

Supplementary Information

TANDEM TRAPPED ION MOBILITY SPECTROMETRY

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1. GENERAL SETTINGS

In all experiments, nitrogen produced by a nitrogen generator (Peak Scientific, NM32-LA-MS-230V) was used as buffer gas. The buffer gas was infused into the instrument via the desolvation gas unit of the electrospray source with a flow rate of 4.0 L min⁻¹. Sample solutions were loaded into a gas tight syringe (Hamilton, 250 μL) and infused into the electrospray source at a flow rate of 180 μL h⁻¹. The ion accumulation time for the TIMS-1 cartridge was set to 28.1 ms. The rf peak-to-peak amplitude was set to 305 V for TIMS-1 and 328 V for TIMS-2 cartridges. All spectra were acquired in positive ion mode.

2. SAMPLES

Bradykinin

Positively charged bradykinin (≥98 %, Sigma-Aldrich) ions were electrosprayed from a 50 μM solution in 50:50 water:methanol (LC/MS grade, Fisher Scientific, and LC/MS grade, Acros Organics).

Ubiquitin

Positively charged ubiquitin (≥98 %, Sigma-Aldrich) ions were electrosprayed from a 100 μM aqueous solution (LC/MS grade, Fisher Scientific) containing 1 v% acetic acid (LC/MS grade, Fisher Scientific).

3. PRESERVATION OF NATIVE-LIKE PROTEIN CONFORMATIONS

TIMS-TIMS spectra of ubiquitin employing “soft” settings (Figure 3a and 3b)

The spectrum in Figure 3a was recorded with mobility-separation mode in TIMS-1 while TIMS-2 transmits ions to the mass spectrometer region (Mode 1A). The desolvation gas temperature was kept at 304 K and the electrospray voltage was set to 1700 V. The pressures at p1/p2/p3/p4 were kept at 3.2/1.6/0.9/0.3 mbar. In TIMS-1, a dc bias of 20 V was applied between deflector and entrance funnel, while the dc potential difference across the entrance funnel was kept at 10 V. For mobility separation, the potential at the entrance of analyzer tunnel of TIMS-1 was gradually increased over scan range of 215 V with a rate of 3.0 V ms⁻¹. The dc potential at the exit funnel of TIMS-1, aperture-1, aperture-2, and deflector-2 were set to 70/70/65/65 V. In TIMS-2, a dc bias of 20 V was applied between deflector-2 and entrance funnel-2, and 5 V was kept across the entrance funnel-2. The dc potential at analyzer tunnel-2 and exit funnel-2 were set to 40 V and 39 V at all times.

The spectrum in Figure 3b was recorded with TIMS-1 employed to transmit ions while mobility-separation occurs in TIMS-2 (Mode 1C). In TIMS-1, a dc bias of 10 V was applied between deflector and entrance funnel, while the dc potential difference across the entrance funnel was kept at 5 V. The dc potential at analyzer tunnel-1 and exit funnel-1 were set to 0 V and -5 V at all times. The dc potential at the aperture-1, aperture-2, and deflector-2 were set to -5/-5/-5 V. In TIMS-2, a dc bias of 0 V was applied between deflector-2 and entrance funnel-2, and 5 V was kept across the entrance funnel-2. For mobility separation, the potential at the entrance of analyzer tunnel of TIMS-1 was gradually increased over scan range of 35 V with a rate of 0.5 V ms⁻¹. The dc potential at exit funnel-2 were set to 39 V at all times.

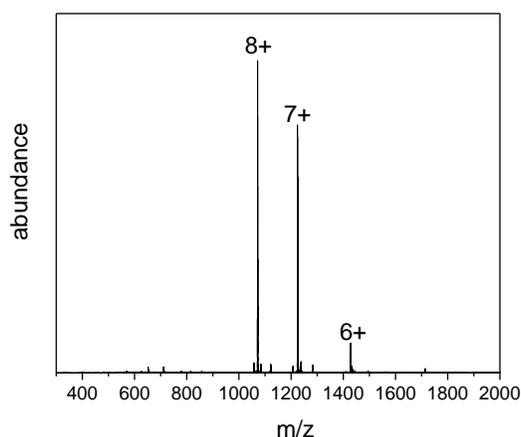


Figure S1. Mass spectrum obtained from electrospraying aqueous ubiquitin solution (1 v% acetic acid) using “soft” TIMS-TIMS settings.

