TOP-DOWN SEQUENCE ANALYSIS OF ANTIBODY FRAGMENTS BY AN ION-MOBILITY TIME -OF-FLIGHT MASS SPECTROMETER

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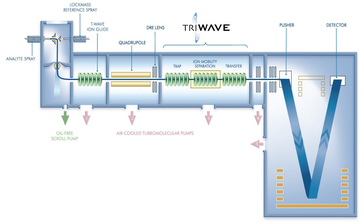
OVERVIEW

- Top-or middle-down MS methodology directly fragments intact proteins to obtain information for protein characterization or identification.
- However, direct fragmentation of large proteins leads to many different types of fragments, making the spectra interpretation and sequence deduction very difficult.
- Waters Synapt™ HDMS™ possesses a unique ion-mobility separation (IMS) function and is capable of separating/fractionating fragment ions by size, shape, and charge prior to mass spectrometric detection.
- In this presentation, we have demonstrated the utility of IMS coupling with CID in the top-down analysis of antibody subunit (LC) or fragments (Fd) for the characterization of a commercial recombinant monoclonal antibody.

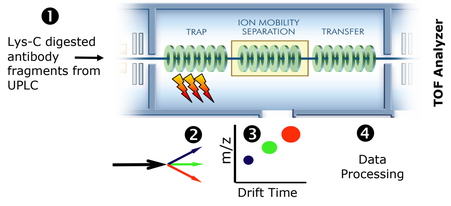
- The LC/MS system was configured with a Waters ACUITY UPLC™ chromatography system and a Waters Synapt™ HDMS™ quadrupole ion-mobility time-of-flight mass spectrometer.
- Synapt HDMS was operated in the mobility-TOF mode for all analyses. MassLynx 4.1 software was used for instrument control and data processing.
- Chromatographic separations were accomplished on a 2.1 x 50 mm BEH C4, 1.7 µm column. Antibody fragments from limited Lys-C digestion and DTT reduction were resolved using a linear acetonitrile gradient in 0.1% formic acid.

ADDING ION MOBILITY TO A Q-TOF MS



- The ion-mobility section is comprised by three Traveling Wave-enabled Stacked Ring Ion Guides (SRIG).
- The TRAP ion guide is used to accumulate ions and release them as packets for ion mobility separation.
- The TRANSFER ion guide conveys the mobility separated ions to the oa-TOF for mass analysis.
- Fragmentation can take place either in the TRAP or in the TRANSFER ion guide or both.

CID FOLLOWED BY IMS SEPARATION OF FRAGMENT IONS



❶ Select few charge states of the light chain or Fd fragment using quadrupole

❷ Fragment LC or Fd using CID in the TRAP cell

❸ Separate CID fragment ions based on the charges, sizes and masses by ion mobility

❹ Process data to simplify the fragmentation data

LYS-C PROTEOLYSIS AND REDUCTION

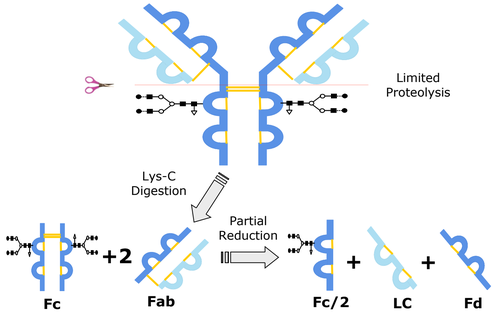


Figure 1. Fragments generated by limited proteolysis of monoclonal antibody with Lys-C followed by partial reduction.

