Electronic Supplementary Material (ESI) for Analyst.

On the Structural Denaturation of Biological Analytes in Trapped Ion Mobility Spectrometry- Mass Spectrometry.

Fanny C. Liu[a], Samuel R. Kirk[a], Christian Bleiholder[a,b,*]

[a] Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306-4390

[b] Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306-4390

[*] Correspondence to: cbleiholder@fsu.edu

SUPPORTING INFORMATION

S1. Details on the TIMS settings used to record ubiquitin spectra when varying the conditions in regions 1 and 2.

Table S1.

Potentials [V]				Potential differences [V]		Electric field E_{DC}[b] [V/m]		ΔE_{lab}^{DC} [b,c] [kJ/mol]	
Deflector V_{def}	**Funnel** V_{ent}	**Ramp** V_{ramp}	**RF**[a]	**1**	**2**	**1**	**2**	**1**	**2**
-80	-130	-200	150	50	70	2631.6	1495.7	100.9	32.6
-80	-130	-200	230	50	70	2631.6	1495.7	100.9	32.6
-30	-80	-200	150	50	120	2631.6	2564.1	100.9	95.8
-30	-80	-200	230	50	120	2631.6	2564.1	100.9	95.8
20	-30	-200	150	50	170	2631.6	3632.5	100.9	192.3
20	-30	-200	230	50	170	2631.6	3632.5	100.9	192.3
70	20	-200	150	50	220	2631.6	4700.9	100.9	322.0
70	20	-200	230	50	220	2631.6	4700.9	100.9	322.0
120	70	-200	150	50	270	2631.6	5769.2	100.9	485.0
120	70	-200	230	50	270	2631.6	5769.2	100.9	485.0
-150	-180	-200	150	30	20	1578.9	427.4	36.3	2.7
-150	-180	-200	230	30	20	1578.9	427.4	36.3	2.7
-130	-180	-200	150	50	20	2631.6	427.4	100.9	2.7
-130	-180	-200	230	50	20	2631.6	427.4	100.9	2.7
-80	-180	-200	150	100	20	5263.2	427.4	403.6	2.7
-80	-180	-200	230	100	20	5263.2	427.4	403.6	2.7
-30	-180	-200	150	150	20	7894.7	427.4	908.2	2.7
-30	-180	-200	230	150	20	7894.7	427.4	908.2	2.7

[a] Peak-to-peak amplitude in Volts.

[b] 1 and 2 refer to the regions 1 and 2, respectively (see Figure 1 in the main text). The strengths of the electric fields are estimated from the values of the electric potentials set at the electrode plates within the TIMS device (Deflector, Funnel, and Ramp, see also Figure 1 in the main text), the electric resistances between the plates, and their distances.[1–3] The resulting estimates for the field strengths within the TIMS analyzer agree with SIMION calculations[4] for the purpose of this work.

[c] for details see Section S2 in the Supporting Information.

S2. Calculation of translational energies gained from the DC electric field between two collisions.

The gain in translational energy $\Delta E_{\mathrm{lab}}^{DC}$ arising from the DC electric field between two collisions was estimated as the translational energy that a point mass with mass and charge equal to the analyte mass and charge would gain in the applied electric field and buffer gas according to equation (4) in the main text:

$$\Delta E_{lab}^{DC} = \frac{m}{2}\Delta v^2 = \frac{m}{2}\left[\int_0^{\delta t} \frac{F_{DC}}{m} dt\right]^2 = \frac{(qE_{DC}\delta t)^2}{2m}$$

The following parameters were used for analysis of the ubiquitin charge state 7+:

1. mass $\mathrm{m} = 8568\mathrm{g/mol}$ for ubiquitin.
2. charge $q = 7\mathrm{F}$ for the ubiquitin charge state 7+ where F is the Faraday constant (96485 C/mol).
3. the strengths of the electric fields are estimated from the values of the electric potentials set at the electrode plates within the TIMS device (see Figure 1 in the main text), and the electric resistances between the plates, and their distances (see Table S1).
4. the time period between two collisions was computed to $\delta t = \frac{\lambda}{v}$ where $v = \sqrt{\frac{8RT}{\mu}}$ is the mean thermal relative velocity at temperature $T = 304\ K$, $\mu = \frac{m \cdot m_{bg}}{m + m_{bg}}$ the reduced mass, $R = 8.314\ \mathrm{J\ K^{-1}\ mol^{-1}}$ the ideal gas constant, and λ the mean free path of a mass point with charge q in a nitrogen bath gas ($m_{bg} = 28.01\ \mathrm{g/mol}$).
5. The mean free path was estimated according to

$$\lambda = \frac{RT}{\pi \sqrt{2}\,(2r)^2\,N_A\,p}$$

where $N_A = 6.0221413 \cdot 10^{23}\ \mathrm{mol}^{-1}$ is the Avogadro constant, p the pressure (2.9 mbar), and the van der Waals radius of molecular nitrogen $r = 0.21\ \mathrm{nm}$.[5]

S3. Details on the TIMS settings used to record ubiquitin spectra when varying the conditions in region 3.

