Sequencing Grade Tandem Mass Spectrometry for Top-Down Proteomics Using Hyrbrid Electron Capture Dissociation Methods in a Benchtop Orbitrap Mass Spectrometer

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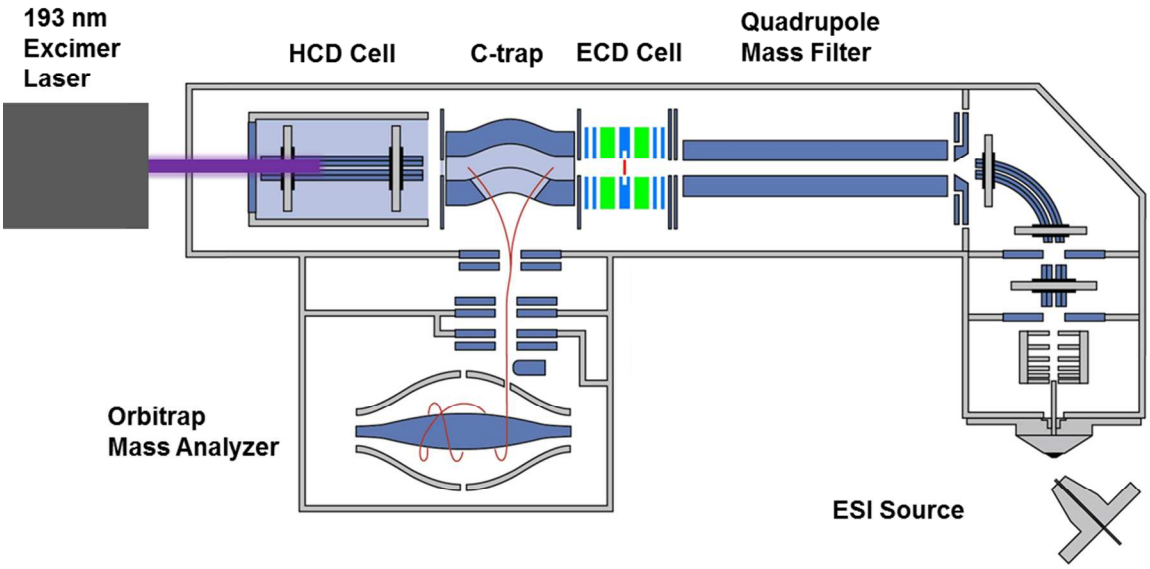


Figure S1. Instrument schematic showing the incorporation of the electromagnetostatic ECD cell in the Q Exactive instrument platform.

