Implementation of Fragment Ion Protection (FIP) during Ultraviolet Photodissociation (UVPD) Mass Spectrometry

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Supporting Information

Supporting information includes a graphical proof-of-principle illustrating the application of a resonant excitation waveform to move an ion out of the path of the laser beam during UVPD and its impact on production of CAD and UVPD fragment ions; a schematic of a representative waveform applied during UVPD-FIP; ion profiles produced from UVPD of myoglobin with and without FIP; graphical display of the impact of activation time and waveform amplitude to cause loss of 15% of precursor ions; theoretical isotope distribution of a_{71} ion from UVPD of ubiquitin; graphical displays of sequence coverage and number of fragment ions generated from UVPD of myoglobin as a function of the number of laser pulses with and without FIP; distribution of deconvolved fragment ions from UVPD of myoglobin based on mass bins; and graphical displays of sequence coverage and number of fragment ions generated from UVPD of cytochrome c as a function of the number of laser pulses with and without FIP.

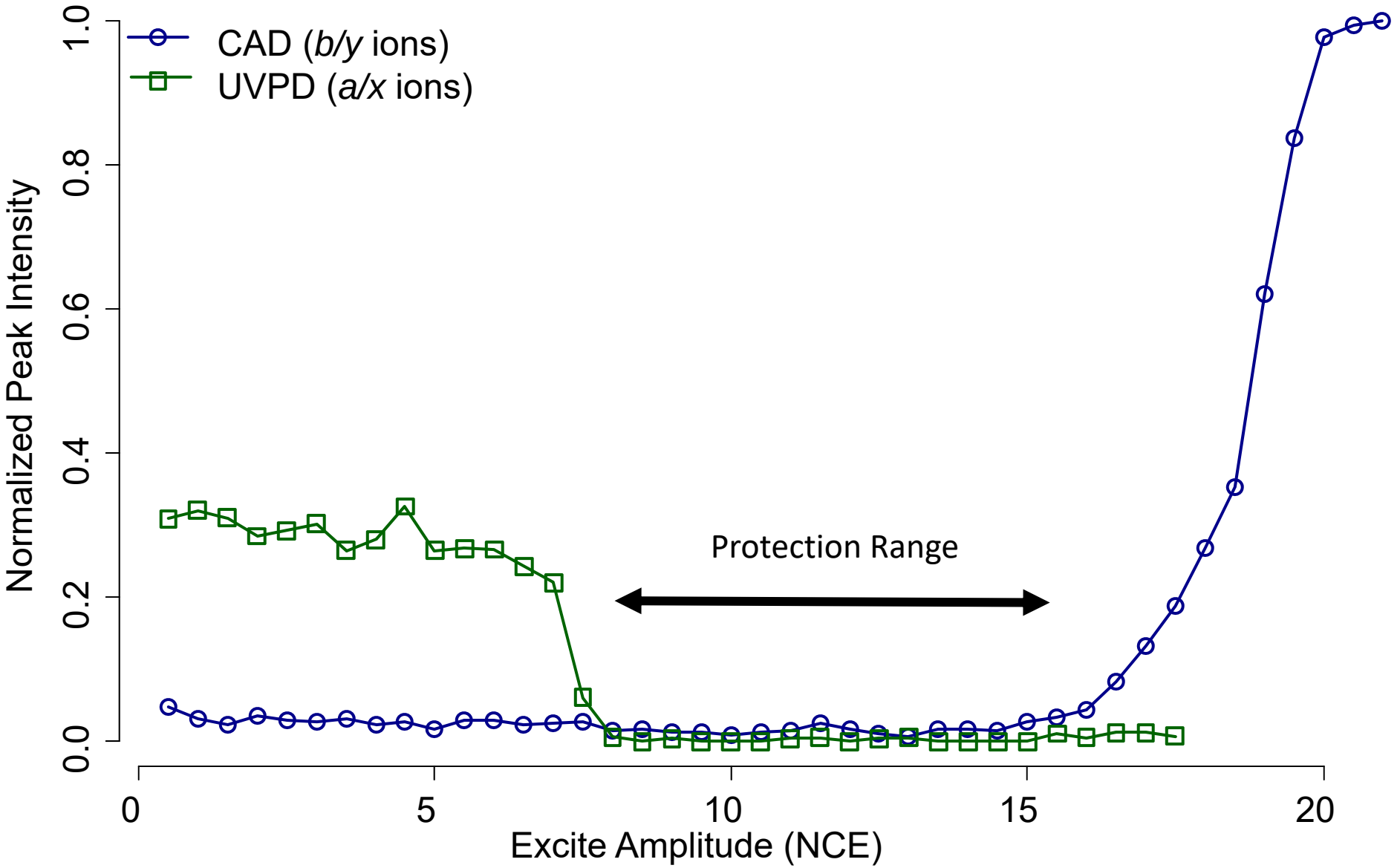

Figure S1. Proof-of-principle illustrating the application of a resonant excitation waveform to move an ion out of the path of the laser beam during UVPD and its impact on production of CAD and UVPD fragment ions. The selected ion is angiotensin I (3+). The amplitude of excitation was varied from 0 to 20 NCE. Ten pulses were used for UVPD. The abundances of all *b/y* ions generated by CID were summed and plotted as blue circles. The abundances of all *a/x* ions generated by UVPD were summed and plotted as green squares. At low excitation amplitudes, angiotensin undergoes UVPD and is converted to fragment ions. At medium amplitudes, angiotensin is moved outside of the laser beam path and does not undergo UVPD. This represents the range of protection. At high amplitudes, angiotensin undergoes collisional activation and is primarily converted to CID-type fragment ions.

