

Supporting Information

Perturbation-induced high-frequency pulsing of nano-ESI with facile ion selection at atmospheric pressure

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Chemicals & Reagents

Analytical solutions in aqueous solvent (18.2 M Ω water, Elga Veolia Purelab Flex 1 system – High Wycombe, UK) were prepared by diluting reagents (source: Sigma Aldrich, St. Louis, MO, USA) to the concentrations detailed in Table S1. Additionally, solutions for experiments shown in Figures 7-9 and S1-S6 were spiked with formic acid (Fisher Scientific (Optima LC/MS), Pittsburgh, PA, USA) and sodium chloride (EM Science (Merck KGaA) – Darmstadt, Germany) to promote protonation and sodiation, respectively.

Table S1. Details regarding sample preparation.

Analyte, Purity	Conc. in Analytical Solution	Ion
Tetraethylammonium chloride, $\geq 98\%$	3 μM	[TEtA] ⁺
Tetrabutylammonium chloride, $\geq 97\%$	2 μM	[TButA] ⁺
Tetraheptylammonium bromide, $\geq 99\%$	3 μM	[THepA] ⁺
L-Arginine, $\geq 99.5\%$	15 μM	[Arg+H] ⁺
4-Acetamidophenol (Acetaminophen), 98%	25 μM	[Act+H] ⁺ [Act+Na] ⁺
Sucralose, NA ^a	50 μM	[Suc+Na] ⁺
Cortisol, $\geq 98\%$	30 μM	[Crt+H] ⁺

^aInformation not provided.

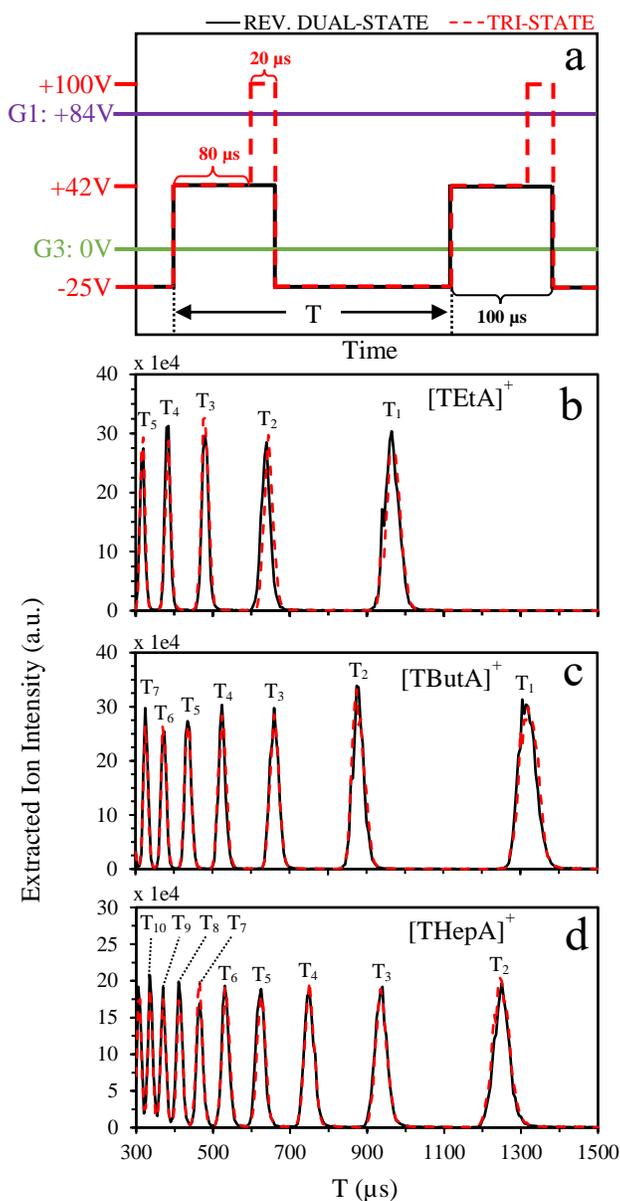


Figure S1. (a) Pulse scheme for reverse dual-state (solid black trace) and tri-state (dashed red trace) operation of G2 using 100 μs total pulse widths. The ion arrival curves using these pulses are shown for (b) [TEtA]⁺, (c) [TButA]⁺, (d) [THepA]⁺. Data were generated by scanning T at 5 μs increments with the following experimental parameters: E1= +500 V, G1= +84 V, G3= 0 V, spray voltage= +1380V, tip-to-G1 distance = 12.5 mm, N₂ counter flow gas (entering MS)= 3 LPM.

