

Optimizing Native Ion Mobility Q-TOF in Helium and Nitrogen for Very Fragile Noncovalent Structures

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SUPPLEMENTARY FIGURES AS DESCRIBED IN TEXT

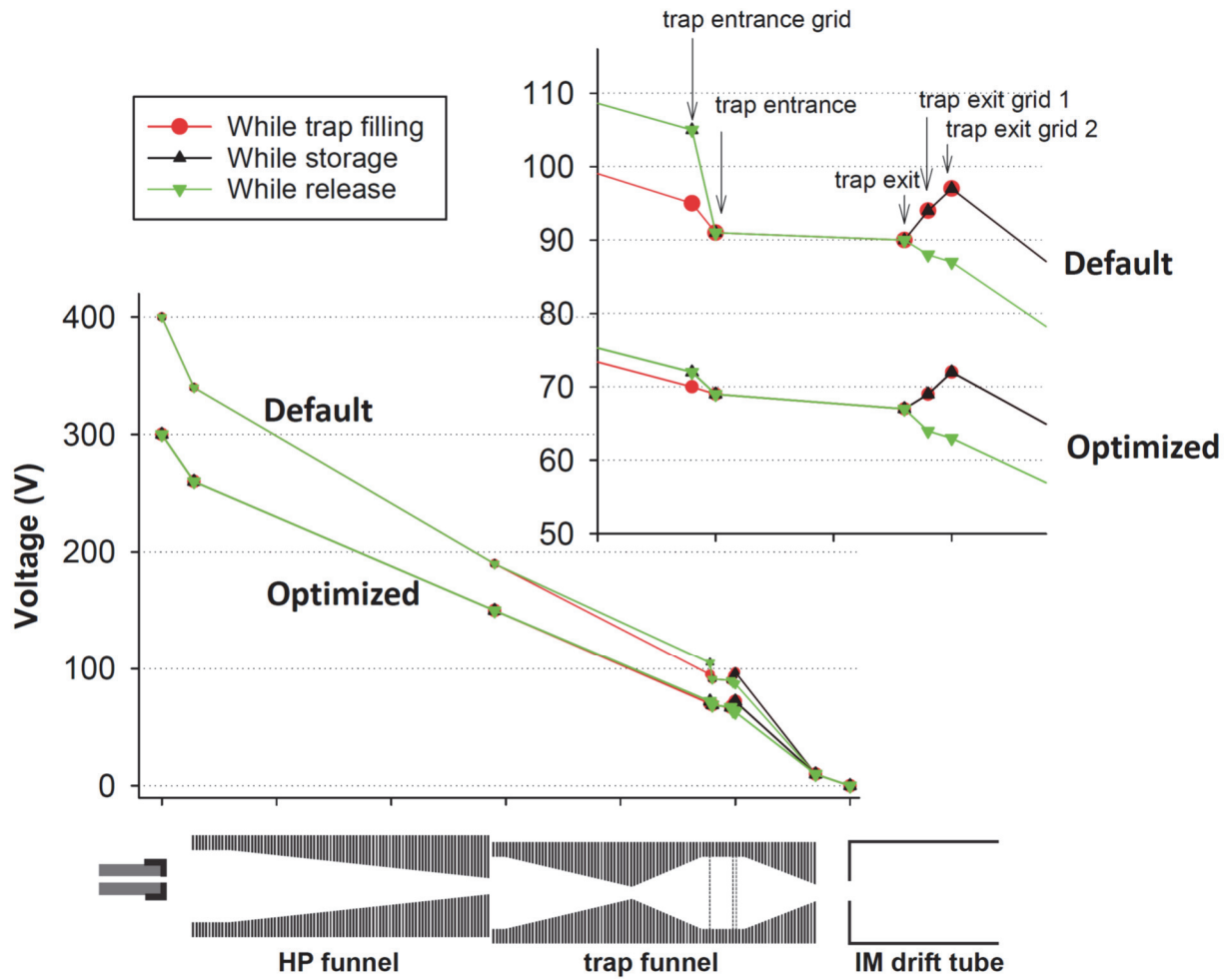


Figure S1: Comparison of the pre-IM DC voltage gradient in the default tuning parameters and in the tuning parameters optimized for soft conditions when the drift tube is operated in 3.89 Torr helium and the trapping funnel pressure is 3.73 Torr.

