Structural characterization of carbonic anhydrase•arylsulfonamide complexes using ultraviolet photodissociation mass spectrometry

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Supporting Information

Figure S1. A) Crystal structure of human carbonic anhydrase II bound to zinc and complexed with ethoxzolamide (PDB 3CAJ) with sheets in yellow, loops in green, and α-helices in red. The residues that form the hydrophilic pocket (light blue), the hydrophobic pocket (pink), and the residues that interact with zinc (yellow) are shown as sticks. Loop 1 is shaded in a pink circle, and loop 2 is shaded in a peach circle. Zinc is depicted as an orange sphere, and ethoxzolamide is the bright blue structure. B) β-strands and α-helices along hCAII sequence.

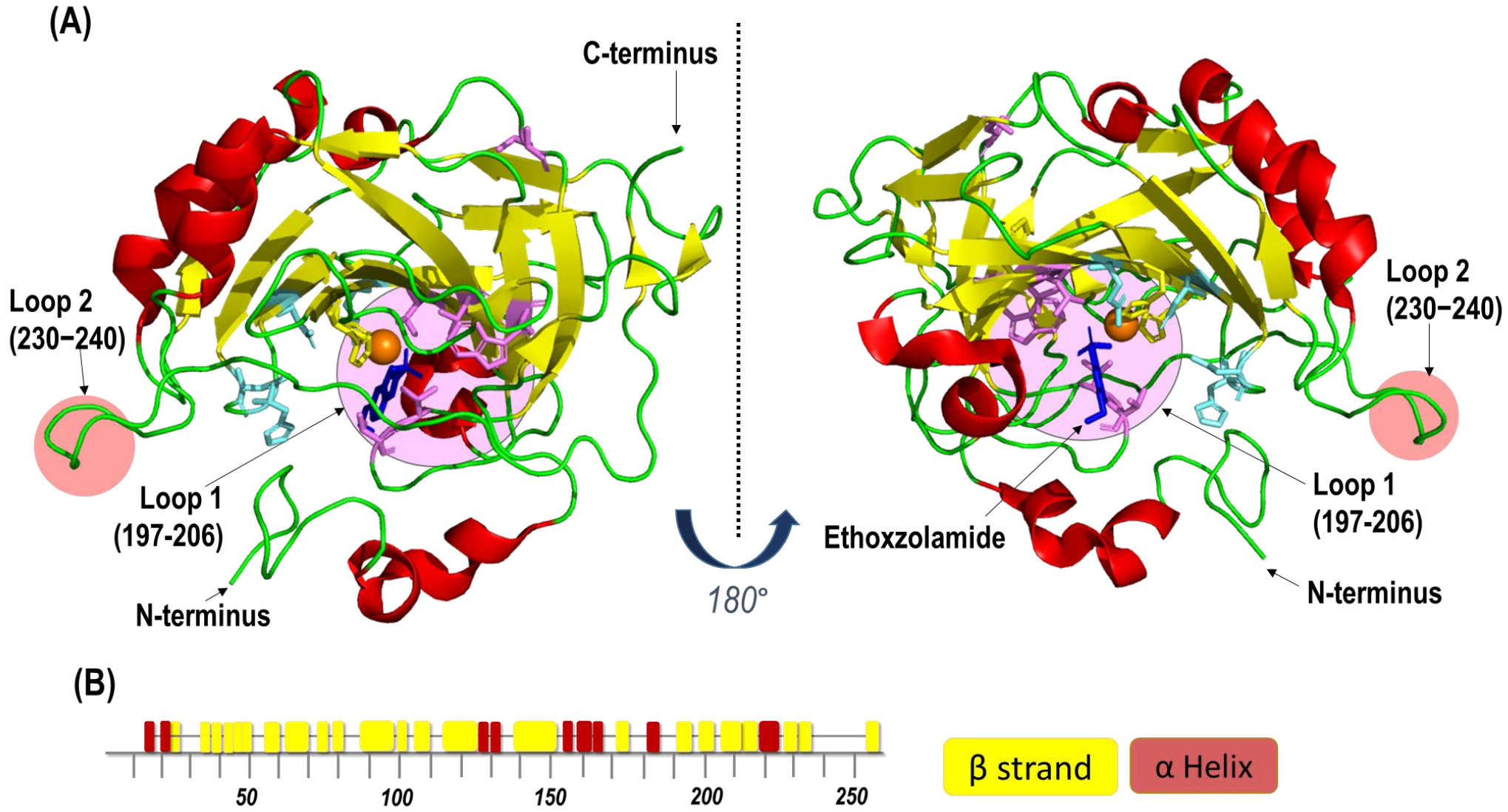

Figure S2. Sequence coverage map of hCAII for the 9 + charge state that corresponds to fragment ions produced upon UVPD (1 p 2 mJ).

