Supporting Information:

Variable-Temperature-Electrospray Ionization for Temperature-Dependent Folding/Refolding Reactions of Proteins and Ligand Binding

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Expression and preparation of fraxatin:

A *His_6-GST-TEV-FXN* construct was generated by subcloning the FXN (Δ1-81) gene into a pET-28a(+) vector containing *His_6-GST-TEV-CyaY* [1] using the MEGAWHOP [2] protocol, and was transformed into the *E. coli* strain BL21(DE3) for expression. After growing at 37 °C to an OD_{600} of 0.5, 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added into the cell culture to induce protein expression at 18 °C. Cells were harvested by centrifugation the following morning, and stored in a -80 °C freezer until use. The cell pellet from a 3 L culture was thawed and resuspended in GST buffer A (50 mM Hepes, 150 mM NaCl, pH = 7.8). The cells were lysed by 2 cycles of French press at 18,500 psi, followed by centrifugation at 16,420 RCF for 30 min. The clarified lysate was loaded onto a manually packed GST-column (Prometheus) at 4 °C and bound proteins were eluted with GST buffer B (50 mM Hepes, 150 mM NaCl, 10 mM glutathione, pH = 7.8). The GST tag was cut by an overnight TEV digestion at 4 °C, and the products were loaded onto a Ni-NTA column (5 mL; GE Healthcare) to remove the TEV protease. The flow-through from the Ni-NTA column was concentrated to 20 mL, diluted to 150 mL with size exclusion buffer (50 mM Hepes, 250 mM NaCl, pH = 7.5), and loaded onto a HiPrep 26/60 Sephacryl S100 HR column. Fractions containing FXN were pooled, concentrated, frozen in liquid nitrogen, and stored at -80 °C until use. Concentration was determined using an extinction coefficient of 26,930 M^{-1} cm^{-1} at 280 nm as estimated by ExPASy ProtParam.

Analysis of experimental data

MS data were collected at a determined heat exchanger temperature based on a solution temperature determined from prior calibration. The average charge state (Z_{avg}) was calculated following the method of El-Baba et al.[3],

$$Z_{avg} = \frac{\sum_j^n z_j i_j}{\sum_j^n i_j}$$

where Z_{avg} is the average charge state, *j* is a single temperature, *i* is the normalized intensity of the ion signal. To determine the melting temp (T_{melt}), the inflection point was calculated by fitting the experimental data with a sigmoidal curve generated in SigmaPlot 10 using a sigmoid, 4 parameter fit,

$$Z_{avg}(T) = \frac{a}{1 + e^{-\left(\frac{T - T_{melt}}{b}\right)}}$$

where Z_{avg} is the average charge state and T_{melt} is the temperature of melting, and *a* and *b* are fitting parameters.

Custom Power Supply Fabrication

The TEC controller and additional electronics are housed in a small aluminum enclosure (22.86 cm L x 17.78 cm W x 5.08 cm H). An AC/DC power supply (Meanwell LRS-100 24) provides 24 VDC to the TEC controller, 24 VDC fans, and the DC/DC converter (Texas Instruments PTN78020WAH). The DC/DC converter is mounted on a custom PCB, designed using EAGLE 9.6.2 (Autodesk), and fabricated by OSHPark (www.oshpark.com). The DC/DC converter steps down the 24V DC from the power supply to the max voltage of the TEC of 5.3V at 6A. The DC/DC converter allows for the TEC to operate at the optimal voltage and current to

quickly and efficiently change the temperature. In addition, the voltage output of the DC/DC converter can be changed by changing the resistance value if a different TEC is ever selected. The small footprint and preset output voltage allow users who are not familiar with the system to operate the VT-ESI device using included software safely and quickly with the TC-720 (TE Technology). A wiring schematic of the power supply can be found in **Figure S1**.