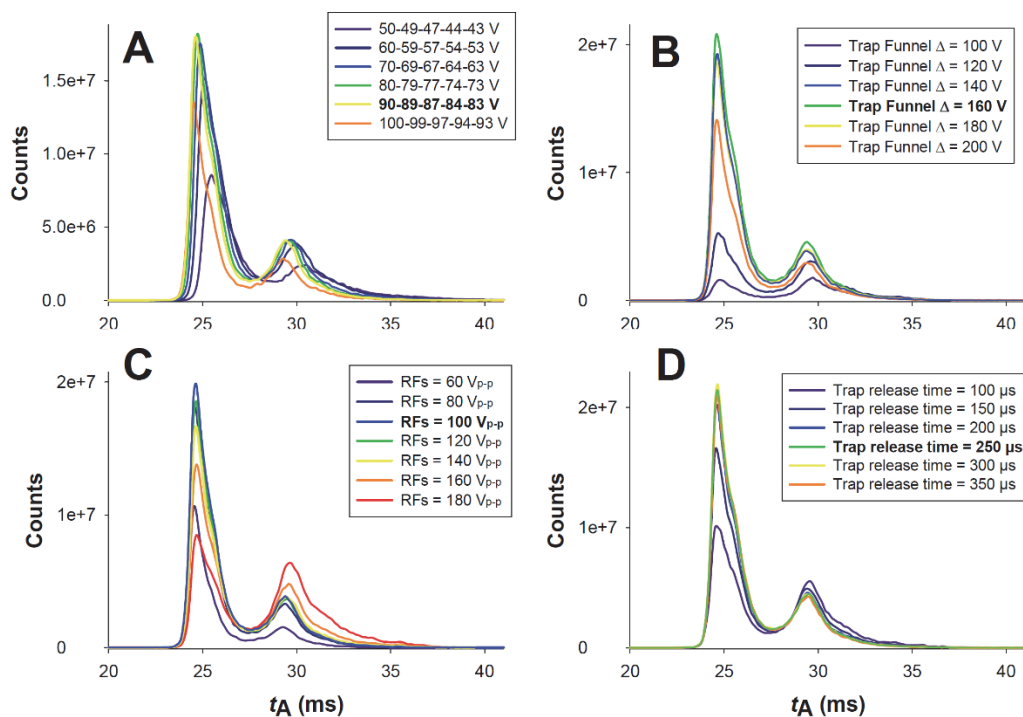


Figure S2: Influence of tuning parameters on the arrival time distribution of Ubiquitin⁷⁺, sprayed from a 0.75 μM solution in 99% H₂O and 1% acetic acid, with the drift tube operated in 3.89 Torr **nitrogen**. The drift tube was operated with $\Delta V = 1390$ V. The peak at ~ 25 ms corresponds to the native form ($CCS_{N_2} = 1262 \text{ \AA}^2$; standard deviation was 9 \AA^2 from 5 independent measurements in the optimum conditions, which are indicated in bold). Effects of A) voltages on the whole trapping region (see inset of Figure 1), the values written being the trap entrance grid low-trap entrance-trap exit-trap exit grid 1 low-trap exit grid 2 low; B) trap funnel delta; C) HP funnel RF and front funnel RF, both set at the same value; D) trap release time.