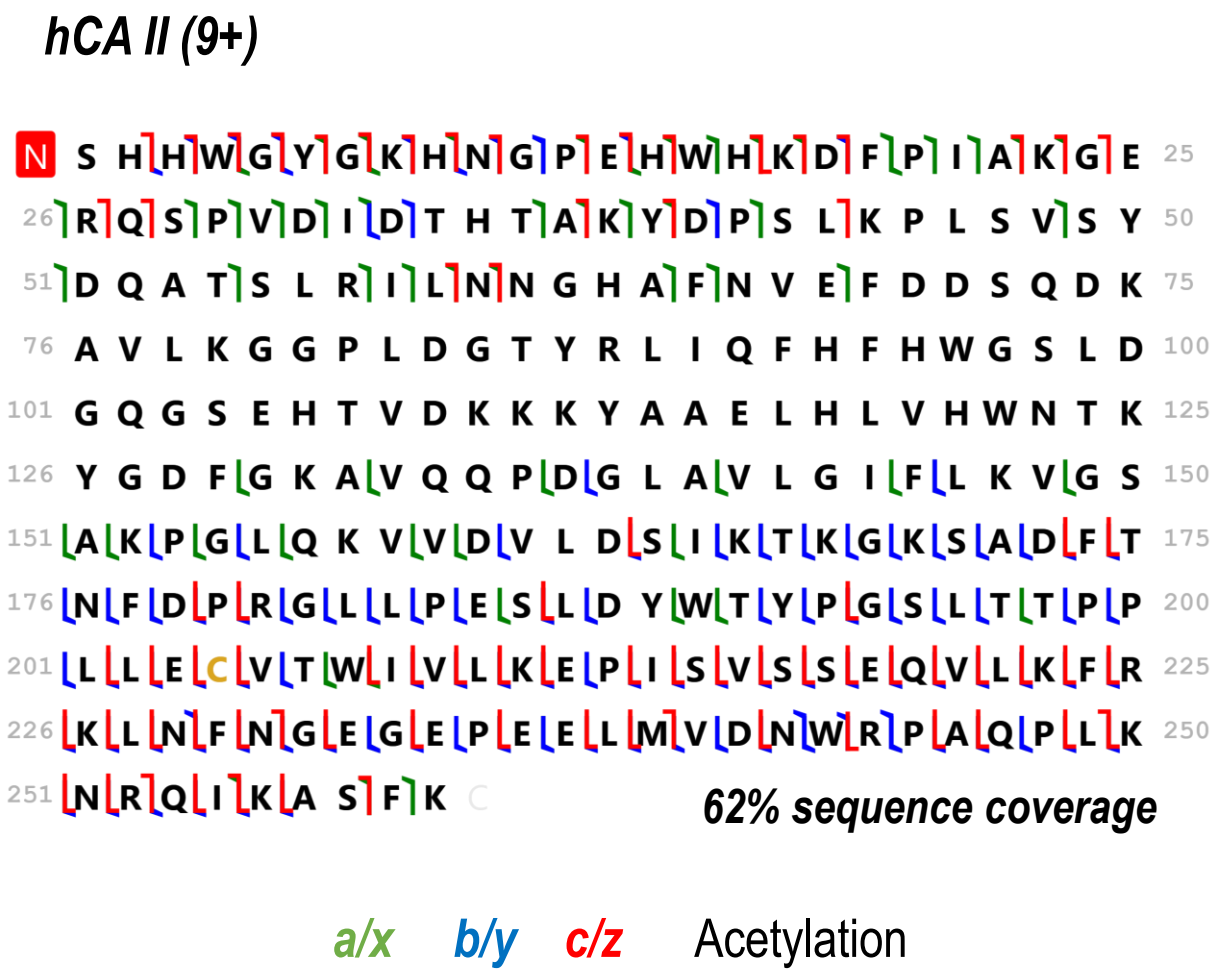

Figure S3. Percent sequence coverage (including all fragment ions with or without Zn), number of apo (without Zn) fragment ions, and number of holo (Zn containing) fragment ions generated from UVPD of all of the charge states based on analysis of both supercharging and native solutions for human carbonic anhydrase II. Error bars equal to standard deviation of triplicate data.

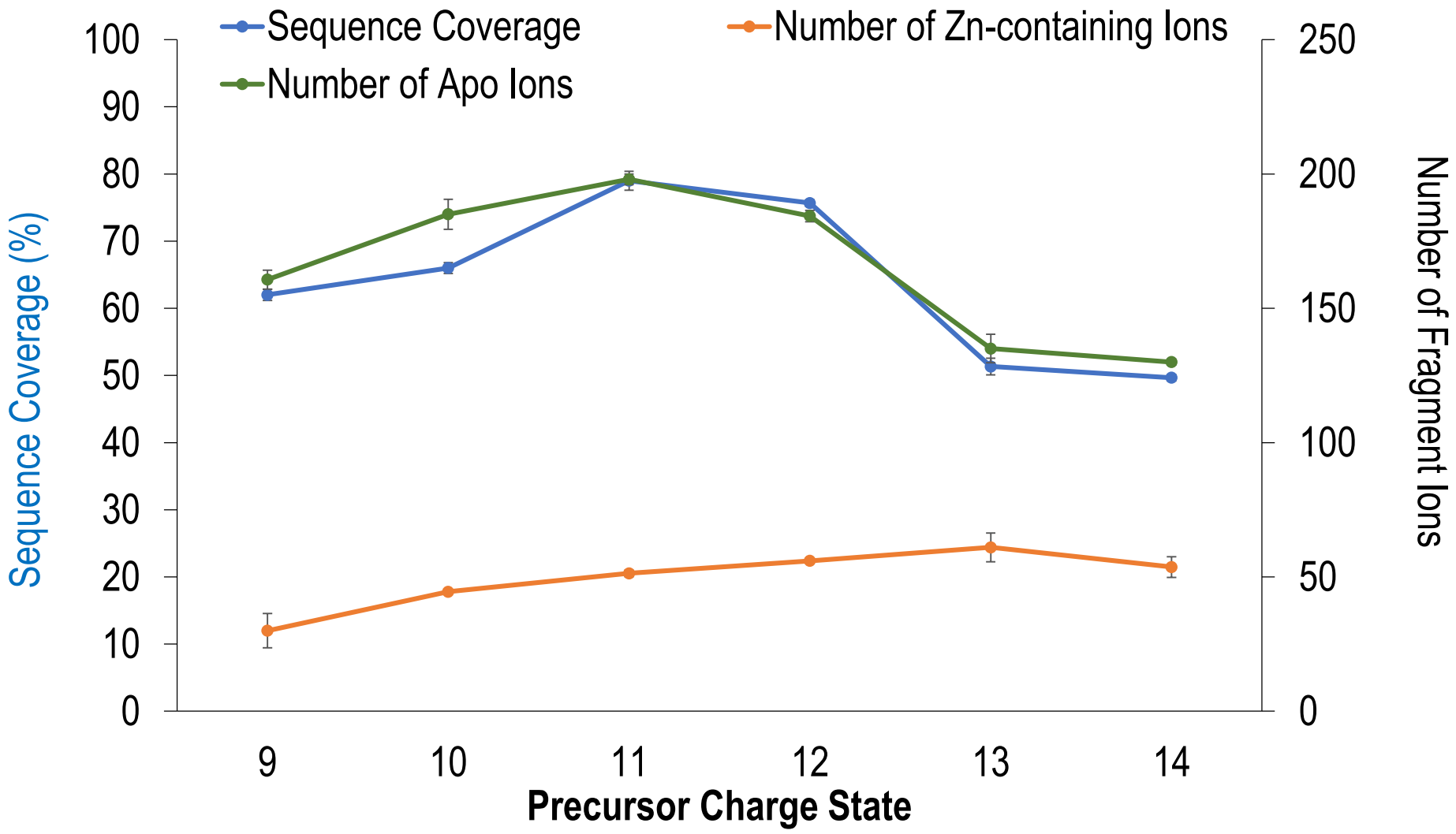

Figure S4. Supercharged ESI mass spectra of (A) hCAII (B) hCAII bound to ethoxzolamide (1:1 ratio). Highlighted is the 11+ charge state that was isolated and activated with UVPD. P represents the protein, and L indicates the bound ligand.

