: 500° C;
Desolvation Temp: 350 ° C;
Desolvation Gas Flow: 800 L/h
Scan Rate 0.5 Sec; Mass Range: 500-4000 *m/z*
Targeted MS/MS Conditions
Scan Rate 1 Sec; Mass Range: 100-4000 *m/z*
Isolation Window: *2 m/z*
CE: 32 eV

Software: UNIFI Scientific Information System V1.8 and MassLynx 4.1

RESULTS AND DISCUSSION

Subunit MS Analysis with UNIFI (Xevo G2-XS)



Top Down Analysis with UNIFI (Xevo G2-XS)

Precursor Ions Selected for Targeted MSMS

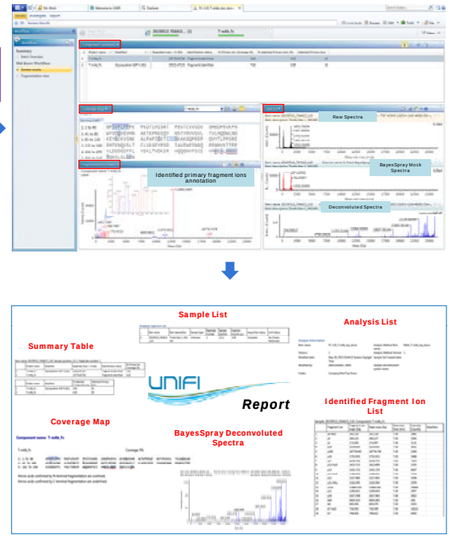


Figure 3: Tmab subunits MS analysis followed by top down analysis using the Biopharmaceutical platform solution with UNIFI. MaxEnt1 deconvolution confirms the molecular weight of the subunits and their modified forms. The most abundant precursor ions were selected for CID fragmentation followed by data processing and reporting. Fc/2 top down analysis data and UNIFI report is shown here.

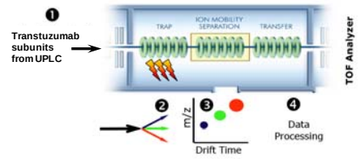
Option to Export to ProsightPTM Analysis

Figure 4: LC and Fd top down analysis data (BayesSpray deconvoluted fragment ions with intensity) were exported as .PUF file followed by ProsightPTM analysis. Matched sequence coverage of Tmab LC and Fd subunits are shown here. 10 b- and 22 y-ions were assigned for LC and 22 b- and 9 y-ions were assigned for Fd respectively, corresponding to approximately 11% of the theoretical number of fragments.

CONCLUSION

- The Biopharmaceutical Platform Solution with UNIFI provides a streamlined workflow from mAb intact/subunit MS analysis and subsequent protein top/middle down MSMS analysis.
- The developed UNIFI workflow enables automate targeted MSMS acquisition, data processing and report generating.
- Top down data can be exported to ProsightPTM analysis for additional confirmation.
- The post-fragmentation IMS separation enabled the separation of different charge states of fragment ions, produced easily interpretable spectra from selected ion fragments, and increased sequence coverage

Top Down Fragmentation of LC followed by IMS Separation (SYNAPT G2-Si HDMS)



1. Select few charge states of Tmab subunits using quadrupole
2. Fragment using CID in the TRAP cell
3. Separate CID fragment ions based on the charges, size and masses by ion mobility
4. Process data to simplify the fragmentation data

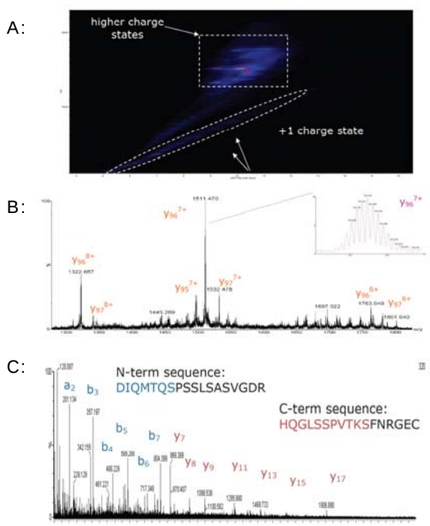


Figure 5: A) IMS display (Driftscope) of all fragment ions after ion mobility separation; B) Spectrum of higher charge state fragment ions selected from the IMS display; C) Spectrum of 1+charged fragment ions (precursor m/z 1079.2, 22+) following ion mobility separation.

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