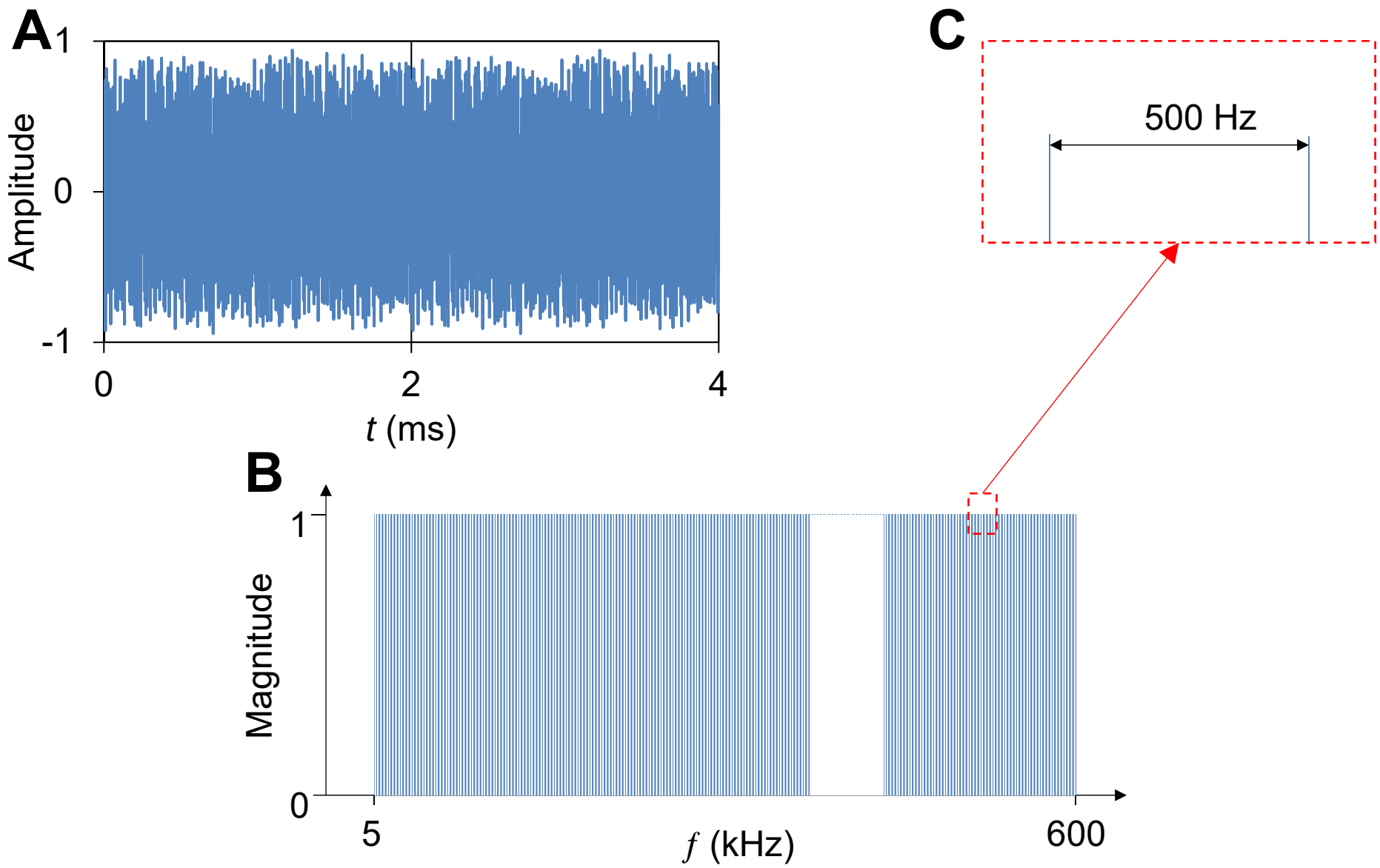

Figure S2. Schematic of a representative waveform applied during UVPD-FIP. With a frequency of 500 Hz (2 ms period), the waveform was repeated for however long was necessary during UVPD-FIP. A) A typical UVPD-FIP activation time of 4 ms would require 2 repeats of the waveform. B) For every waveform repeat the frequency domain of a waveform is composed of frequencies ranging from 5 – 600 kHz. For the UVPD-FIP experiments a flat magnitude frequency profile waveform was used. A