Supporting Information

Soft Picosecond Infrared Laser Extraction of Highly Charged Proteins and Peptides from Bulk Liquid Water for Mass Spectrometry

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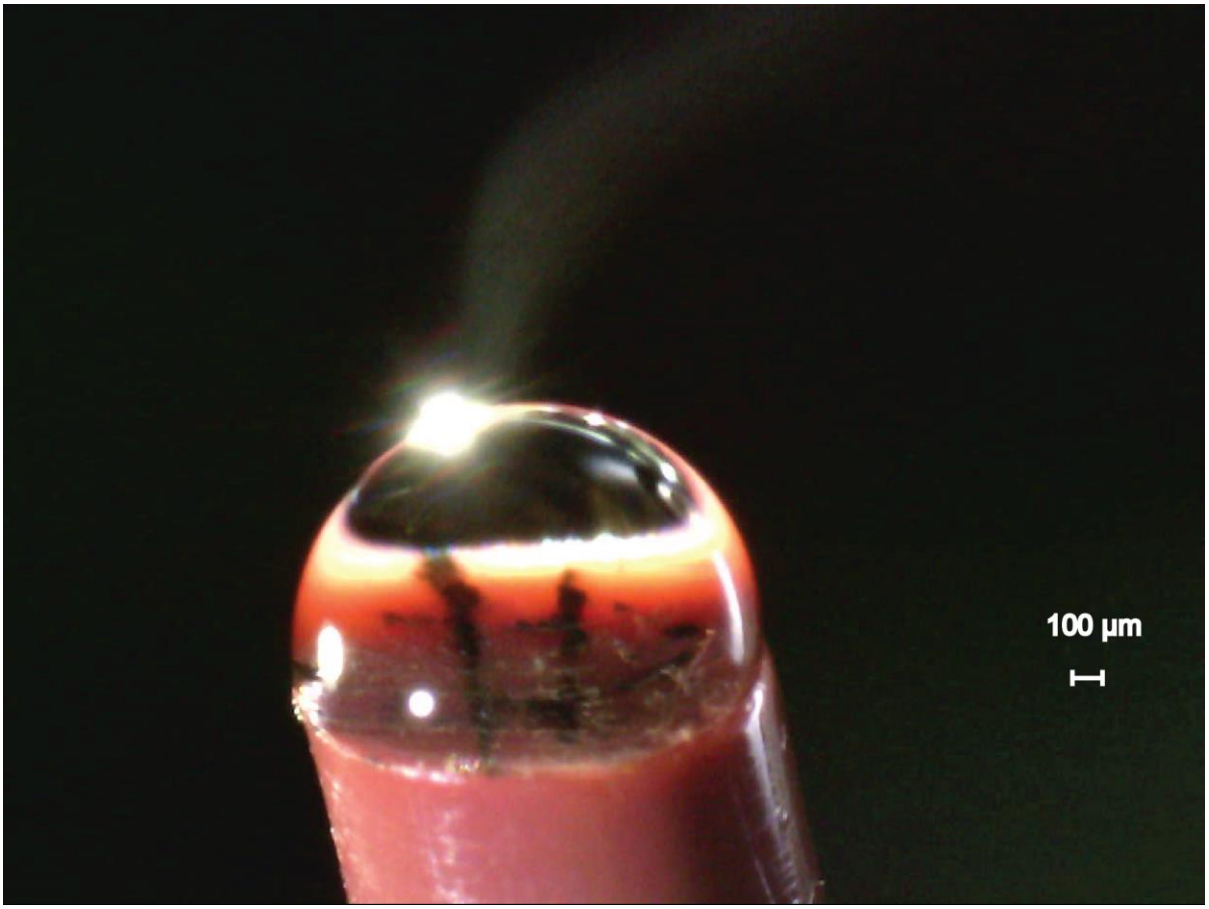

Figure S1. Image of sample delivery capillary with the sample bead undergoing 1 kHz PIRL-DIVE ablation. Scale bar shown is 100 µm.

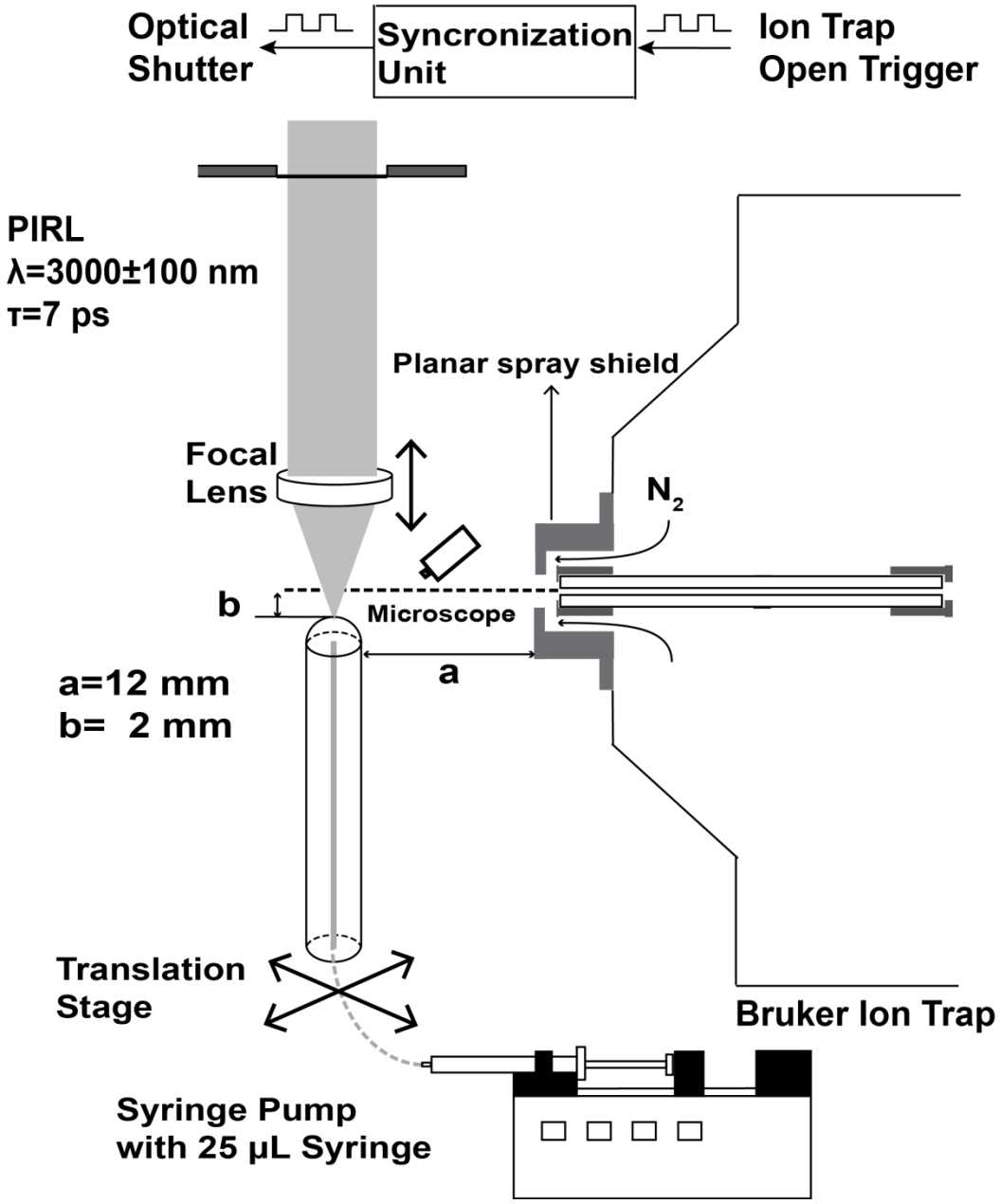

***Figure S2*.** A schematic representation of the sample delivery system, ablation, and modified ion trap MS with standard planar shield end plate.

