Supplemental Information

Fourier Transform-Ion Mobility-Orbitrap Mass Spectrometer: A Next-generation Instrument for Native Mass Spectrometry

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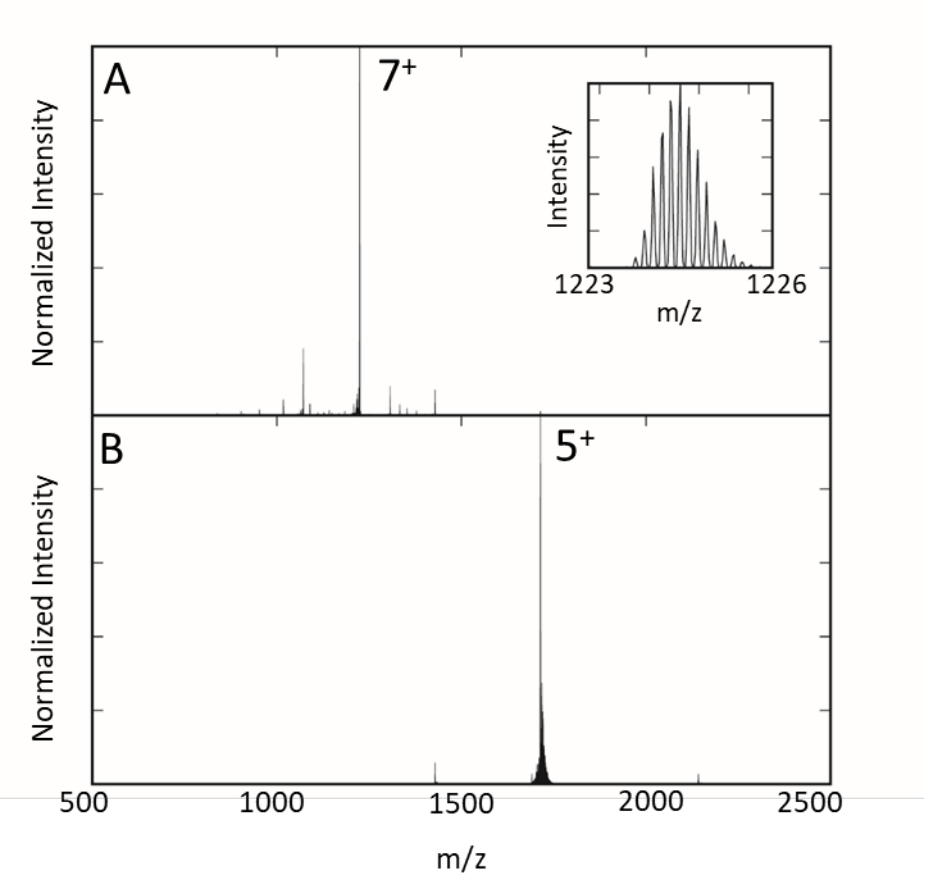

Figure S1. Mass spectrum of ubiquitin in (A) water with 1% acetic acid and (B) water with 100 mM ammonium acetate. Ubiquitin charge states in water with 1% acetic acid are centered at $[M + 7H^+]^{7+}$, but in water with 100 mM ammonium acetate the charge state is centered about $[M + 5H^+]^{5+}$. The Orbitrap mass analyzer is able to achieve a max $R_{m/z}$ greater than 35000 for ubiquitin allowing for isotopic resolution as shown in the inset of panel A.

