



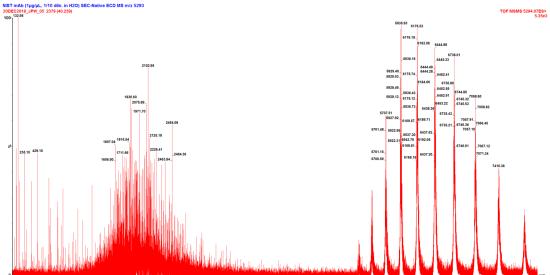
Electron-Capture Dissociation for Top-Down Analysis on Synapt

Traditionally, top-down analysis of intact proteins is one of the most common applications of ECD fragmentation. The reason is that ECD, in contrast to CID, allows one to efficiently fragment intact proteins and protein complexes. While CID loses efficiency with bigger molecules due to their ability to get rid of additional energy by vibration and conformational changes, ECD activation is of an electronic nature and can not be lowered by vibration. Also, the fragmentation process is faster than vibrational rearrangements. Together, this leads to an efficient fragmentation process which has been successfully applied to molecules in excess of 100kDa molecular weight.

The most common molecules in biopharmaceutical research are immunoglobulins, in particular IgG antibodies. These molecules consist out of two pairs of identical protein chains of 25 and 50 kDa molecular weights adding up to a total of 150 kDa. Typical questions concern glycosylation heterogeneity, disulfide bridge location (scrambling), deamidation or pyroGlu formation, or other posttranslational modifications.

ECD fragmentation analysis of the NIST antibody

Assignment of the observed ions on the expected sequences confirm all three CDR regions of the light chain:



Data courtesy of John Williams, Waters Corp., acquired on a Waters Synapt system equipped with the ExD WS-25x option

DIQMÍTÍQSPSÍTLSÁSVGDÍRÍV TITTCSÁSSRVGYMHWJYQQKPGKAPKLLJYDTSKLASGVPSÍRFSGSGSGTEFT LTJSSLQPDDFATYYCFQGSGYPFTFGGGGTKVEJKRÍTÍVAA PSVFJFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC Data annotation using e-MSion's ExDProcess viewer software

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Top-down of non-covalent complexes on Native Synapt

MS Vision's dedicated Native Synapt systems are modified particularly for non-covalent complex and very high mass analysis. This involves optimized pressure and potential settings as well as a modified quadrupole for isolation of precursor ions in excess of m/z 30.000. This makes the systems uniquely suited for the characterization of non-covalent assemblies and the study of their conformational changes. By the addition of eMSion's ExD WS-25x option, the system is now also able to perform top-down analysis of the isolated complex constitutents.

