Are Internal Fragments Observable in Electron Based Top-Down Mass Spectrometry?

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Funding Grants: K. M. Amm (NSF DMR-1644779 and DMR-2128556); K. Håkansson (NSF CHE-2004043, NIH S10OD021619); A. I. Nesvizhskii (NIH T32CA140044, R01GM094231, and U24CA271037); J. A. Loo (NIH R35GM145286 and DOE DE-FC02-02ER63421); B. T. Ruotolo (Agilent Technologies); D. F. Hunt (NIH GM037537)

Tandem mass spectrometry of intact proteins, or “top-down” proteomics, provides a molecular understanding of phenotype that is otherwise unobtainable. Successful experiments provide the highest amino acid sequence coverage possible to pinpoint sites of mutation and protein modifications. Internal fragment ions, which are produced when the protein backbone undergoes multiple cleavage events, can provide valuable information by increasing the sequence coverage of a protein. However, with electron-based dissociation techniques, there are tens of thousands of possible internal fragments, many of which are isomers or isobars, and their signal-to-noise ratios are too low to be reliably detected.

In 2017, MagLab researchers published an ultrahigh quality spectrum of a 29 kDa protein fragmented by electron transfer dissociation (**Figure 1**) to demonstrate the maximum sequence coverage achievable by 21 tesla Fourier transform ion cyclotron resonance tandem mass spectrometry. More recently, this spectrum was exhaustively annotated, and terminal fragments accounted for virtually all of the signals in the spectrum, resulting in 91% sequence coverage of the protein. None of the signals could be reliably assigned to internal fragments.

These findings, along with extensive additional data from multiple laboratories, demonstrate that internal fragments are not formed at a detectable level from electron-based fragmentation techniques. We urge great caution when assigning such fragments due to their innate potential for false discovery. Elimination of false assignments will enhance the integrity of top-down proteomics data and prevent misidentification of proteoforms involved in phenotypes of interest.

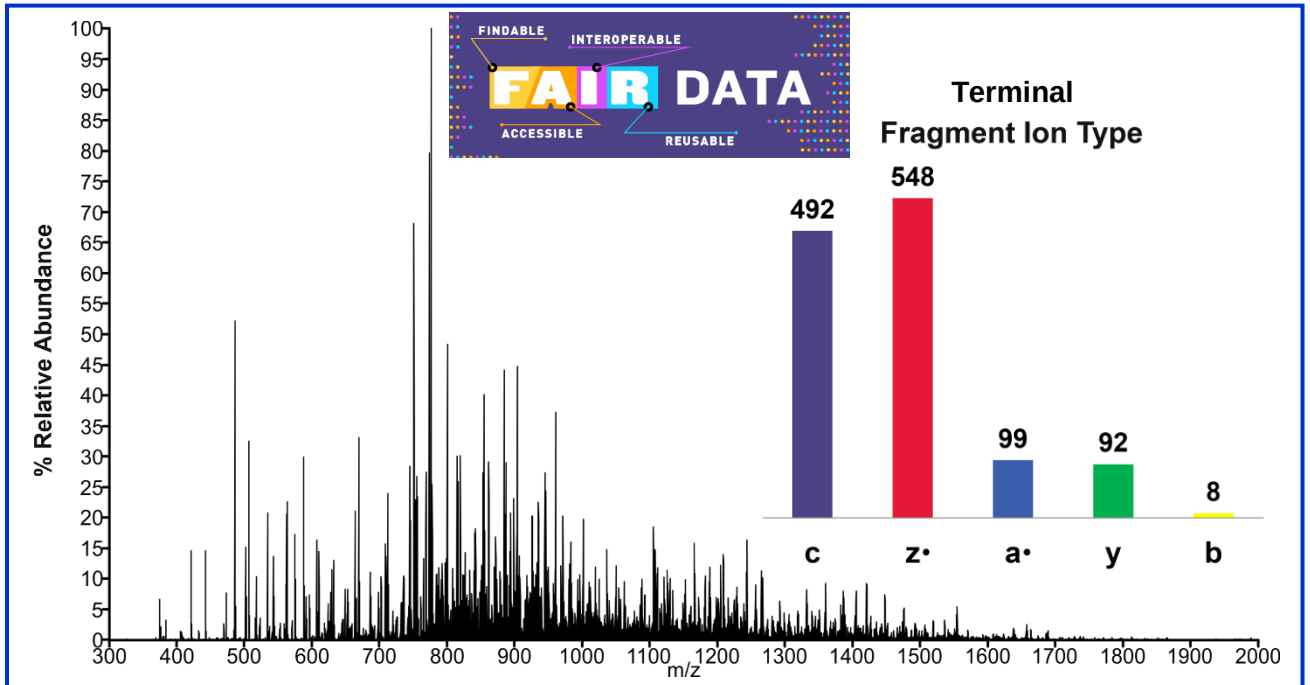

Figure 1: 21 T Fourier transform ion cyclotron resonance tandem mass spectrum of carbonic anhydrase II (34+, 29 kDa) following 6 milliseconds of electron transfer dissociation. The signal was summed over 1600 acquisitions (600,000 resolving power at m/z 400). The inset shows the terminal fragment ion types identified in the spectrum. No internal fragments were found.

Facilities and instrumentation used: Ion Cyclotron Resonance (21 T FT-ICR); FAIR data

Citation: Mikawy, N.N.; Ramírez, C.R.; DeFiglia, S.A.; Szot, C.W.; Le, J.; Lantz, C.; Wei, B.; Zenaidee, M.A.; Blakney, G.T.; Nesvizhskii, A.I.; Loo, J.A.; Ruotolo, B.T.; Shabanowitz, J.; Anderson, L.C.; Hakansson, K., *Are Internal Fragments Observable in Electron Based Top-Down Mass Spectrometry?,* Molecular and Cellular Proteomics, **17** (23), 100814 (2024) **doi.org/10.1016/j.mcpro.2024.100814 Data Set – DOI: 10.1007/s13361-017-1702-3 (Weisbrod et al. J. Am. Soc. Mass Spectrom. 2017; 28:1787-1795)**