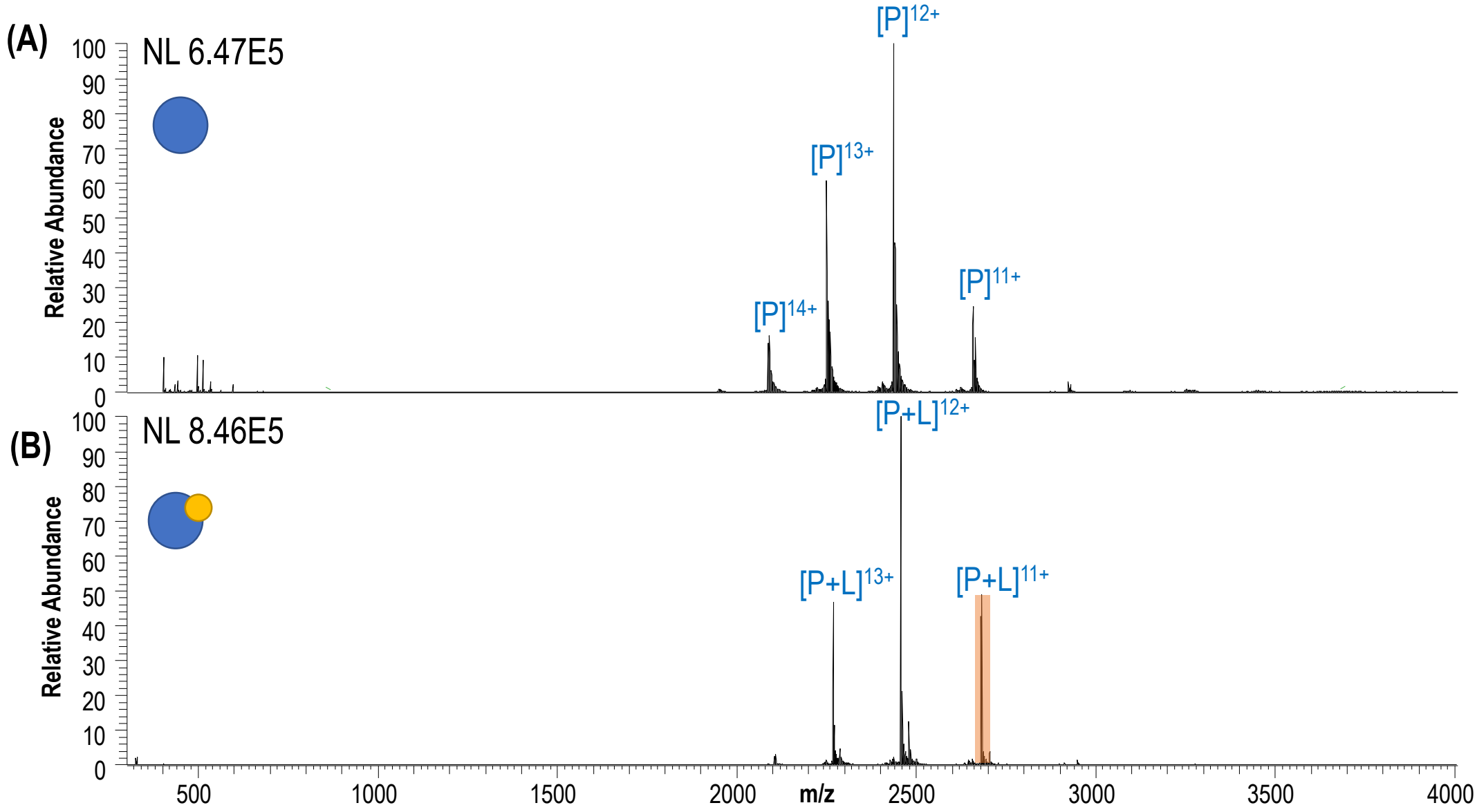

Figure S5. Supercharged ESI mass spectra of (A) hCAII (B) hCAII bound to furosemide (1:1 ratio). Highlighted is the 11+ charge state that was isolated and activated with UVPD. P represents the protein, and L indicates the bound ligand

