Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information to Accompany:

Obtaining Complementary Polypeptide Sequence Information from a Single Precursor Ion Packet *via* Sequential Ion Mobility Resolved Electron Transfer and Vibrational Activation

Deepali Rathore, Forouzan Aboufazeli, and Eric D. Dodds

Department of Chemistry, University of Nebraska – Lincoln

Lincoln, NE 68588-0304 USA

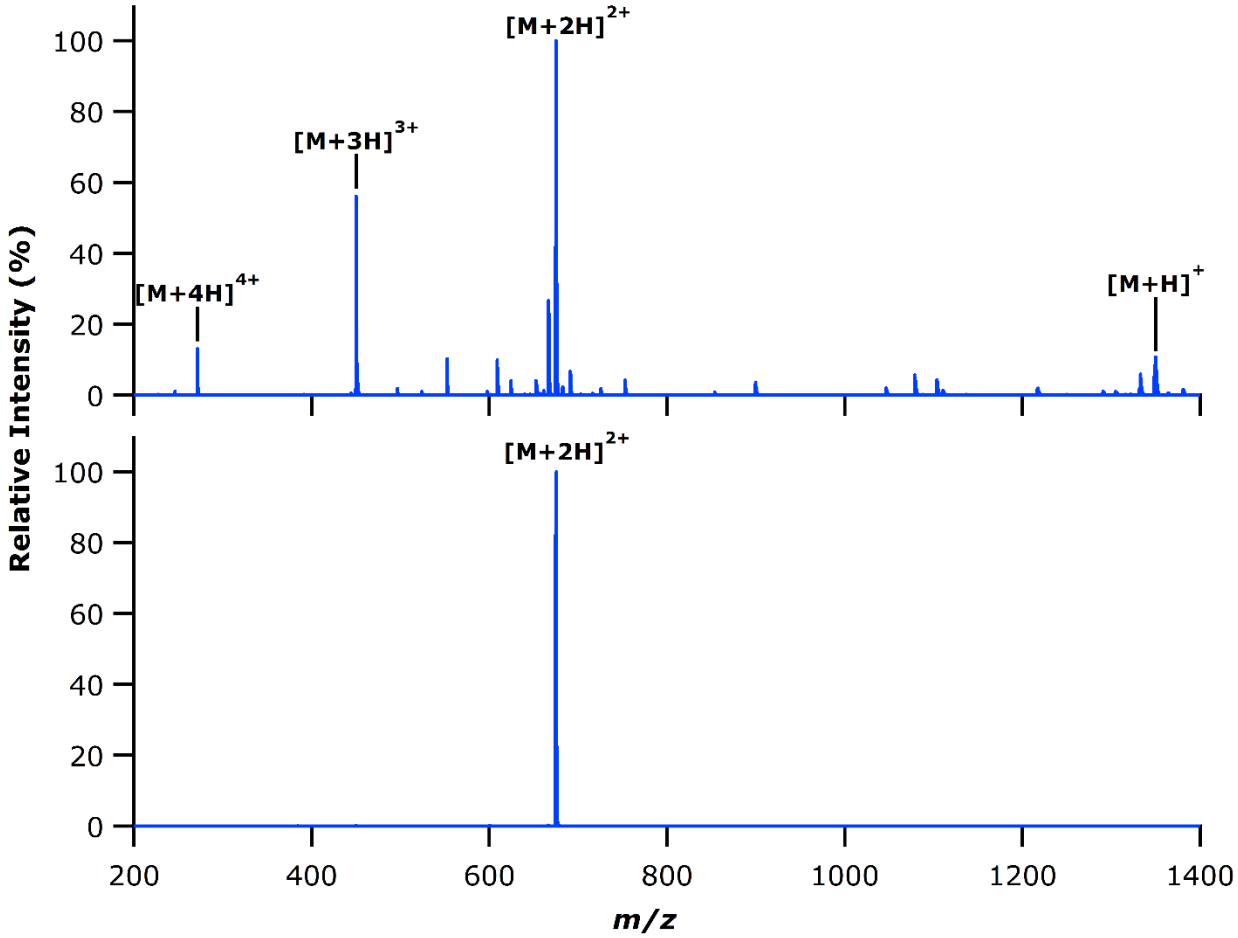

Figure S1. TOF-MS spectrum of the described substance P solution (upper trace), and Q-TOF-MS selection of the $[M+2H]^{2+}$ molecular ion at *m/z* = 674 (lower trace). The subsequent ET-IM-VA analysis of this precursor ion is shown in ***Figure 2*** of the main article.