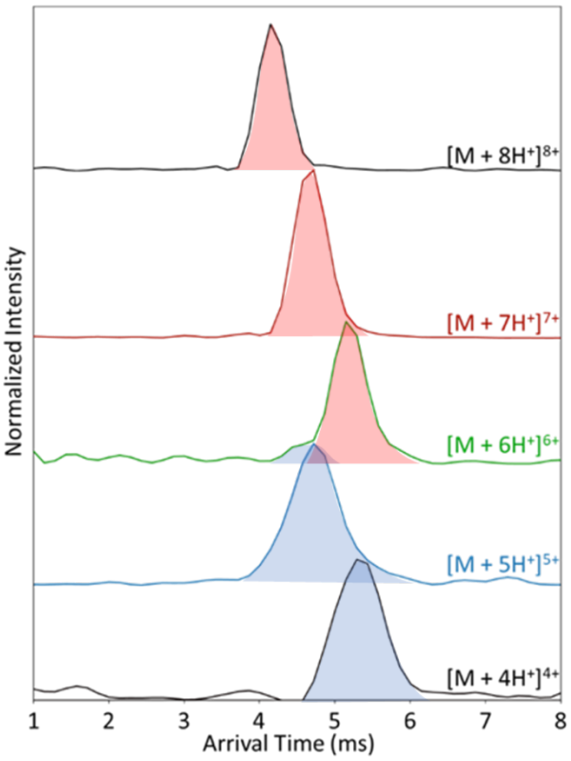

Figure S2. Extracted ATDs of the $[M + 4H^+]^{4+}$ through $[M + 8H^+]^{8+}$ ions of ubiquitin. The ATD for $[M + 4H^+]^{4+}$ and $[M + 5H^+]^{5+}$ were extracted from the mass spectrum collected in water with 100mM ammonium acetate whereas the ATD for $[M + 6H^+]^{6+}$ through $[M + 8H^+]^{8+}$ ions were extracted from the mass spectrum collected in water with 1% acetic acid. Low charge states of $[M + 4H^+]^{4+}$ and $[M + 5H^+]^{5+}$ exhibit compact, native-like conformers (blue). Additional charges lead to a charge state dependent unfolding where the $[M + 6H^+]^{6+}$ ion populates the compact conformer and the slightly unfolded intermediate conformer (red), and the $[M + 7H^+]^{7+}$ and $[M + 8H^+]^{8+}$ populate only the intermediate conformers. These data match those reported by a number of studies.[1-3]

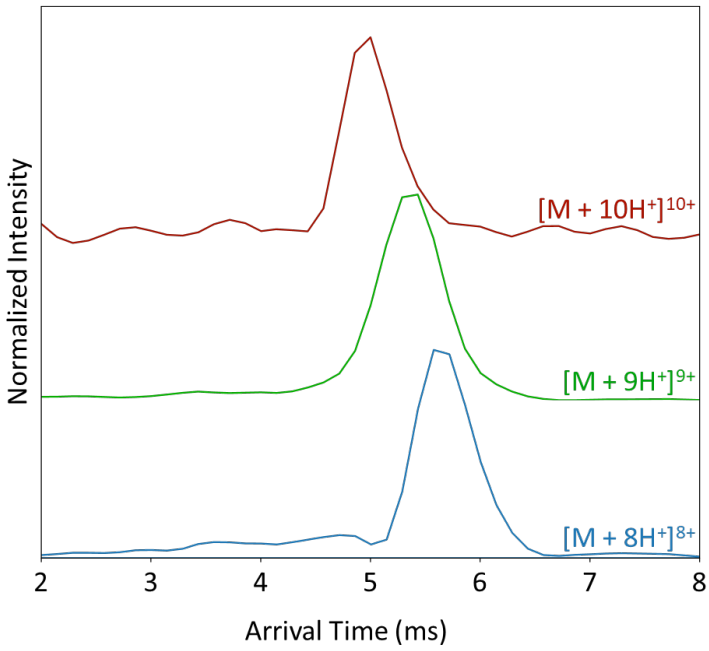

Figure S3. Extracted ATDs of lysozyme in water and 1% acetic acid for $[M + 8H^+]^{8+}$ through $[M + 10H^+]^{10+}$ ions. Compact conformers are observed for all three charge states, and no charge state-dependent unfolding was observed. This is interpreted as evidence that the ions are formed with low internal energies.[4-5]