notch was placed in the waveform to minimize resonant excitation of precursor ions centered at a q value of 0.25. C) Frequency components within the waveform were spaced by 500 Hz. Amplitude and magnitude are for reference and not to scale.

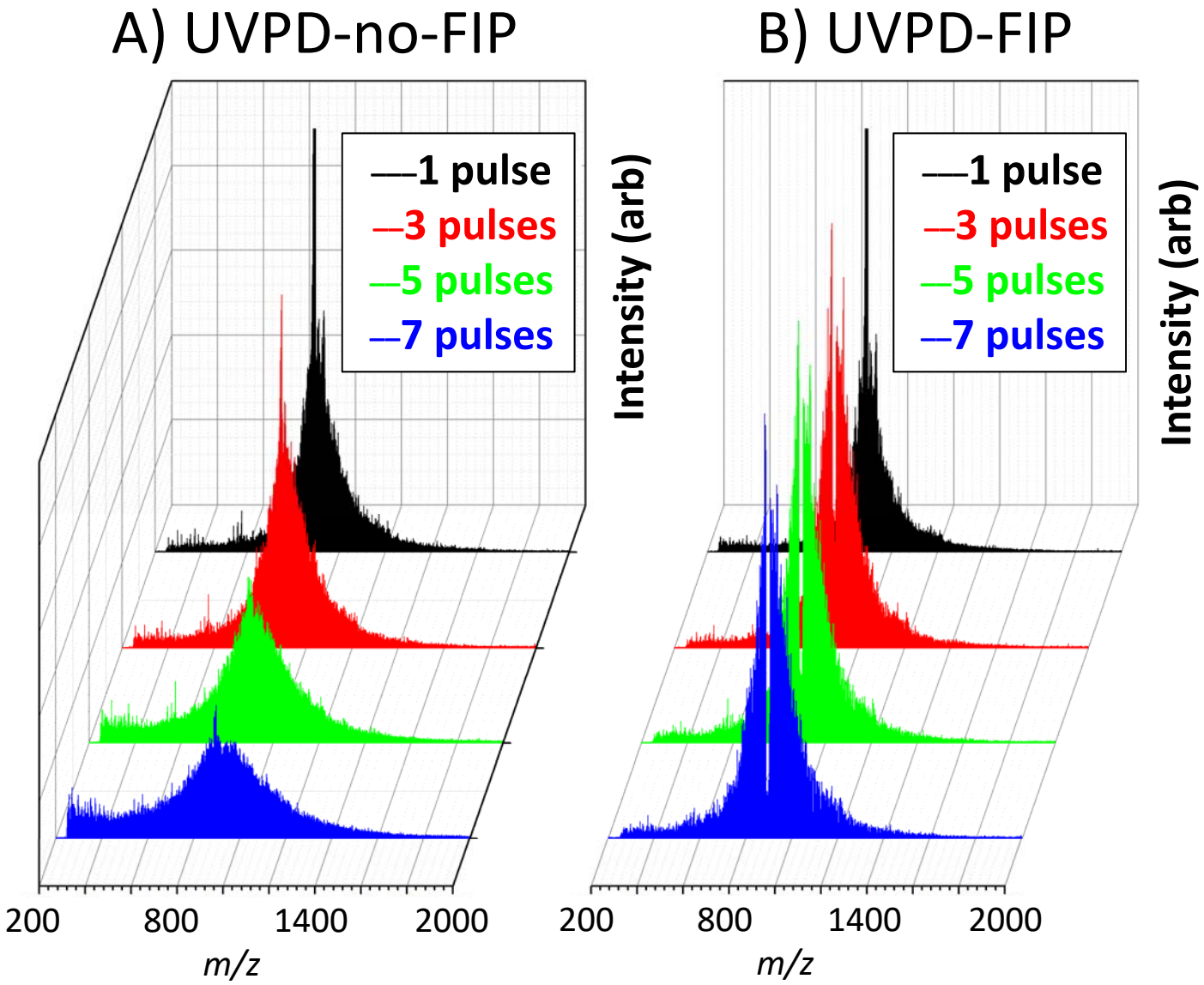

Figure S3. Apomyoglobin $[M+19H]^{19+}$ subjected to UVPD using a variable number (N) of pulses where N = 1,3, 5, or 7. Products were analyzed in the linear ion trap. A) Fragment ion protection disabled. B) Fragment ion protection enabled.