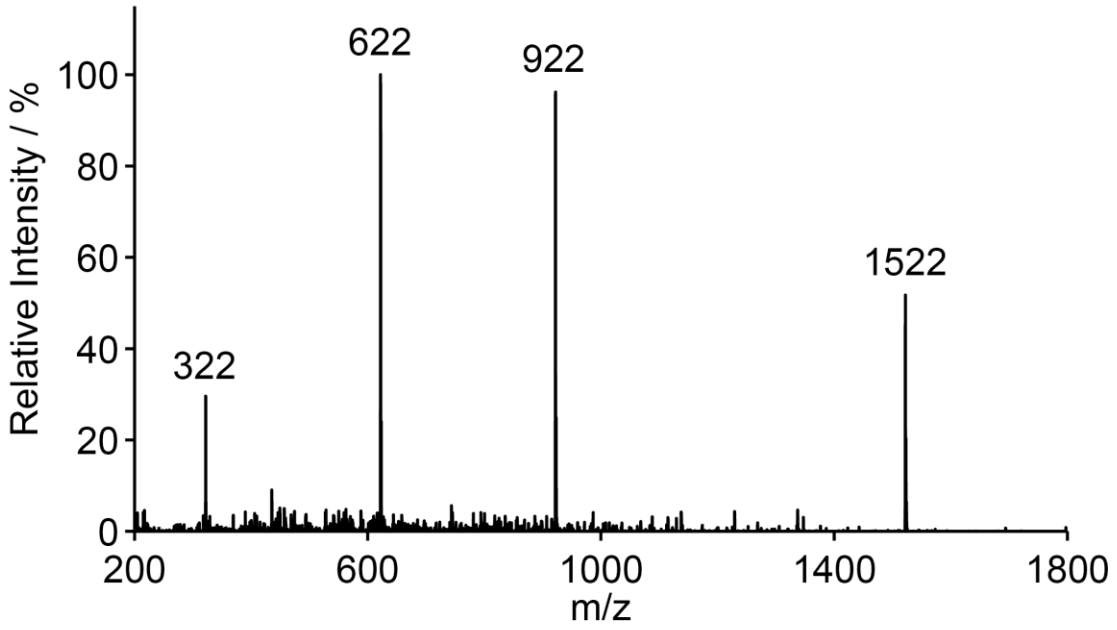

Figure S3. The DIVE-MS spectra of singly charged state, $[M+H]^+$, of hexamethoxyphosphazene (*m/z* = 322), hexakis(2,2-difluoroethoxy)phosphazene (*m/z* = 622), hexakis(1*H*, 1*H*, 3*H*-tetrafluoropropoxyphosphazene (*m/z* = 922) and hexakis(1*H*, 1*H*, 5*H*-octafluoropentoxy)phosphazene (*m/z* = 1522). The analyte consumption for the spectra are, 48 amol, 240 amol, 639 amol, 639 amol, respectively. Spectrum is averaged for 1 second, DIVE ablation shutter frequency 6 Hz, 4 pulse bursts, flow rate 167 nL min^{-1}. Water sample containing trifluoroacetic acid ammonium salt (TFA) (93.1 µM) and 5% acetonitrile. Transfer capillary temperature was 139 °C.

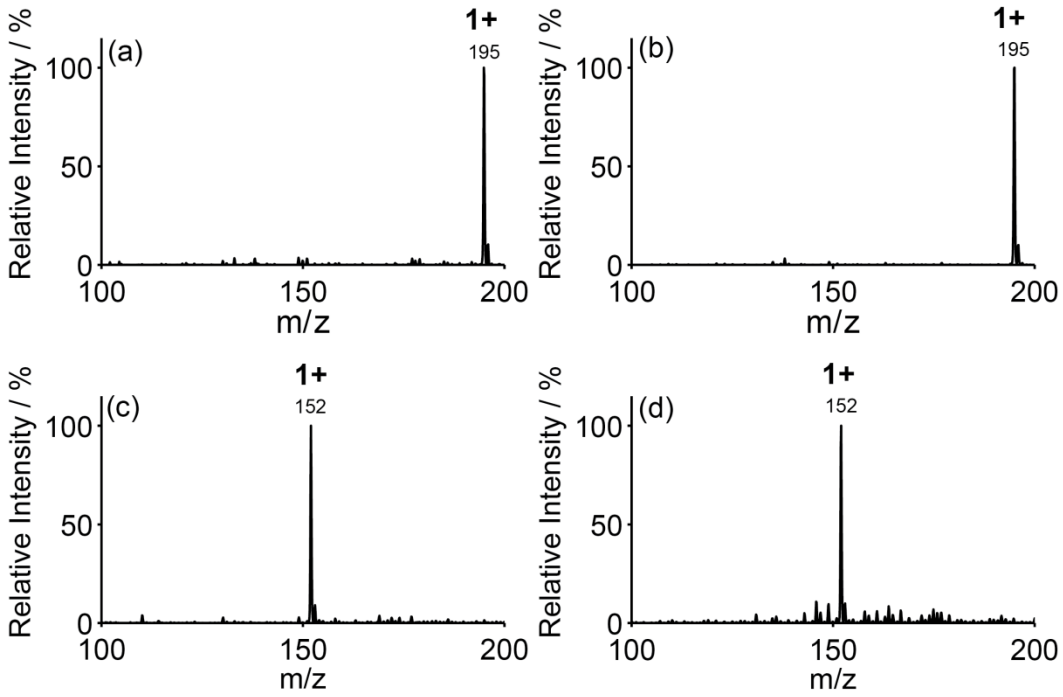

Figure S4. Mass spectra of caffeine (100 µM) in water containing 0.1% acetic acid (v/v) showing the singly charged state, $[M+H]^+$. (a) DIVE-MS (flow rate 835 nL min^{-1}, shutter frequency 2 Hz, 100 pulse bursts, without gas diverting heated transfer extension) and (b) ESI (flow rate 3 µL min^{-1}). Mass spectra of acetaminophen (10 µM) in water containing 0.5% formic acid (v/v) showing the singly charged state, $[M+H]^+$. (c) DIVE-MS (flow rate 417 nL min^{-1}, shutter frequency 5 Hz, 150 pulse burst, without gas diverting heated transfer extension) and (d) ESI (flow rate 3 µL min^{-1}). Transfer capillary temperature was measured to be 36 °C.