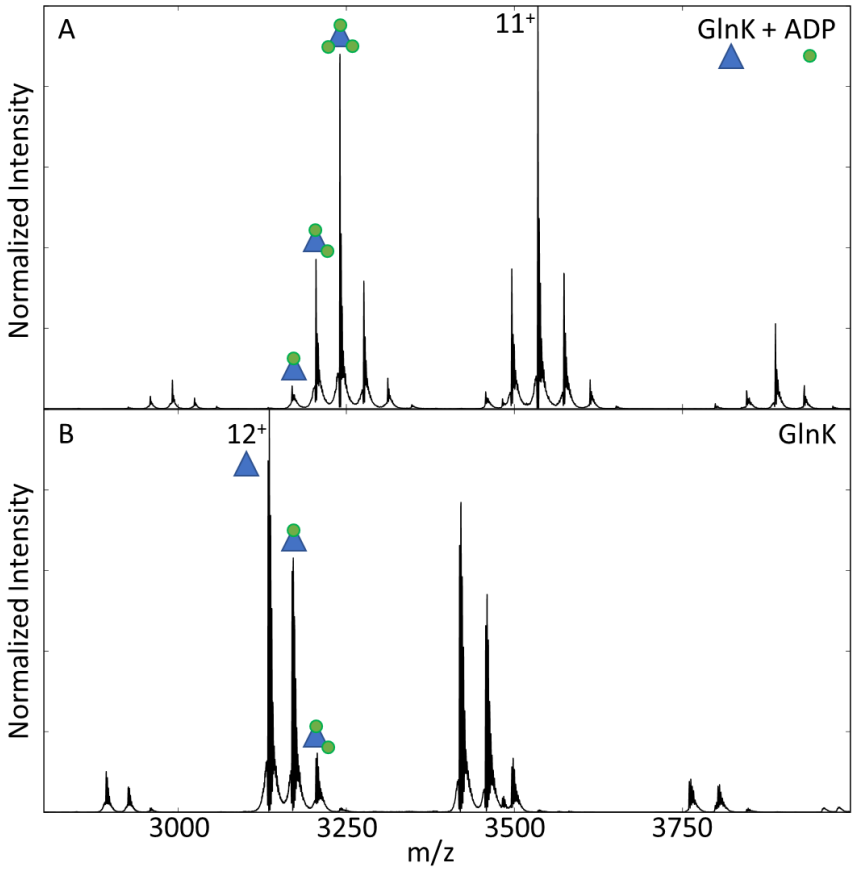

Figure S4. (A) Mass spectrum of the GlnK•ADP protein complex in the presence of excess ADP. The peaks are centered at GlnK with 3 ADPs bound corresponding to the holo-GlnK complex. Additional non-specific ADP binding is also observed. (B) Mass spectrum of GlnK without ADP. Up to two ADPs are bound to the complex and are attributed to endogenous ADP bound after protein purification.

