Supporting Information:

Melting Proteins Confined in Nanodroplets with 10.6 µm Light Provides Clues about Early Steps of Denaturation

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Experimental and Instrumentation

Sample preparation. Ubiquitin and all other chemical used were of the highest purity (Sigma Aldrich, St. Louis, MO). Stock solutions were made to 100 μM in MilliQ water and diluted to 10 μM for analysis. Each solution was pH adjusted with acetic acid immediately prior to use. All optical components were purchased from ThorLabs (Newton, NJ).

Infrared Laser-Electrospray Source. Ions were produced by electrospray ionization using filamented borosilicate emitters (1.5 mm o.d./0.78 mm ID) pulled to 2.0±0.9 and 19 ± 0.4 μm using a micropipette puller (Flaming/Brown P-97, Sutter Instruments, Novato, CA). Droplet sizes are estimated to be between 1/17th and 1/20th of the diameter of the electrospray emitter, as previously described.[1] A platinum wire was inserted into the analyte solution through the back of the capillary to apply an electrospray potential of 0.8 –1.2 kV. The electrospray capillary situated concentric, and placed 3-5 mm from the instrument entrance.

The continuous wave CO_2 laser beam (25 W, Synrad, Mukilteo, WA) was directed, using several gold-coated mirrors, orthogonal to the instrument orifice. Light was focused to a ~500 μm spot using a plano-convex lens (f = 10 cm). The focal point of the lens was positioned at the immediate entrance of the instrument. The laser power was controlled by pulse-width modulation. After irradiating electrospray droplets, the laser line was terminated at a power meter.

Instrumentation. Experiments were performed on a home-built 4-meter ion mobility spectrometry (IMS)-mass spectrometry (MS) instrument, similar to previous designs.[2] Briefly, accumulated packets of ions are released (100 μs pulse width) into the drift tube held at ~12 V cm^{-1} where they are separated based on their mobilities through the buffer gas (2.50 Torr He), measured experimentally as a drift time (t_D). An ions' mobility can be related to its rotationally-averaged collision cross section (CCS, Ω) using:[3]

$$\Omega = \frac{(18\pi)^{1/2}}{16}\frac{ze}{(k_{\mathrm{b}}T)^{1/2}}\left[\frac{1}{m_{\mathrm{I}}}+\frac{1}{m_{\mathrm{B}}}\right]^{1/2}\frac{t_{\mathrm{D}}E}{L}\frac{760}{P}\frac{T}{273.2}\frac{1}{N}$$

where ze, and k_b, the charge on the ion and Boltzmann's constant, and T, P, and N are the temperature, pressure, and number density of the buffer gas, respectively. Parameters E and L are the applied electric field and the drift tube length. Mobility separated ions exiting the drift tube are pulsed orthogonally into a time-of-flight mass analyzer for *m*/*z* analysis, collected in a nested t_D(*m/z*) fashion.[4] Tandem IMS techniques have been described in detail previously.[2]

Supplemental data

Conformations produced by droplet irradiation are distinct from those produced by gas-phase heating.

IR heating of ions *in vacuo* is a means to induce fragmentation via infrared multiphoton dissociation.[5] To explore the differences between heating naked ions *in vacuo* and heating molecules immersed in droplets, we compared the structures formed via IR heating in droplets, IR irradiation of ions in the drift tube, and collisional activation of ions via tandem IMS. Figure S1 shows the $[M+8H]^{8+}$ cross section distributions obtained by irradiation of molecules in droplets. At 0 W, the sharp peaks at Ω ~ 1010 Å^2, corresponding to the compact, folded state, dominates the spectrum. By 11 W, the relative population of Ω ~ 1010 Å^2 has significantly decreased, and by 13 W this peak has decayed completely and new structures between Ω ~ 1100 – 1500 Å^2 have formed.

Also shown in Figure S1 are the cross section distributions obtained upon heating naked ions with IR light after they have undergone 1 m of mobility separation. As shown, there is no change to the cross section distribution between the spectrum obtained at 0 W and 20 W. This result is not unexpected, as other groups have observed efficient collision relaxation following vibrational excitation at pressures above ~10^{-5} Torr.[6]

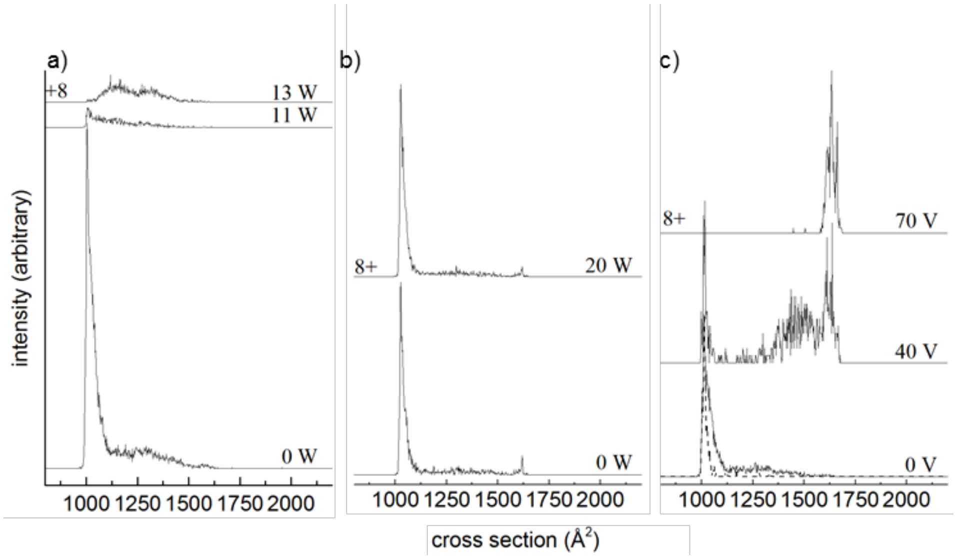

Figure S1. Cross section distributions for $[M+8H]^{8+}$ ions obtained by (a) droplet excitation with infrared light, (b) infrared excitation of naked ions following 1 m of drift separation, and (c) heating of ions through energetic collisions with the buffer gas after 1 m of drift separation.

The cross section distributions obtained by tandem IMS techniques (Figure S1c) shows ions subjected to energetic collisions with the buffer gas isomerize to form a broad feature centered at $\Omega \sim 1500$ Å^2 and three elongated peaks centered at $\Omega \sim 1635$ Å^2. At 70 V, the elongated structures centered at $\Omega \sim 1635$ Å^2 dominate the spectrum, indicating that the entire population of [M+8H]8+ ions have undergone a compact→extended transition. These peaks are not observed with IR heating of ubiquitin within droplets, nor are they observed with IR heating of ubiquitin *in vacuo*. This provides evidence for a distinct set of structures forming from changes to the droplet (or solution) temperature (i.e., solution transitions) as compared to those which result from *in vacuo* activation.

In summary, droplet excitation by IR light results in the depletion of compact $\Omega \sim 1010$ Å^2 structures with increasing laser power to form several broad features at $\Omega \sim 1150$ and 1330 Å^2. Excitation of naked ions in the drift tube after partial mobility separation did not produce a noticeable change in the structure of the gas-phase ion. The cross section distributions obtained from tandem IMS show that a different set of conformers are accessible to the *in vacuo* ion as compared to the solvated species.

References

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