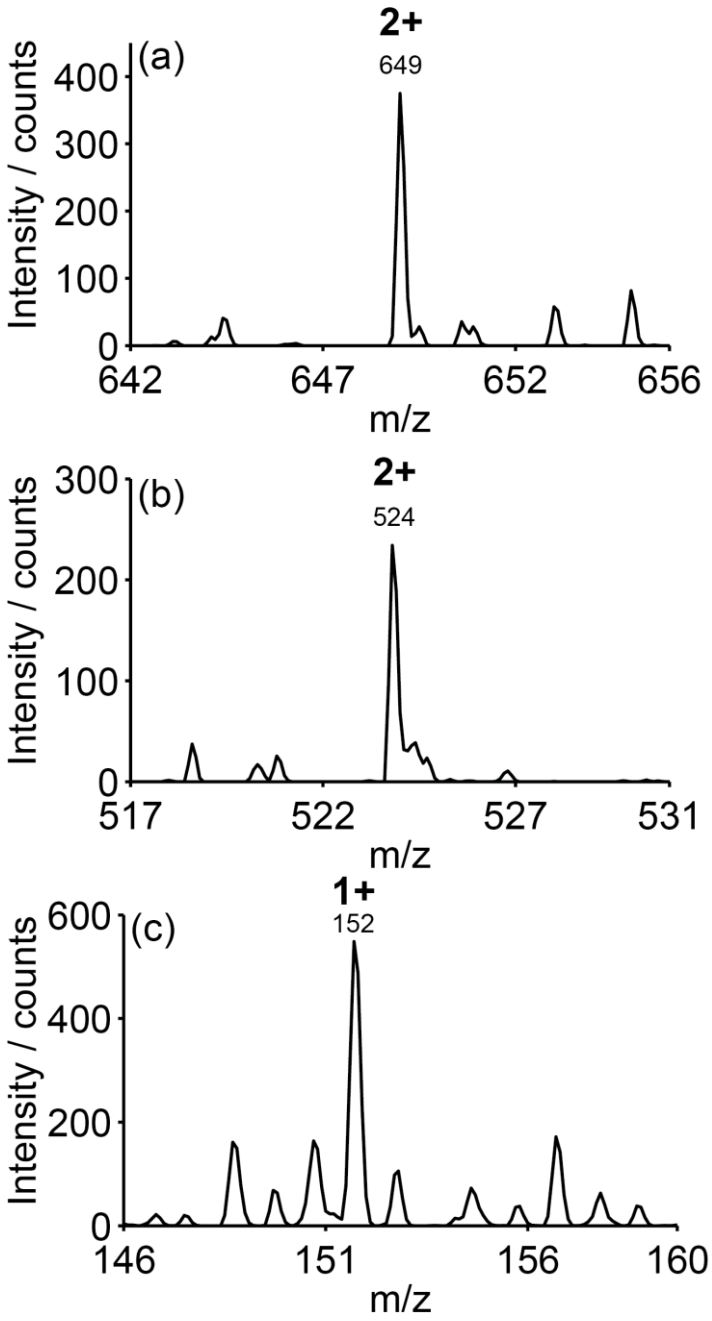

Figure S5. DIVE-MS measurement for 39 amol (a) $[M+2H]^{2+}$ of 10 nM angiotensin I dissolved in water containing 0.1% formic acid. (b) $[M+2H]^{2}$ of 10 nM angiotensin II dissolved in water containing 0.1% formic acid. (c) $[M+H]^{+}$ of 10 nM acetaminophen in pure water. Mass spectra are the average of 6 s of data collection, and transfer capillary temperature was 139 °C.

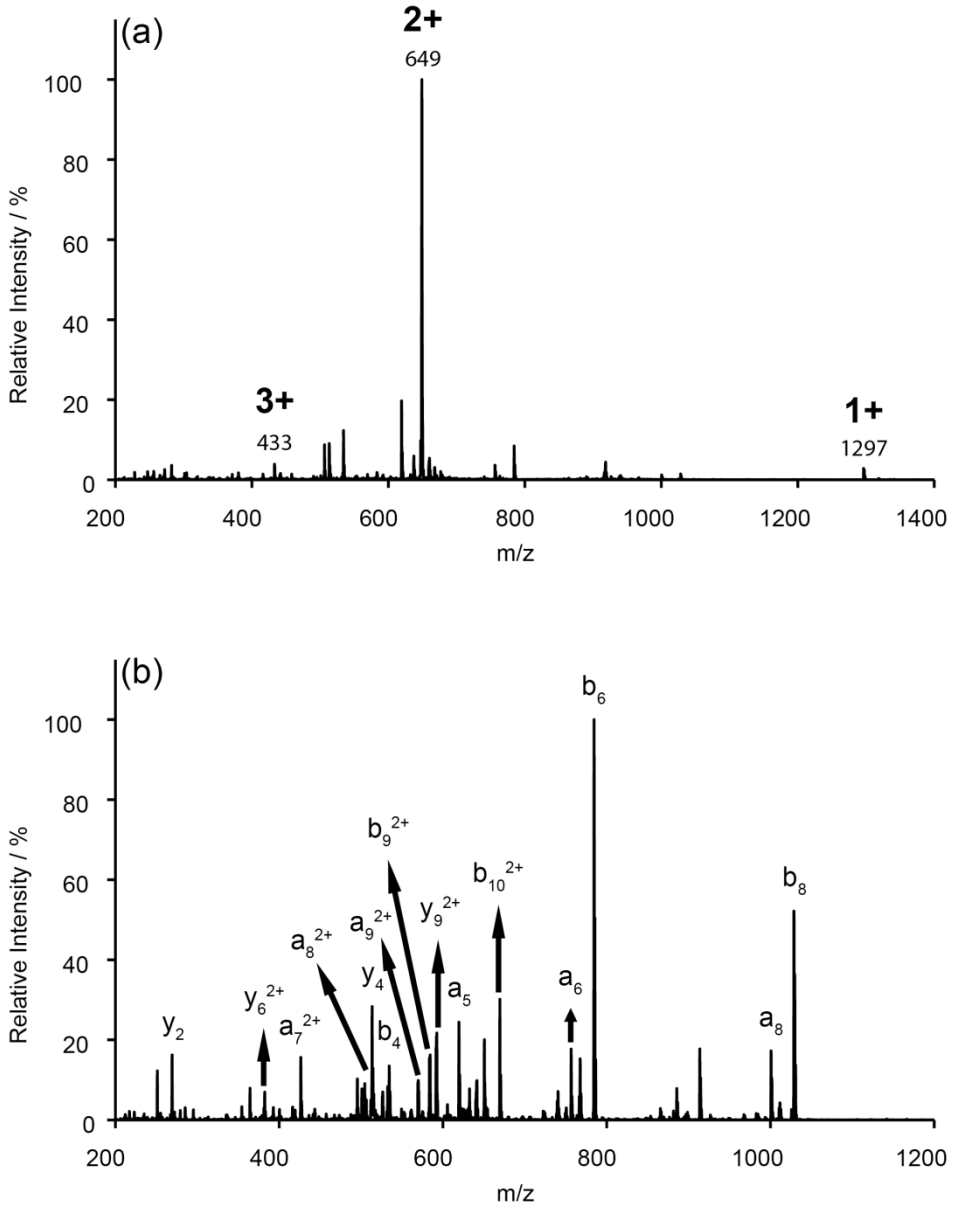

Figure S6. (a) ESI mass spectra of the peptide angiotensin I (10 μM) in water containing 0.1% formic acid (v/v). (b) Collisionally induced dissociation spectra of the doubly charged species of angiotensin I, $[M+2H]^{2+}$, produced by ESI-MS. The precursor ion mass isolation window was set to 4 mass units (m/z of the precursor ion ±2) and the fragmentation time was 40 ms. The collision gas was helium and the fragmentation amplitude was varied to achieve the required degree of fragmentation. No gas diverting heated transfer extension was applied. Transfer capillary temperature was 199 °C. ESI flow rate was 3 μL min^{-1}.