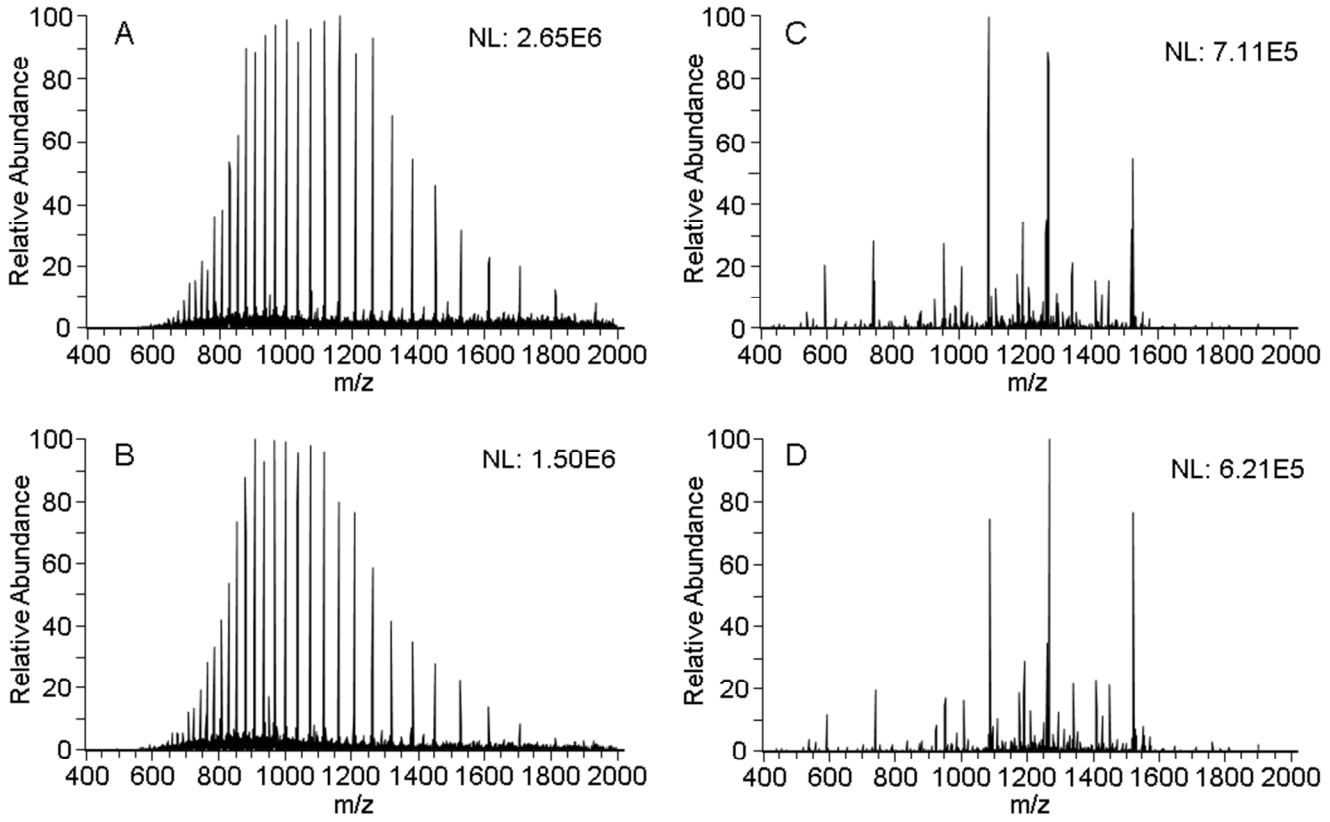


Figure S2. Transmission through the electromagnetostatic ECD cell was characterized with bovine carbonic anhydrase II and the instrument set to protein mode. MS1 spectra of carbonic anhydrase acquire without (A) and with (B) the ECD cell installed show approximate 50% reduction in transmission base on NL value (intensity of the most intense peak divided by ion injection time) with the ECD cell installed. However, nearly identical S/N (43.4 and 44.7 without and with the ECD cell, respectively) was achieved for the 30+ charge state (m/z=968) in the MS1 spectrum. This loss of transmission is likely not due to ECD cell. The measurements with the ECD cell were performed directly after bakeout of the vacuum system and UHV pressure was still approximate 2-3X higher than for the measurements performed without the ECD cell. Comparison of HCD spectra without (C) and with (D) the ECD cell installed show ~10% reduction in transmission. The y_{67}^{6+} ion (m/z=1267) was observed with S/N of 484 and 402 with and without the ECD cell, respectively. Once again this is likely due to the the pressure in the UHV portion of the vacuum chamber. Higher UHV pressure had a lower impact on detection of smaller fragments compared to the intact protein as expected due to the small collision cross section of the smaller product ions.