4. MOBILITY-SELECTION OF IONS IN THE INTERFACE AND TRAPPING OF MOBILITY-SELECTED IONS

TIMS-TIMS spectra of bradykinin using Mode 1A and 2A (Figure 4b)

The total ion distribution was obtained using Mode 1A, and the selected ion mobility distribution was obtained using Mode 2A. The desolvation gas temperature was kept at 304 K and the electrospray voltage was set to 1700 V. The pressures at p1/p2/p3/p4 were kept at 2.9/1.4/1.1/0.4 mbar. In TIMS-1, a dc bias of 50 V was applied between deflector and entrance funnel, while the dc potential difference across the entrance funnel was kept at 20 V. For mobility separation, the potential at the entrance of analyzer tunnel of TIMS-1 was linearly ramped from -100 V to 50 V at a rate of 2.15 V ms⁻¹. The dc potential at the exit funnel of TIMS-1, aperture-1, aperture-2, and deflector-2 were set to 80/75/65/60 V. In TIMS-2, a dc bias of 20 V was applied between deflector-2 and entrance funnel-2, and 5 V was kept across the entrance funnel-2. The dc potential at analyzer tunnel-2 and exit funnel-2 were set to 40 V and 39 V at all times. The rf peak-to-peak amplitude was set to 265 V for TIMS-1 and 328 V for TIMS-2 cartridges. To select the monomer peak (mode 2A), aperture-2 was set to a transmitting voltage (65 V) only for a duration of 10.1 ms after a delay time of 7.2 ms, and to a blocking potential of 165 V otherwise.

Ion trapping (Figure 4c)

Mode 2B was utilized to perform mobility analysis in TIMS-1, mobility selection in the interface, and a second mobility analysis in TIMS-2. The settings in TIMS-1 were set to maintain the same electric fields as described for Mode 1A and 2A (see above). For mobility separation at the TIMS-2 analyzer, the potential at the entrance of analyzer tunnel was gradually increased from -50 V to 15 V at a rate of 0.9 V ms⁻¹. The dc potential at the exit funnel of TIMS-1, aperture-1, aperture-2, and deflector-2 were set to -15/-20/-25/-30 V. To select the monomer peak, aperture-2 was set to a transmitting voltage (-25 V) only for a duration of 10.1 ms after a delay time of 7.2 ms, and to a blocking potential of 75 V otherwise. The measurement time is increased from 154 ms to 298, 442, and 1450 ms. These values translate to ion trapping times in the analyzer tunnel-2 of 199, 343, and 1350 ms.

5. COLLISIONAL ACTIVATION OF MOBILITY-SELECTED PROTEIN IONS

TIMS-TIMS spectra of ubiquitin 7+ using Mode 1A and 2A (Figure 5a)

The total ion distribution was obtained using Mode 1A, and the selected ion mobility spectrum was obtained using Mode 2A. For mobility separation, the potential at the entrance of analyzer tunnel of TIMS-1 was gradually increased over scan range of 155 V with a rate of 2.15 V ms⁻¹. The dc potential at the exit funnel of TIMS-1, aperture-1, aperture-2, and deflector-2 were set to 70/70/70/70 V. In TIMS-2, a dc bias of 10 V was applied between deflector-2 and entrance funnel-2, and 5 V was kept across the entrance funnel-2. The dc potential at analyzer tunnel-2 and exit funnel-2 were set to 55 V and 46 V at all times. To select the native-like ubiquitin 7+ ions (Mode 2A), aperture-2 was set to a transmitting voltage (70 V) only for a duration of 12.2 ms after a delay time of 47.5 ms, and to a blocking potential of 120 V otherwise. All other settings were set to the ones used for recording the “soft” TIMS-TIMS spectrum of ubiquitin (see above).

TIMS-TIMS spectra of ubiquitin 7+ using Mode 2B (Figure 5b)

For mobility separation at the TIMS-2 analyzer, the potential at the entrance of analyzer tunnel-2 was gradually increased over scan range of 25 V with a rate of 0.9 V ms⁻¹. The dc potential at the exit funnel of TIMS-1, aperture-1, aperture-2, and deflector-2 were set to 30/30/30/30 V. To select the native-like ubiquitin 7+ ions, aperture-2 was set to a transmitting voltage (30 V) only for a duration of 12.2 ms after a delay time of 47.5 ms, and to a blocking potential of 80 V otherwise. All other settings were set to the ones used for recording Mode 1A spectrum (see above).