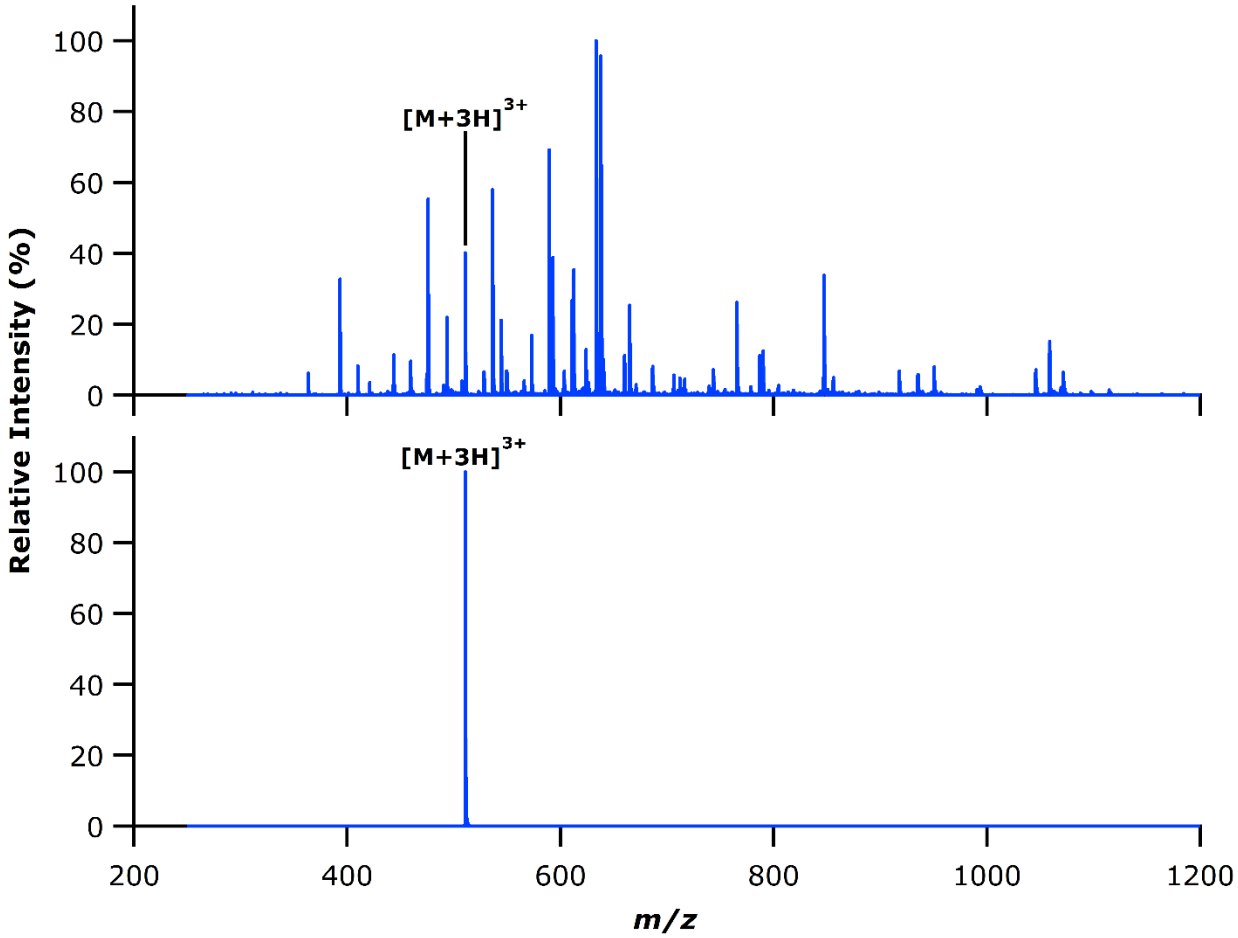

Figure S2. TOF-MS spectrum of the described hemoglobin tryptic digest solution (upper trace), and Q-TOF-MS selection of the $[M+3H]^{3+}$ tryptic peptide at *m/z* = 510 (lower trace). The subsequent ET-IM-VA analysis of this precursor ion is shown in ***Figure 3*** of the main article.

