Electronic Supplementary Information

for

Gas-Phase Microsolvation of Ubiquitin: Investigation of Crown Ether Complexation Sites using Ion Mobility-Mass Spectrometry

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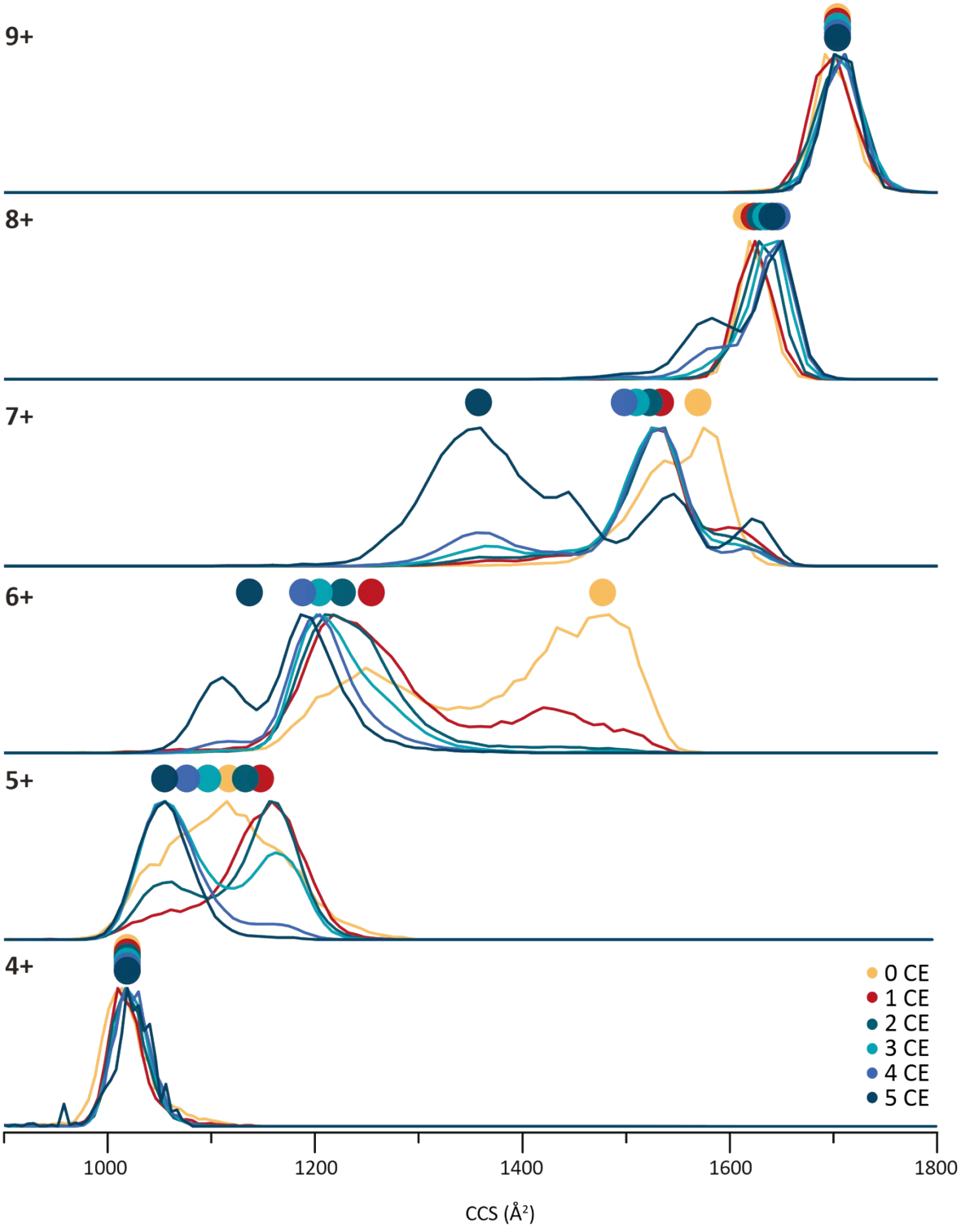

Figure S1. Collision cross sections (CCSs)[1,2] of ubiquitin wild type for different charge states and different numbers of attached crown ether molecules (CE). The average size increase of the protein per additional CE molecule (1.6% to 1.7%) is subtracted from the CCS of the complex. Intermediate charge states (5+ to 7+) undergo a compaction, whereas globular (4+) and unfolded (8+ and 9+) ions do not show this effect upon crown-ether attachment. The compaction of the intermediate charge states differs slightly depending on the charge state: 5+ ions adopt a compact form (~1050 Å²) with 2CE to 3CE attached, whereas 6+ and 7+ both first adopt an intermediate form (~1250 Å² for 6+ and

~1350 Å^2 for 7+) with 1CE to 4CE bound and only 6+ also shows the compact form with 5CEs attached (~1100 Å^2).