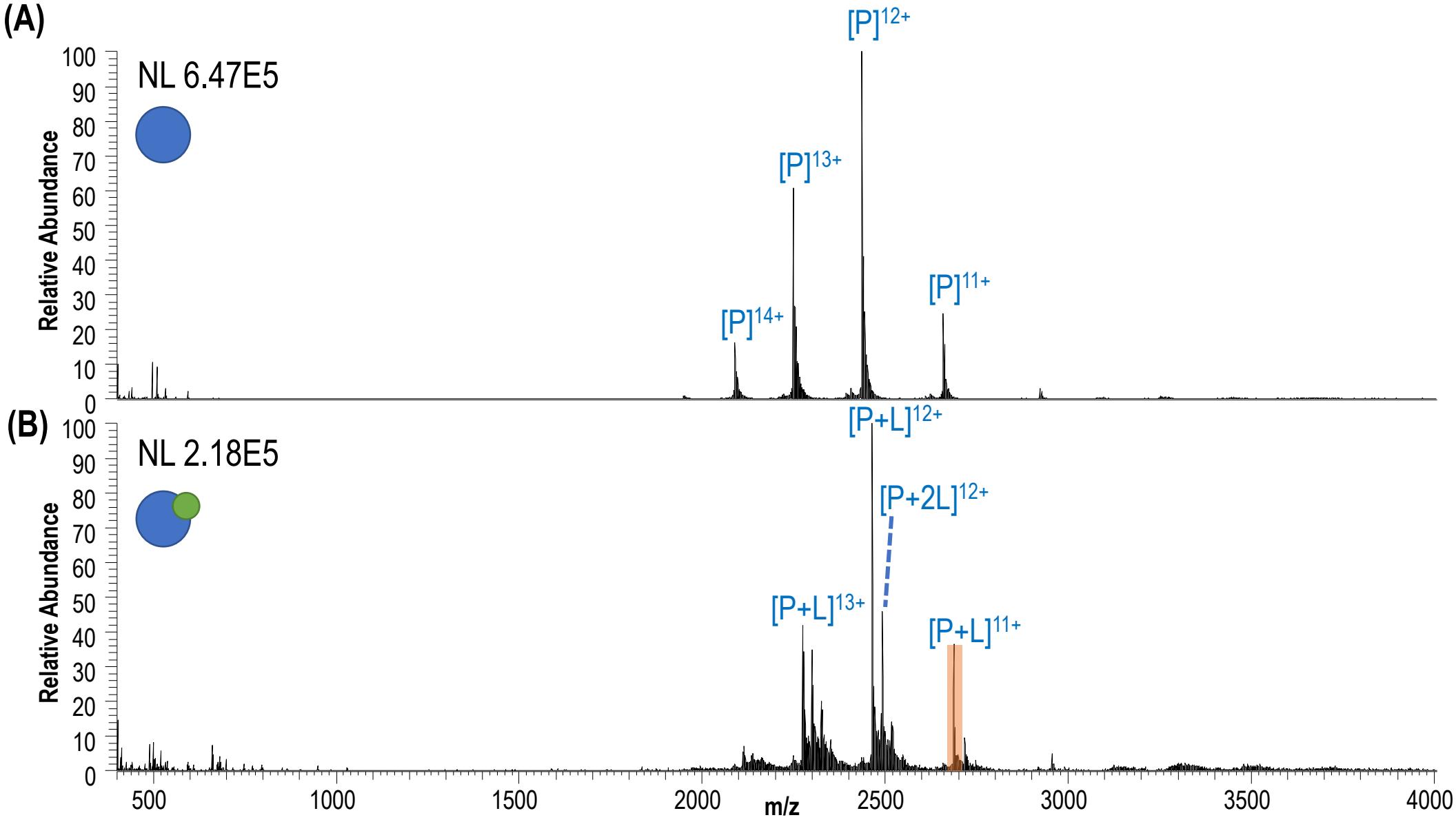

Figure S6. Supercharged ESI mass spectra of (A) hCAII (B) hCAII bound to chlorothiazide (1:1 ratio). Highlighted is the 11+ charge state that was isolated and activated with UVPD. P represents the protein, and L indicates the bound ligand

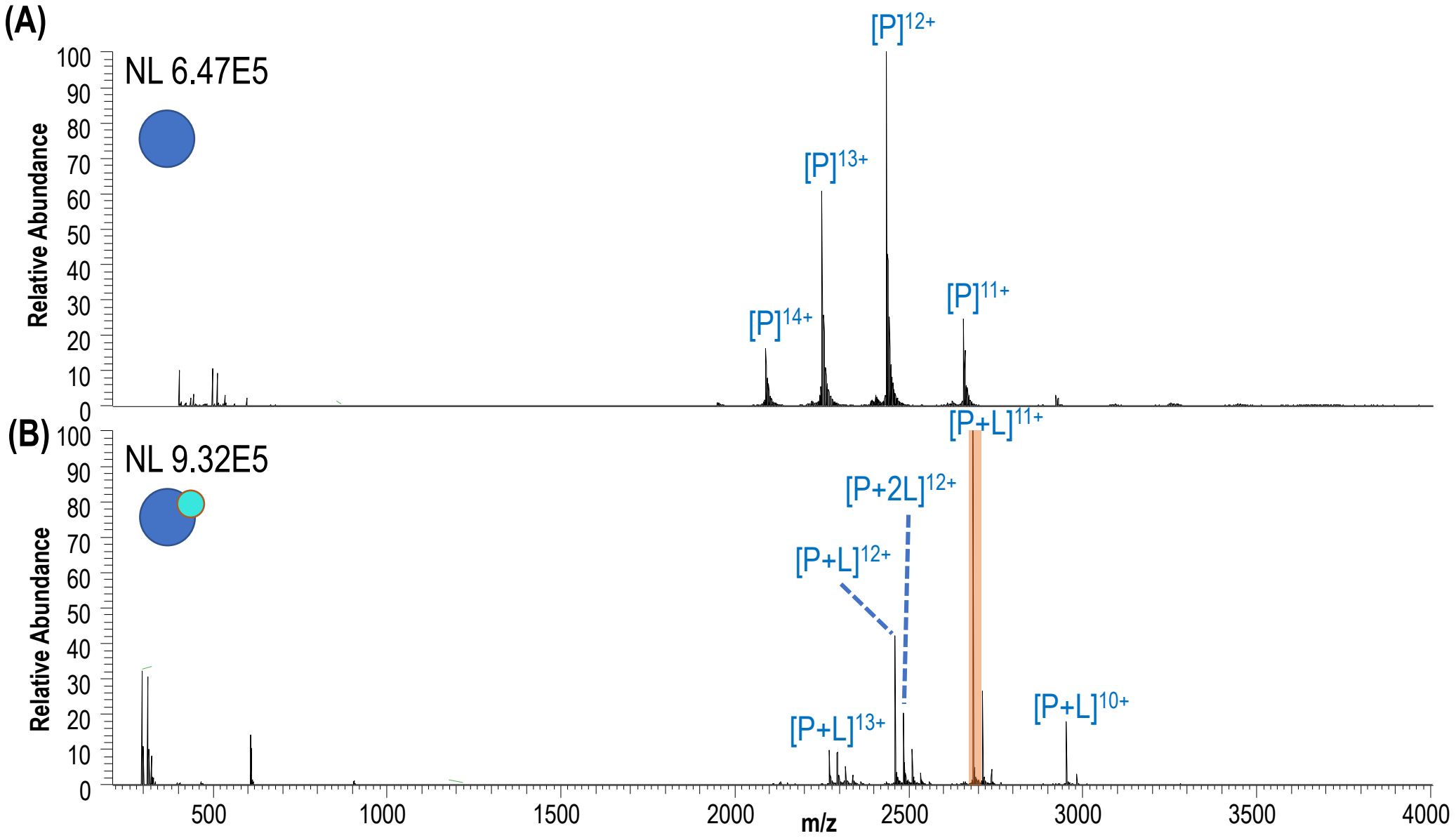

Figure S7. Native ESI mass spectra of (A) hCAII (B) hCAII bound to ethoxzolamide (1:1 ratio). P represents the protein, and L indicates the bound ligand