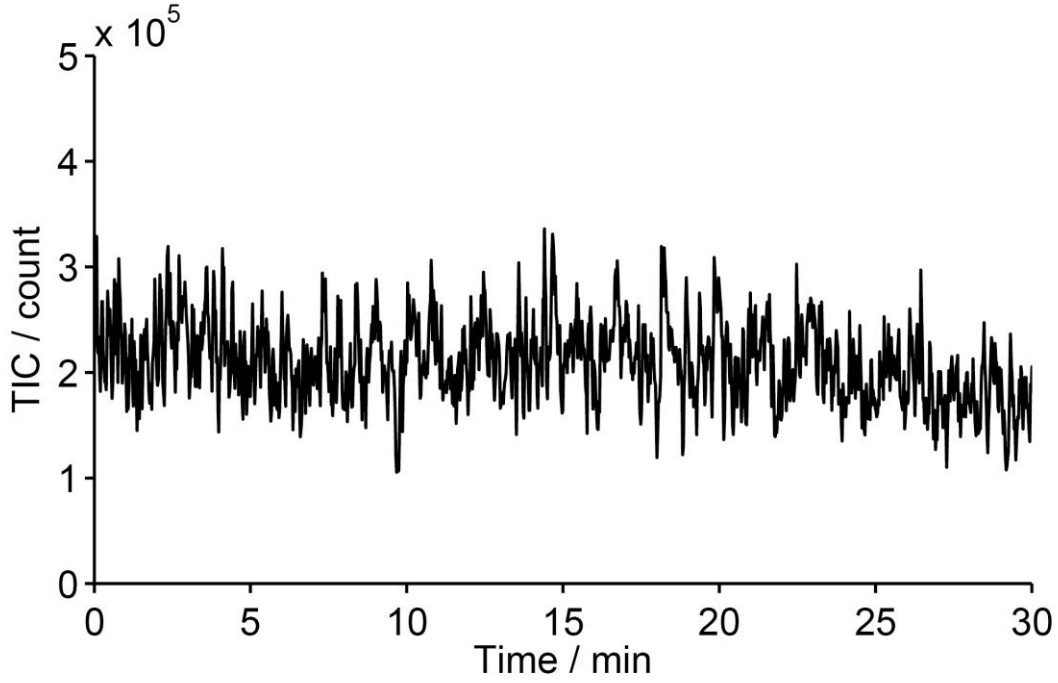

Figure S7. DIVE-MS total ion current (TIC) of angiotensin I (10 μM) in water with 0.1% formic acid. DIVE ablation was performed at flow rate 167 nL min^{-1}, shutter frequency 6 Hz, 4 pulse bursts. Transfer capillary temperature was 199°C.

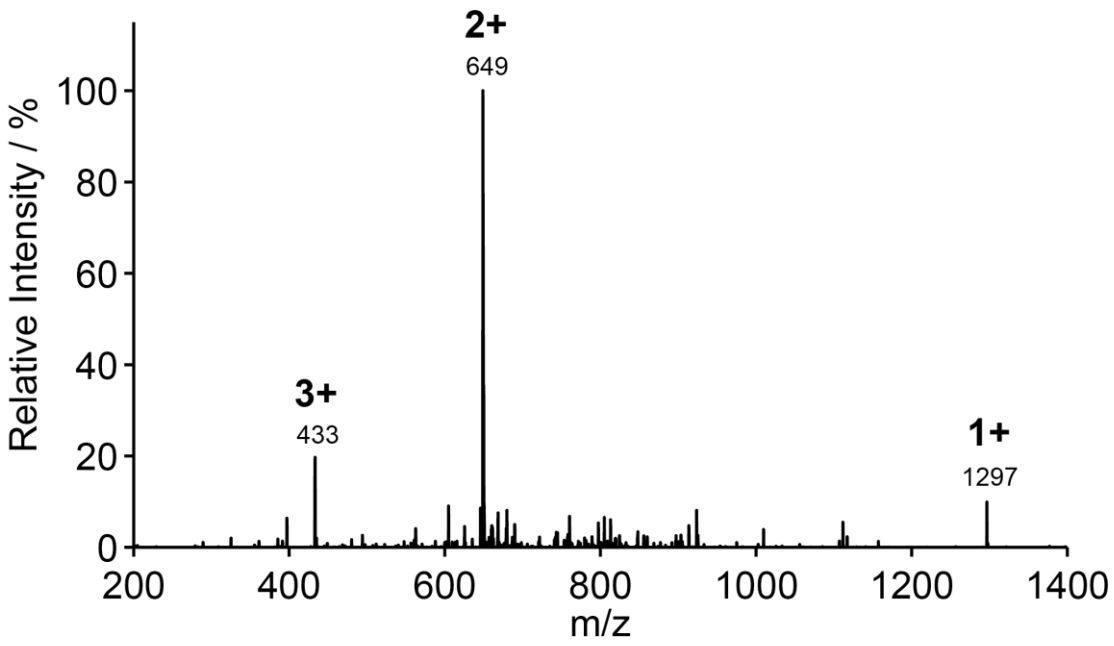

Figure S8. DIVE-MS spectrum of angiotensin I (10 μM) in pure water from a single PIRL laser pulse (~27 pL volume extraction). Transfer capillary temperature was 199°C.