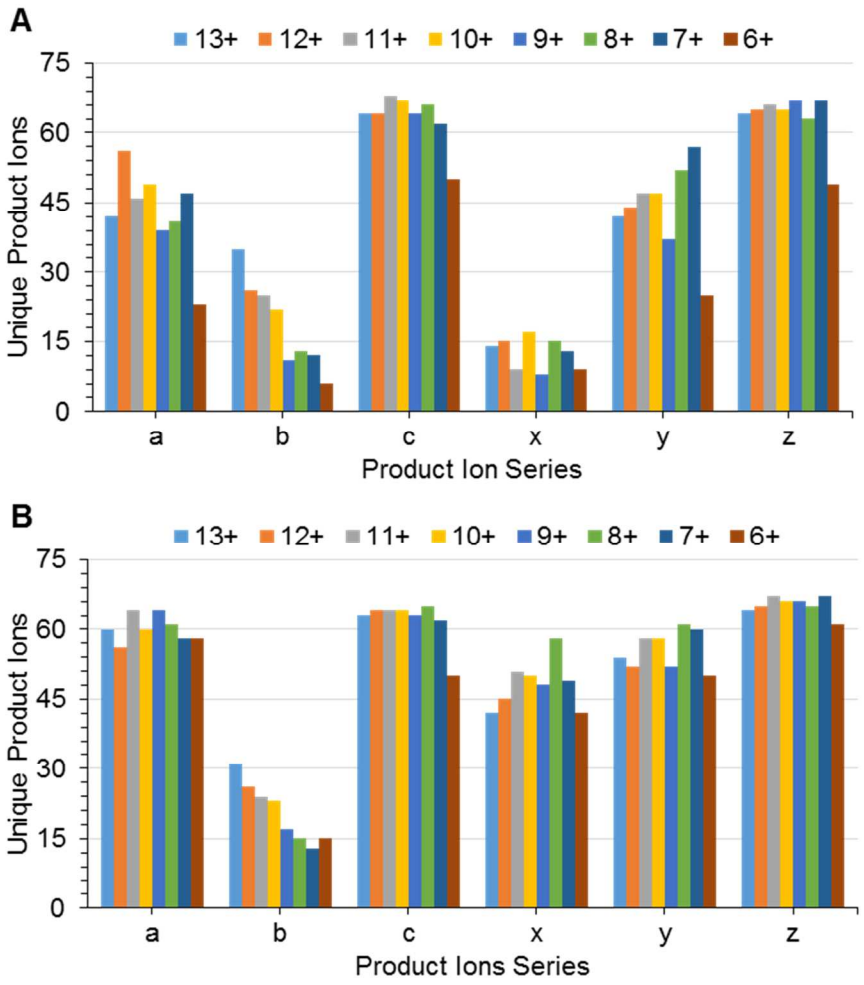


Figure S3. The number of unique product ions observed for ECD (A) and ECuvPD (B) as a function of precursor ion charge state for ubiquitin.