TIMS-TIMS spectra of ubiquitin 7+ using Mode 3B (Figure 5c-f)

The dc potential at the exit funnel of TIMS-1, aperture-1, aperture-2, and deflector-2 were set to (30+x)/(30+x)/(30+x)/30 V, with x as the dc voltage bias set between aperture-2 and deflector-2 (20, 30, 40, and 50 V). All potentials in TIMS-1 analyzer are increased by x V. All other settings were set to the ones used for recording Mode 2B.

6. COLLISIONAL DISSOCIATION OF MOBILITY-SELECTED IONS

TIMS-TIMS spectra of ubiquitin using Mode 1A (Figure 6a)

The total ion mobility spectrum was obtained using Mode 1A. The pressures at p1/p2/p3/p4 were kept at 3.2/1.6/0.9/0.3 mbar and the flow rate of the syringe pump at 80 $\mu\text{l/h}$. To generate extended ubiquitin conformation, the desolvation gas temperature was kept at 334 K and the electrospray voltage was set to 4000 V. In TIMS-1, a dc bias of 80 V was applied between deflector and entrance funnel, while the dc potential difference across the entrance funnel was kept at 100 V. For mobility separation, the potential at the entrance of analyzer tunnel of TIMS-1 was gradually increased over scan range of 150 V with a rate of 2.08 V ms^{-1} . The dc potential at the exit funnel of TIMS-1, aperture-1, aperture-2, and deflector-2 were set to 50/50/50/50 V. In TIMS-2, a dc bias of 5 V was applied between deflector-2 and entrance funnel-2, and 5 V was kept across the entrance funnel-2. The dc potential at analyzer tunnel-2 and exit funnel-2 were set to 40 V and 39 V at all times.

TIMS-TIMS spectra of ubiquitin using Mode 2A (Figure 6b)

The selected ion mobility spectrum was obtained using Mode 2A. Aperture-2 was set to a transmitting voltage (50 V) only for a duration of 4.3 ms after a delay time of 25.9 ms, and to a blocking potential of 100 V otherwise. All other settings were set to the ones used for recording Figure 6a, top (see above).

TIMS-TIMS spectra of ubiquitin using Mode 3B (Figure 6c, d, e)

Spectra of collisionally dissociated ubiquitin were obtained using Mode 3B. Here, a second mobility analysis was performed at TIMS-2 analyzer. For mobility separation at the TIMS-2 analyzer, the potential at the entrance of analyzer tunnel-2 was gradually increased over scan range of 35 V with a rate of 0.5 V ms^{-1} . The dc potential at the exit funnel of TIMS-1, aperture-1, and deflector-2 were set to $(-5+x)/(-5+x)/-5$ V, with x as the activation potential set between aperture-2 and deflector-2 (100, 170, 180, 190, 200, 220, 250, and 260 V). Aperture-2 was set to a transmitting voltage $(-5+x)$ only for a duration of 4.3 ms after a delay time of 25.9 ms, and to a blocking potential of $(-5+x+50)$ V otherwise. All potentials in TIMS-1 analyzer are increased by x V. All other settings were set to the ones used for recording Mode 2A.