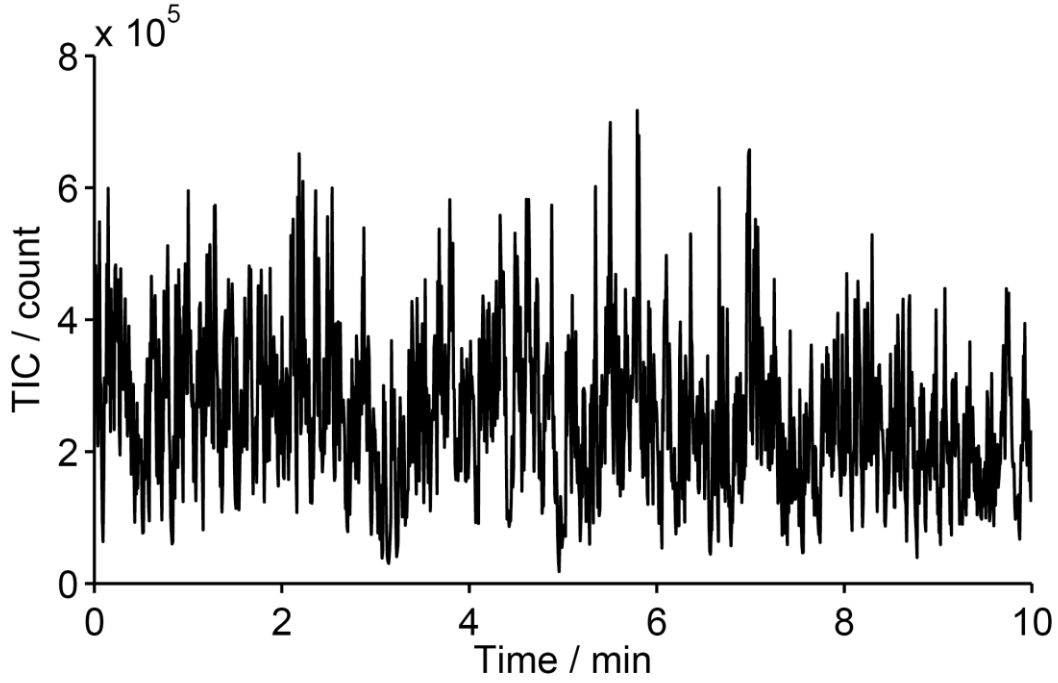

Figure S9. DIVE-MS total ion current (TIC) MS spectra of angiotensin I (10 μM) in pure water for single PIRL laser pulse extraction. DIVE ablation shutter frequency 1 Hz, 1 pulse burst, flow rate 167 nL min^{-1}, and transfer capillary temperature of 199 ºC.

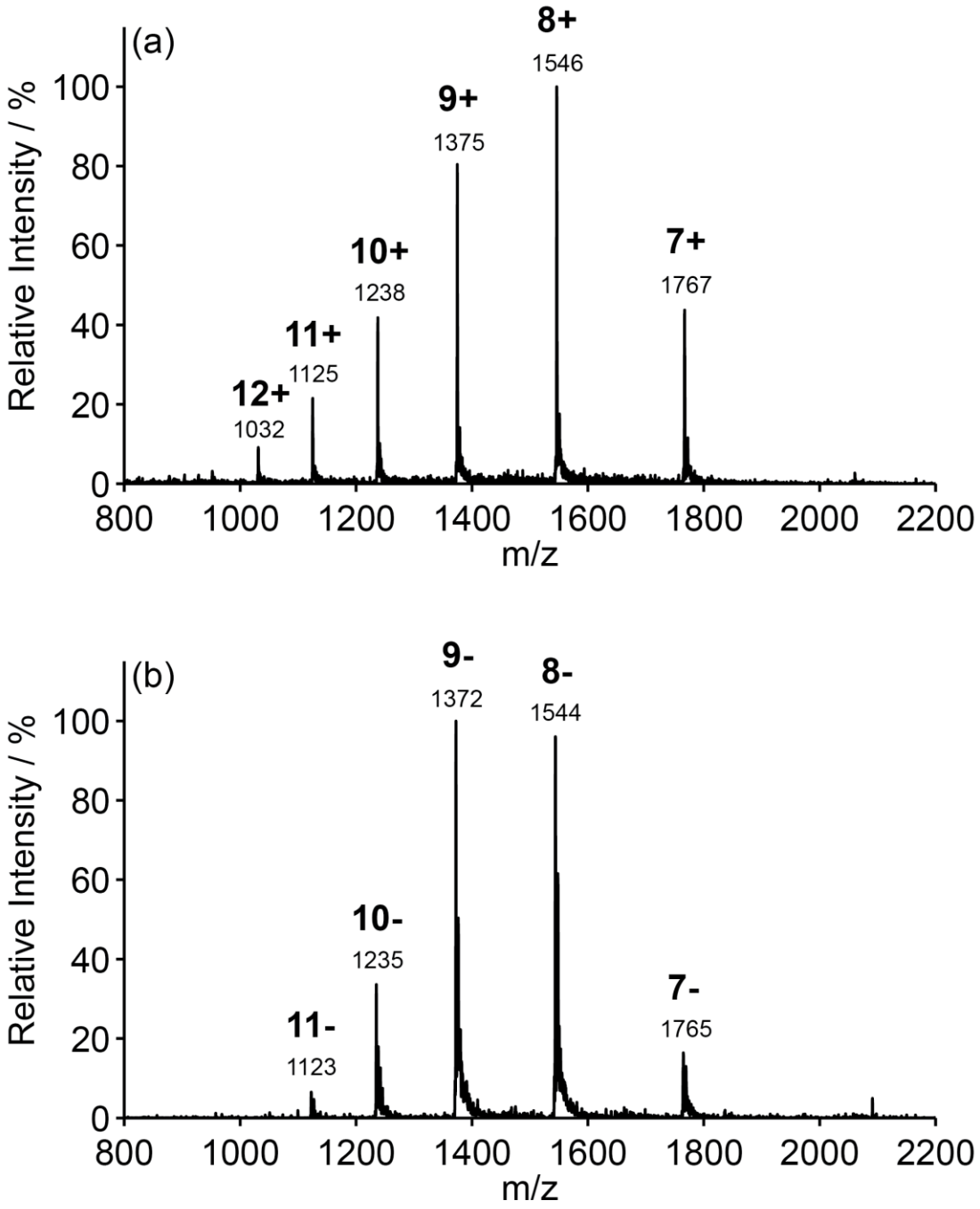

Figure S10. DIVE-MS spectra of cytochrome c (10 µM) for (a) positive and (b) negative ion mode. Cytochrome c was prepared in distilled water with 0.5% acetic acid (v/v). DIVE ablation shutter frequency 2 Hz, 100 pulse burst, flow rate 833 nL min^{-1}. The spectra shown is the average of 1 min of data collection without background correction. No gas diverting heated transfer extension was applied and capillary transfer temperature was 36 °C.