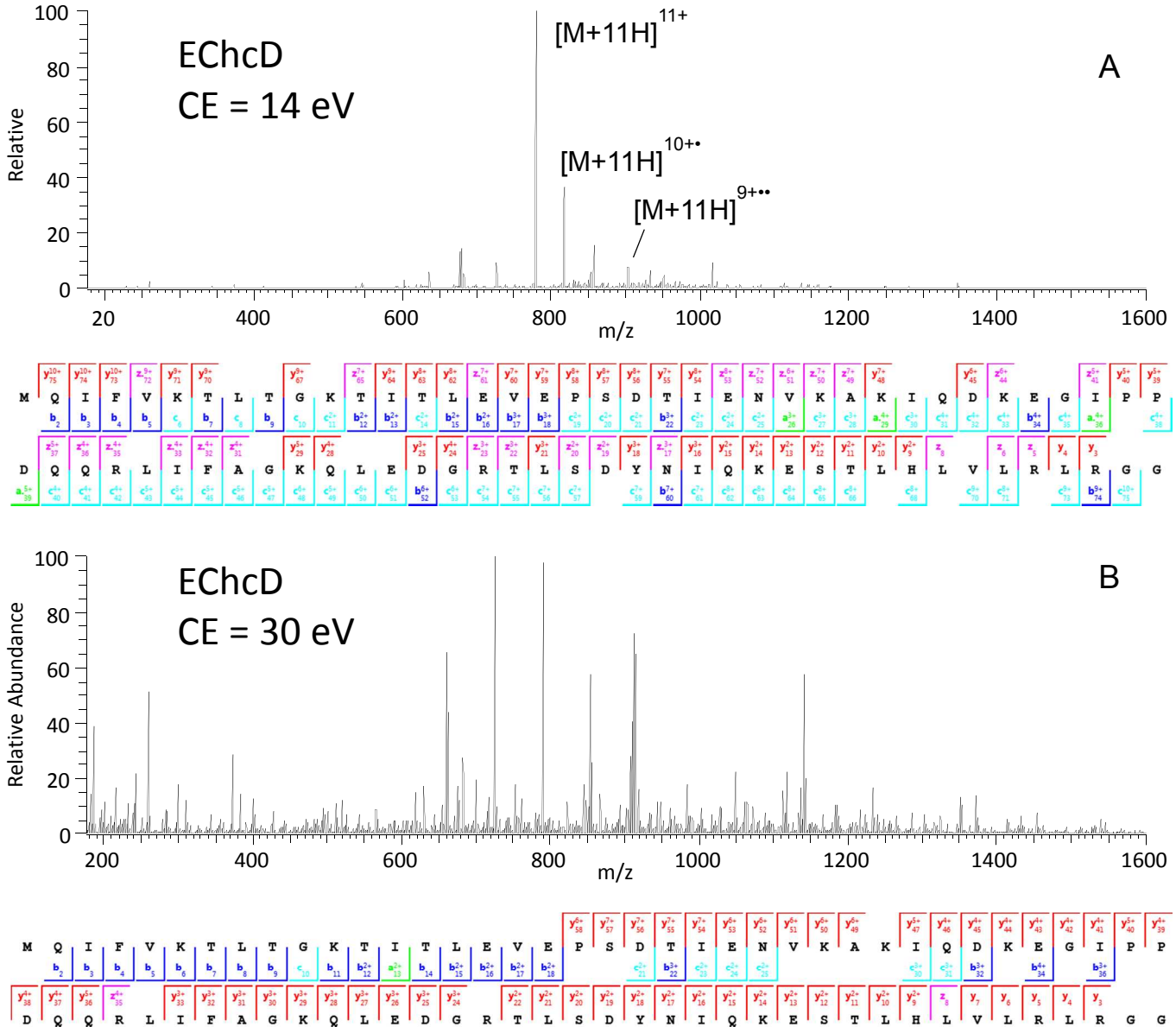


Figure S4. EChcD spectra and product ion maps generated with HCD collision energies of 14 eV (A) and 30 eV (B) for the 11+ charge state of ubiquitin.