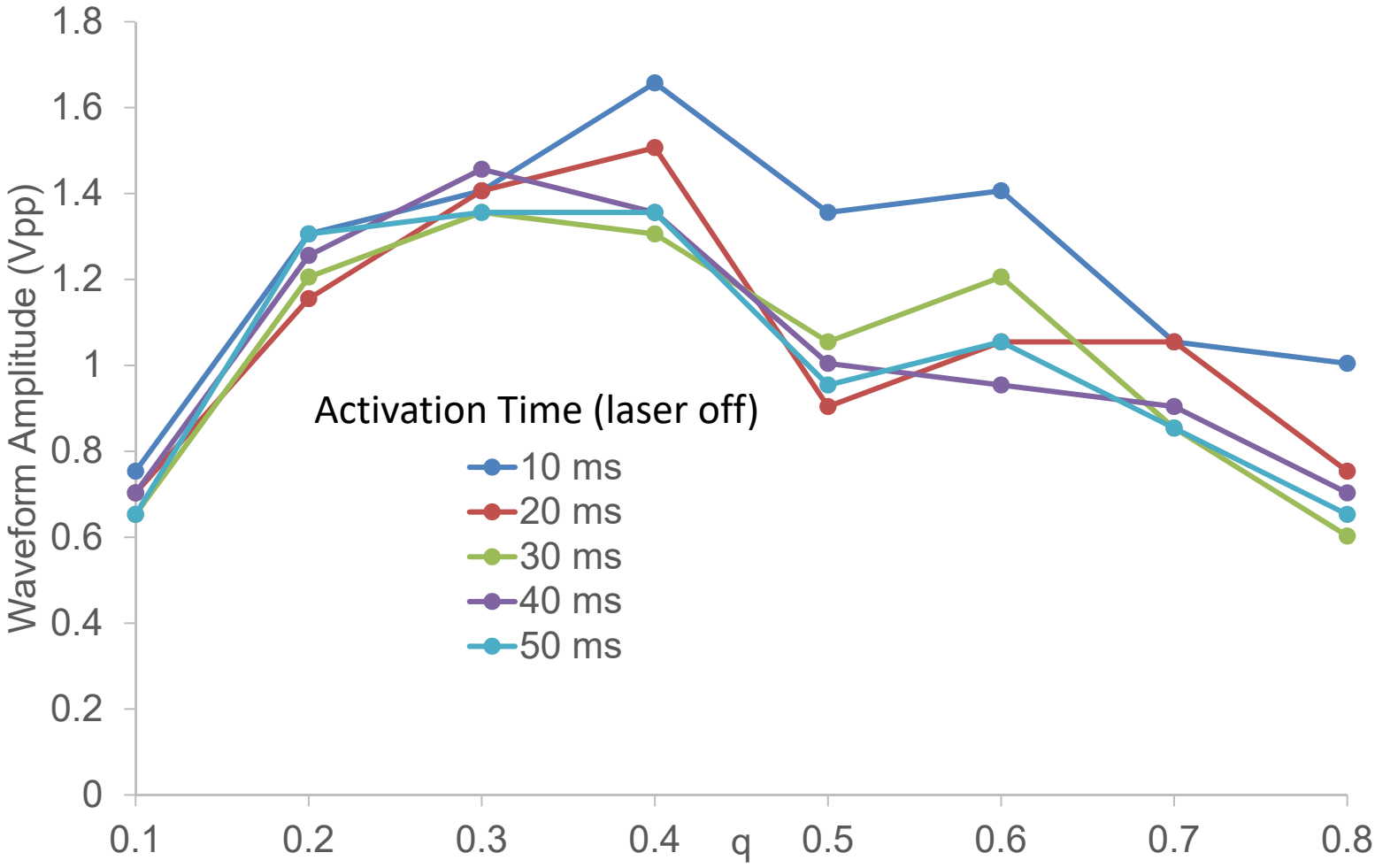

Figure S4. Ubiquitin ions (12+) were subjected to auxiliary waveform resonance excitation in the low pressure trap at various trapping conditions (q value = 0.1 to 0.8). With the laser turned off, activation time was incremented from 10 to 50 ms, and for each activation time step the amplitude of the excitation waveform was ramped. For each activation step the excitation amplitude required to cause loss of 15% of the precursor ions owing to collisional dissociation or ejection was recorded. For this assessment, a dipolar waveform with frequency components of equal magnitude ranging from 5 kHz to 600 kHz, encompassing the entire *m/z* range occurring between stability q values 0.908 and 0.012, was applied to the ion trap as described in **Figure S2**. For this