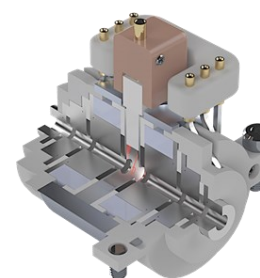
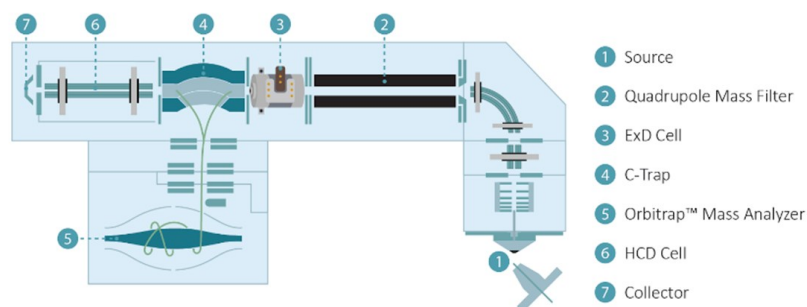


## ECD upgrades for ThermoFisher QExactive for top down mass spectrometry

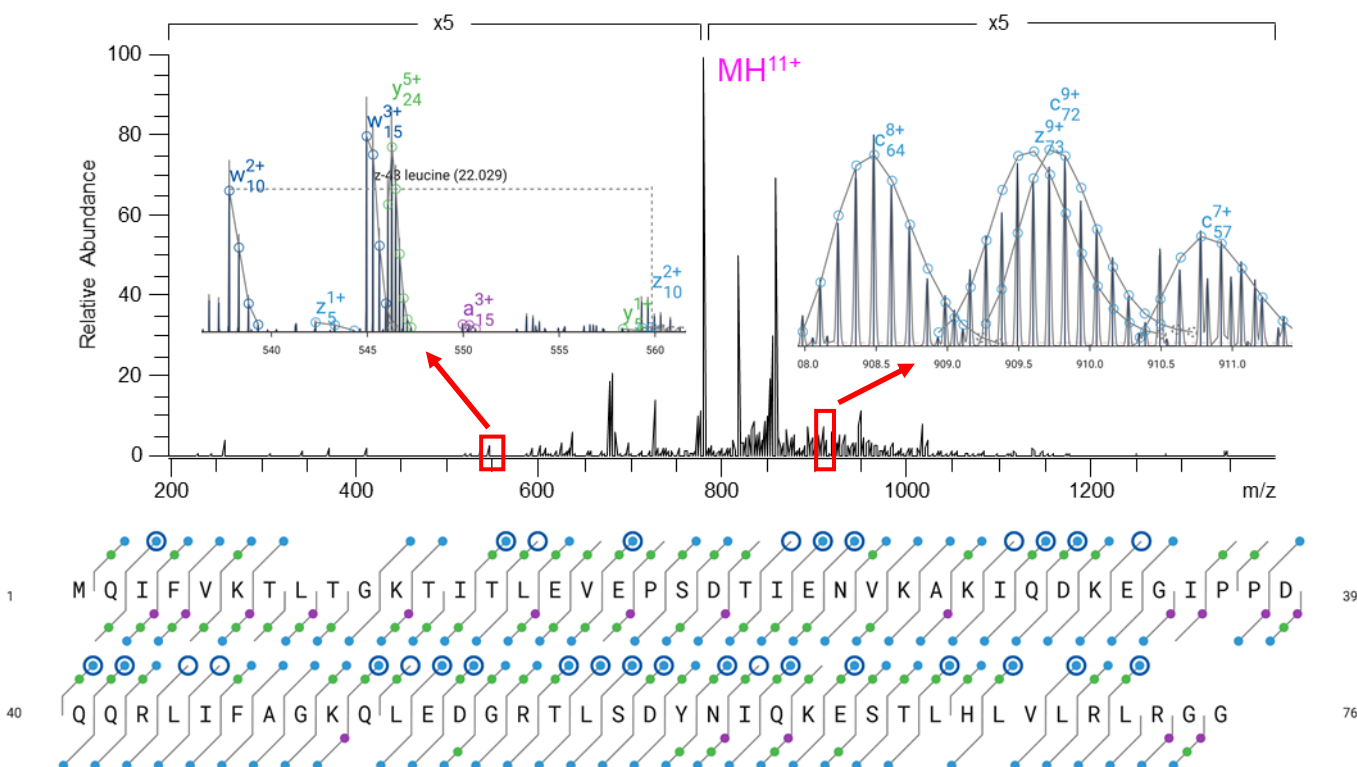
### Technical setup



e-MSION ExD cell for the ThermoFisher QExactive

The ECD cell is being located between the quadrupole and the C-trap. Additional HCD fragmentation can be applied for ETHcD. HCD collision energies in the range of 10-15V support the dissociation of ECD fragment pairs to increase sequence coverage, whereas higher HCD values will lead to additional b/y-type fragmentation which may increase sequence coverage