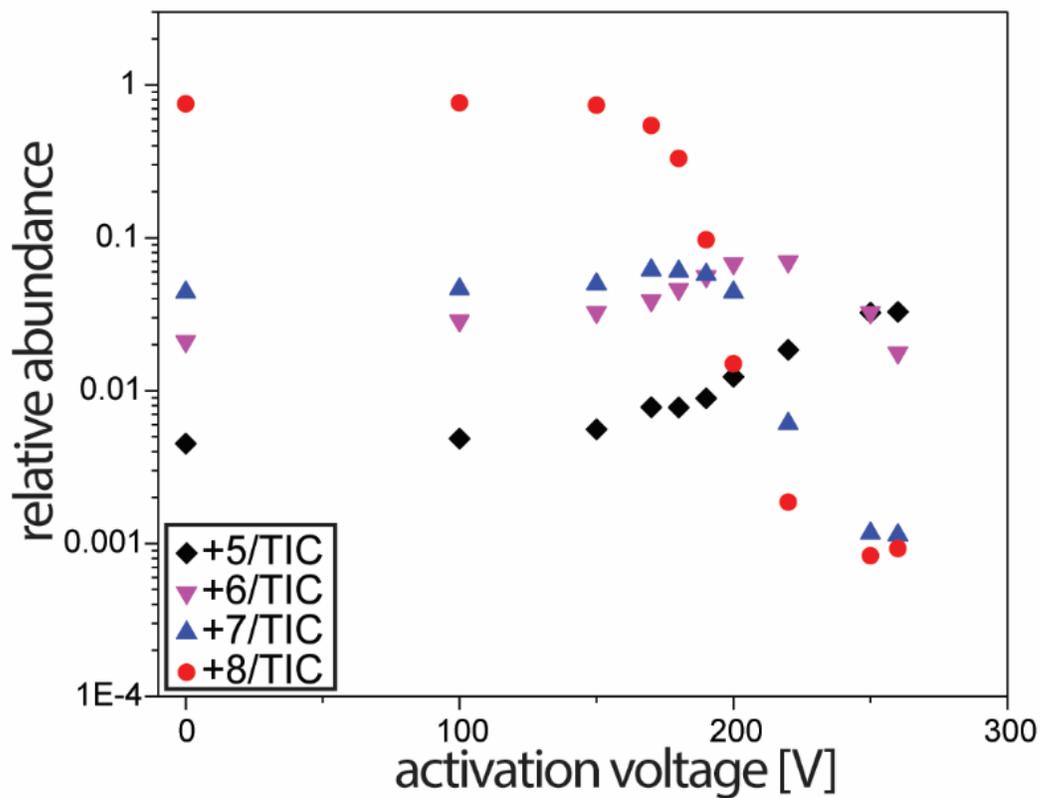


Figure S2. Relative abundances of ubiquitin +5, +6, +7, and +8 ions as a function of activation voltage obtained from collisional induced dissociation in the interface of TIMS-TIMS. The abundance of charge state +8 drops by three order of magnitudes between ~170 and 200 V. The abundance of charge state +7 decreases by two orders of magnitudes between ~200 and 250 V. We observe only a slight increases of +6 ions between ~200 and 250 V, suggesting that charge stripping is not of significance.

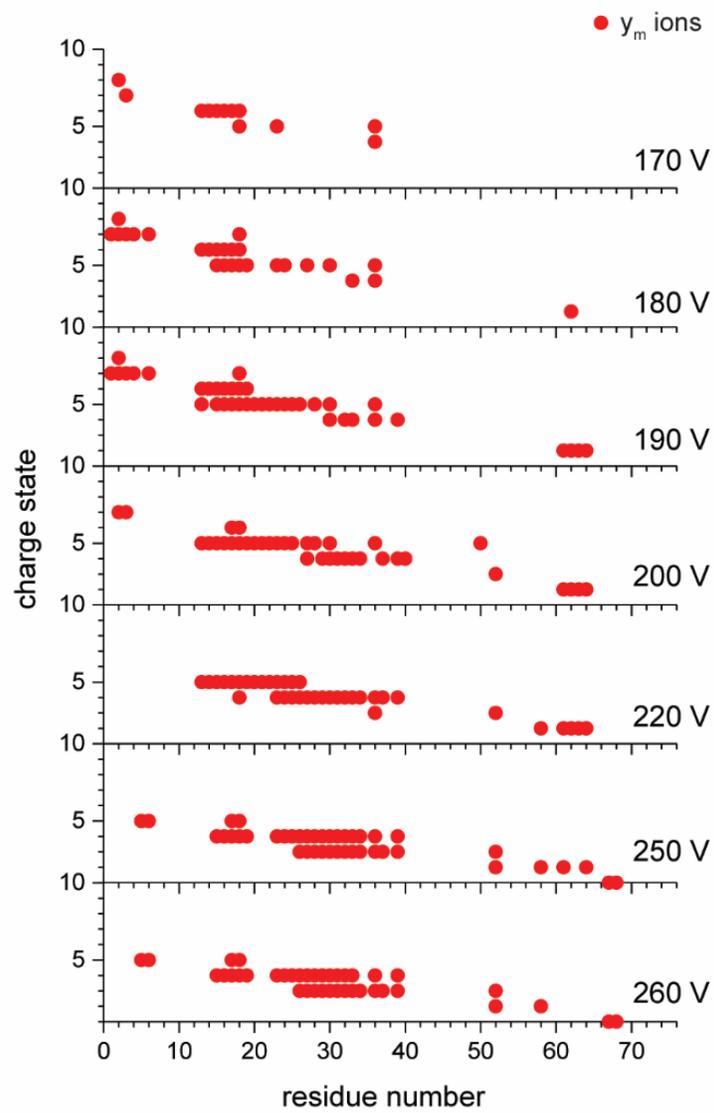


Figure S3. y_m^{n+} fragment ions of ubiquitin as a function of charge state and residue numbers obtained by collisional induced dissociation in TIMS-TIMS