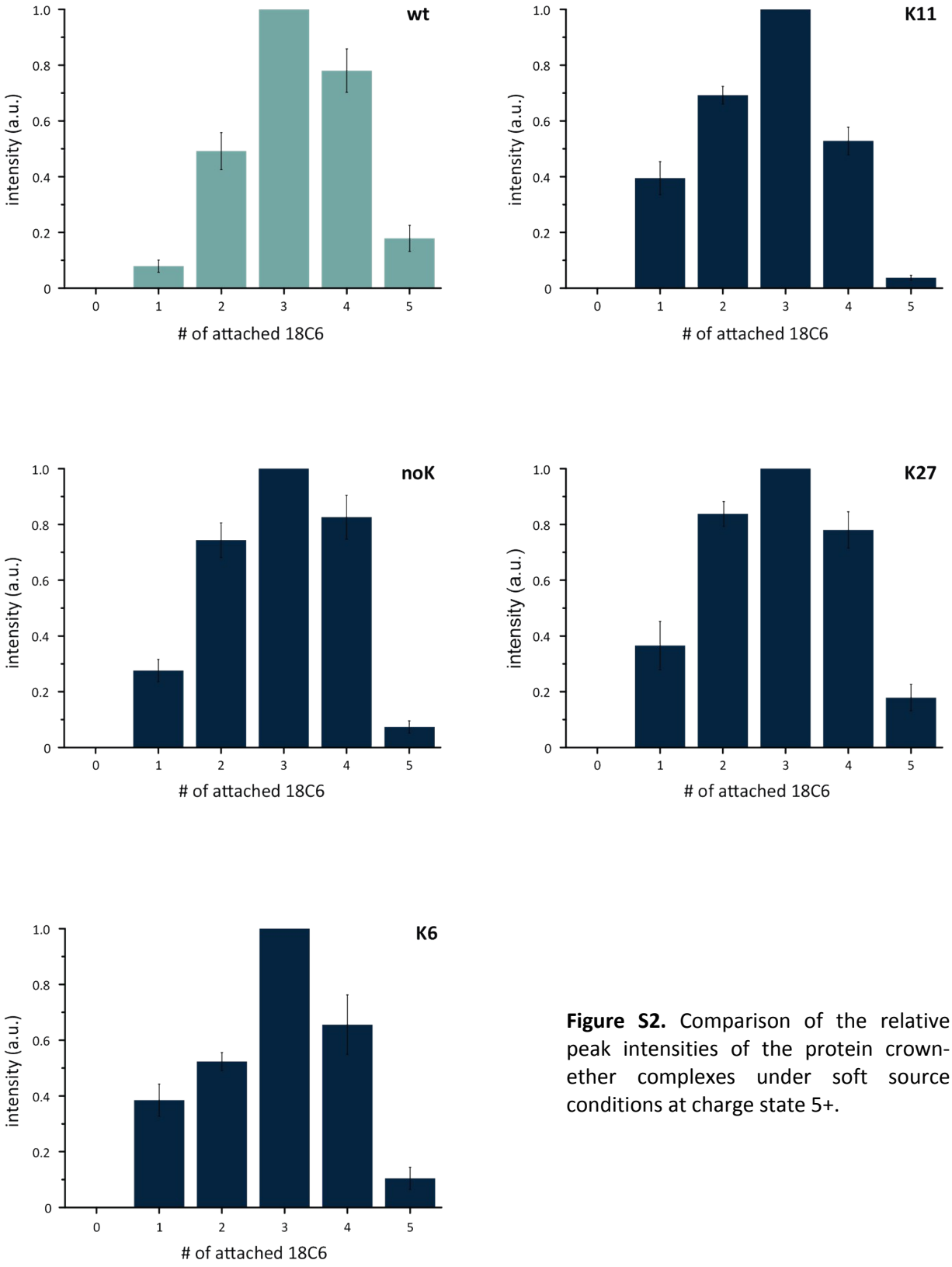

Figure S2. Comparison of the relative peak intensities of the protein crown-ether complexes under soft source conditions at charge state 5+.