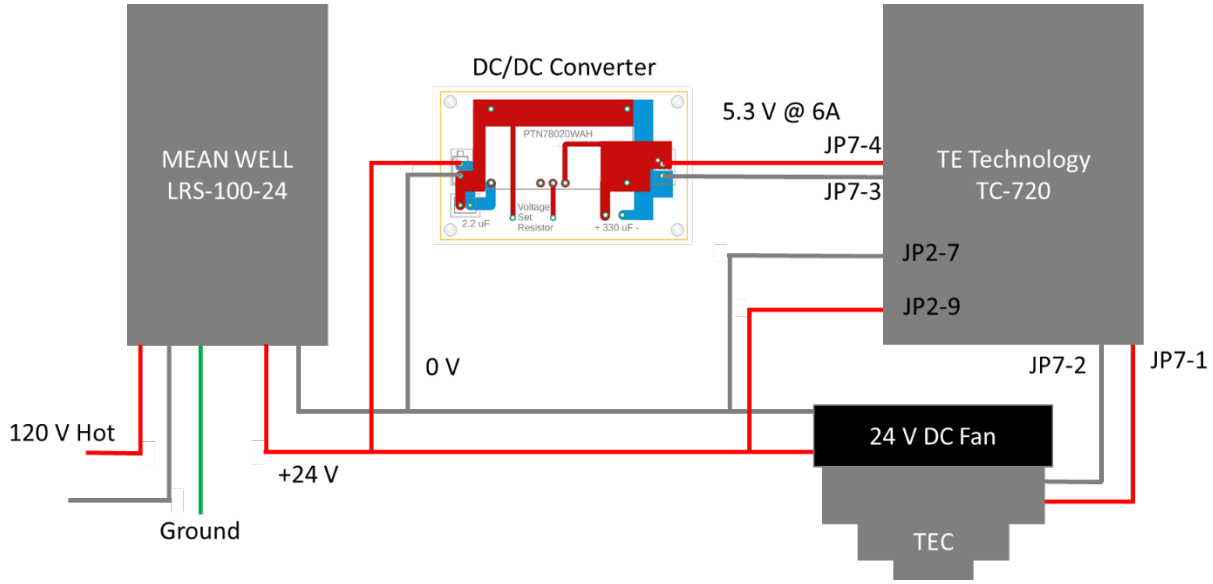

Figure S1. Wiring schematic of the Peltier TEC.