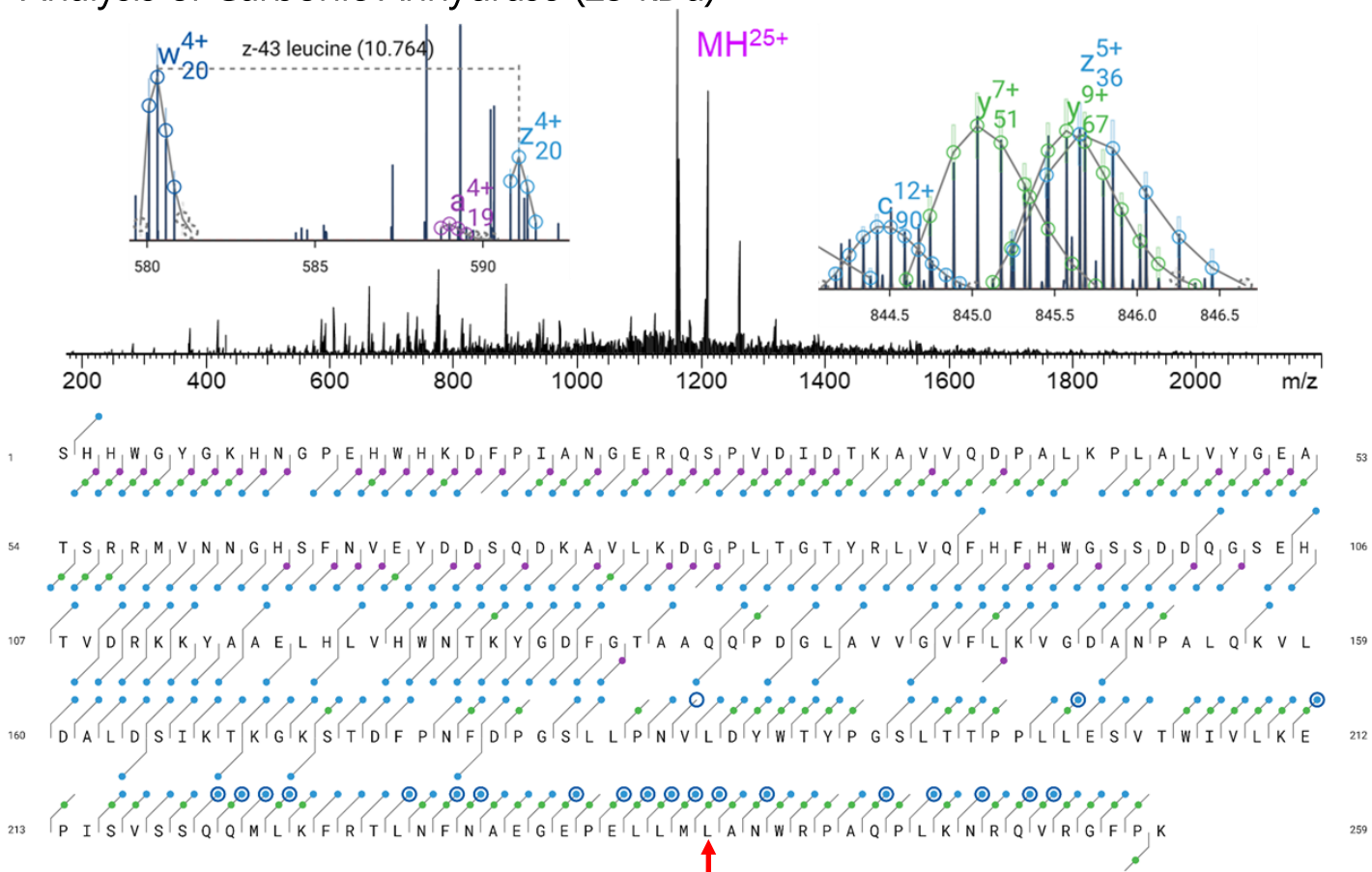
### Ubiquitin analysis



Fragmentation of 11+ Ubiquitin, achieving 100% sequence coverage. Data was acquired at a resolution of 140,000. Data processing and annotation using ExDPProcess software.

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# Analysis of Carbonic Anhydrase (29 kDa)



ECD fragmentation of the denatured 25+ charge state of Carbonic Anhydrase at 140,000 resolution. An MS/MS sequence coverage of 90% was achieved using ECD. Several isomeric residues could be confirmed by side chain fragmentation such as the marked Leucine residues leading to the shown  $w_{20}^{4+}$  ion. Data processing and sequence annotation using e-MSion's ExDProcess software.

The analysis of the native Carbonic Anhydrase 11+ ion results in less congested spectra with very clear sequence assignments. A low mass quadrupole cutoff of 2400 Da was used in combination with ECD and HCD. From the native protein an MS/MS sequence coverage of 64% was achieved. Data was acquired at 140,000 resolution.