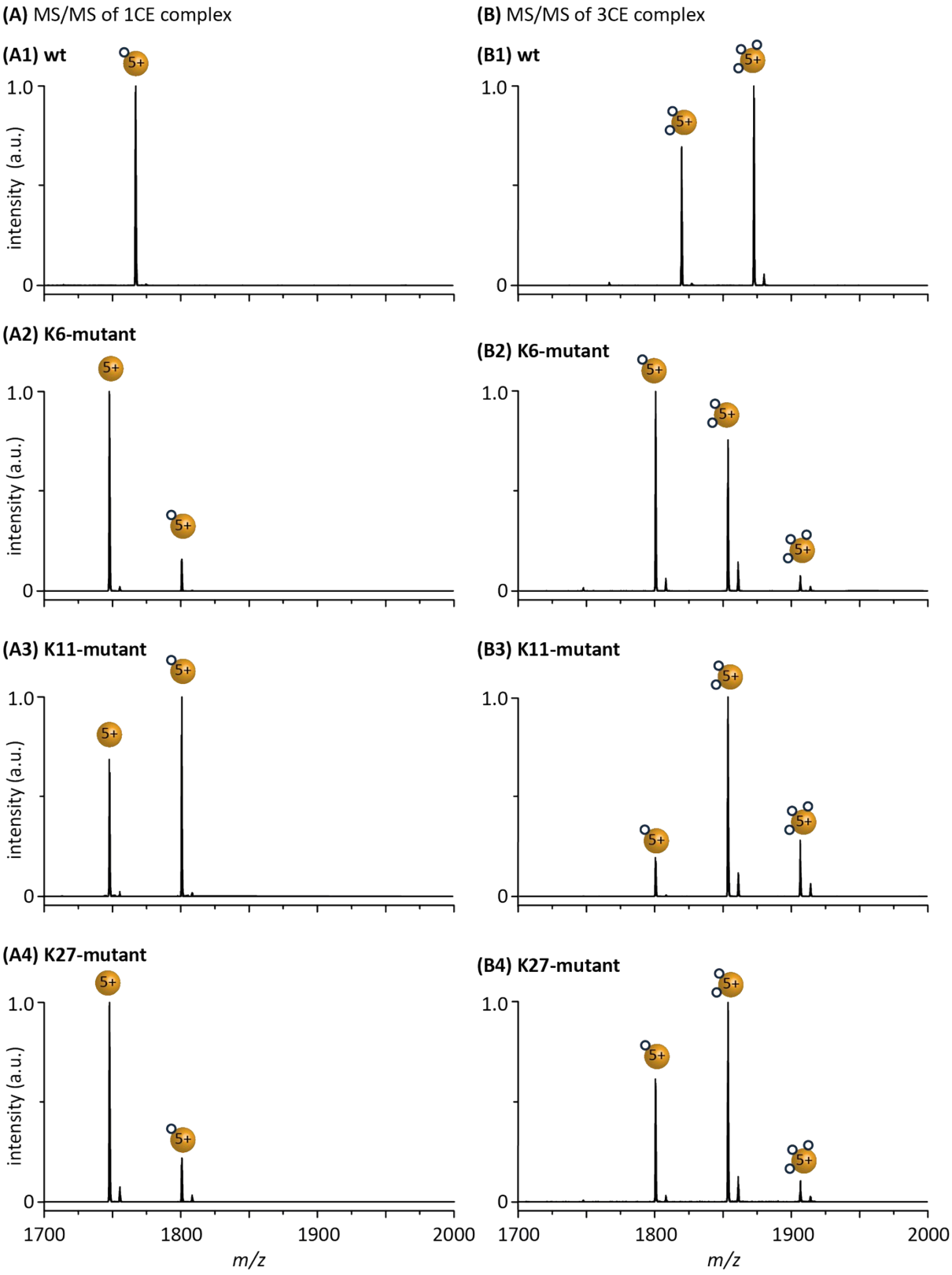

Figure S3. MS/MS-experiments of both wild type and mutants 1CE complexes at 16V collision voltage (A1 - A4) and 3CE complexes at 10V collision voltage (B1 - B4). At 9 V collision voltage (Figure 4, noK) very low fragmentation is observed for the wt and the other variants.