experiment the waveform did not contain a notch to assure that ions would experience resonance excitation by the auxiliary waveform independent of q. This graphical display establishes acceptable boundary conditions for application of waveforms without causing excessive loss of ions owing to CAD or ejection.

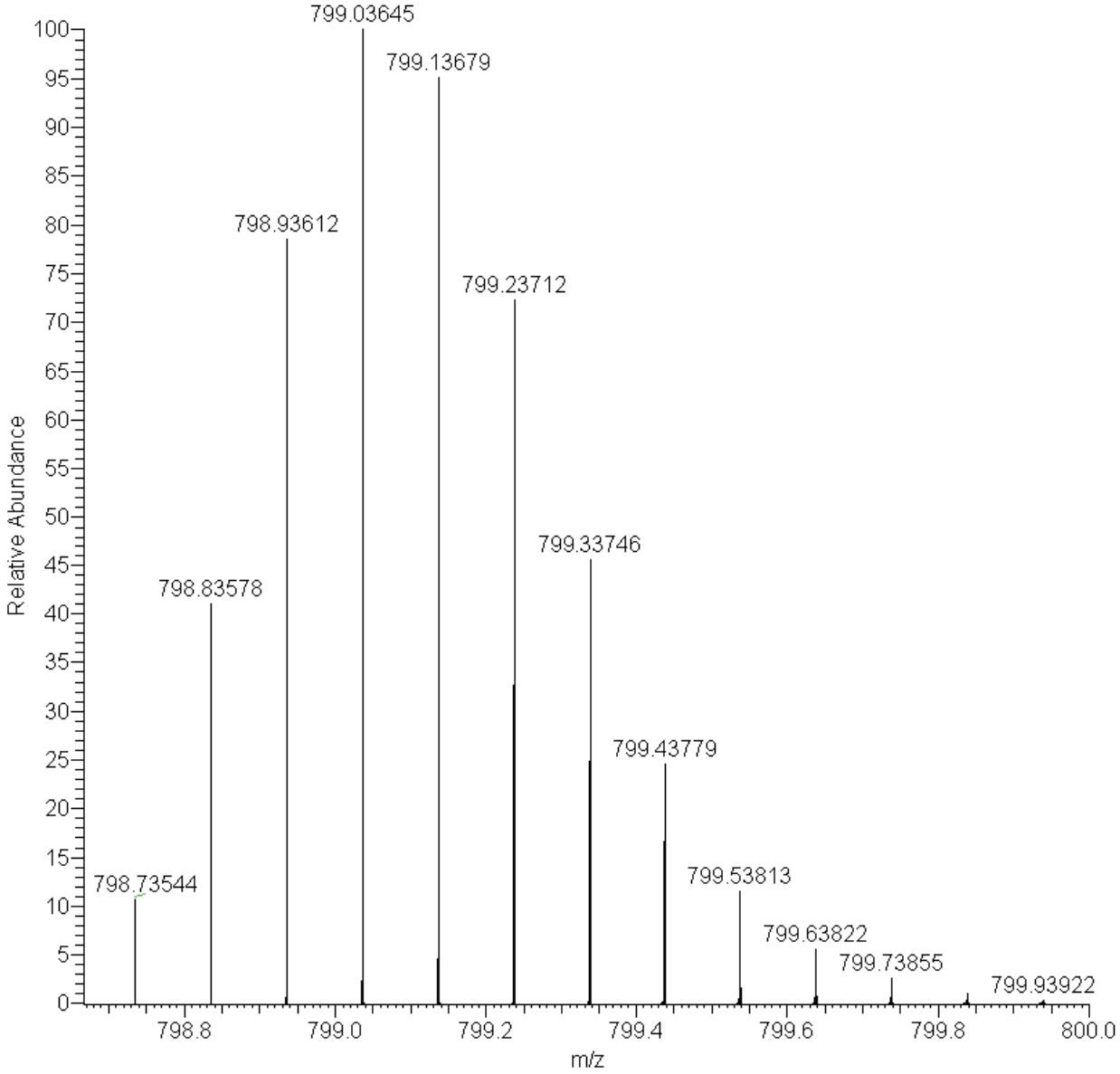

Figure S5. Example of the theoretical isotope distribution of the a_{71} (10+) ion from UVPD of ubiquitin (12+). The experimentally observed profiles without and with FIP are shown in Figure 3A. The formula of this ion is C355H587N94O113S with a monoisotopic mass of 7975.289868 Da. Given acceptable S/N and without overlapping background ions and other isobaric analyte ions, the profile of the a_{71} (10+) ion would be expected to mirror this profile.