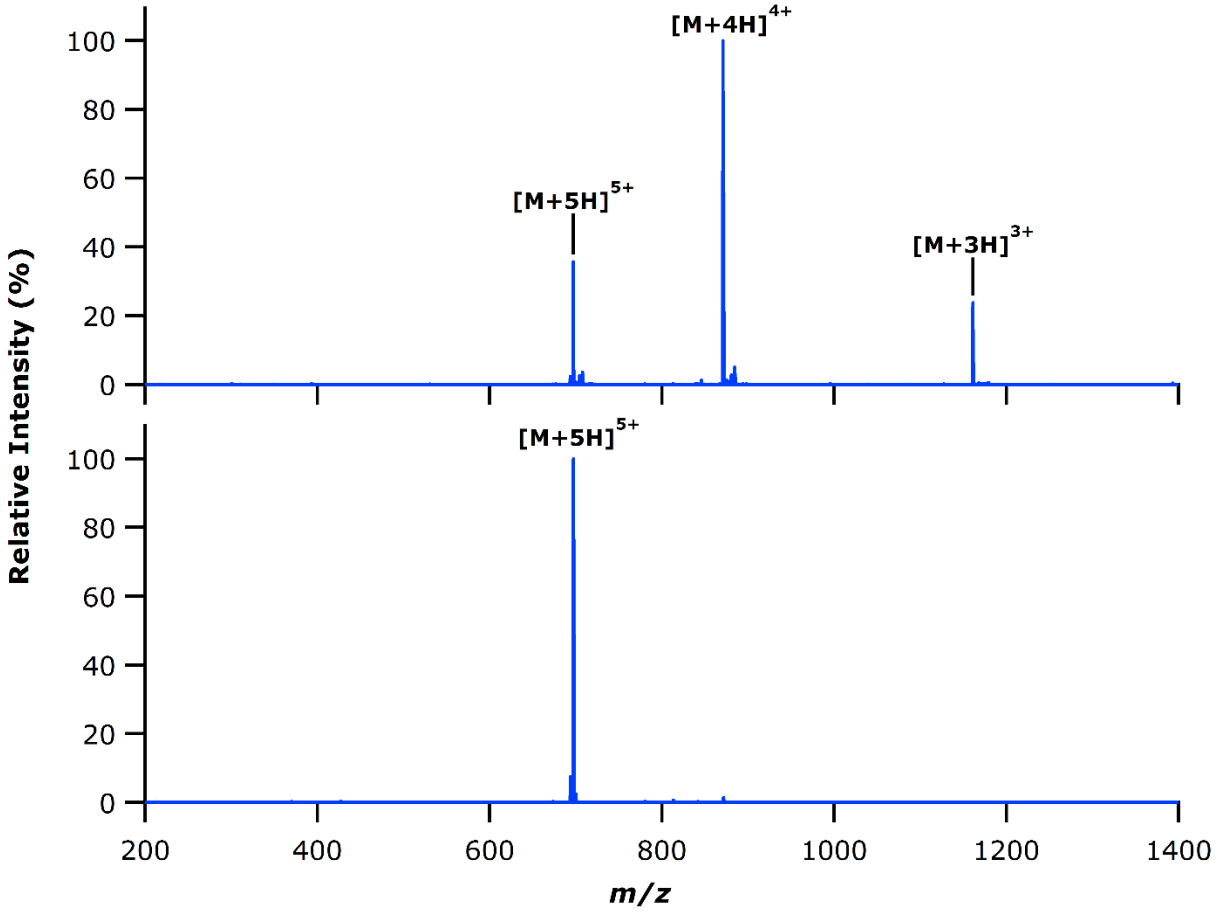

Figure S3. TOF-MS spectrum of the described glucagon solution (upper trace), and Q-TOF-MS selection of the $[M+5H]^{5+}$ molecular ion at *m/z* = 693 (lower trace). The subsequent ET-IM-VA analysis of this precursor ion is shown in ***Figure 4*** of the main article. In the lower trace, the appearance of a small peak (~1% relative intensity) corresponding to the $[M+5H]^{4+\bullet}$ ion is attributed to ET reactions occurring during a short co-residence time of precursor ions with ET reagent ions in the trap cell of the instrument. This occurs even when the trap cell traveling DC wave height is elevated with the intent of minimizing co-residence time in order to prevent ET reactions. Because the proportion of the ET product ion is so small in this precursor selection spectrum, this had essentially no impact on the overall analysis.