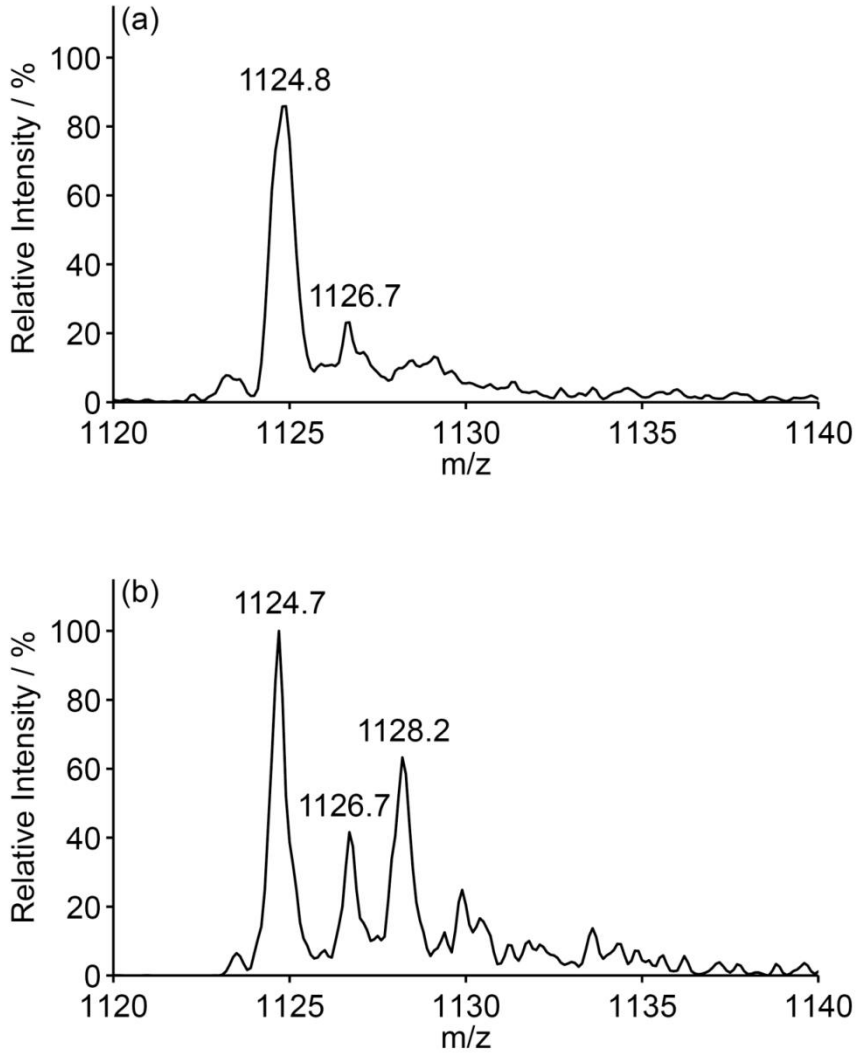

Figure S11. Comparison of DIVE-MS of adducts for the +11 charge states of cytochrome c between (a) water solution with 0.1% formic acid and (b) pure water. The 1126.7 and 1128.2 m/z peaks correspond to sodium and potassium adducts respectively. DIVE ablation was performed at flow rate 167 nL min^{-1}, shutter frequency 6 Hz, 4 pulse bursts and an averaging time for each spectra of 1 min. Transfer capillary temperature was measured to be 74 °C.

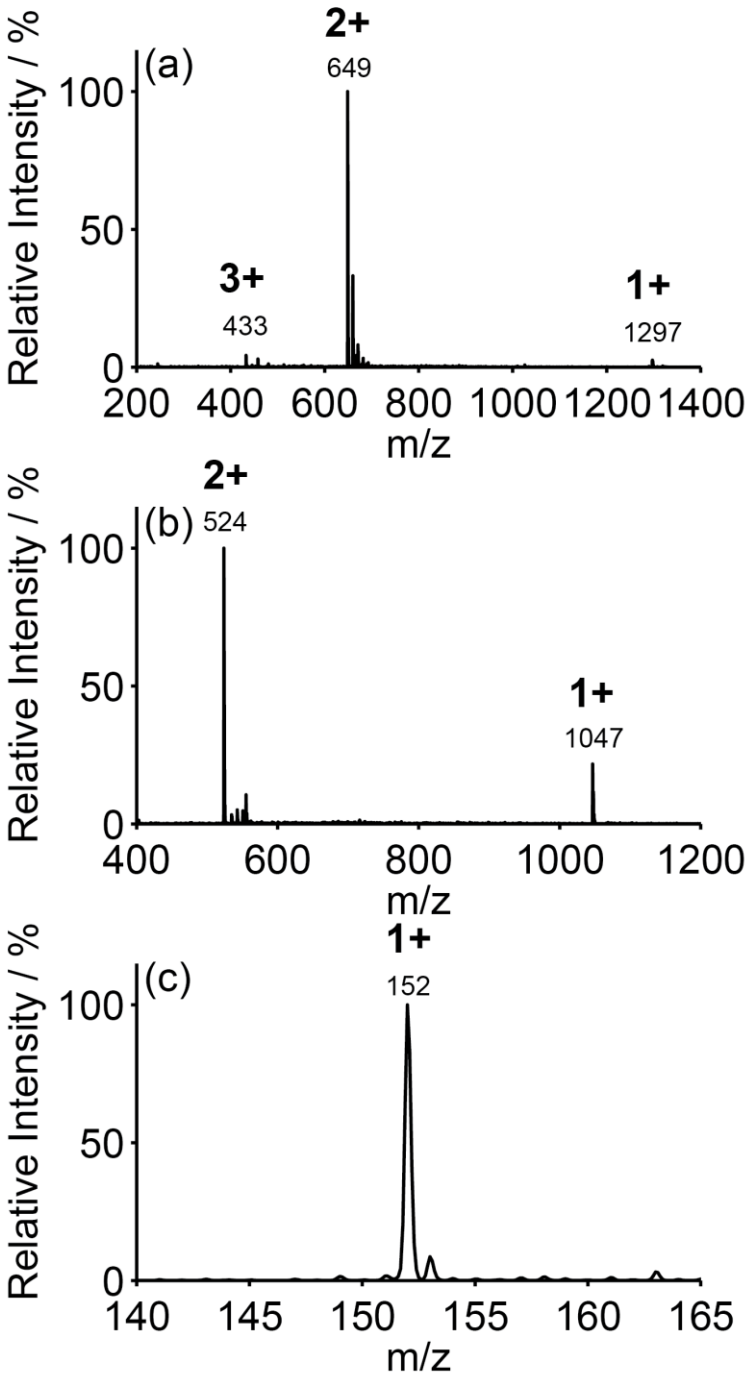

Figure S12. DIVE-MS spectra in pure water without the addition of acid. (a) DIVE-MS spectrum, $[M+H]^{1+}$, $[M+2H]^{2+}$ and $[M+3H]^{3+}$, of 10 µM angiotensin I dissolved in water. (b) DIVE-MS spectrum, $[M+H]^{1+}$ and $[M+2H]^{2+}$, of 10 µM angiotensin II dissolved in water. (c) DIVE-MS spectrum, $[M+H]^{+}$, of 10 µM acetaminophen dissolved in water. DIVE ablation shutter frequency 6 Hz, 4 pulse bursts, flow rate 167 $nLmin^{-1}$. Mass spectra was the average of 1 min of data collection. Transfer capillary temperature was 139 °C.