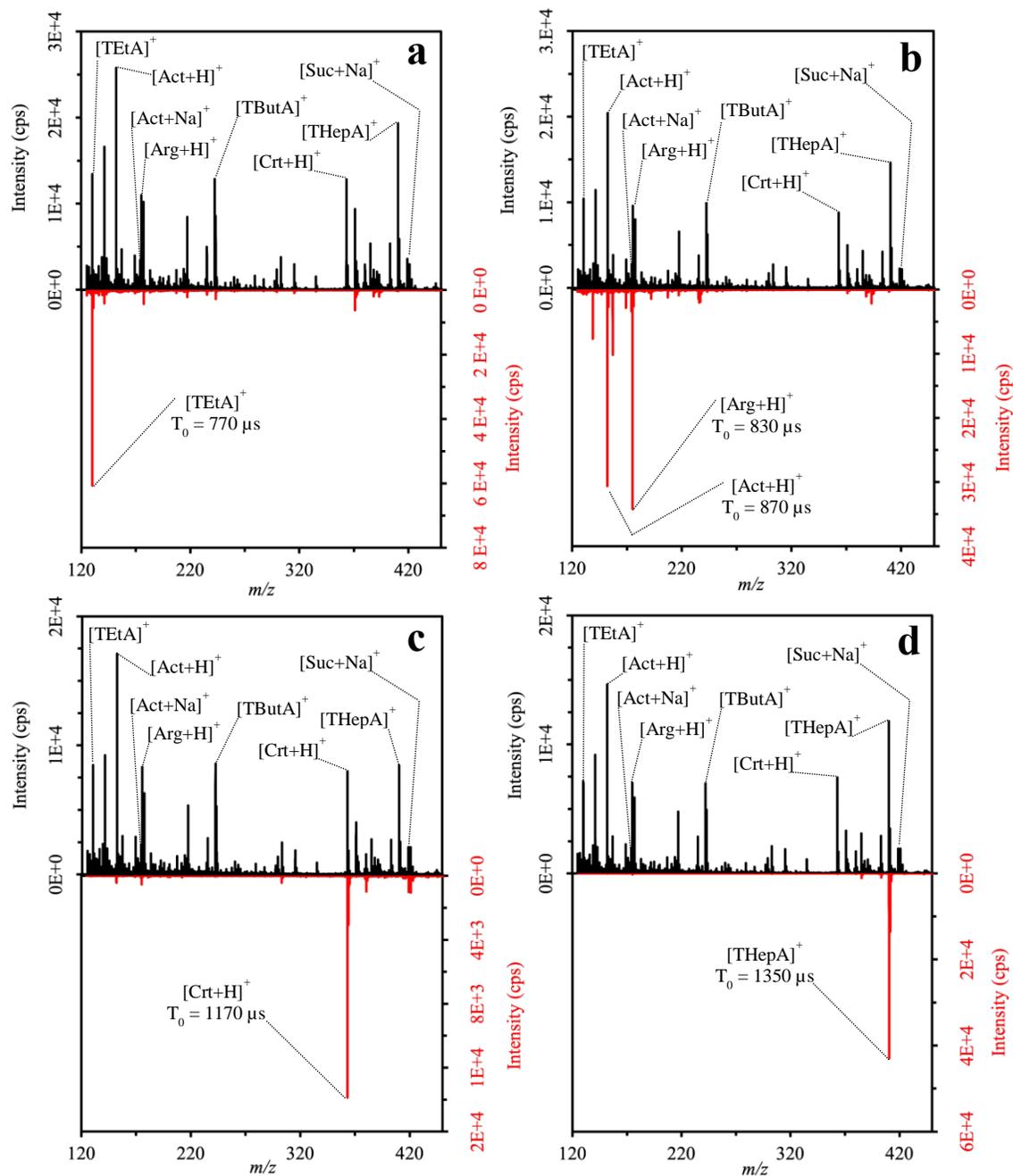


Figure S2. Mass spectra obtained at emitter voltages of +1375 V (top, black panels) and +1325 V (bottom, red panels) at pulse periods of: (a) $T = 770 \mu\text{s}$, (b) $T = 830 \mu\text{s}$, (c) $T = 1170 \mu\text{s}$, and (d) $T = 1350 \mu\text{s}$. Experimental conditions can be found in Figure 7 caption. The spectra using +1375 V do not change with T while the spectra at +1325 V show selective transmission of ions with T .