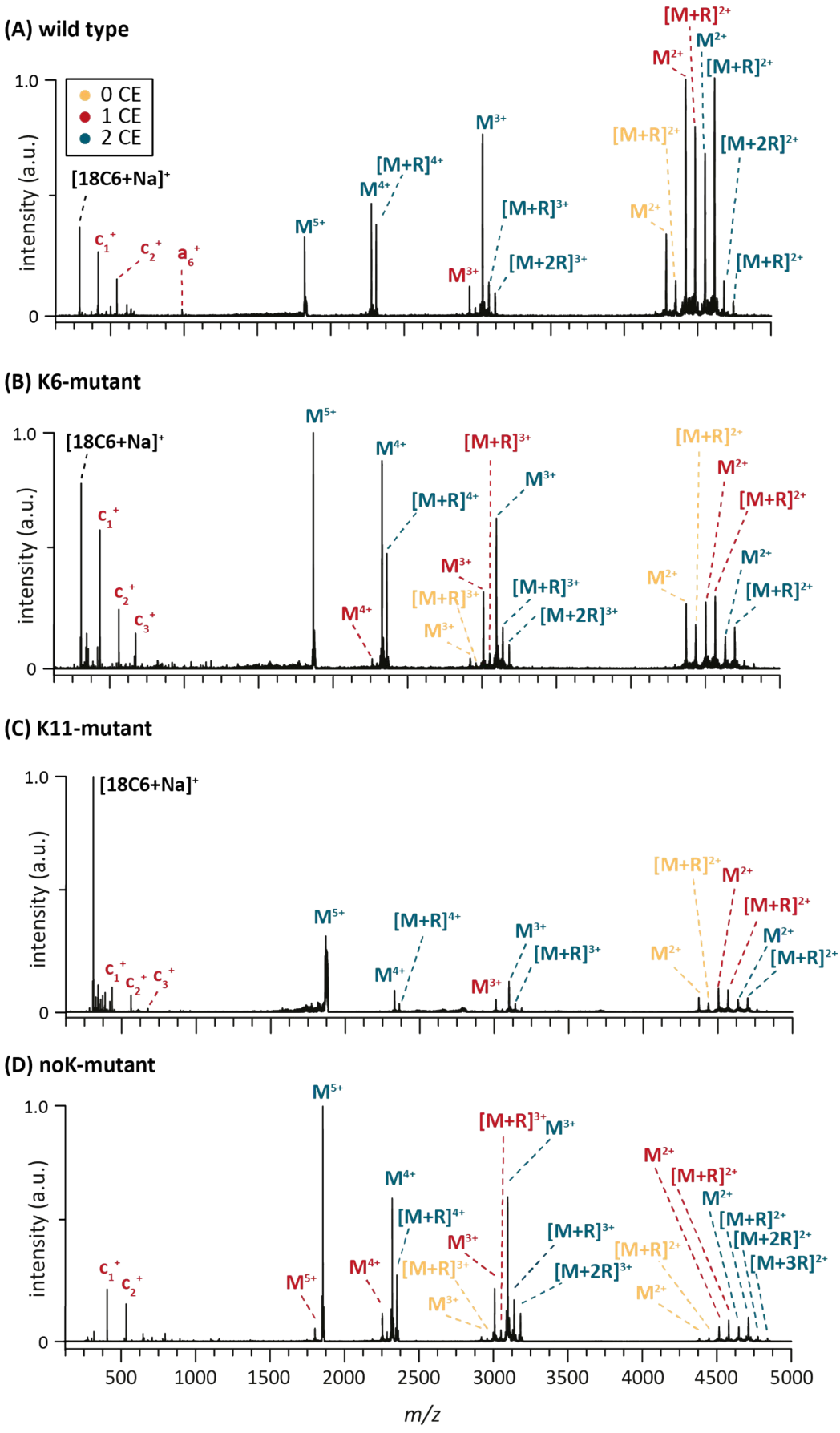

Figure S4. Complete ETD spectra for a 5+ precursor complex (M^{5+}) with two CEs. (A) wild type ubiquitin, (B) K6-mutant, (C) K11-mutant, (D) noK-mutant.