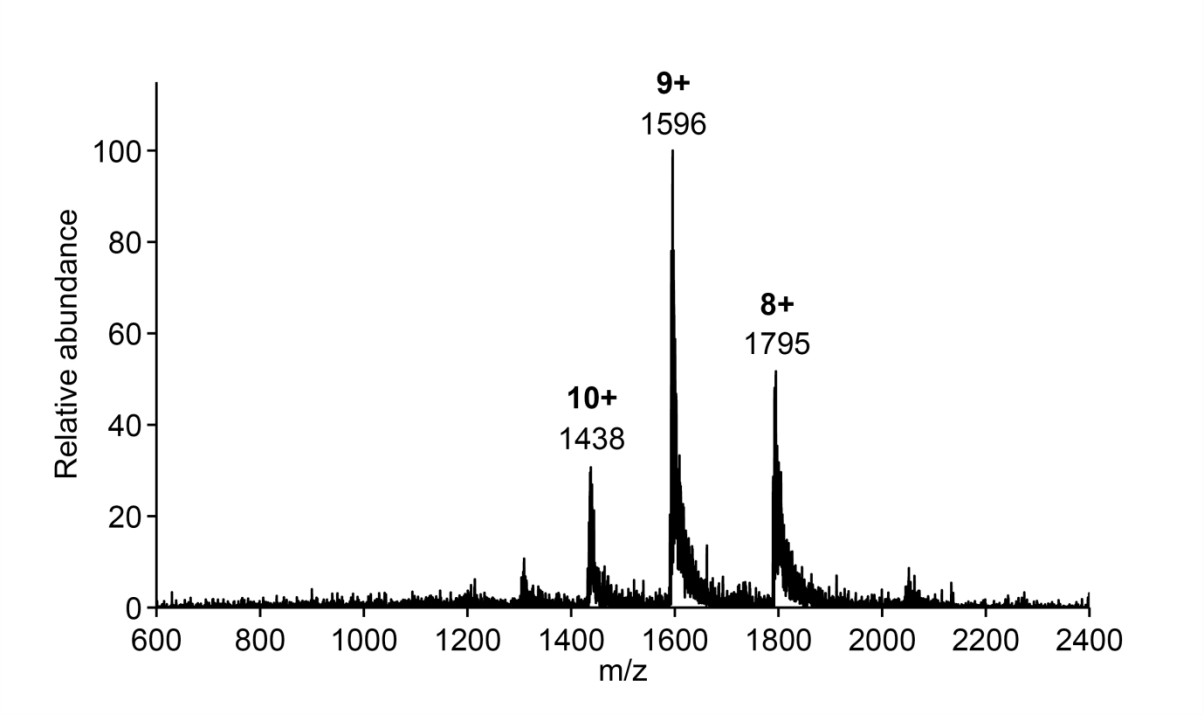

Figure S13. DIVE-MS spectrum of 10 µM chicken white lysozyme in 50 mM ammonium bicarbonate buffer. DIVE ablation shutter frequency 6 Hz, 4 pulse burst, flow rate 167 nL min^{-1}. The spectra shown was the average of 1 min of data collection without the use of the additional gas diverting heated transfer extension and a transfer capillary temperature of 36 °C

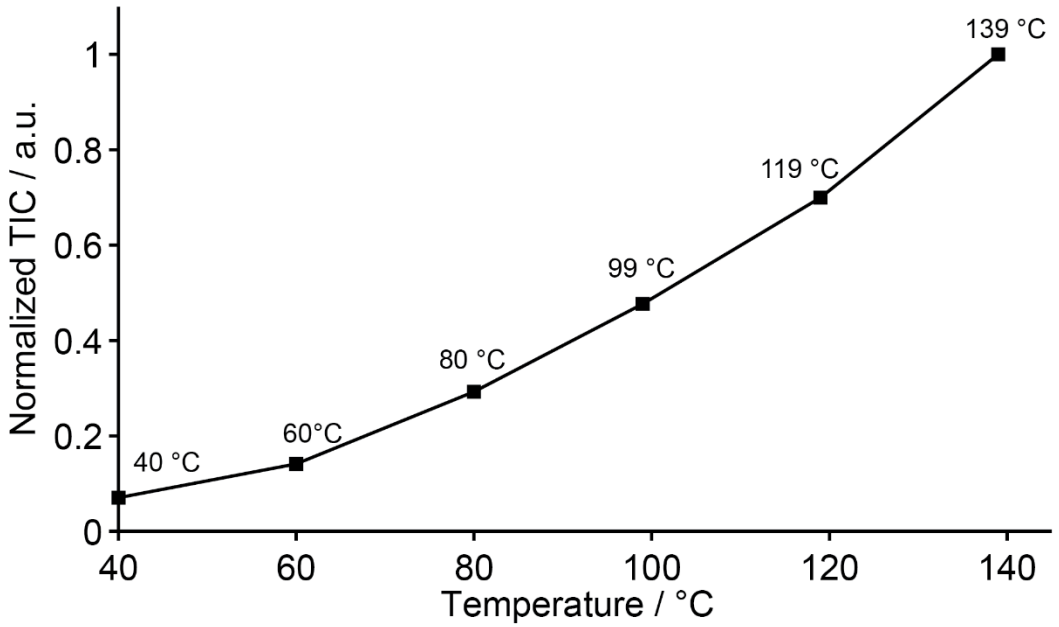

Figure S14. Temperature dependence of TIC of cytochrome c. The gas diverting heated capillary extension was used and allowed to come into thermal equilibrium before data collection. DIVE ablation was performed on 10 µM cytochrome c in pure water without addition of acid, flow rate 167 nL min^{-1}, shutter frequency 6 Hz, 4 pulse bursts and an averaging time for each data point of 1 min.