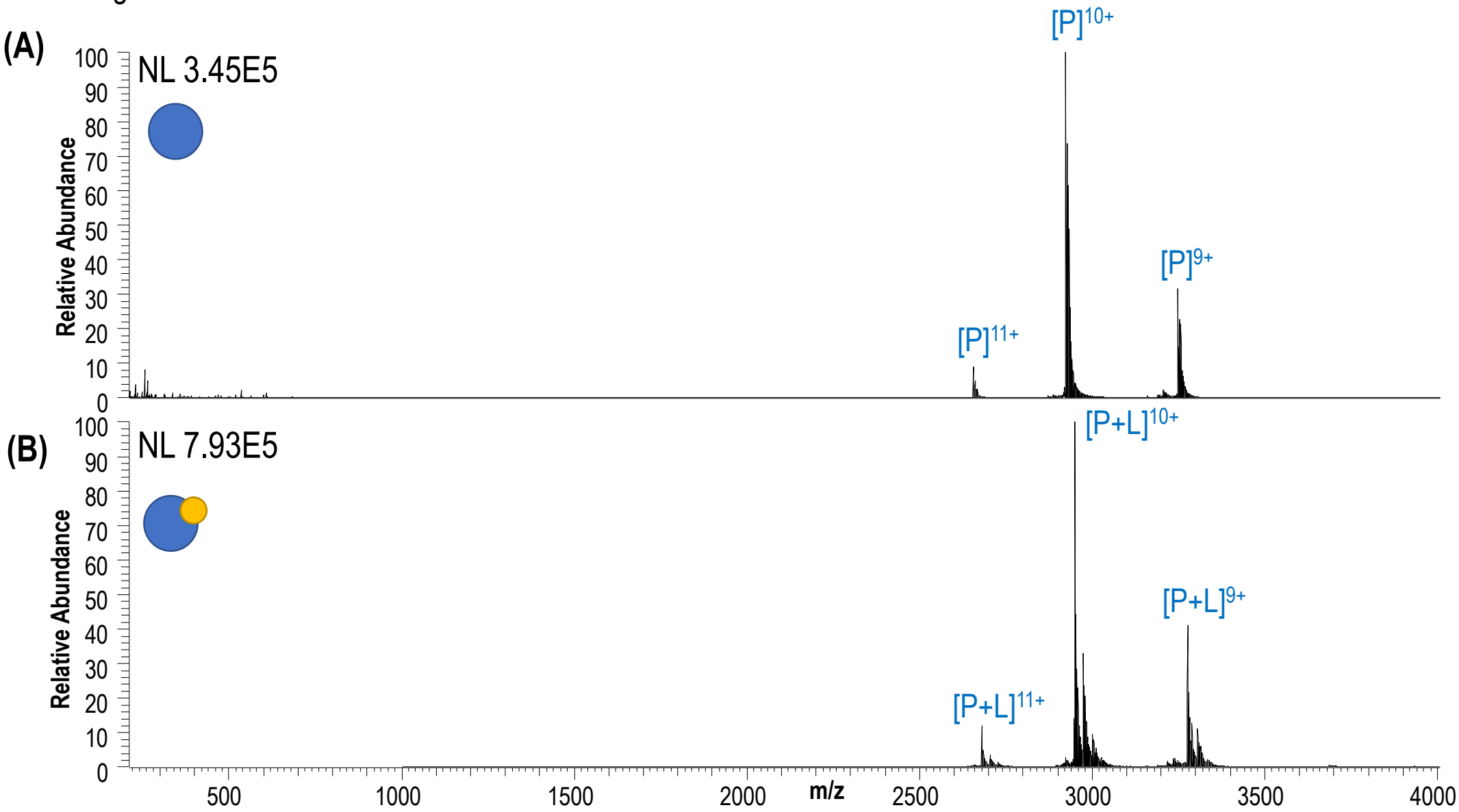

Figure S8. UVPD mass spectra of the 11+ charge state of (A) hCAII and (B) hCAII bound to ethoxzolamide.