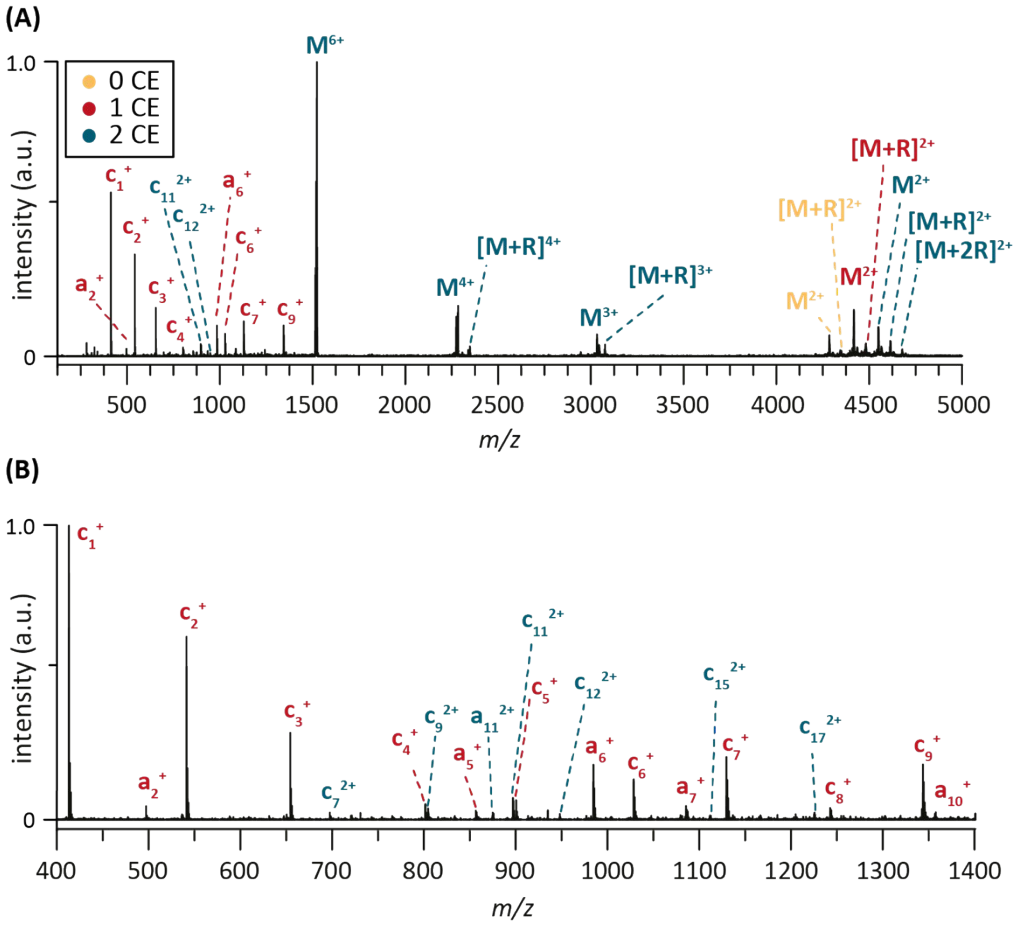

Figure S5. (A) Complete ETD spectrum for a 6+ precursor wt-complex with two CEs (M^{6+}). (B) Extension of the *m/z* range 400 to 1400. Fragments with one and two CEs attached to wt ubiquitin are observed. Intense peaks are formed, which indicate N-terminal binding of the first CE (c_1^+, a_2^+, c_2^+, c_3^+, a_4^+, c_4^+, a_5^+, c_5^+), but also fragments with one CE indicating attachment to the N-terminus or to K6 (a_6^+, c_6^+, a_7^+, c_7^+, c_8^+, c_9^+, a_{10}^+) appear with reasonable intensity. The fragments $c_7^+$and c_9^+ with two CEs (green) show N-terminal and K6-binding, whereas the longer sequenced fragments (a_{11}^{2+}, c_{11}^{2+}, c_{12}^{2+}, c_{15}^{2+} and c_{17}^{2+} could also indicate binding to K11.