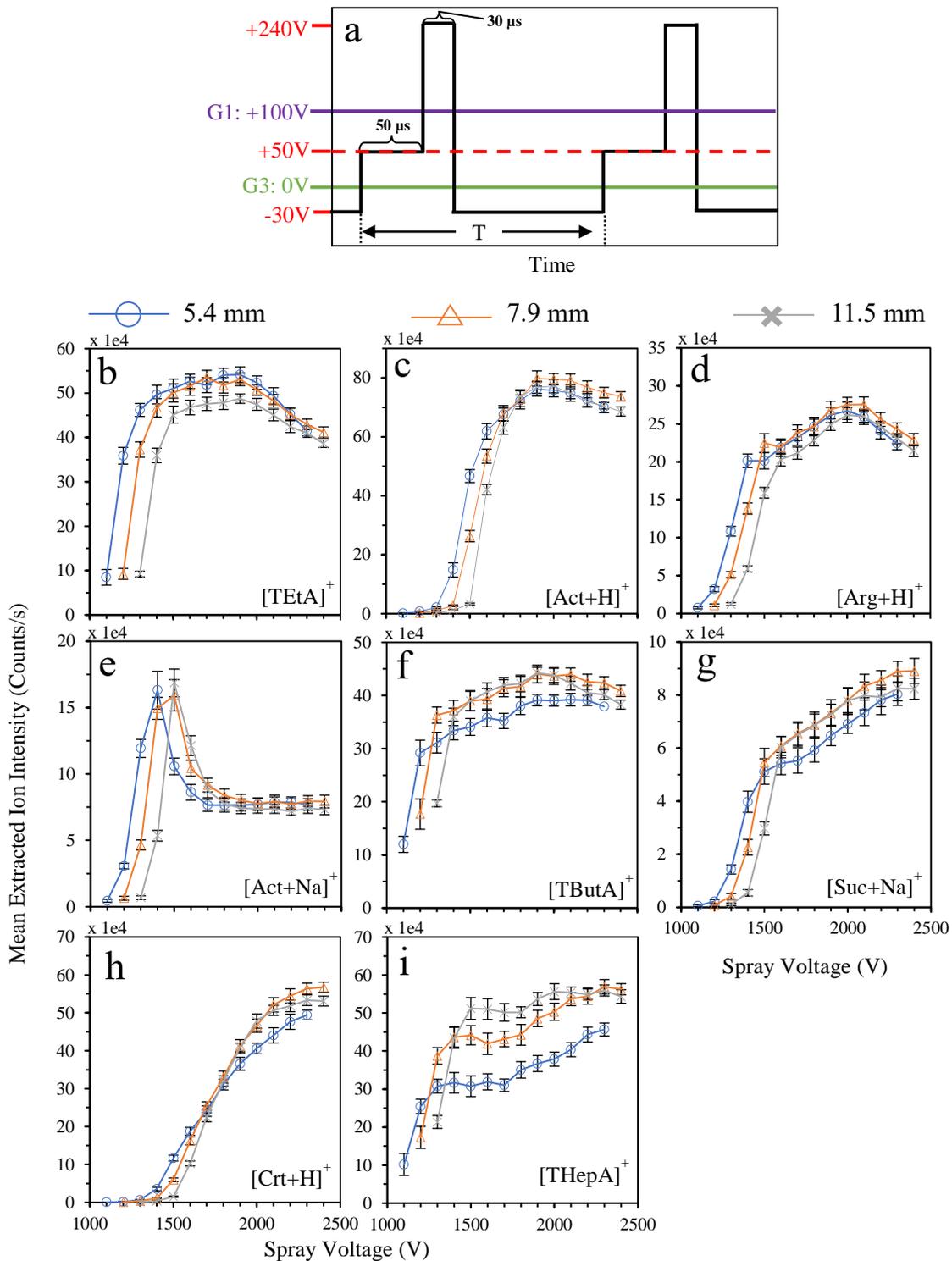


Figure S3. (a) Tri-state pulse scheme (solid black line) applied to G2. (b)-(i) Ion intensities using continuous nano-ESI mode by applying a constant +50 V to G2 as indicated by the dashed red line. Error bars indicate standard deviation of intensities measured over 30 seconds.

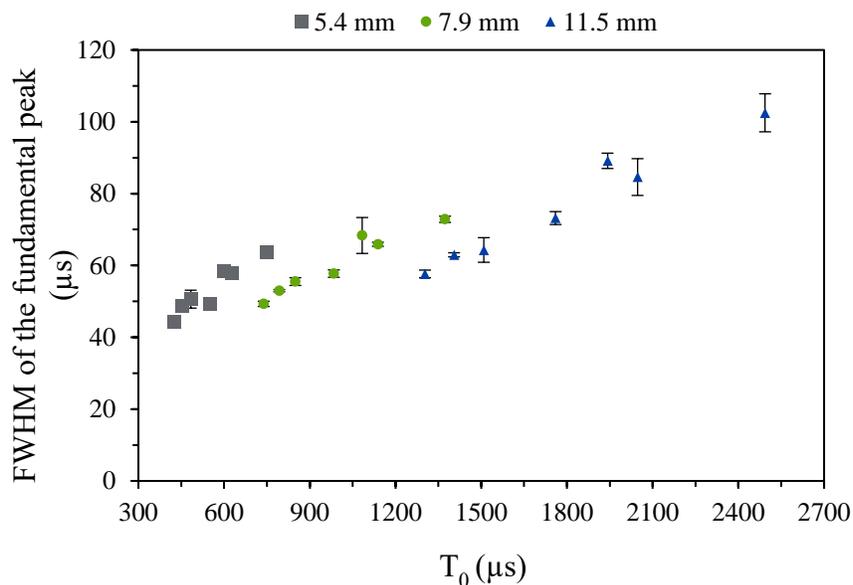


Figure S4. Full widths at half maxima (FWHM) for the fundamental peaks (T_0) as a function of T_0 at three tip-to-G1 distances. For experimental details, see Figure 8 caption. Error bars represent standard deviations of triplicate measurements.

Acetaminophen Aggregation

The contribution of solvent evaporation between pulses to the higher-than-expected pulsed nano-ESI ion intensities was investigated by quantifying the extent of aggregation for acetaminophen. Protonated dimer and monomer ions were monitored, and the ratios of the intensities were used as a metric for aggregation in both pulsed and continuous modes. Importantly, the protonated monomer and multimers are sampled simultaneously in the continuous mode, and the observed dimer:monomer ratio includes declustering of aggregates upon ion sampling into the MS. On the other hand, aggregate ions traverse the tip-to-gate distance at varying times in pulsed mode. They appear at different T_s in pulsed mode, and their declustering products would not appear simultaneously with the monomer. Therefore, a careful examination of the

arrival peaks in the pulsed mode is needed to account for declustering effects in the observed dimer:monomer ratio.