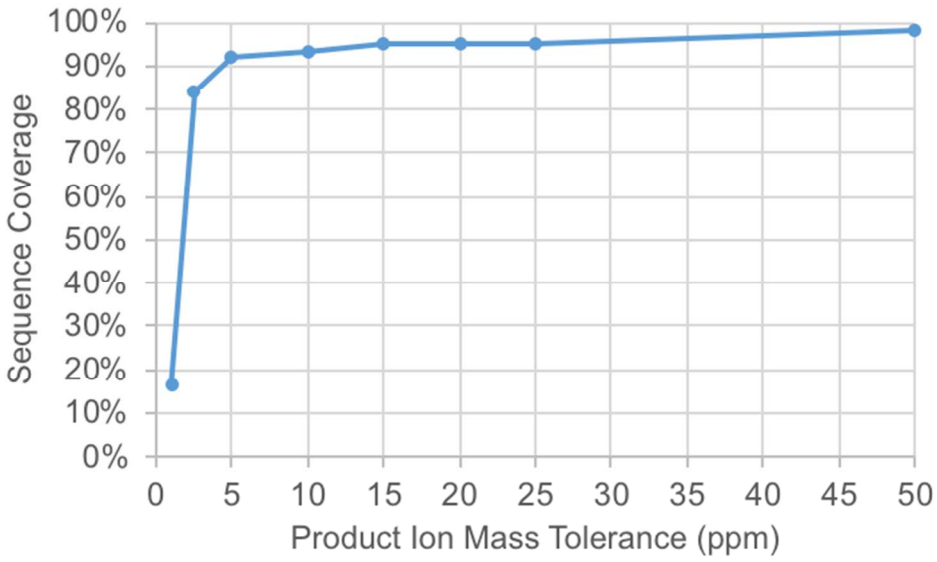
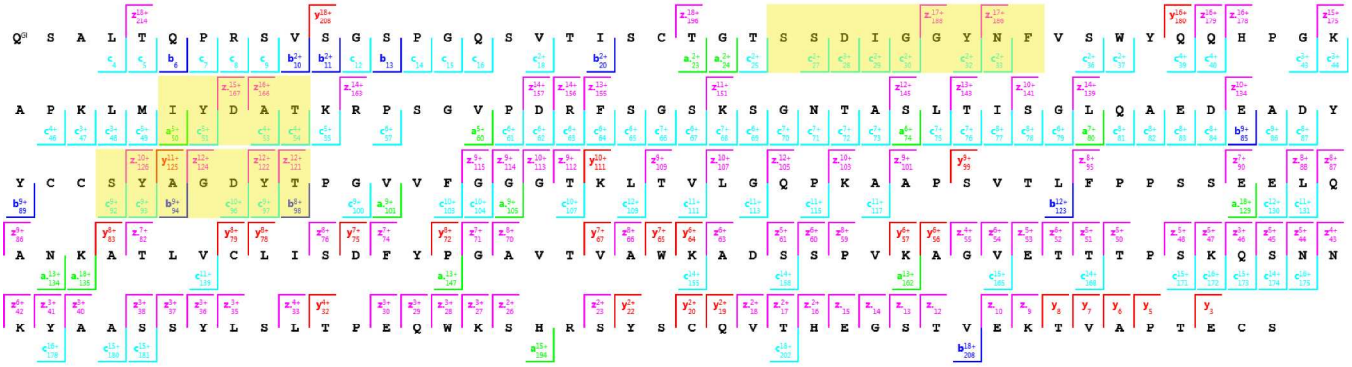
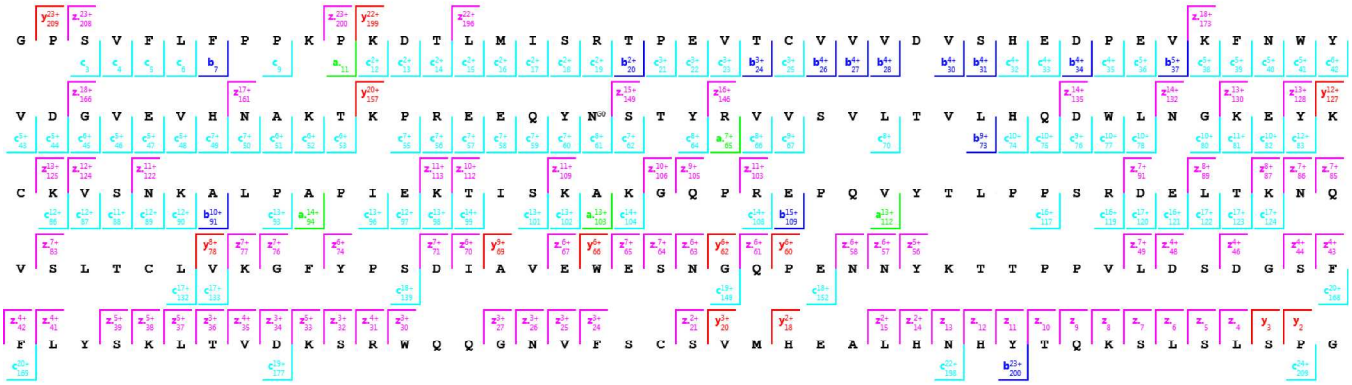


Figure S5. Sequence coverage as function of product ion mass tolerance for ECD of the 25+ charge state of bovine carbonic anhydrase II. The rms mass error for product ions annotated with 5 ppm mass tolerance was 2.68 ppm.