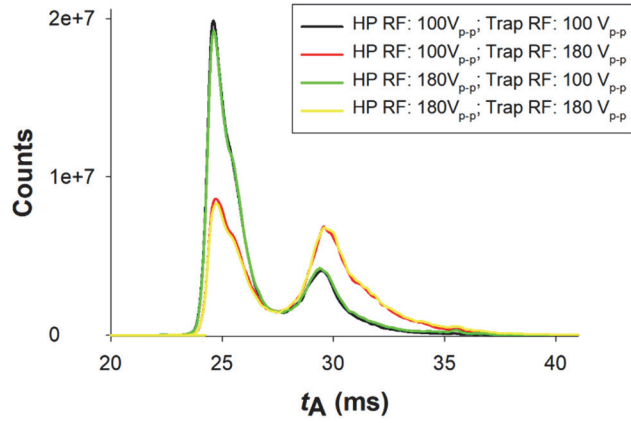


Figure S3: Influence of the high pressure funnel RF amplitude and trap funnel RF amplitude on the arrival time distribution of ubiquitin⁷⁺ ions, sprayed from a 0.75 μ M solution in 99% H₂O and 1% acetic acid, with the drift tube in nitrogen. Only the trapping funnel RF amplitude is responsible for the ion activation.

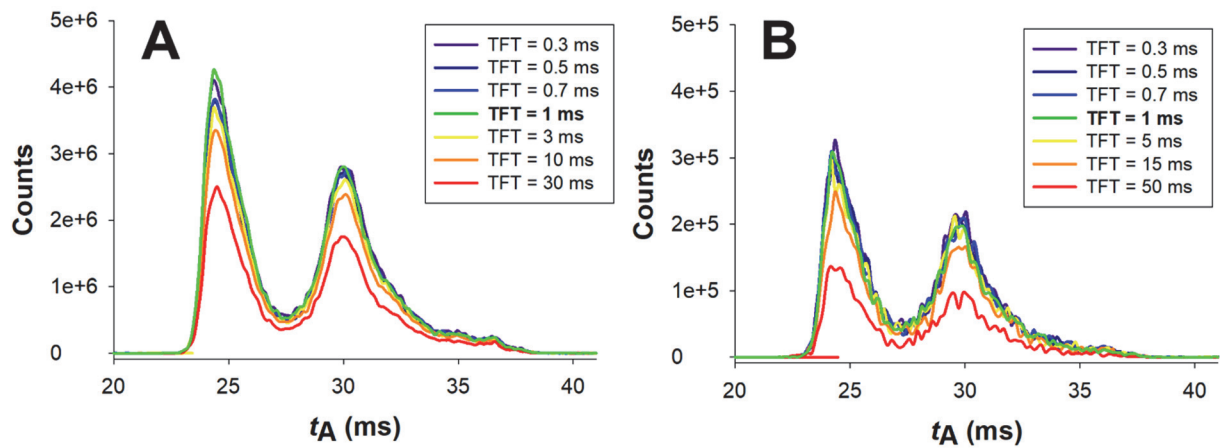


Figure S4: Influence of the trap fill time on the arrival time distribution of ubiquitin⁷⁺ ions, sprayed from (A) a 0.75 μ M or (B) a 0.075 μ M solution in 99% H₂O and 1% acetic acid, with the drift tube in helium. The same number of scans were summed in each case.