Figure S5a depicts the detection of the protonated acetaminophen monomer and dimer in the pulsed mode. The ion arrival curve via extracting m/z 152 (corresponding to protonated monomer $[\text{Act}+\text{H}]^+$) shows two distinct ion populations for the fundamental peak of this m/z . The peak at the longer period corresponds to declustering of the protonated dimer ($[2\text{Act}+\text{H}]^+$) within the MS, evident from the overlap in arrival of the dimer (m/z 303) at the same T . Moreover, the arrival curve for m/z 303 indicates a broad feature for the fundamental peak denoting the formation of multimers and subsequent declustering to form the protonated dimer. To account for declustering effects in intensity ratios, the total peak areas at the fundamental peaks for m/z 152 and 303 were utilized, resulting in a dimer:monomer intensity ratio of 0.091 ± 0.001 ($\bar{x} \pm \text{s.d.}$, $n=3$) in the pulsed mode. Values of 0.069-0.081 (Figure S5b) were observed for the continuous mode depending on the operating voltage. The 0.069 ratio was obtained at the voltage corresponding to the maximum intensity of acetaminophen in Figure S3c.

To verify the expectation of increased dimer formation at higher concentrations, we conducted a separate experiment in continuous mode. Aqueous solutions of 25 μM and 75 μM acetaminophen containing 0.1% formic acid were sprayed consecutively using the same 5- μm emitter by placing the tip 5.4 mm from the gate with $G1=+100$ V, $G2= +50$ V, and $G3=0$. A platinum wire was inserted into the back of the capillary, and an emitter voltage of +1400 V was applied to the solution. The 25- μM solution yielded a dimer:monomer ratio of 0.154 ± 0.005 based on a 30-second MS acquisition at 1 Hz. The 75- μM solution yielded a dimer:monomer ratio of 0.265 ± 0.006 using the same acquisition parameters. The dimerization ratio increased by a factor

of 1.72 using the three-fold more concentrated solution, confirming the expected aggregation of acetaminophen at higher concentrations.