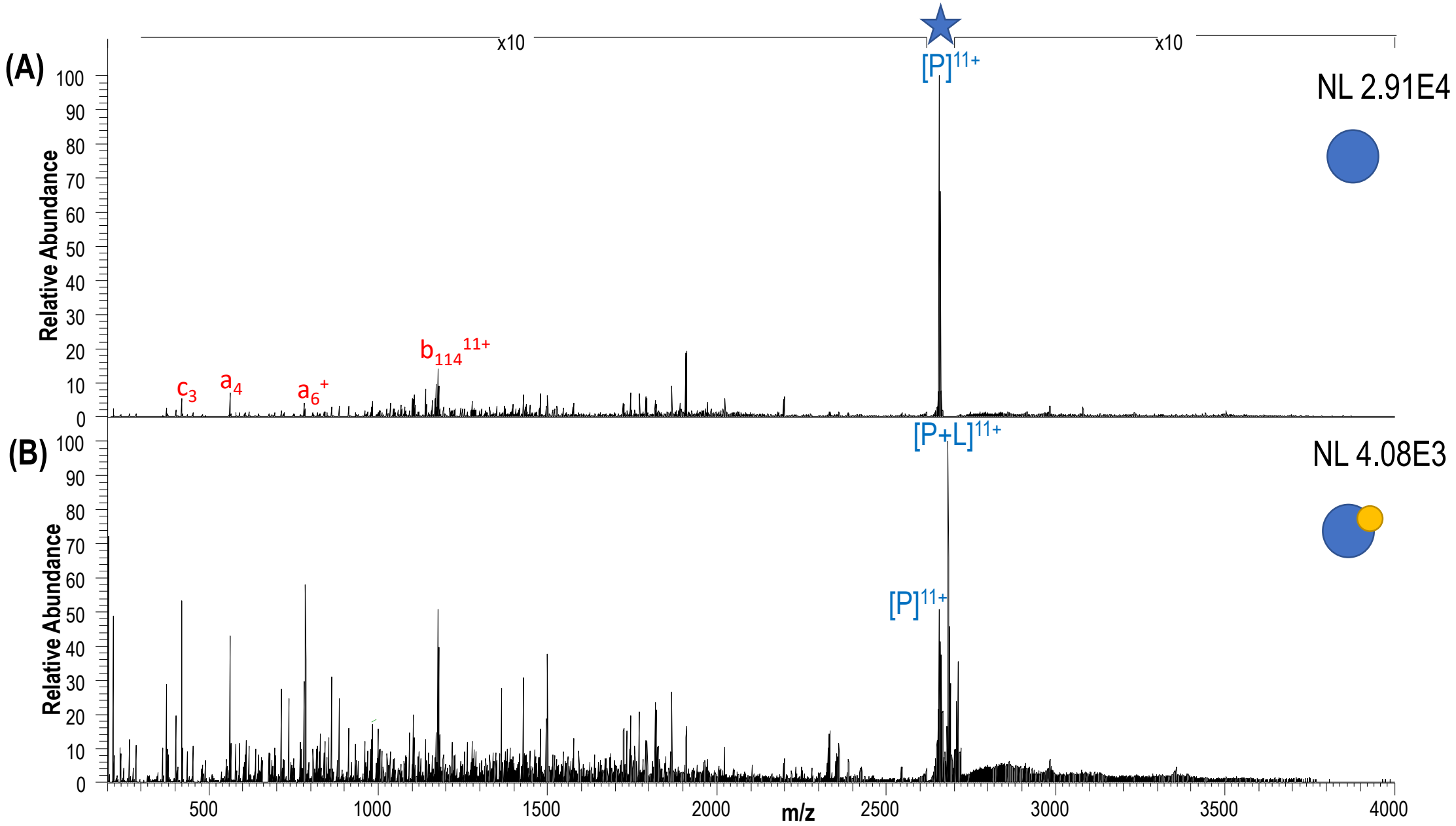

Figure S9. UVPD mass spectra of the 11+ charge state of (A) hCAII and (B) hCAII bound to furosemide.

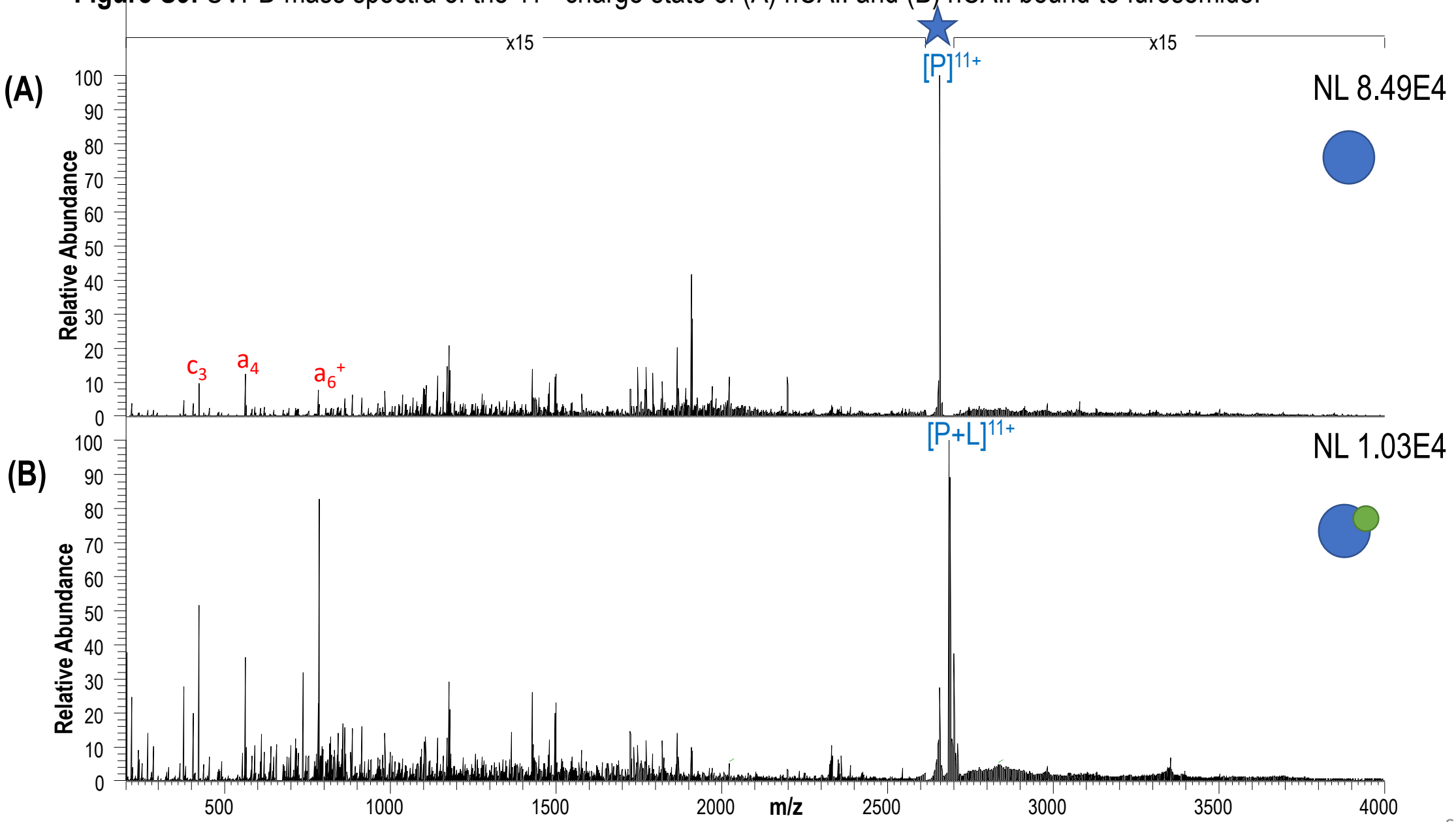

Figure S10. UVPD mass spectra of the 11+ charge state of (A) hCAII and (B) hCAII bound to chlorothiazide.

