Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

for

Overcoming Aggregation with Laser Heated Nanoelectrospray Mass Spectrometry:

Thermal Stability and Pathways for Loss of Bicarbonate from Carbonic Anhydrase II

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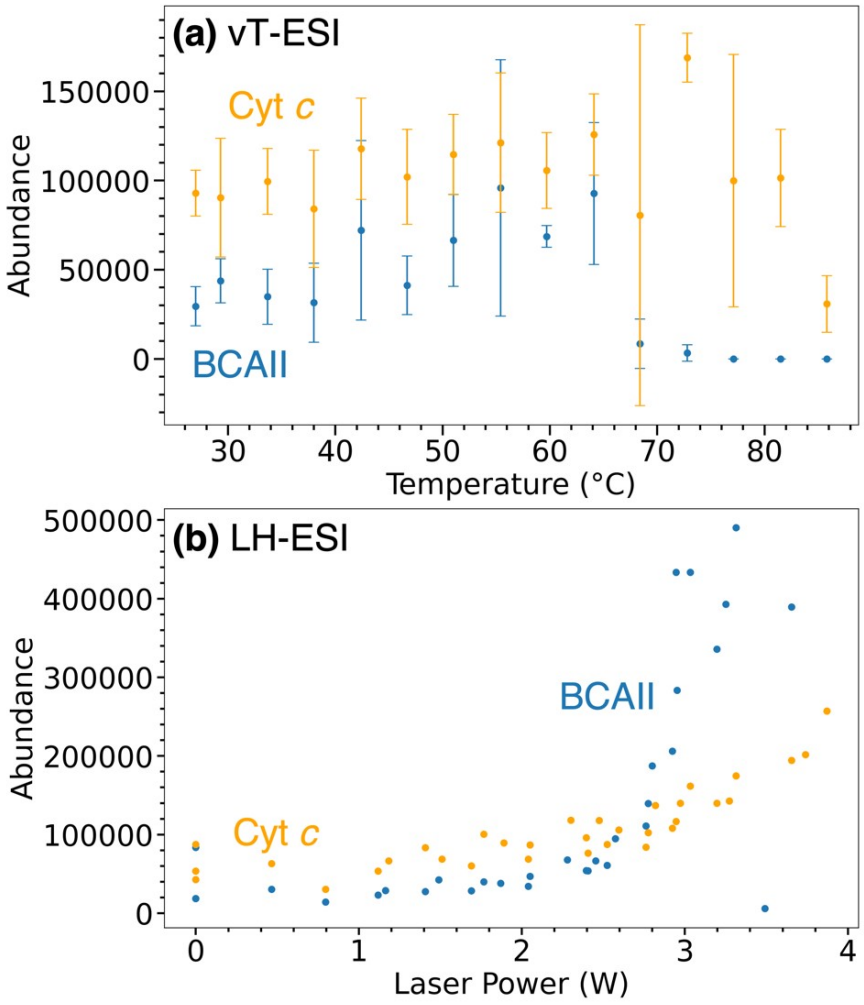
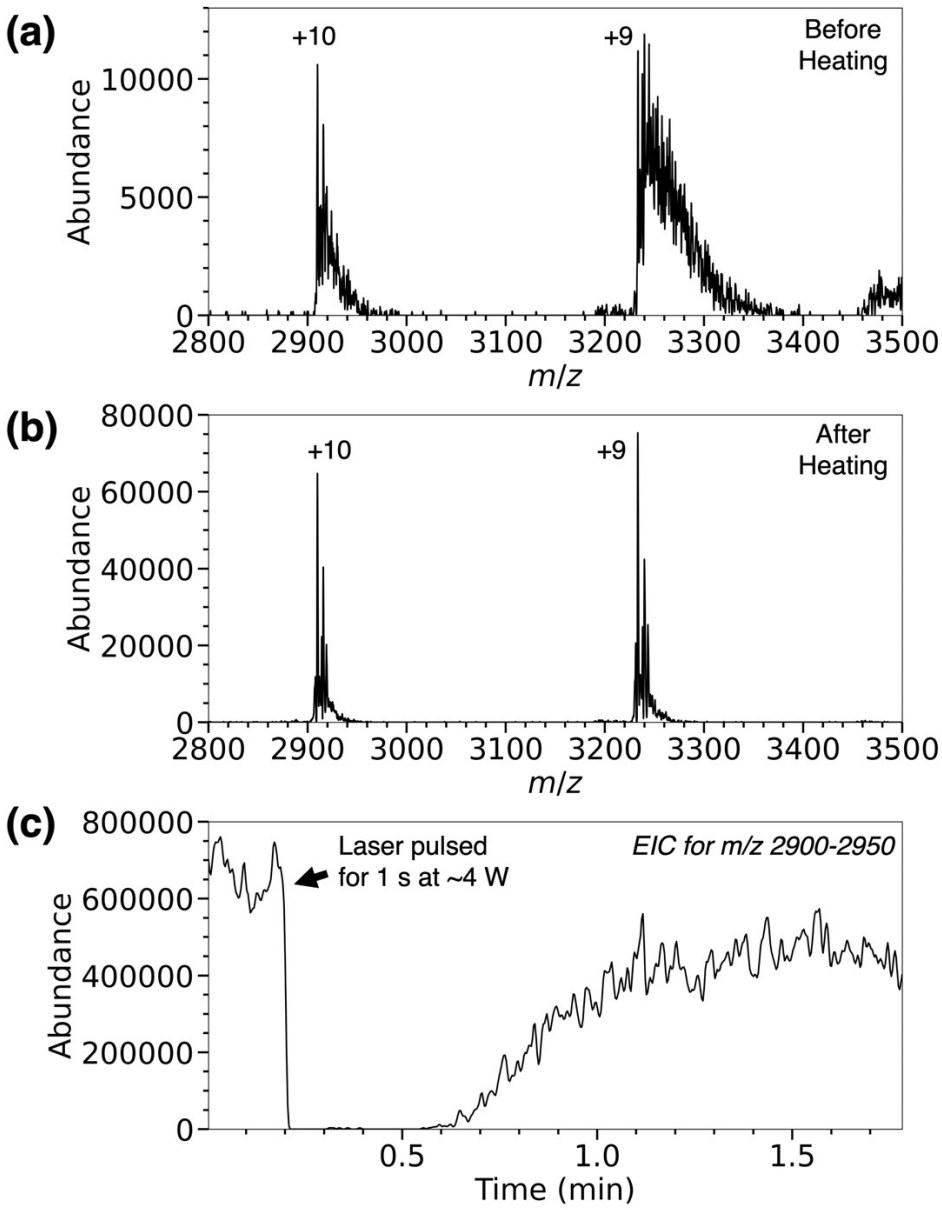
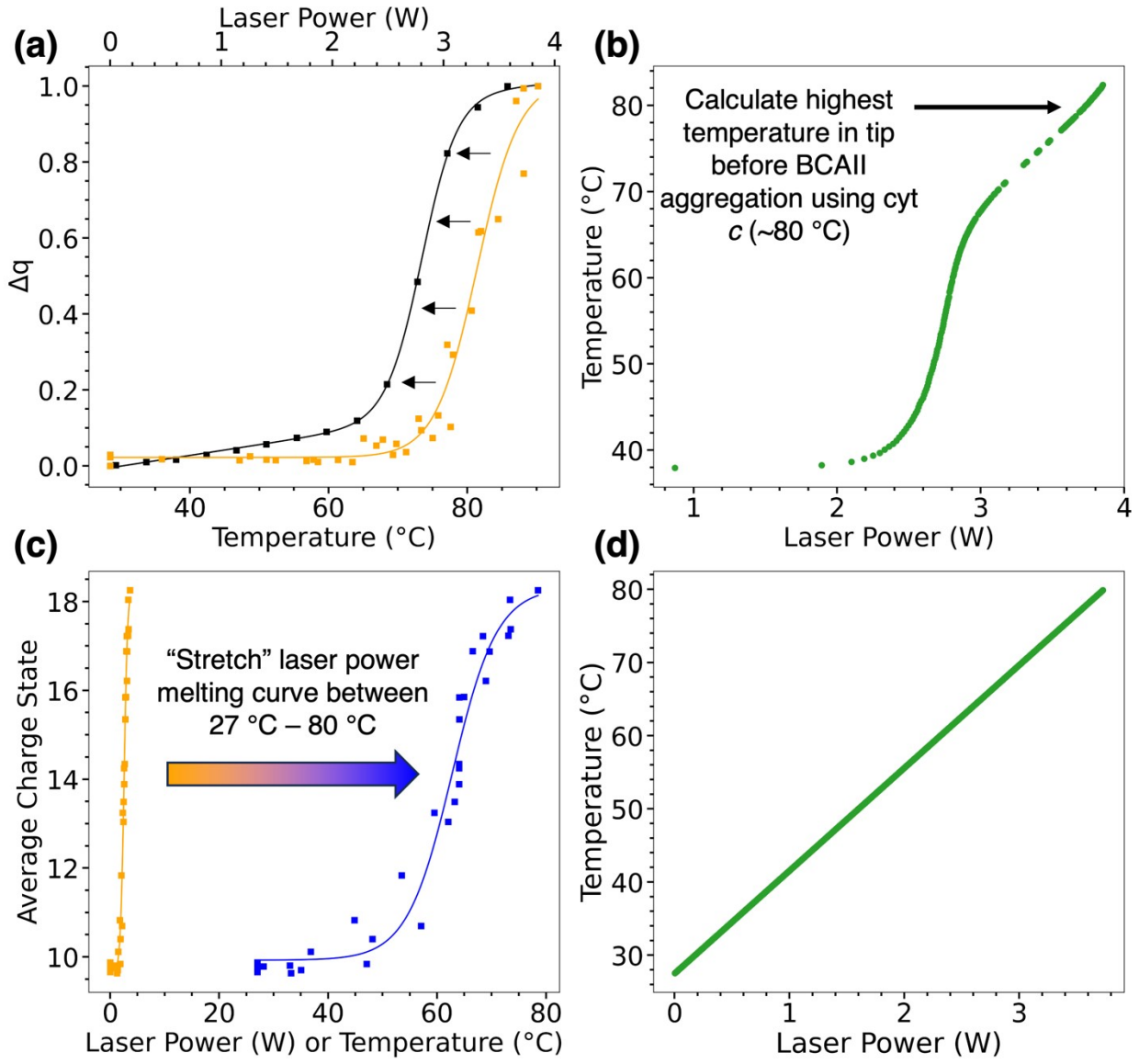