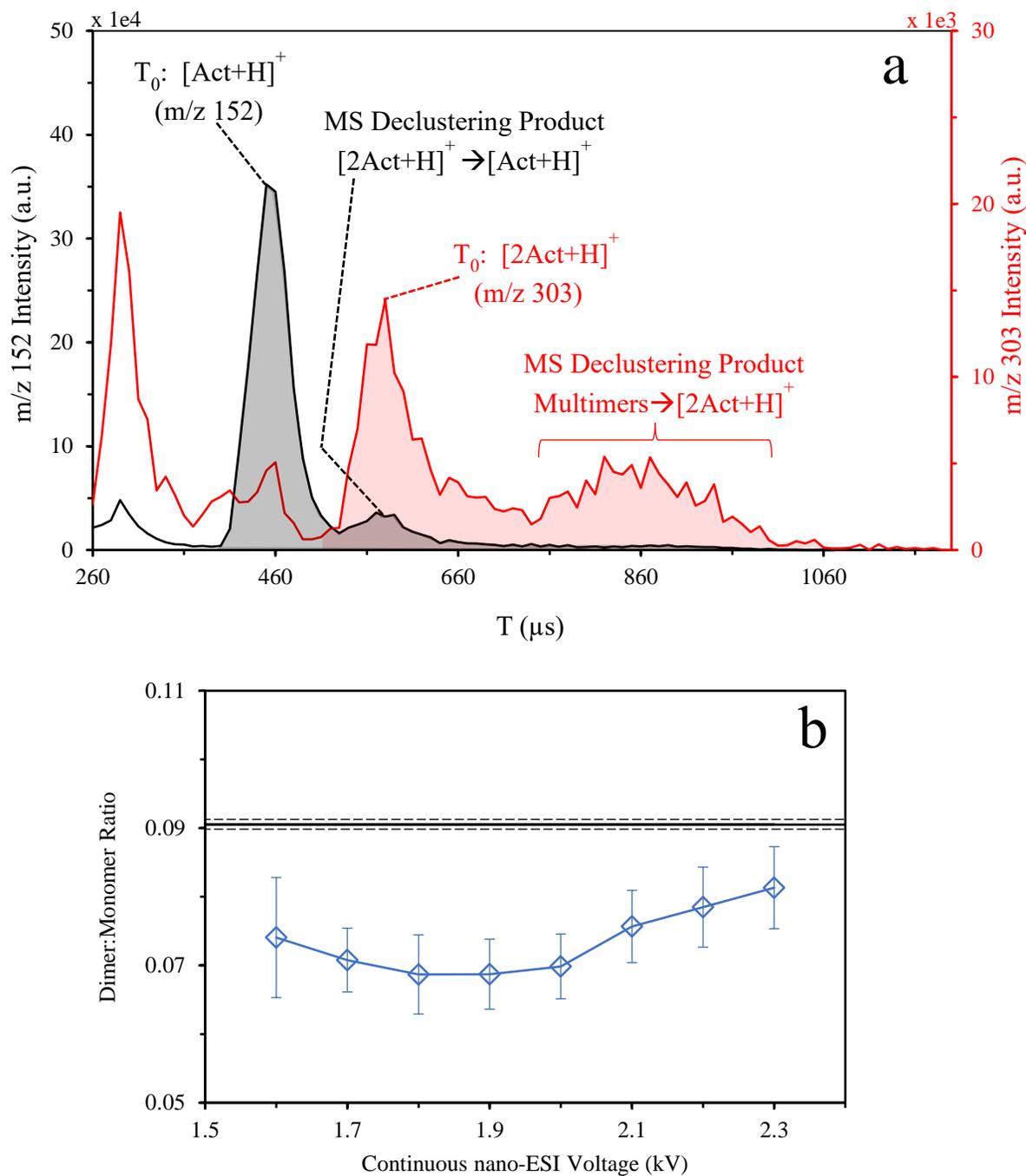


Figure S5. (a) Ion arrival curves in pulsed mode at a tip-to-gate distance of 5.4 mm for $[\text{Act}+\text{H}]^+$ (black trace) and $[2\text{Act}+\text{H}]^+$ (red trace) via extracting m/z 152 and m/z 303, respectively

(see Figure 8 caption for experimental details). Peak areas at the fundamental peaks were used to determine dimer:monomer ratio – grey shaded area corresponds to peak area for monomer (m/z 152), while red shaded area corresponds to the peak area for the dimer (m/z 303). (b) The diamonds mark the dimer:monomer ratio in continuous mode as a function of continuous nano-ESI emitter voltage. Error bars represent standard deviations of ratios calculated from standard deviations of ion intensities within 30-second acquisitions in continuous mode. The solid black line shows the dimer:monomer ratio in pulsed mode, calculated from the arrival curves in 5a. Dotted black lines indicate standard deviation of the dimer:monomer ratio in pulsed mode based on triplicate measurements. Note that a constant emitter voltage of 1600 V is used in the pulsed mode, but for ease of comparison, the dimer:monomer ratio is shown across all emitter voltages for continuous mode.

