Supplemental Information for:

Native Mass Spectrometry Beyond Ammonium Acetate: Effects of Nonvolatile Salts on Protein Stability and Structure

Zijie Xia, Joseph B. DeGrandchamp, and Evan R. Williams*

Department of Chemistry, University of California, Berkeley, California 94720-1460

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*Corresponding Author: Evan R. Williams:

Phone: (510) 643-7161
e-mail: erw@berkeley.edu

Table S-1. Tyrosine emission intensity (in arbitrary units) at 302 nm for AAAYGGFL and RA-RNase A at 25 °C in 175 mM AA, 25 mM AA with 150 mM NaCl, and 25 mM Tris with 150 mM NaCl.

	AAAYGGFL		RA-RNase A	
Solution	**Average**	**Standard Deviation**	**Average**	**Standard Deviation**
175 mM AA	**1589.82**	**110.04**	**6079.50**	**454.39**
25 mM AA 150 mM NaCl	**1635.00**	**247.28**	**6892.07**	**488.72**
25 mM Tris 150 mM NaCl	**1790.65**	**82.76**	**6919.80**	**229.32**

Collisional Cross Section Measurements

The lowest charge state for each protein or protein complex with a previously reported collisional cross section is used in the calibration curve. The corrected drift times (t_D'') are plotted as a function of cross section measured with helium gas in a static or radio-frequency confining drift tube (Figure S-1).[1,2] A previous study showed that similar instrument conditions to ones used in this study minimize ion heating but ion heating still occurs.[3] This can shift the transition between folded and unfolded conformations to lower charge state. This effect can be minimized or eliminated by selecting low charge states and larger proteins or protein complexes that are less affected by ion heating.

The average and highest deviation of these calibrant data from the linear-fitted line is 1.7% and 3.8%, respectively. The collisional cross sections reported in this paper is the average of three measurements from three different submicron electrospray ionization emitters. The precision of three measurements for the same sample ranges from 0.2% to 1%, which is reported as an uncertainty of the measurements in the paper.