$[M+20H]^{20+}$ LC: 74% sequence coverage



$[M+25H]^{25+}$ Fc/2: 78% sequence coverage



$[M+24H]^{24+}$ Fd: 56% sequence coverage

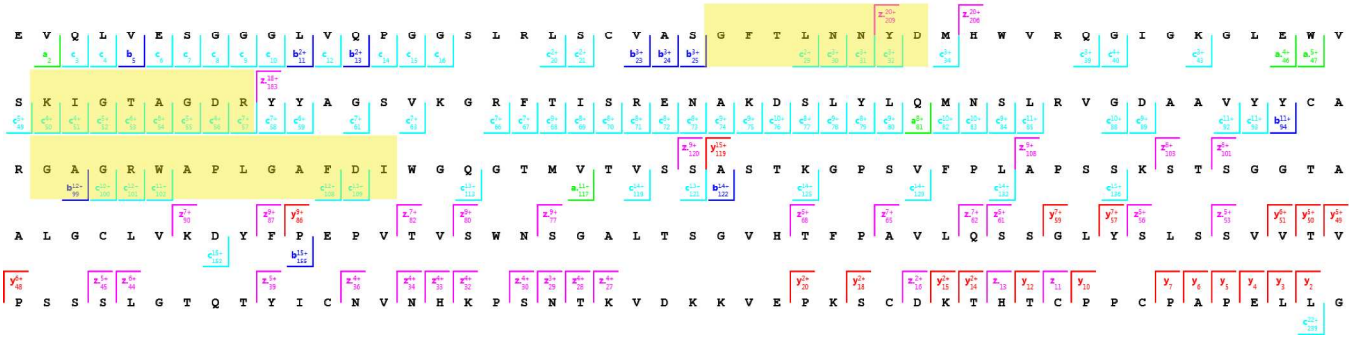
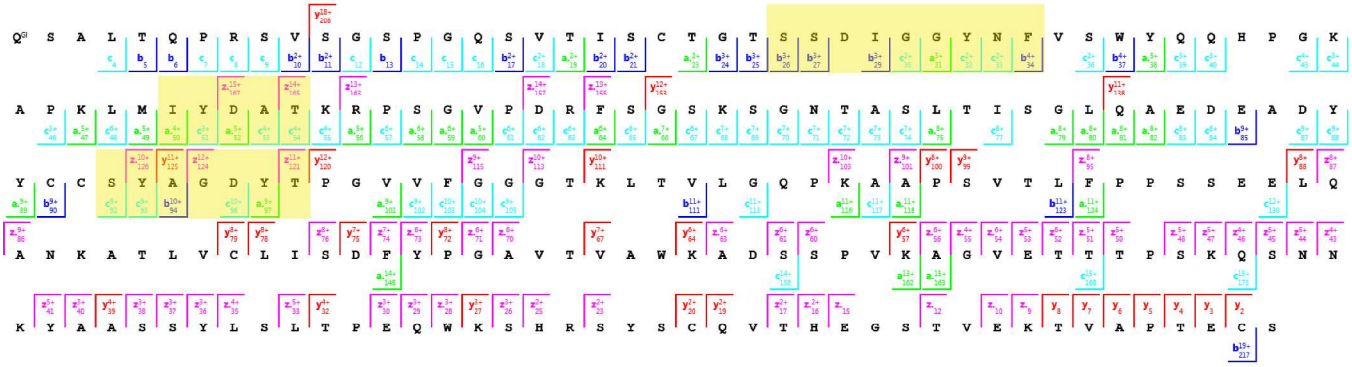
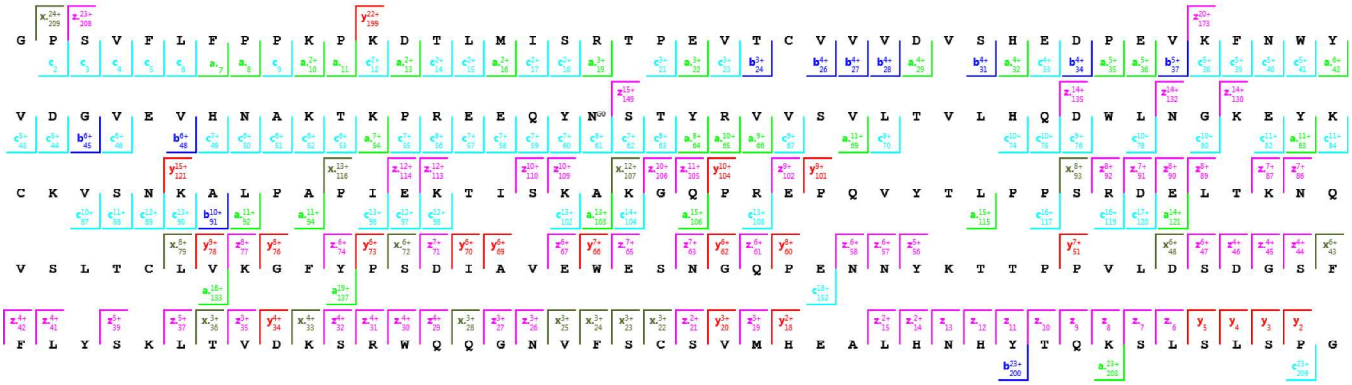