DC potential differences between the deflector plate and the entrance funnel was set to 50 V and the DC potential difference across the entrance funnel was set to 20 V.

Table S2.

Times [ms]					Ion counts per accumulation [counts][a]		
Fill	**Trap**	**Ramp**	**Time**	**RF V_{pp} [V]**	**total ion count (TIC)**	**extended conformation[b]**	**compact conformation[c]**
14.40	3.60	39.60	57.60	150	421	22	266
14.40	133.20	39.60	187.20	150	392	25	175
14.40	277.20	39.60	331.20	150	381	27	137
72.00	3.60	39.60	115.20	150	2158	106	1117
72.00	133.20	39.60	244.80	150	2101	153	675
72.00	277.20	39.60	388.80	150	2067	194	533
216.01	3.60	39.60	259.21	150	4123	420	1506
216.00	133.20	39.60	388.81	150	2971	323	894
216.00	277.20	39.60	532.81	150	2513	248	670
14.40	3.60	39.60	57.60	250	391	35	205
14.40	133.20	39.60	187.21	250	396	41	148
14.40	277.20	39.60	331.20	250	390	49	114
72.00	3.60	39.60	115.20	250	1956	177	861
72.00	133.20	39.60	244.81	250	2009	223	595
72.00	277.20	39.60	388.81	250	1957	256	468
216.01	3.60	39.60	259.21	250	6050	730	1644
216.00	133.20	39.60	388.81	250	6177	993	1060
216.00	277.20	39.60	532.81	250	6063	1180	820

[a] An experiment consists of 1000 accumulations.
[b] Extended ubiquitin conformation in the ion mobility spectra.
[c] Compact ubiquitin conformation in the ion mobility spectra.

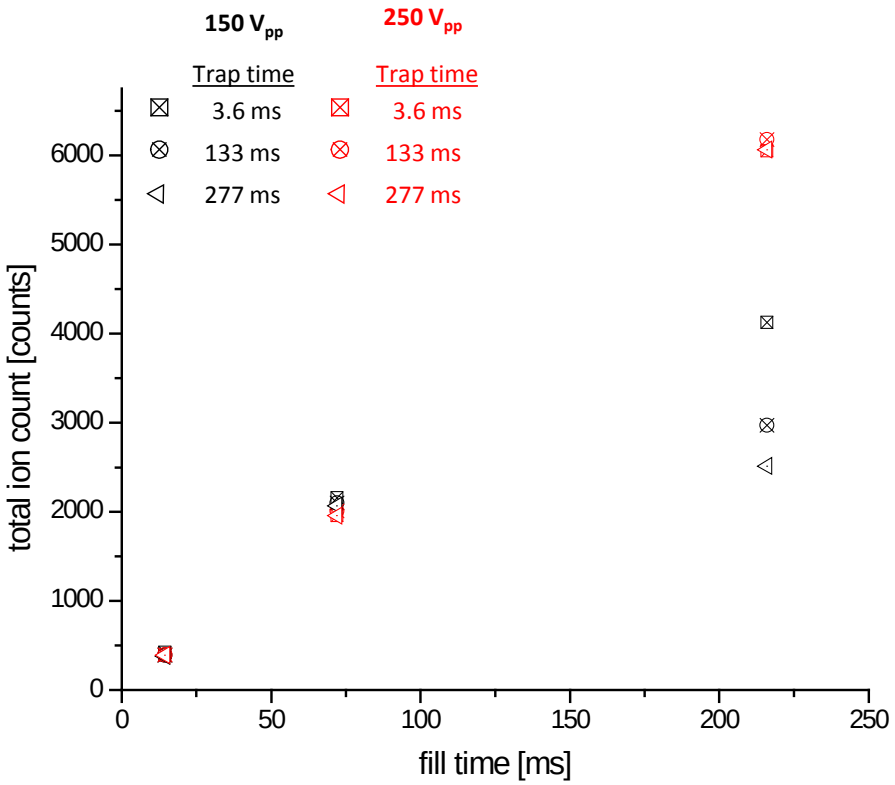

Figure S1. Total ion counts per accumulation observed for ubiquitin charge state 7+ as a function of the fill time, trap time, and RF amplitude of the TIMS experiment. Note that all ubiquitin conformations present in a specific charge state contribute to the TIC. The data show that the ion count increases linearly for the 250 V_{pp} RF amplitude when the fill time is increased. The data further show that the ion counts are largely independent of the RF amplitude or trap time for short fill times (14.4 and 72 ms) but become dependent on the RF amplitude and trapping time when the fill time is increased. The decrease in signal at 150 V_{pp} RF amplitude and long trap and fill time can be rationalized by loss of ions due to charge-charge repulsions and the inability of the electric field to radially confine ubiquitin ions within the TIMS analyzer tunnel when the RF amplitude is 150 V_{pp}, as proposed.[2]

S4. Mass spectrum of ubiquitin obtained under soft-tuned TIMS settings.