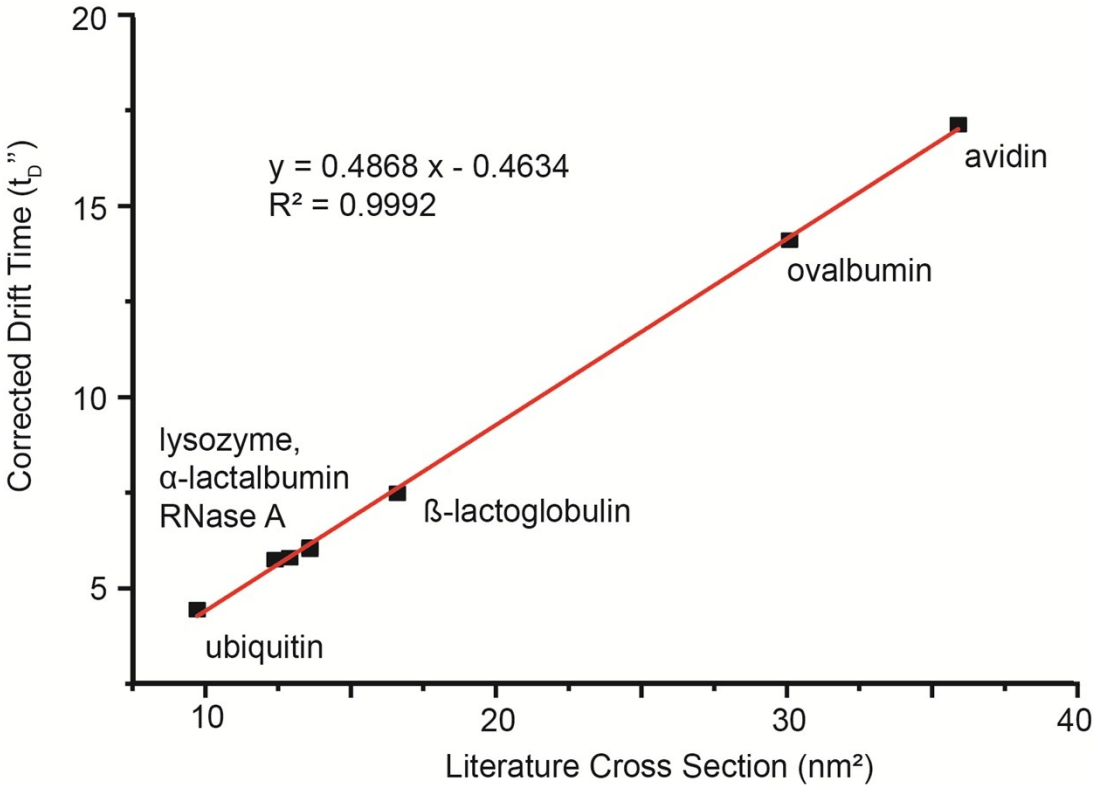

Figure S-1. Calibration curve for converting drift time measured on SYNAPT G2Si to collision cross section.

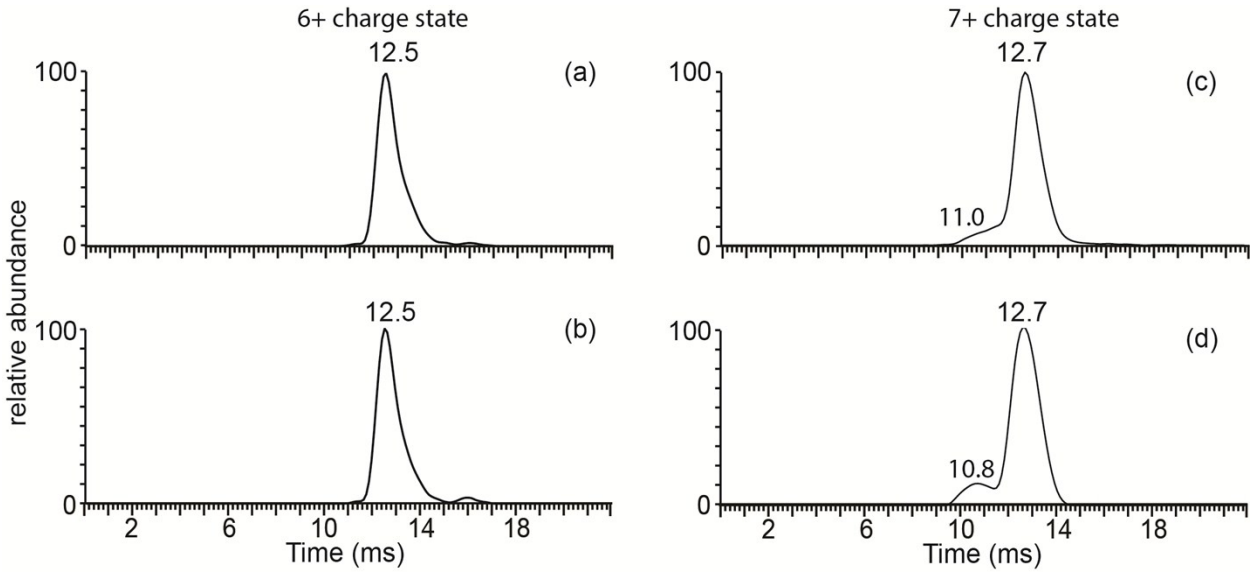

Figure S-2. TWIMS arrival time data for intact RNase A 6+ charge state in (a) conditions identified in Figure 1a. (175 mM AA) and (b) in 200 mM AA, and 7+ charge state in (c) conditions identified in Figure 1a. (175 mM AA) and (d) in 200 mM AA.