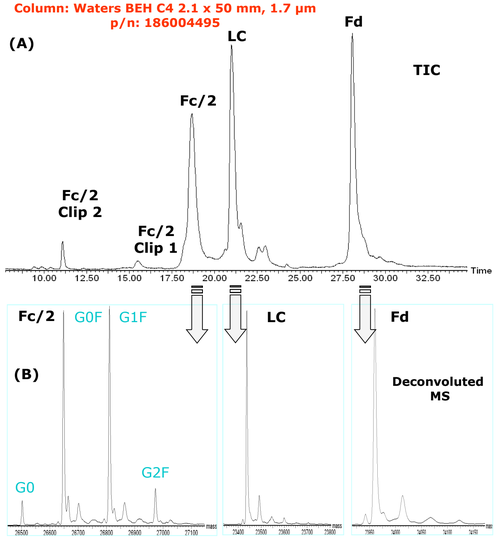


Figure 2. LC/MS analysis of Lys-C digested (limited) and partially reduced monoclonal antibody. Total ion chromatogram (A), MaxEnt 1 deconvoluted MS of Fc/2, LC, and Fd fragments (B) are shown.

CID spectra of LC before IMS Separation

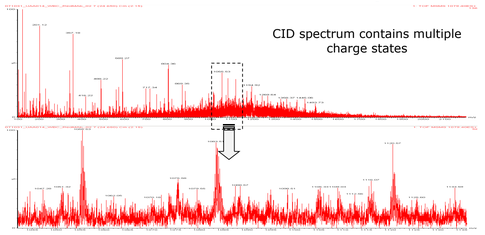


Figure 3. Top-down spectra of light chain (LC, precursor m/z 1079.4, +22). Spectra contain multiple charge states that complicates the sequence interpretation.

Top-down followed by IMS Separation (LC)

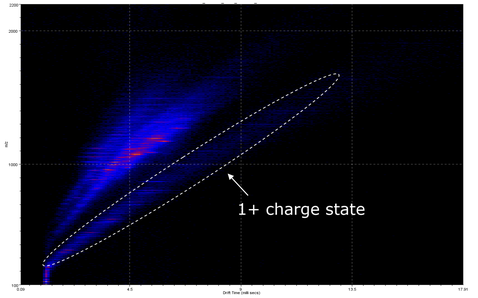


Figure 4. Driftscope of LC fragmentation showing the separation of different charge states.

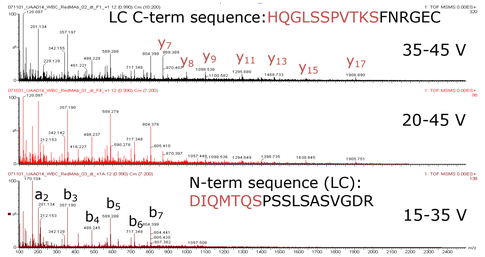


Figure 5. Spectra showing only 1+ charged fragment ions (precursor *m/z*: 1079.4, 22+) generated from ion mobility separation.

Top-down followed by IMS Separation (Fd)

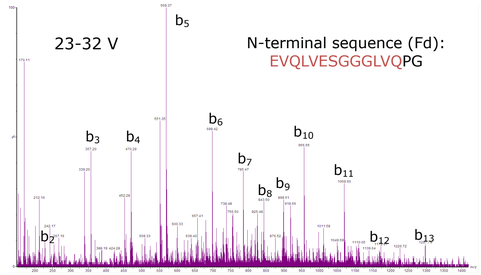


Figure 6. Top-down sequencing of heavy chain fragment, Fd. Spectrum showing only 1+ charged fragment ions (precursor *m/z*: 1262.98, 19+) extracted from ion mobility separation.

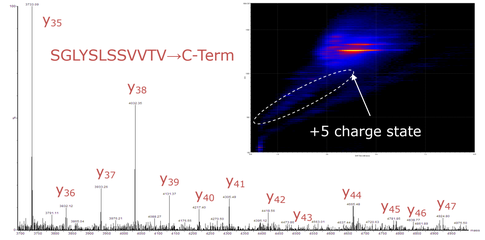


Figure 7. MaxEnt 3 deconvoluted spectrum showing only 5+ charged fragment ions (precursor m/z: 1262.98, 19+) of Fd extracted from ion mobility separation (inset).

CONCLUSIONS

- We have demonstrated the utility of the ion-mobility (IMS) separation in the top-down sequencing of LC and Fd fragments of a monoclonal antibody.
- The post-fragmentation IMS separation enabled the separations of different charge state of fragment ions, produced spectra from ions of mainly one charge state, and thus allowed a segment of antibody sequence to be easily deduced.

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