Figure S6. Product ion maps for SigmaMAB subunits generated from a single targeted LC-MS/MS experiment using ECD. Complementarity determining regions (CDRs) are highlighted in yellow.

$[M+20H]^{20+}$ LC: 75% sequence coverage



$[M+25H]^{25+}$ Fc/2: 79% sequence coverage



$[M+24H]^{24+}$ Fd: 59% sequence coverage

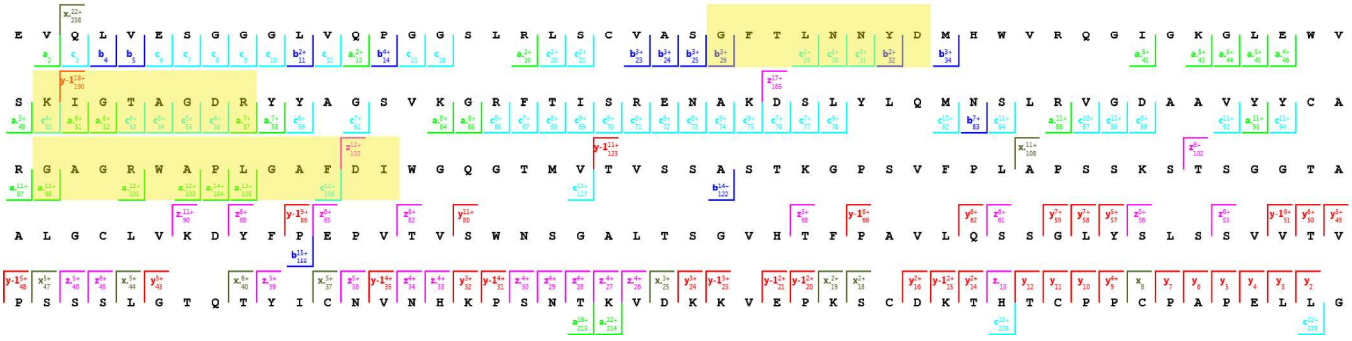
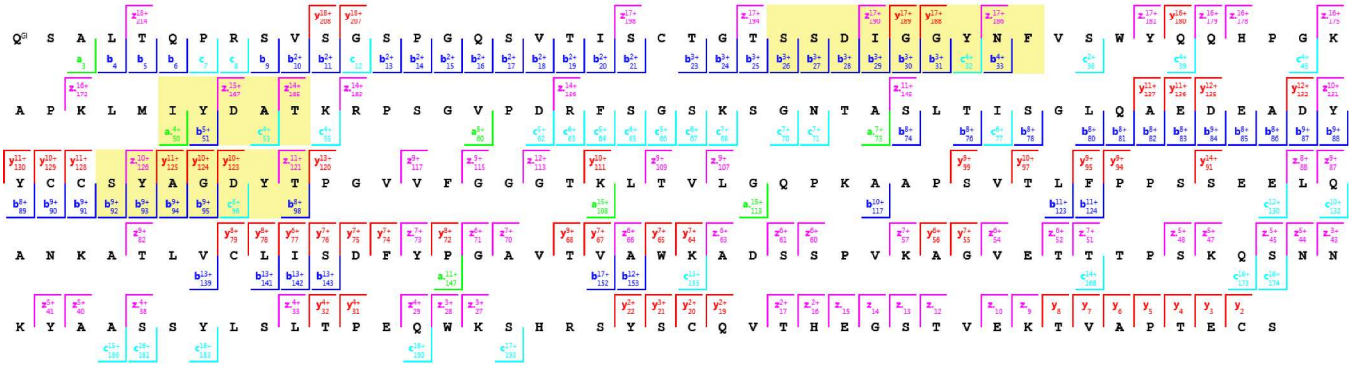
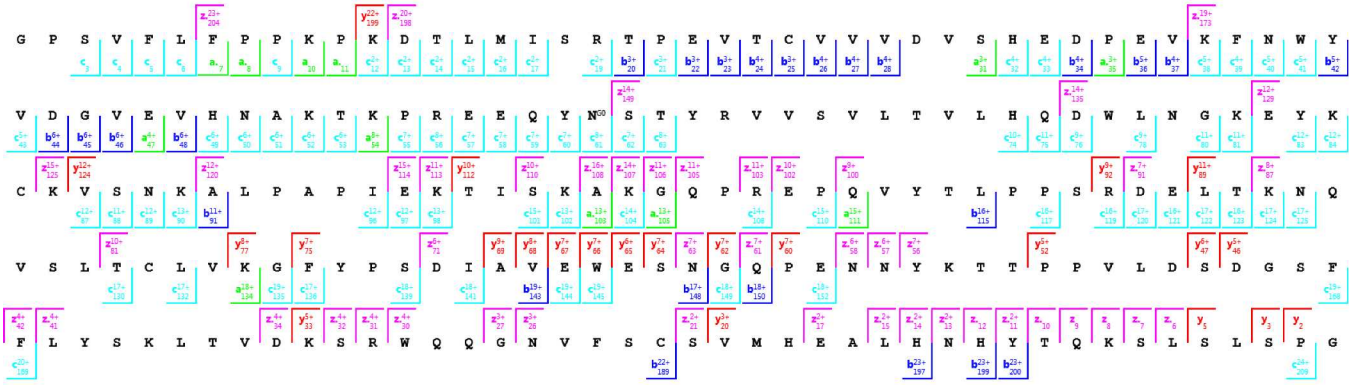