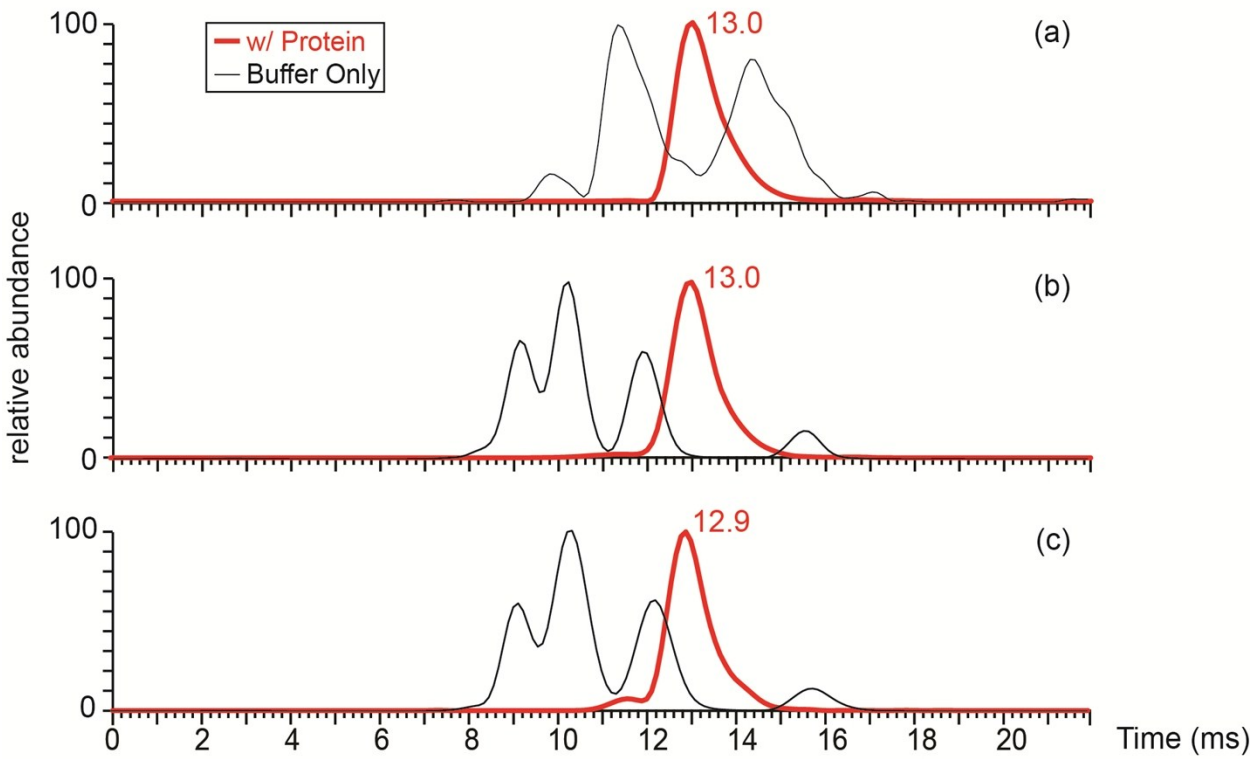

Figure S-3. TWIMS arrival time data for reduced RNase A 6+ charge state (*m*/*z* 2357 – 2366.5) in (a) 175 mM AA, (b) in 165 mM AA 10 mM NaCl, and (c) in 125 mM Tris 50 mM NaCl solutions. Data for the 6+ ion of RA-RNase A (red) are overlaid with data from corresponding solutions without the protein (black) showing the arrival time data for unresolved salt clusters.