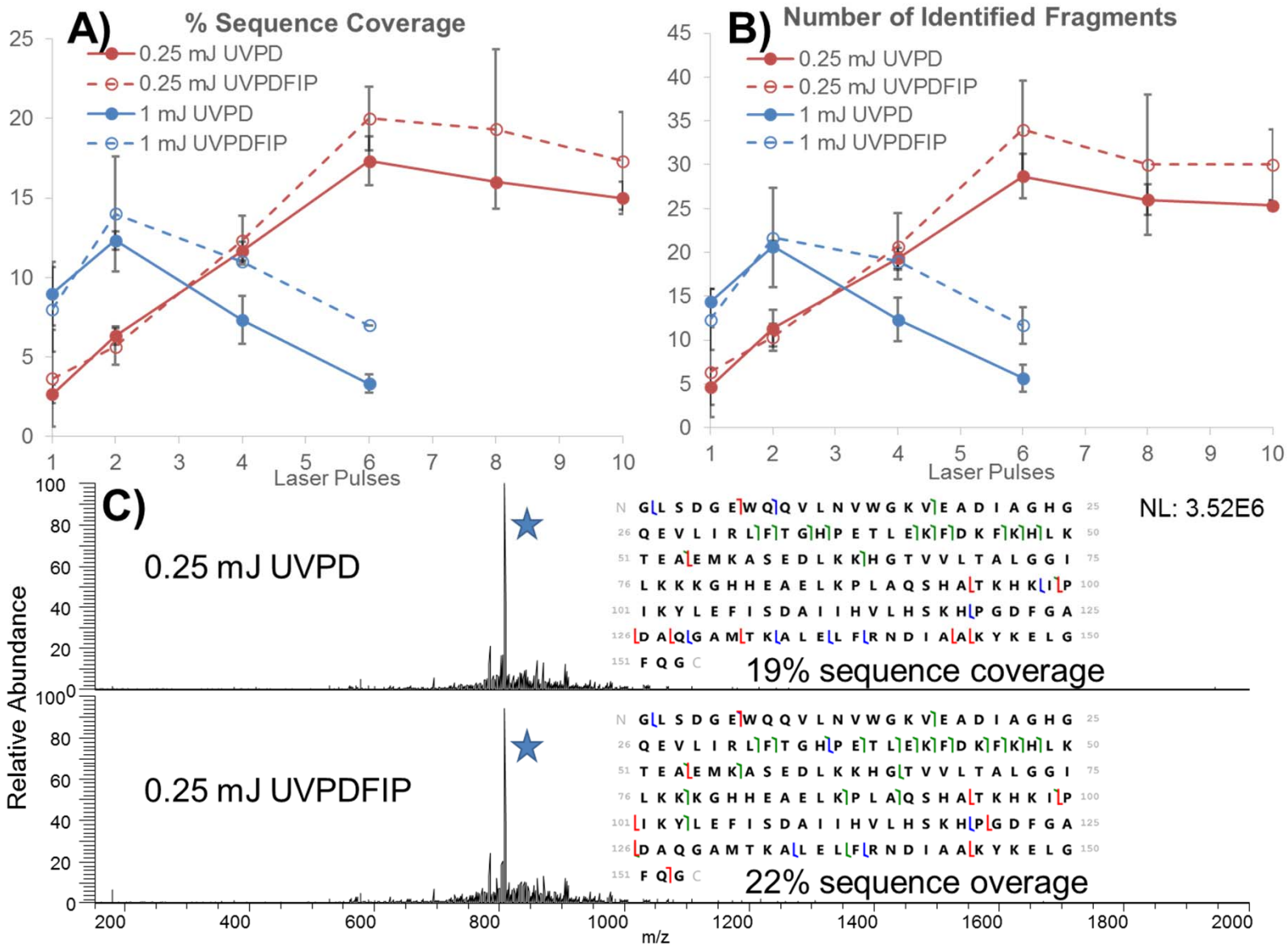

Figure S6: UVPD of apo-myoglobin (21+). A) sequence coverage and B) number of fragment ions identified using 0.25 mJ/pulse (red) or 1 mJ/pulse (blue) upon application of 1 to 10 laser pulses (with solid lines for UVPD and dashed lines for UVPD-FIP). C) UVPD mass spectra (eight 0.25 mJ pulses) and sequence maps without and with FIP. Error bars represent standard deviation of triplicate results.