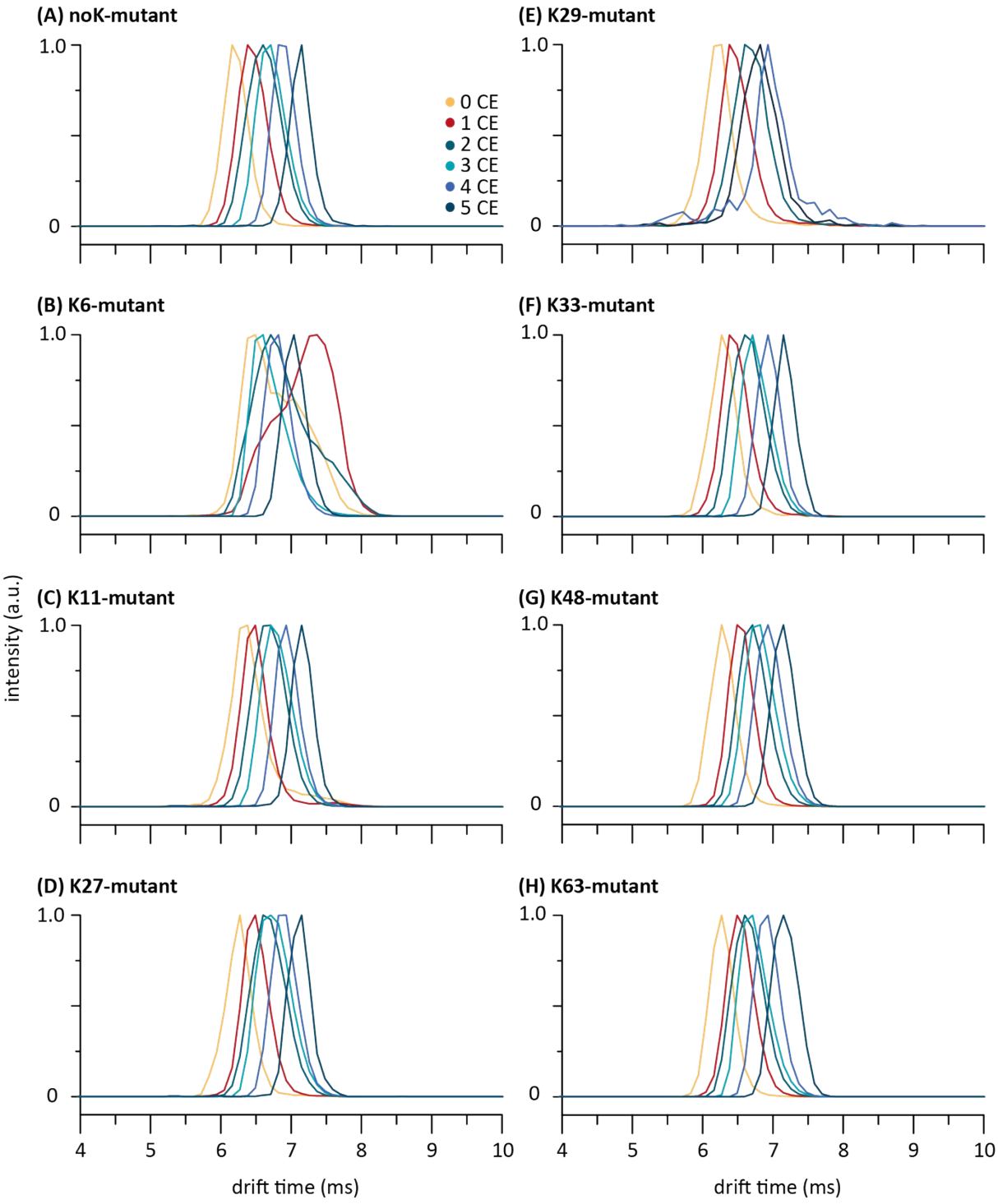

Figure S6. Arrival time distributions (ATDs) recorded with a wave velocity of 700 m/s show charge state 5+ for all possible lysine-to-arginine mutants (A) without lysine and (B) - (H) with one remaining lysine. The different colours indicate different numbers of crown-ether (CE) molecules attached (from 0 to 5 CE complex). Only the K6-mutant shows the same conformational heterogeneity as the wt and structural compaction is observed upon attachment of CEs. ATDs of the other mutants are homogeneous and narrow. They are compact already without the addition of CEs and do not show a significant change in structure when CEs are attached.

[1] The drift times that were recorded with the Synapt G2-S were measured in nitrogen and calibrated with $^{DT}CCS_{He}$ values determined with a drift-tube instrument as described in the manuscript. This generates pseudo helium CCSs.

[2] M. F. Bush, Z. Hall, K. Giles, J. Hoyes, C. V. Robinson and B. T. Ruotolo, *Anal. Chem.* 2010, **82**, 9557-9565.