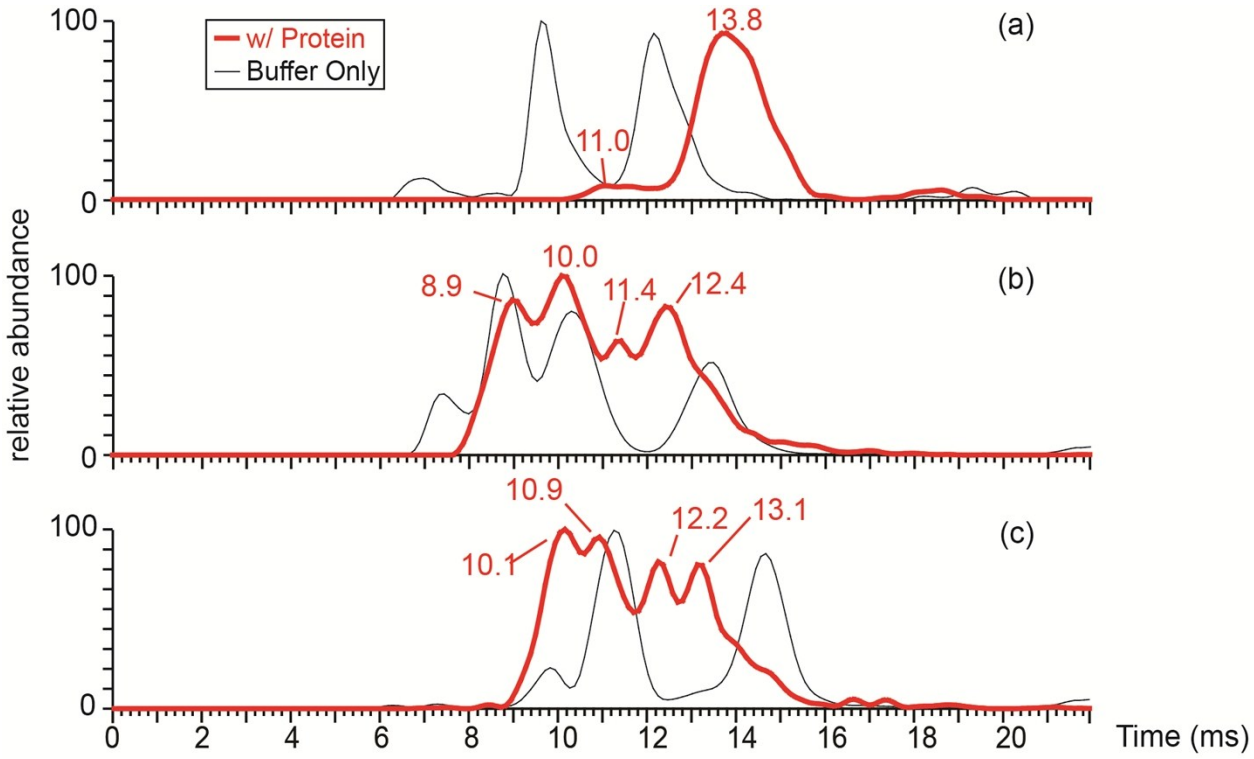

Figure S-4. Ion mobility spectra of reduced RNase A 7+ charge state (*m*/*z* 2020.5 – 2028.5) in (a) 175 mM AA, (b) in 25 mM AA 150 mM NaCl, and (c) in 25 mM Tris 150 mM NaCl solutions. Data for the 7+ ion of RA-RNase A (red) are overlaid with data from corresponding solutions without the protein (black) showing the arrival time data for unresolved salt clusters.