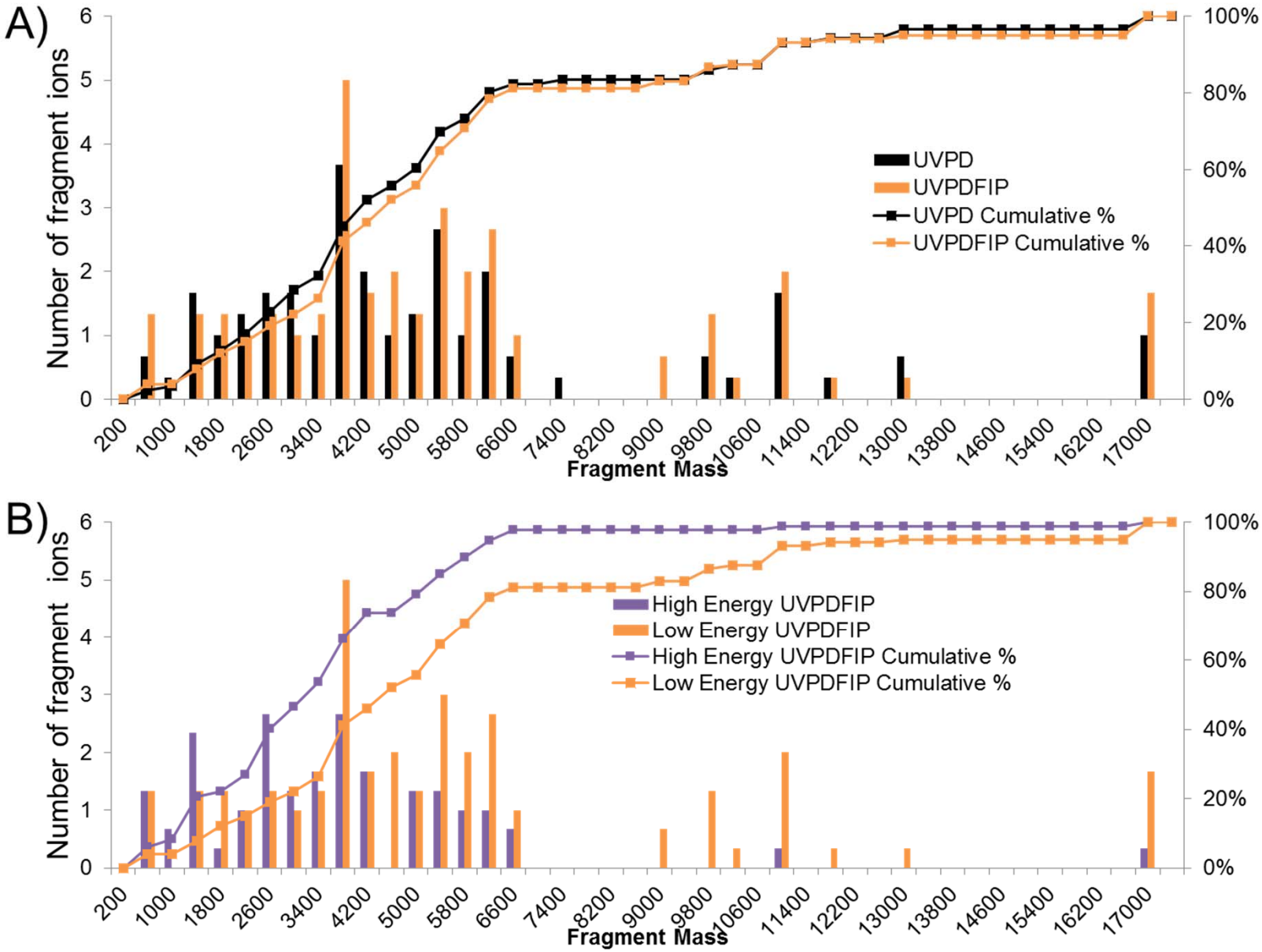

Figure S7: Distribution of deconvoluted fragment ions in 400 Da mass bins for apo-myoglobin (21+) obtained using A) eight 0.25 mJ laser pulses (UVPD versus UVPD-FIP), and B) six 0.25 mJ laser pulses (UVPD-FIP) versus two 1.0 mJ laser pulses (UVPD-FIP). The trend-lines show the cumulative percentage of fragment ions from low to high mass. Results represent the average of triplicate experiments.