Supporting Figure 1. The total ion abundances of BCAII (blue) and cyt *c* (orange) as a function of (a) solution temperature and (b) laser power. Aggregation above ~72 °C results in no BCAII signal and low cyt *c* signal in vT-ESI experiments, whereas high laser powers increase protein signal for both proteins before a sharp decrease in abundance of BCAII at laser powers >~3.3 W.

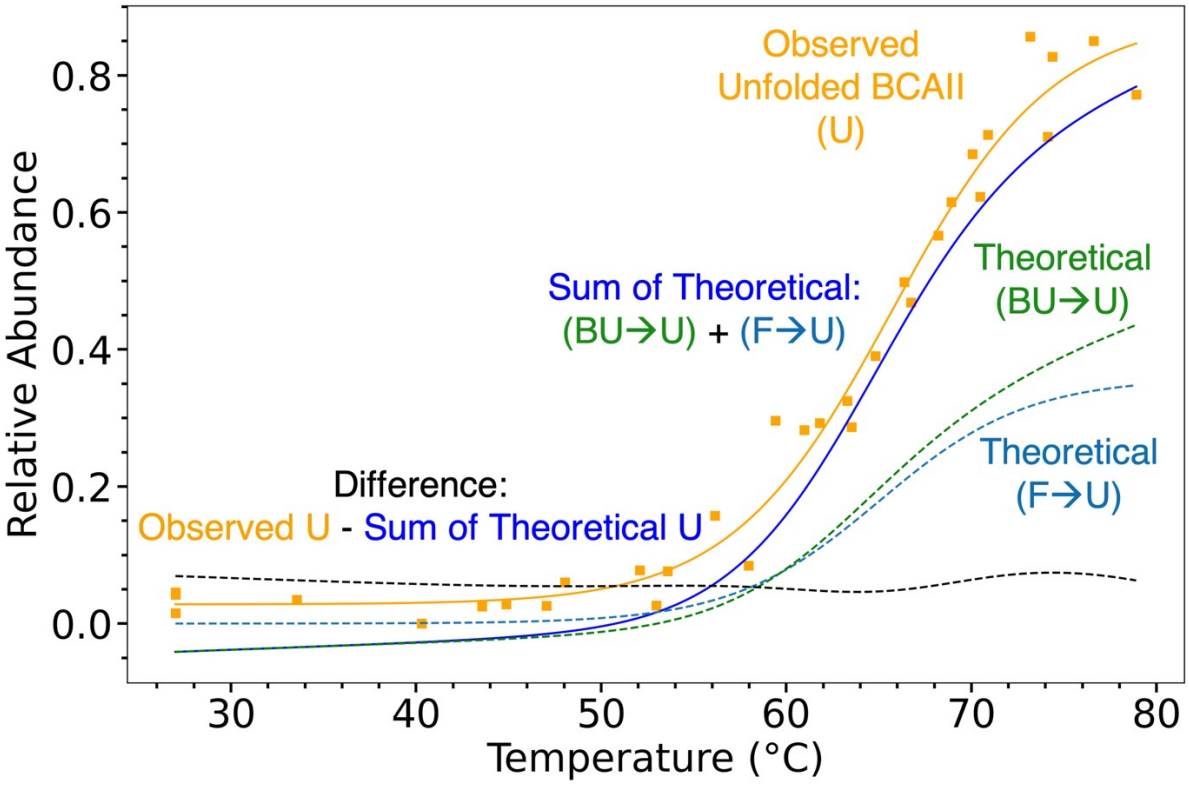


Supporting Figure 2. Small molecule/ion adduction to BCAII results in (a) charge states that tail to higher *m*/*z* before heating, but (b) significantly narrower charge states after heating and a return of the solution to room temperature indicating that a thermal cycle can significantly reduce adduction. A typical (c) extracted ion chromatogram (EIC) for the region of 2900 – 2950 *m/z* (the +10 charge state) demonstrates the time required for aggregated material to be expelled from the heated portion of the emitter and signal to return after laser heating at ~4 W.



Supporting Figure 3. The two-protein calibration procedure consists of (a) aligning the normalized change in the weighted average charge state of cyt *c* measured by vT-ESI and LH-ESI to (b) determine the temperature in the tip at high laser powers. The BCAII melting curve (c) determined by LH-ESI (in yellow) was then mapped between room temperature and ~80 °C (mapped function in blue) to generate (d) a linear calibration function for converting laser power to solution temperature. In contrast to a prior LH-ESI report where cyt *c* was used to establish a relationship between laser power and solution temperature,[1] a linear temperature calibration is

generated here by "stretching" the sigmoidal fit for BCAII determined by LH-ESI between room temperature (27 °C) and the maximum temperature in the tip before BCAII aggregation determined using cyt *c* unfolding (~80 °C). The T_m values determined for BCAII and cyt *c* using this method are 63.4 ± 0.6 °C and 73.9 ± 0.5 °C, in excellent agreement with literature T_m values for these proteins.



Supporting Figure 4. Modeling the theoretical abundance of U originating from the BF → U pathway as a function of temperature using the difference between the summed abundance of the U that originates from the BU → U and F → U pathways and the measured abundance of U.

References

1 J. S. Jordan and E. R. Williams, *Anal. Chem.*, 2022, **94**, 16894–16900.