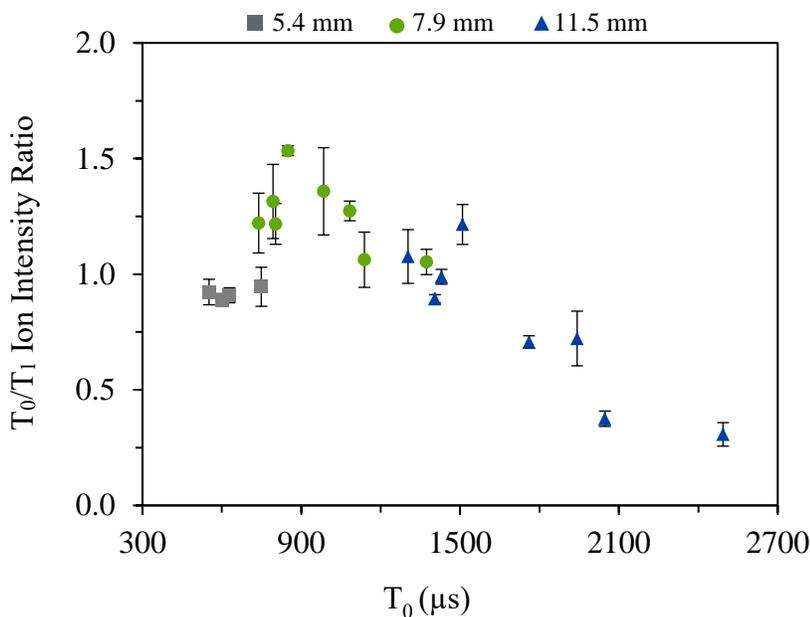


Figure S6. Ratio of ion intensities at T_0 to those at T_1 as a function of T_0 . For experimental details, see Figure 8 caption. Error bars represent standard deviation of triplicate measurements of T_0/T_1 ion intensities.