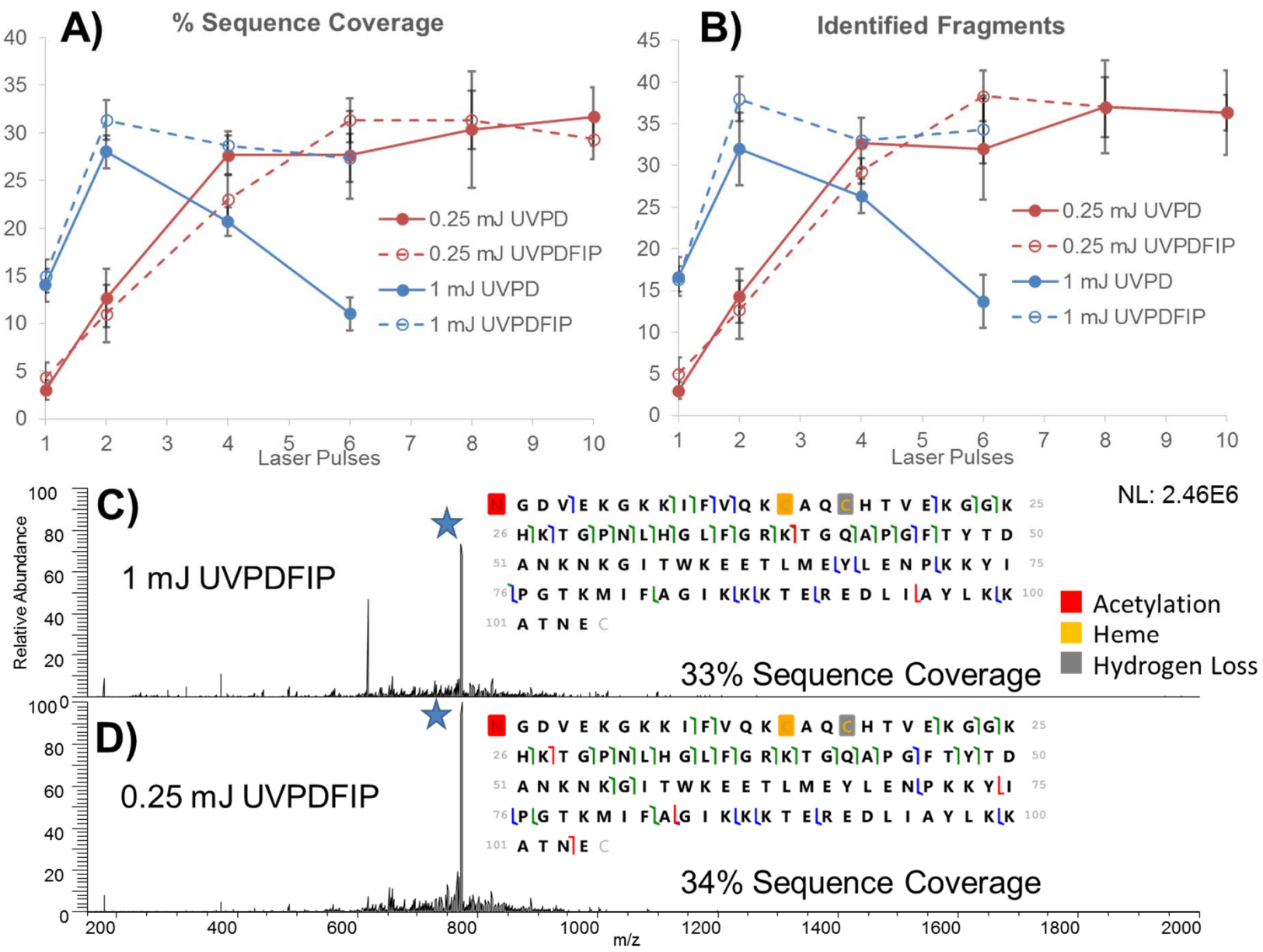

Figure S8: UVPD of cytochrome c (16+). A) sequence coverage and B) number of identified fragment ions using 0.25 mJ/pulse (red) and 1.0 mJ/pulse (blue) UVPD (solid traces) and UVPD-FIP (dotted traces). Spectra and sequence coverage maps using C) two 1.0 mJ/pulse UVPDFIP or D) six 0.25 mJ/pulse UVPD-FIP. Error bars represent standard deviations based on triplicate results.