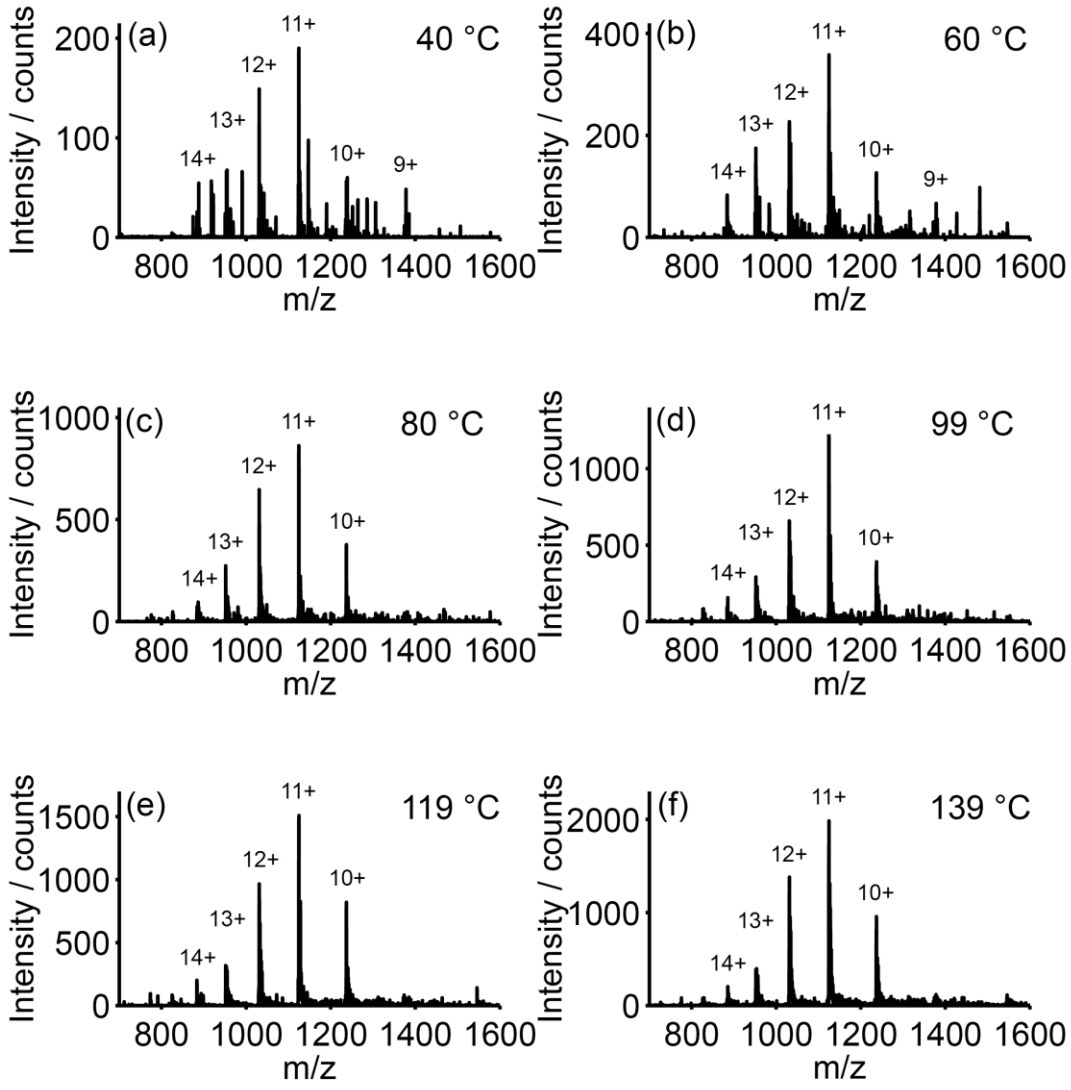

Figure S15. The DIVE-MS spectra of cytochrome c in pure water without the addition of acid, collected at transfer capillary temperatures, (a) 40 ºC, (b) 60 ºC, (c) 80 ºC, (D) 99 ºC, (E) 119 ºC and (f) 139 ºC. The mass spectra are obtained from the TIC shown in Figure SI13 at each temperature. DIVE ablation was performed on 10 μM cytochrome c in pure water without addition of acid, flow rate 167 nL min^{-1}, shutter frequency 6 Hz, 4 pulse bursts and an averaging time for each spectra of 1 min.

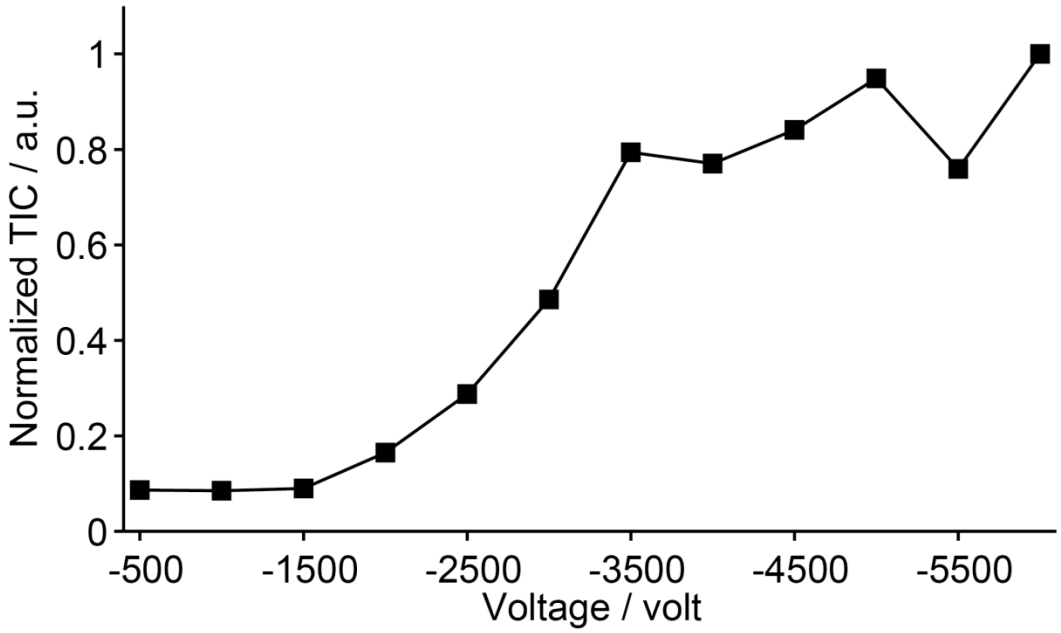

Figure S16. Voltage dependence of the TIC produced by DIVE-MS of cytochrome c versus capillary voltage. The sample conditions were 10 μM cytochrome c in water with 0.1% formic acid, flow rate 167 nL min^{-1}, shutter frequency 6 Hz, 4 pulse bursts, an averaging time for each data point of 1 min and transfer capillary temperature of 74 °C

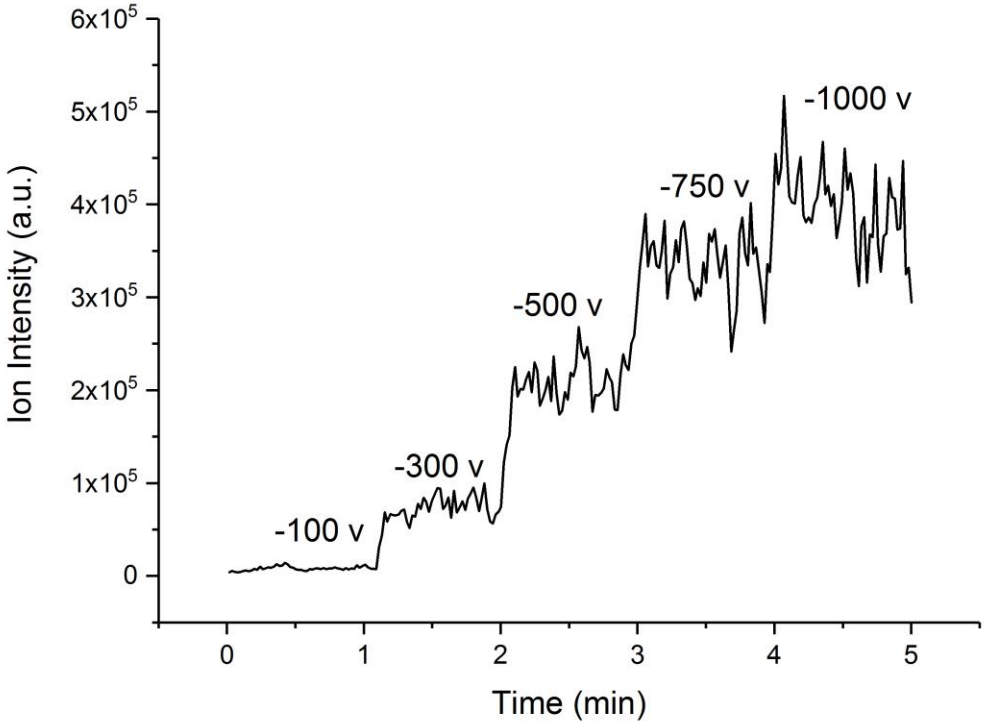

Figure S17. Ion intensity of 10 µM angiotensin I in water with 0.1% formic acid was increased when an electrically isolated, conductive planar mesh grid was placed between the liquid sample and the MS inlet. The voltage of the grid was varied with the voltage of the collection extension and quartz transfer capillary remain constant (-4500 V). Transfer capillary temperature was 199 °C.