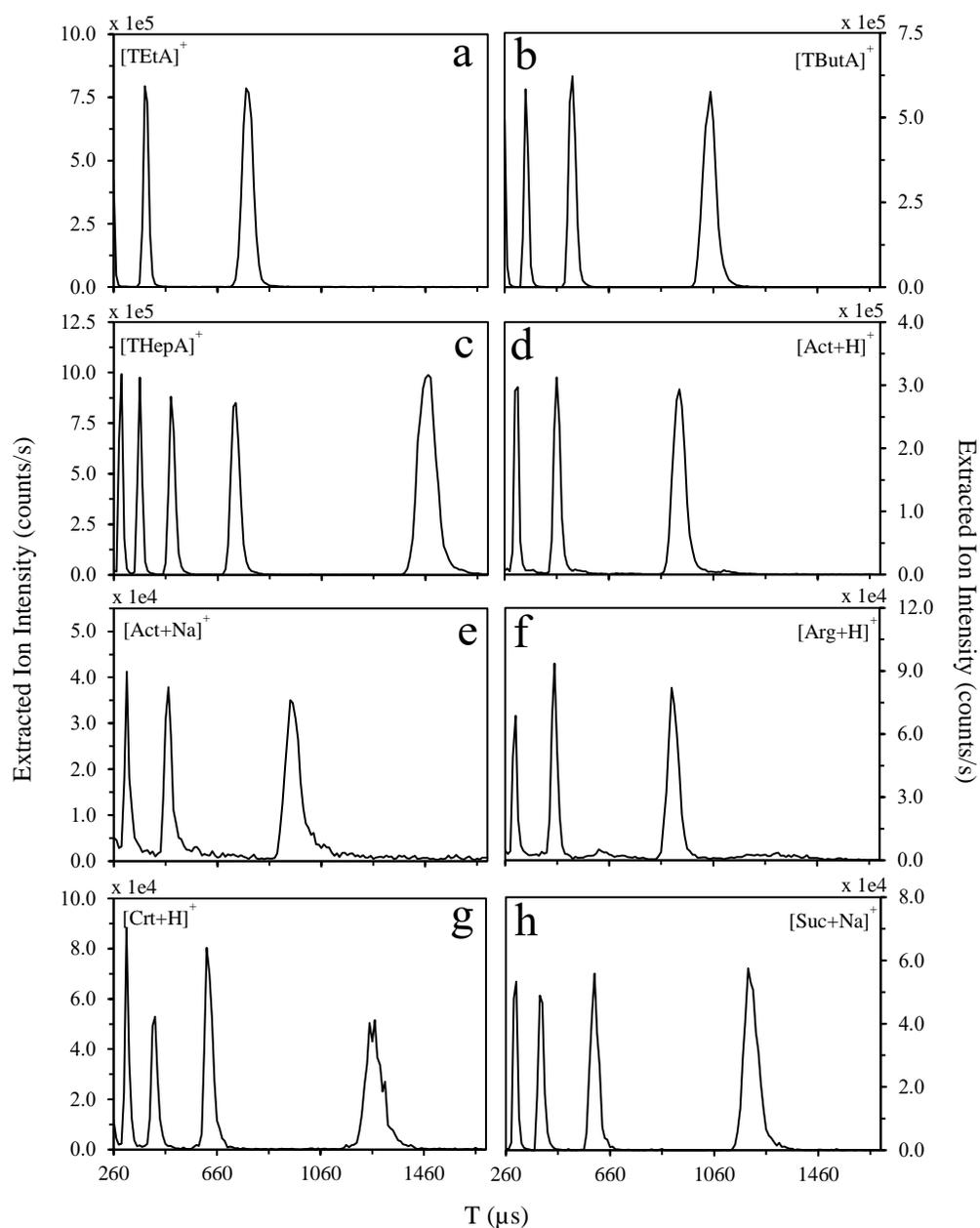


Figure S7. Ion arrival curves for (a) [TEtA]⁺, (b) [TButA]⁺, (c) [THepA]⁺, (d) [Act+H]⁺, (e) [Act+Na]⁺, (f) [Arg+H]⁺, (g) [Crt+H]⁺, and (h) [Suc+Na]⁺ using concentrations in Table S1 and 1:1 water:methanol mixture (LC-MS CHROMASOLV, Sigma Aldrich - St. Louis, MO) as solvent spiked with 0.1% formic acid and 250 μM sodium chloride. Data were collected with 1 LPM counterflow gas through the MS entrance and sample reservoir positioned approximately 19 cm

above the emitter, with a tip-to-G1 distance = 7.2 mm. The spray voltage was tuned to +958 V, while the following tri-state pulse parameters were used: ion blocking voltage = -30 V, ion transmit voltage = +58 V (50 μ s), ion push voltage = +240 V (30 μ s). Additional experimental parameters: E1 = +700 V, G1 = +117 V, G3 = 0 V. Note a representative set of data is depicted above, but three ion arrival curves were acquired for each ion by scanning T between 260-1700 μ s at 10 μ s increments with 4 spectra per setting.