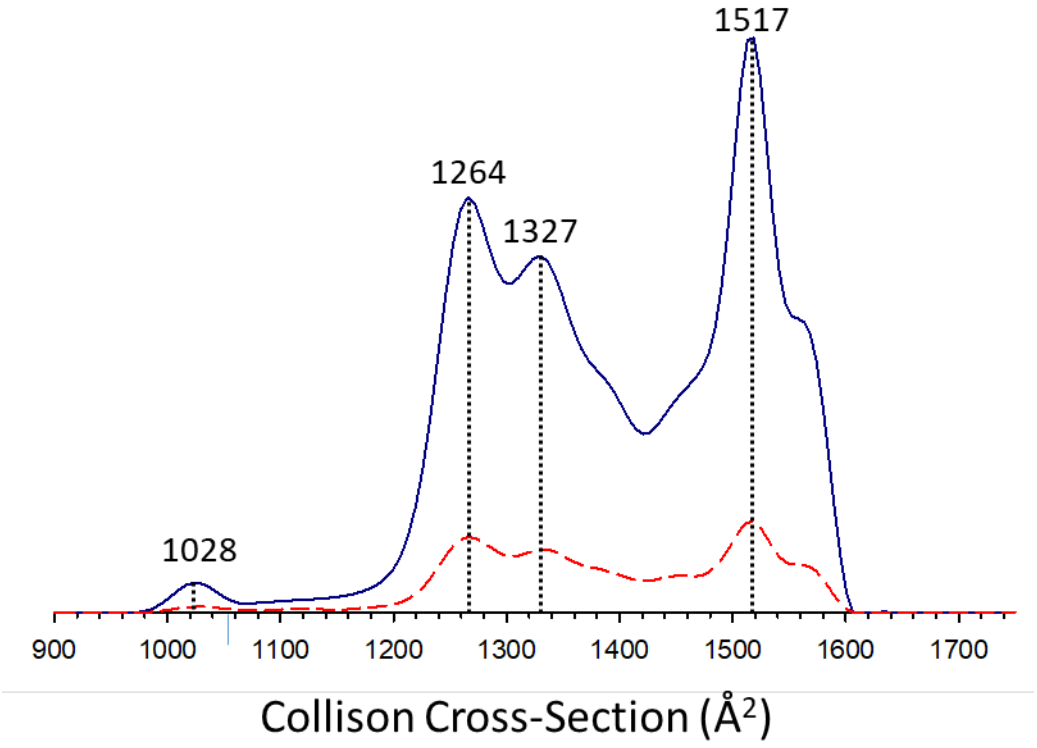

Figure S2. CCS profiles for the A state of ubiquitin from a 49:49:2 MeOH:H_2O:acetic acid solution. Data acquired using the Waters SYNAPT G2 TWIMS and vT-ESI at 5 ^{o}C (blue) and 25 ^{o}C (red).[4]

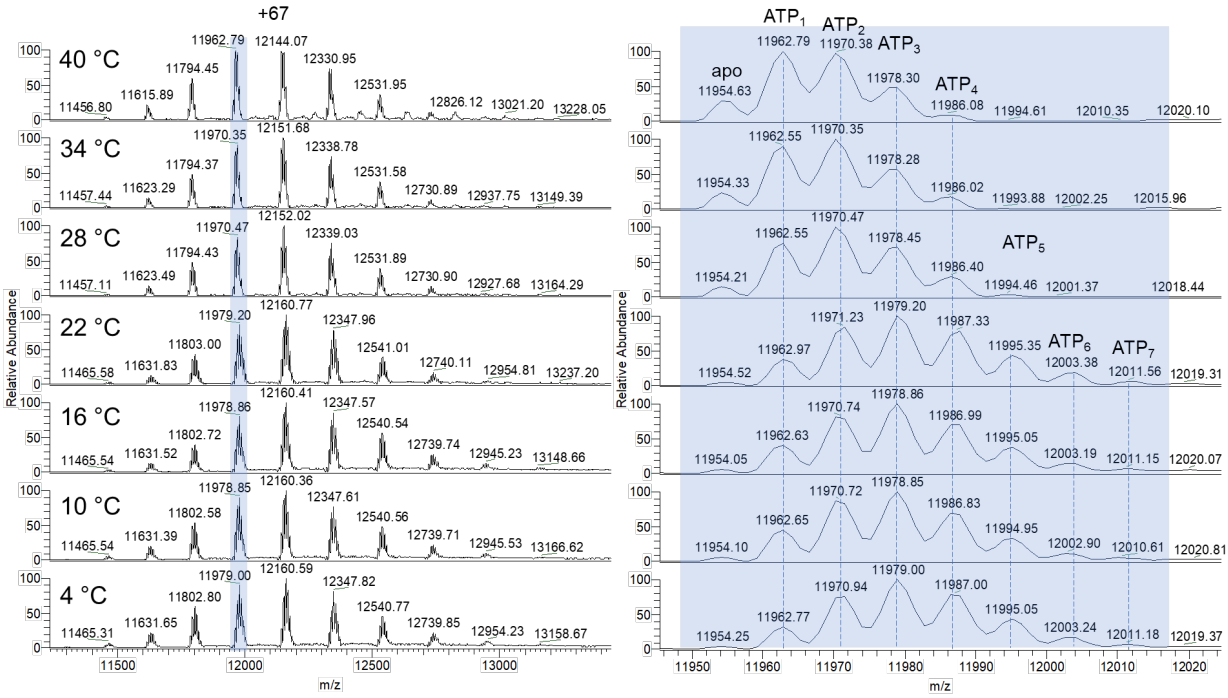

Figure S3. Temperature-dependent MS of 1µM GroEL with ATP (125 uM) in 200 mM ammonium acetate.

Temperature	Z (+)	Average CCS ($Å^2$)	Standard Deviation of CCS	FWHM	R_{IM}
5 °C	6	1540	8	257	6.0
	7	2160	5	176	12.3
37 °C	6	1520	3	220	6.9
	7	2150	4	142	15.1
80 °C	6	1540	10	275	5.6
	7	2160	5	180	12.0

Table S1: Calculated CCS values for acquisitions of FXN at 5 °C, 37 °C, and 80 °C with the peak fitting parameters (n=3). Where R_{IM} is the $CCS/\Delta CCS_{FWHM}$

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