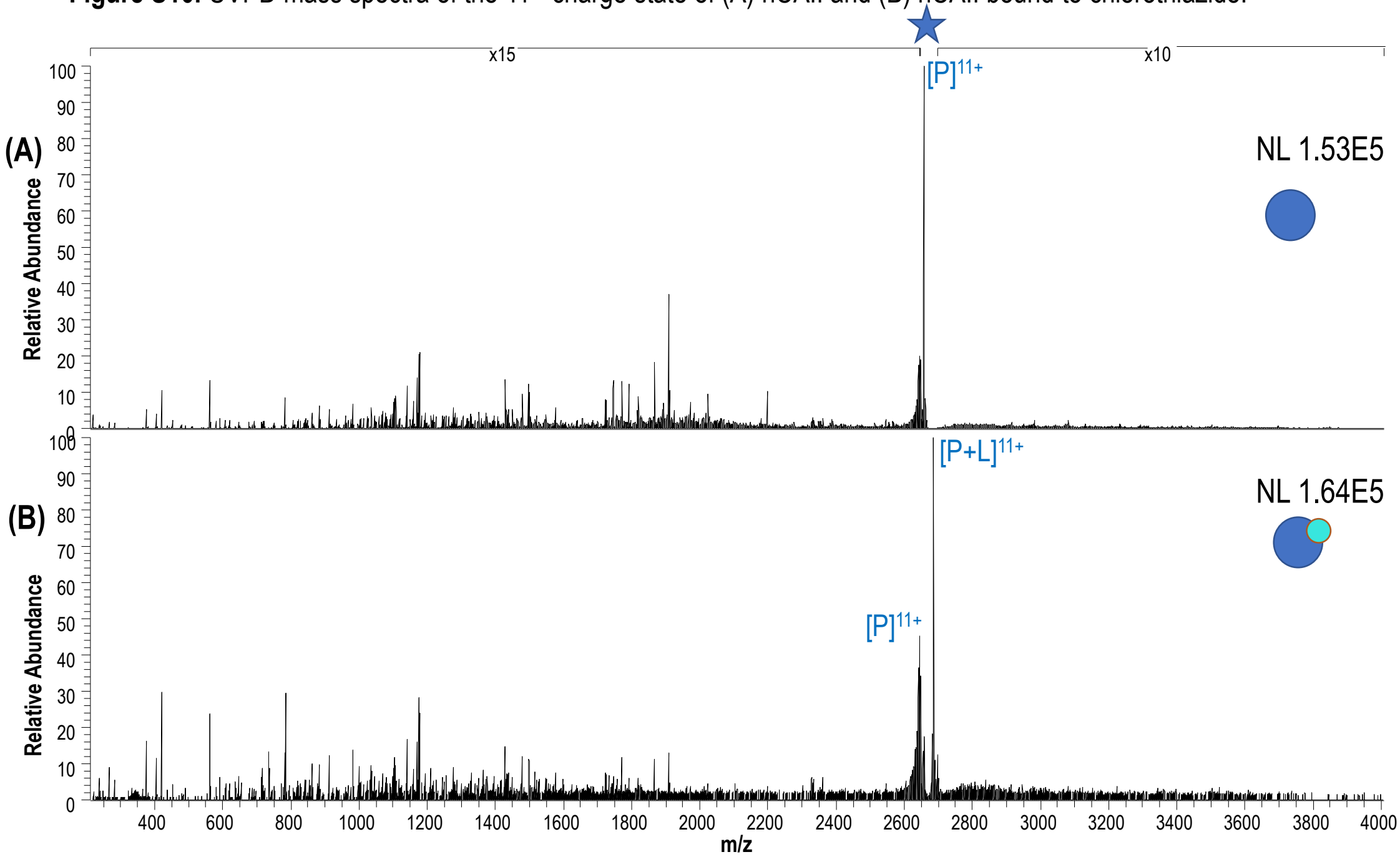

Figure S11. Backbone cleavage propensity maps that correspond to fragment ions (both apo and holo ions) produced upon UVPD (1 pulse, 2 mJ) of (A) hCAII and (B) hCAII bound to furosemide, and respective sequence coverage maps on the right. Backbone cleavages that result in C-terminal ions are depicted in orange and N-terminal ions are depicted in blue. Highlighted are the hydrophobic pocket (pink), the hydrophilic pocket (light blue), and the residues that coordinate zinc (gray). The x-axis shows 1 out of every 7 residues. Shaded regions in the sequence maps highlight decreased sequence coverage for the hCAII complex. β-strands and α-helices are labeled underneath the x-axis using colors corresponding to **Figure S1**.