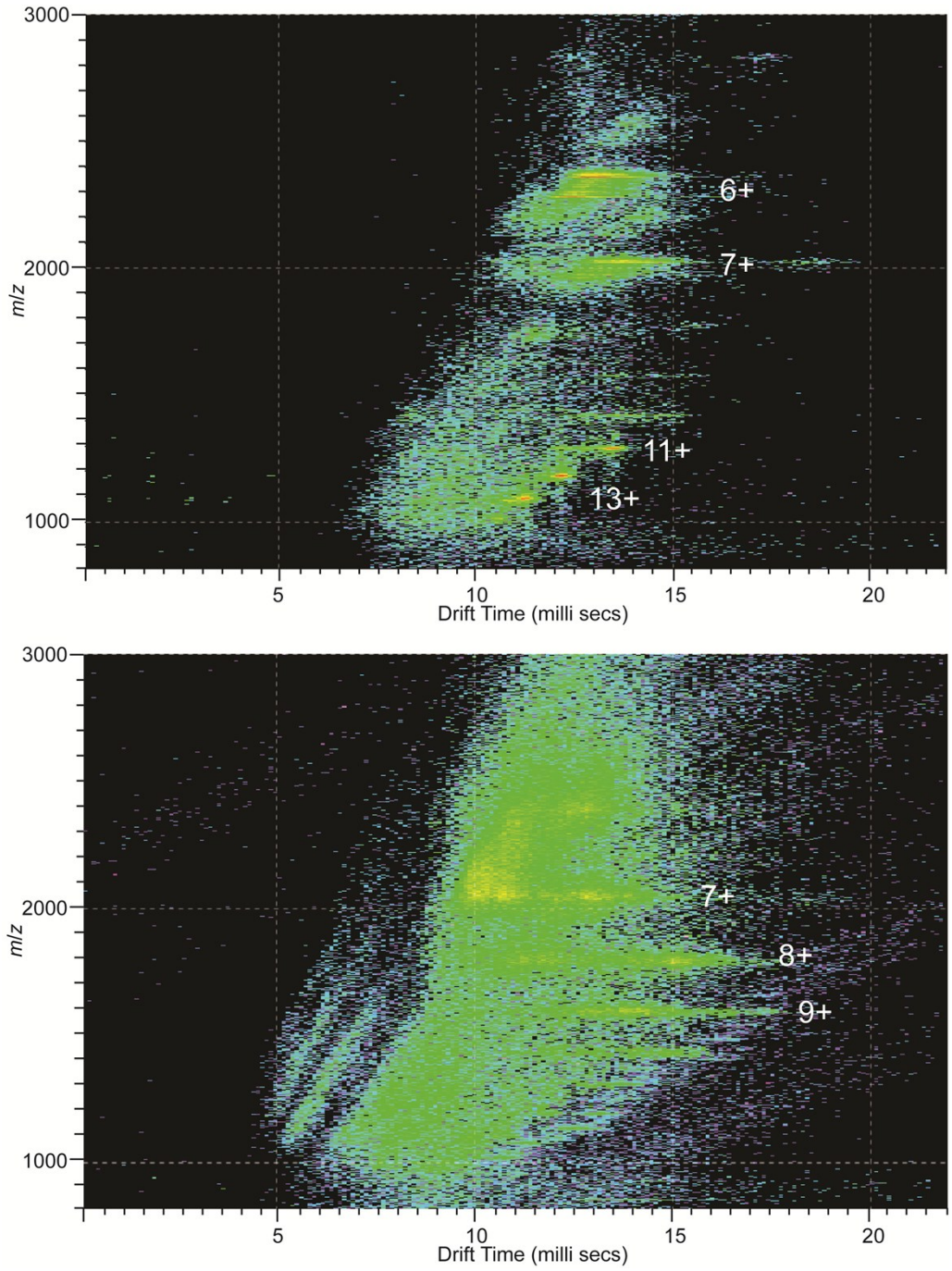

Figure S-5. Ion mobility data for reduced RNase A in 175 mM AA (top) and in 25 mM Tris 150 mM NaCl (bottom). Isolated spots for protein are observed for the data in the top plot. Protein signals and salt signals are overlapped for the data in the bottom plot, which complicates the interpretation of the data.

Reference

1 S. J. Allen, K. Giles, T. Gilbert and M. F. Bush, *Analyst*, 2016, **141**, 884–891.

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

3 S. I. Merenbloom, T. G. Flick and E. R. Williams, *J. Am. Soc. Mass Spectrom.*, 2012, **23**, 553–562.