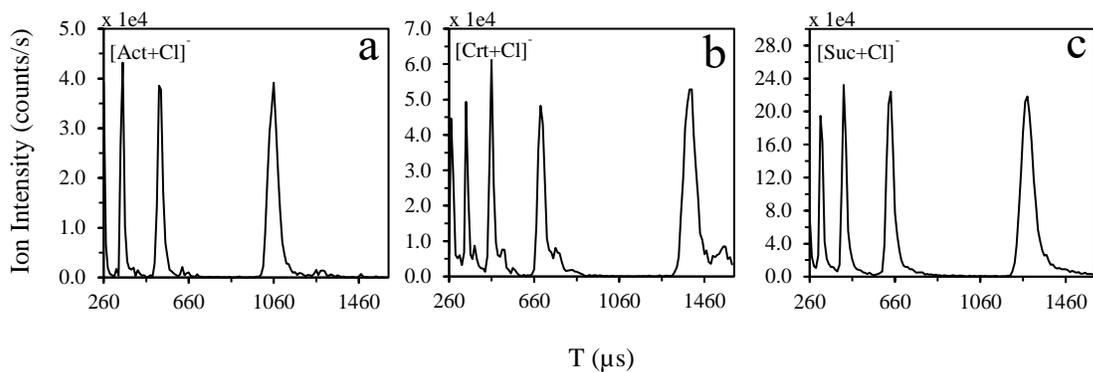


Figure S8. Ion arrival curves for (a) $[\text{Act}+\text{Cl}]^-$, (b) $[\text{Crt}+\text{Cl}]^-$, and (c) $[\text{Suc}+\text{Cl}]^-$ generated using negative polarity. Solution contained acetaminophen, sucralose, and cortisol (concentrations in Table S1) in 18.2 M Ω water with a 250 μ M spike of sodium chloride. MS sampling voltages were the same magnitude used in positive mode but biased to the opposite polarity, and the data were acquired by scanning T at 10 μ s increments using the following experimental parameters: Tri-state pulse voltages: ion blocking = +30 V, ion transmit = -50 V (50 μ s), ion push = -240 V (30 μ s). Spray voltage = -1355 V, E1 = -600 V, G1 = -100 V, G3 = 0 V, tip-to-G1 distance = 8.7 mm, N₂ counter flow gas (entering MS) = 2 LPM.