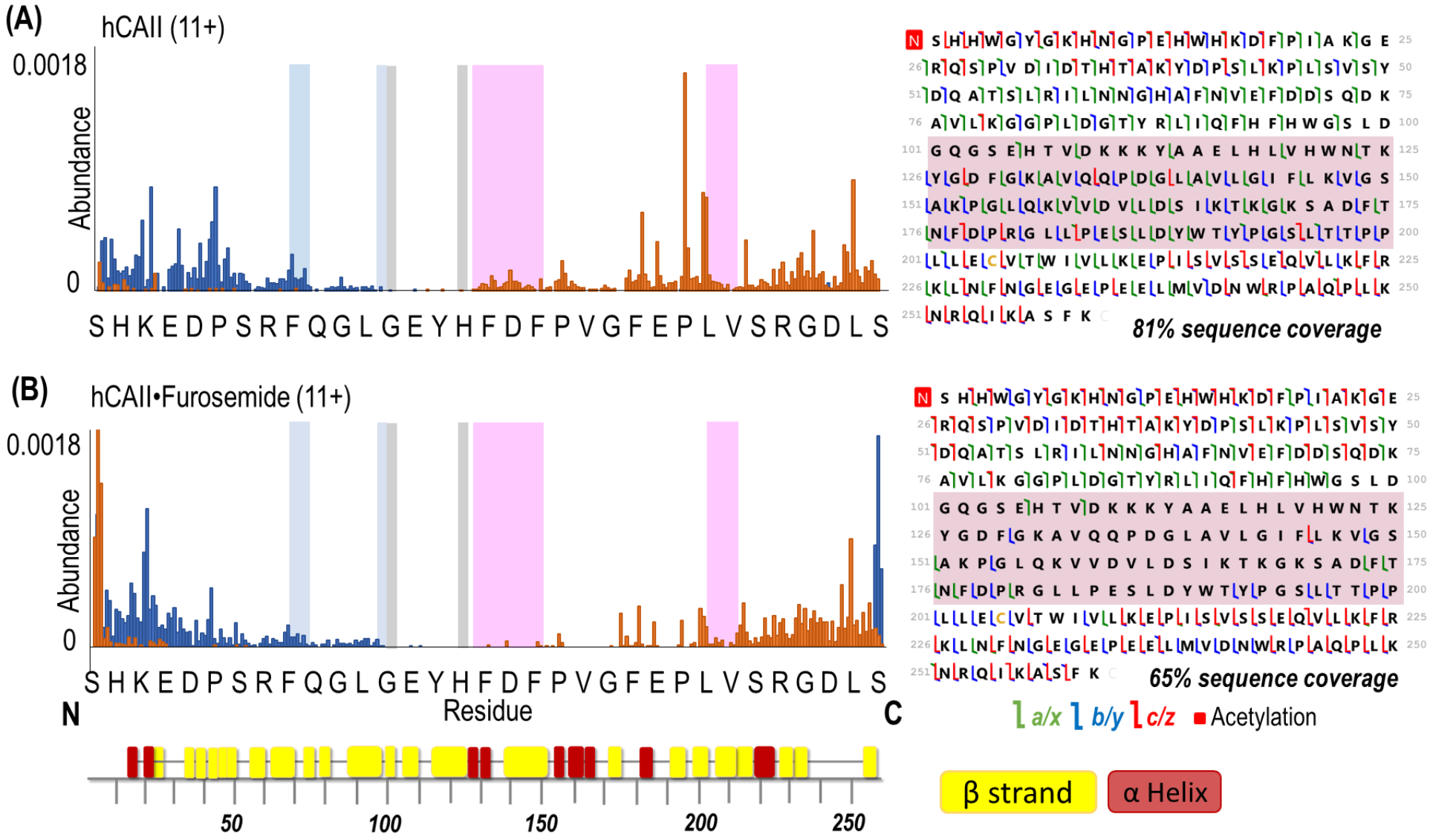

Figure S12. Backbone cleavage propensity maps that correspond to fragment ions (both apo and holo ions) produced upon UVPD (1 pulse, 2 mJ) of (A) hCAII and (B) hCAII bound to chlorothiazide, and respective sequence coverage maps. C-terminal ions are depicted in orange and N-terminal ions are depicted in blue. Highlighted are the hydrophobic pocket (pink), the hydrophilic pocket (light blue), and the residues that coordinate zinc (grey). The x-axis shows 1 out of every 7 residues. Shaded regions in the sequence maps highlight decreased sequence coverage for the hCAII complex. β-strands and α-helices are labeled underneath the x-axis using colors corresponding to **Figure S1**.

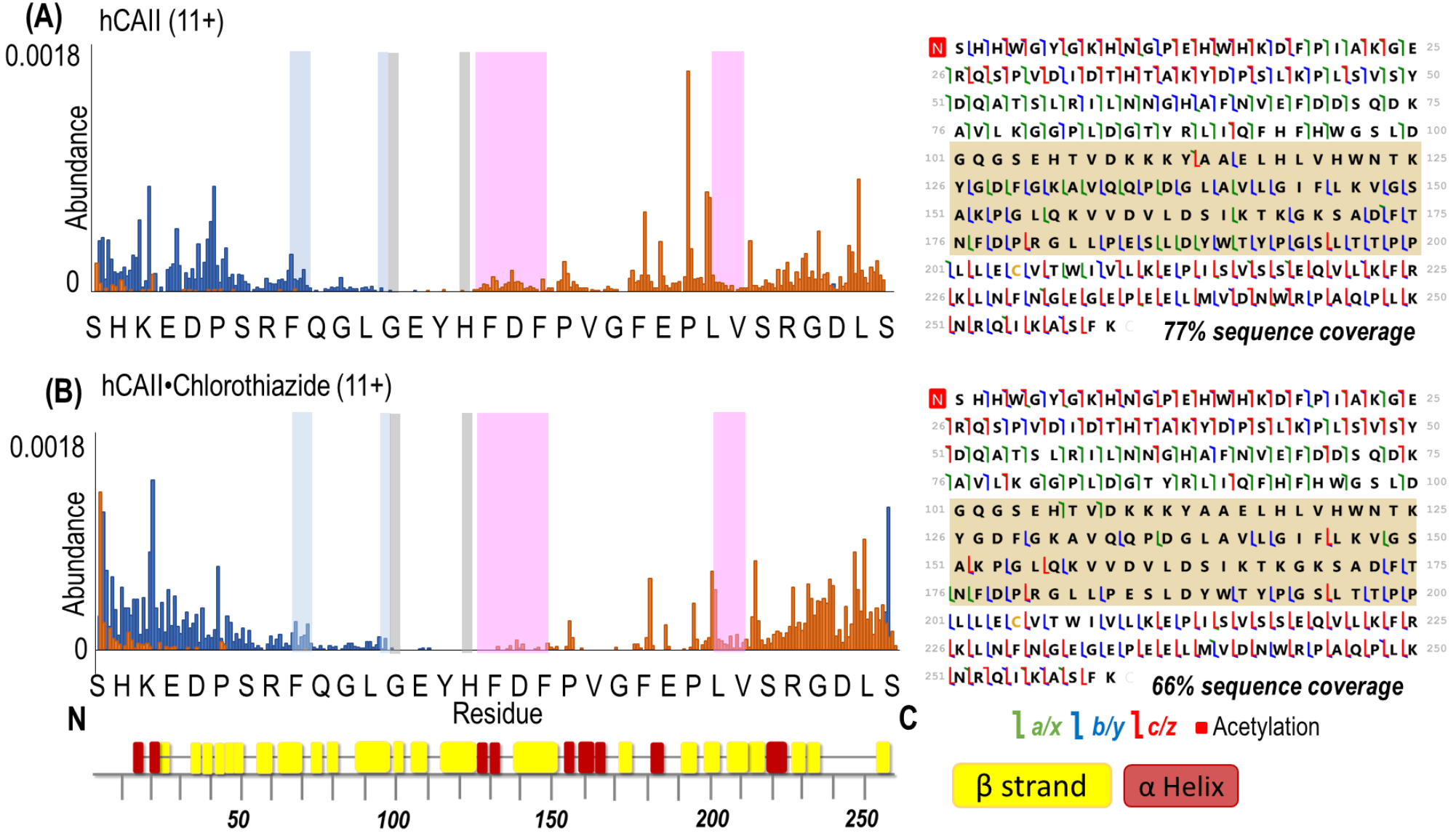

Figure S13. Log of calculated p-values per residue for the differences plotted in Figure 5.The black dotted line represents a confidence threshold at 99%. Pooled standard deviations were used to perform Student's t-test for comparison of UVPD of hCAII-ligand complex to the hCAII. Assuming a two-tailed hypothesis, p-values were determined from calculated t-values. In summary, a p-value smaller than 0.01 at a given residue for a complex indicates that the average measured UVPD intensity within a triplicate measurement is statistically different from the measured average of the hCAII at the 99% confidence level. The letter code along the x-axis shows 1 out of every 5 residues.