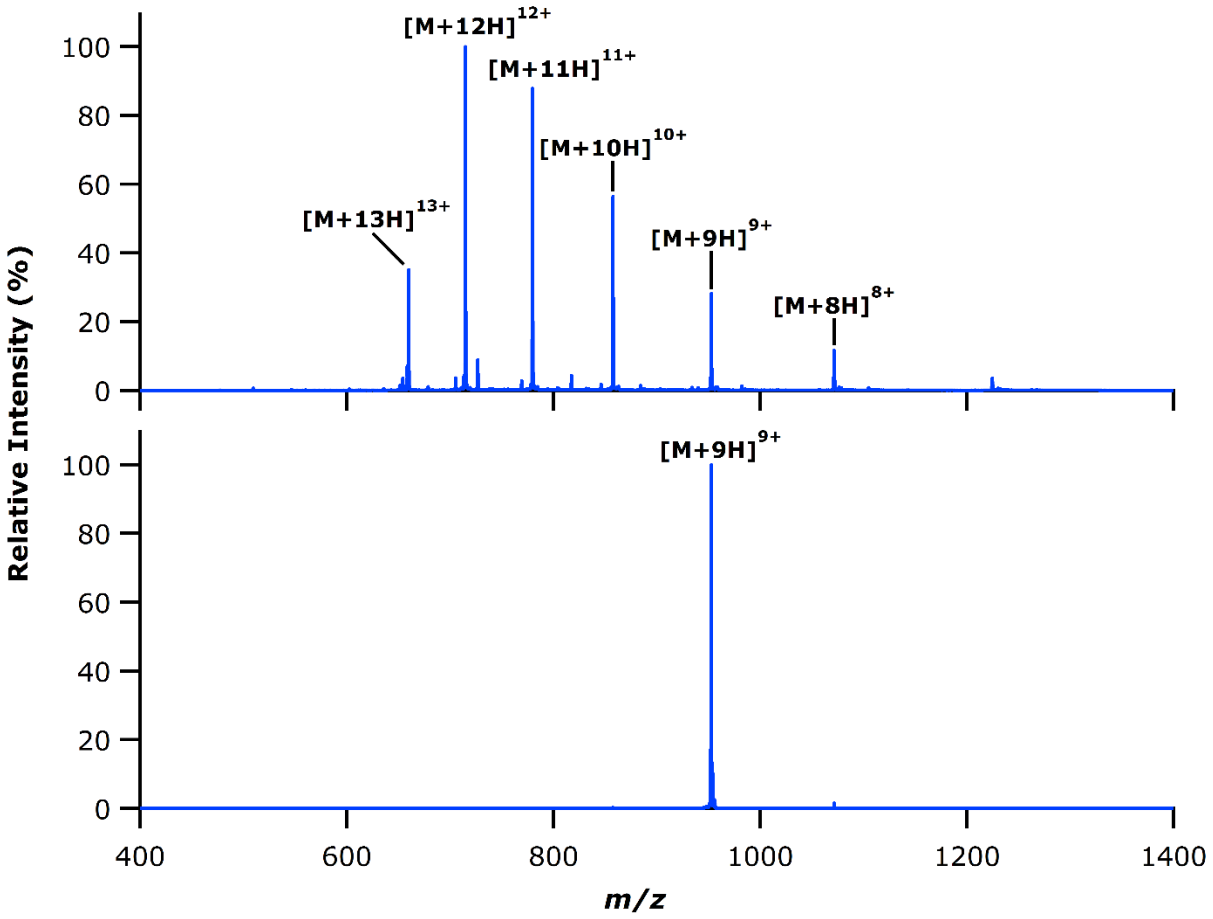

Figure S4. TOF-MS spectrum of the described ubiquitin solution (upper trace), and Q-TOF-MS selection of the $[M+9H]^{9+}$ molecular ion at *m/z* = 952. The subsequent ET-IM-VA analysis of this precursor ion is shown in ***Figure 5*** of the main article. In the lower trace, the appearance of a small peak (~1% relative intensity) corresponding to the $[M+9H]^{8+\bullet}$ ion is attributed to ET reactions occurring during a short co-residence time of precursor ions with ET reagent ions in the trap cell of the instrument. This occurs even when the trap cell traveling DC wave height is elevated with the intent of minimizing co-residence time in order to prevent ET reactions. Because the proportion of the ET product ion is so small in this precursor selection spectrum, this had essentially no impact on the overall analysis.

Table S1. CID and ETD fragmentation efficiency (FE) obtained by ET-IM-VA analysis of each polypeptide, calculated as: [(Fragment Area)(100%) / (Fragment Area + Precursor Area)].

Analyte	CID FE (%)	ETD FE (%)
Substance P	99.8	30.2
BHb Peptide	54.3	18.5
Glucagon	67.8	63.3
Ubiquitin	96.8	82.2