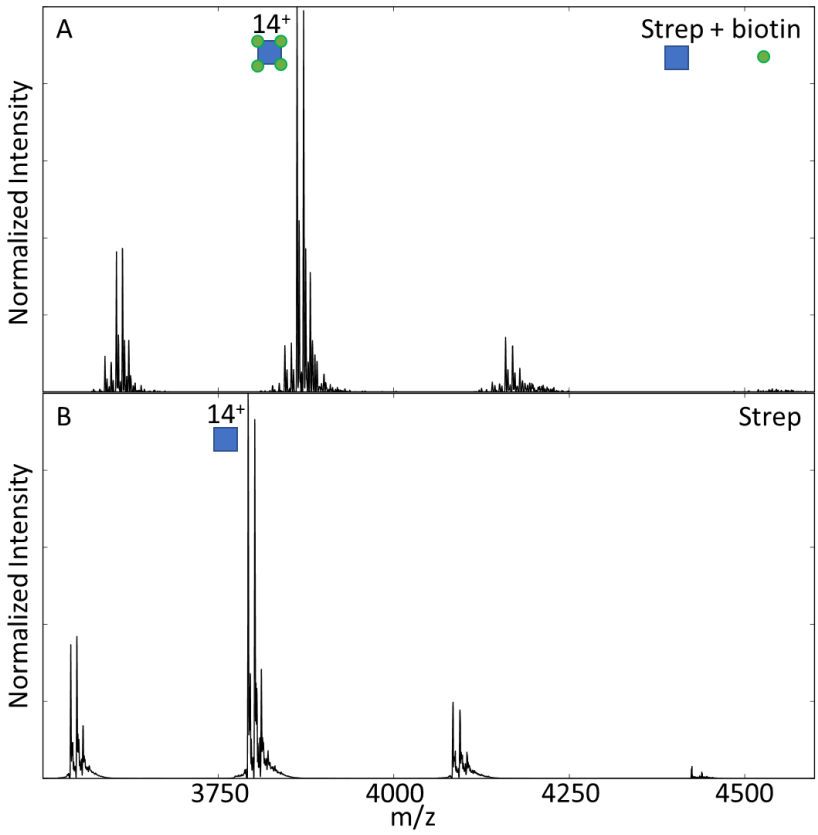

Figure S5. Mass spectrum of the (A) holo streptavidin•biotin complex and (B) apo-streptavidin complex. One biotin binds to each subunit of the tetrameric streptavidin.

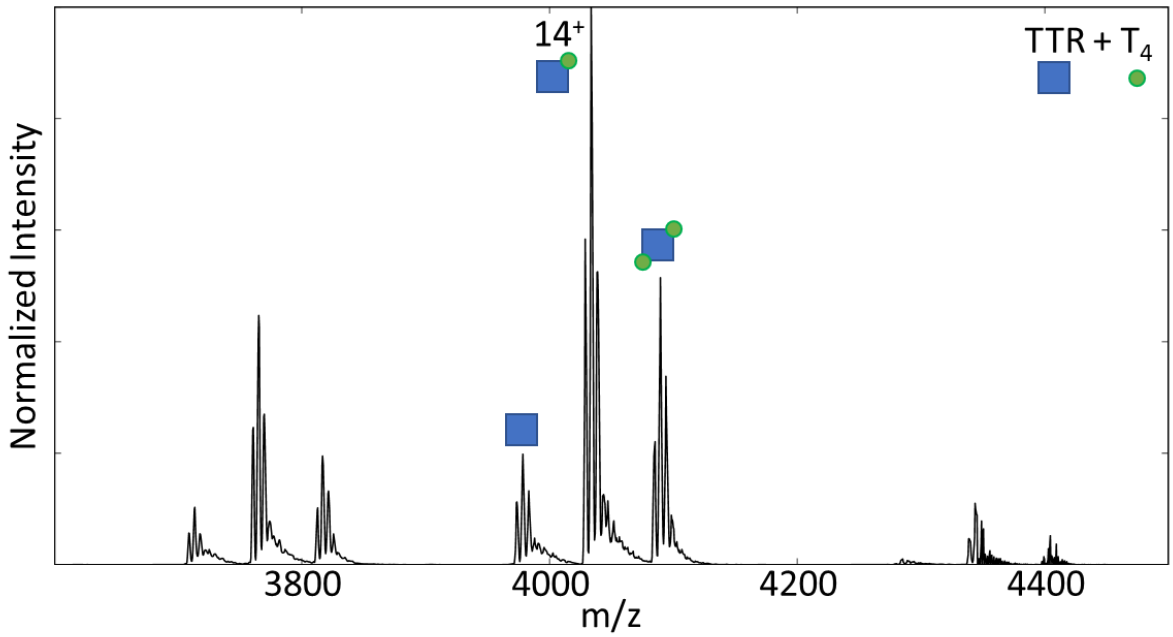

Figure S6. Mass spectrum of the transthyretin•T_4 complex. At the given concentration of T_4, two T_4 molecules are bound to transthyretin.

Table S1. Mass resolution ($R_{m/z}$) for each presented protein.

Protein	max $R_{m/z}$
Ubiquitin	35748
Cytochrome C	29985
Lysozyme	29002
Streptavidin	4618
Transthyretin (TTR)	5018
P-II Transduction Protein (GlnK)	5098

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4. Pringle, S. D.; Giles, K.; Wildgoose, J. L.; Williams, J. P.; Slade, S. E.; Thalassinos, K.; Bateman, R. H.; Bowers, M. T.; Scrivens, J. H., An investigation of the mobility separation of some peptide and protein ions using a new hybrid quadrupole/travelling wave IMS/oa-ToF instrument. *International Journal of Mass Spectrometry* **2007,** *261* (1), 1-12.
5. Clemmer, D. E.; Jarrold, M. F., Ion Mobility Measurements and their Applications to Clusters and Biomolecules. *Journal of Mass Spectrometry* **1997,** *32* (6), 577-592.