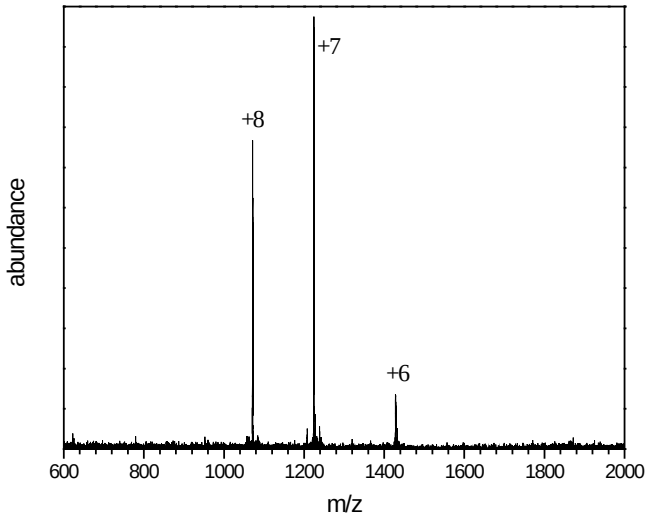

Figure S2. Mass spectrum of ubiquitin obtained with the optimized soft TIMS settings. Intense peaks are observed for charge states 6+,7+, and 8+ whereas charge states ≥ 9 are not observed.

S5. Ion mobility spectrum for charge state 6+ of ubiquitin obtained under soft-tuned TIMS settings.

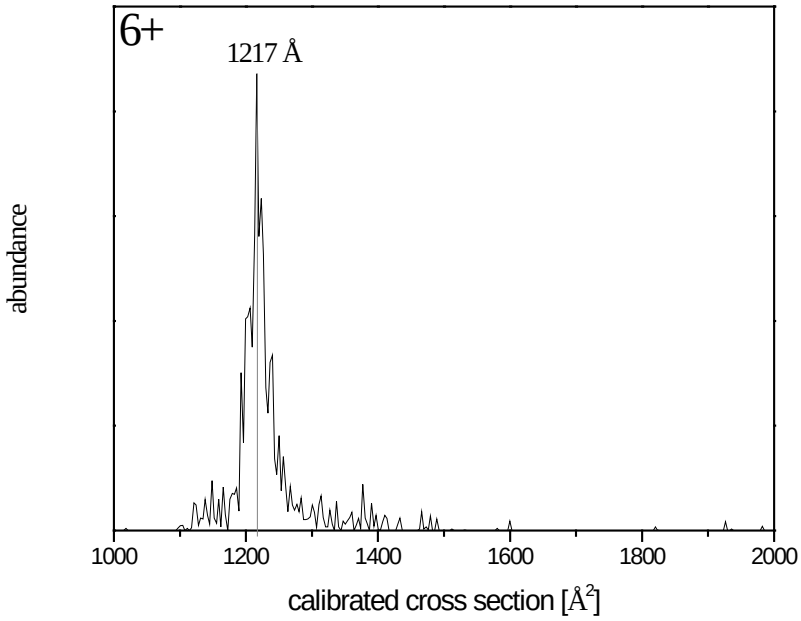

Figure S3. Cross section distribution for charge state 6+ of ubiquitin, obtained with the optimized soft TIMS settings. One dominant peak is found at 1217 Å. Cross sections were calibrated to ESI tune mix as described.[2]

References

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2. Hernandez, D. R. *et al.* Ion dynamics in a trapped ion mobility spectrometer. *The Analyst* **139,** 1913–1921 (2014).
3. Ridgeway, M. E., Silveira, J. A., Meier, J. E. & Park, M. A. Microheterogeneity within conformational states of ubiquitin revealed by high resolution trapped ion mobility spectrometry. *The Analyst* **140,** 6964–6972 (2015).

4. Joshua A. Silvereia. *private communication*.

5. Bondi, A. van der Waals volumes and radii. *J. Phys. Chem.* **68,** 441–451 (1964).