Figure S7. Product ion maps for SigmaMAB subunits generated from a single targeted LC-MS/MS experiment using ECuvPD (one laser pulse at 0.5 mJ). Complementarity determining regions (CDRs) are highlighted in yellow.

$[M+20H]^{20+}$ LC: 71% sequence coverage



$[M+25H]^{25+}$ Fc/2: 71% sequence coverage



$[M+24H]^{24+}$ Fd: 46% sequence coverage

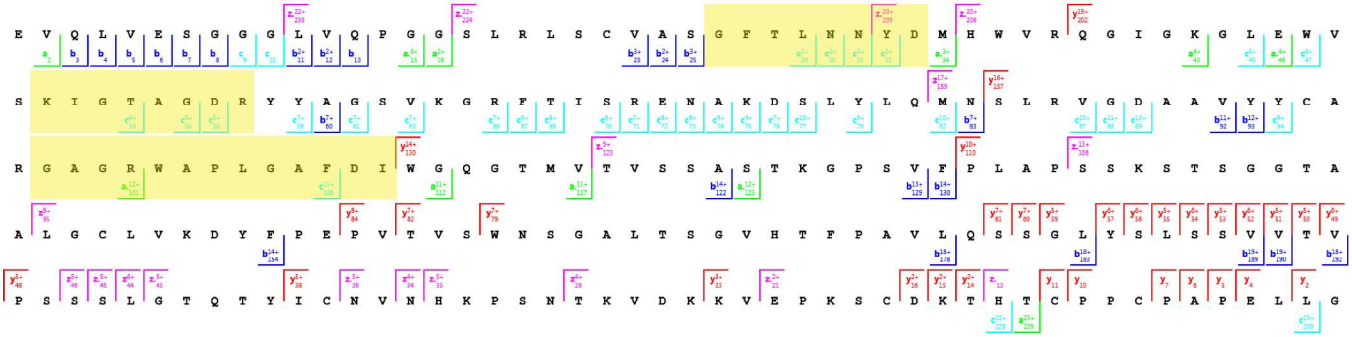


Figure S8. Product ion maps for SigmaMAB subunits generated from a single targeted LC-MS/MS experiment using EChcD (12 eV collision energy). Complementarity determining regions (CDRs) are highlighted in yellow.