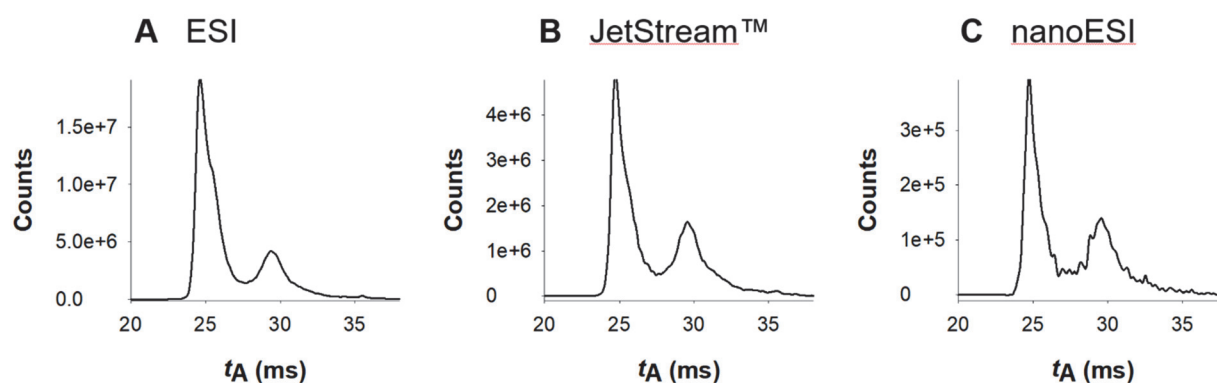


Figure S5: Arrival time distribution of ubiquitin⁷⁺, sprayed from a 0.75 μ M solution in 99% H₂O and 1% acetic acid, with optimization instrumental parameters (see Table 1) and the drift tube in 3.89 Torr nitrogen. (A) Standard ESI source, with drift gas at 1.5 L/min and 220 °C, nebulizer gas at 9 psi, spray voltage at 3500 V. (B) JetStream™ source, with drift gas at 1.5 L/min and 220 °C, nebulizer gas at 9 psi, spray voltage at 3500 V, jet gas flow at 2 L/min and 30°C, and nozzle voltage at 1000 V. (C) Nanospray source with manually cut coated borosilicate capillary, spray voltage at 1100 V, no nebulizer gas, and drying gas at 13 L/min and 30 °C (similar to May et al.²¹). Note that the nanospray cone diverts most of the drift gas flow.

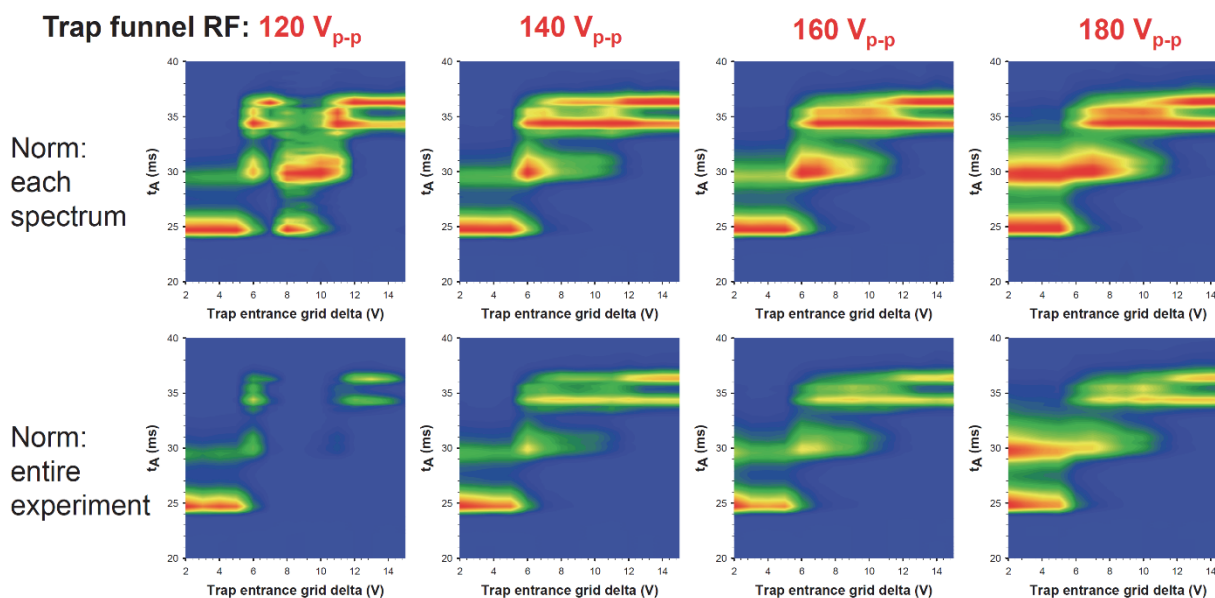


Figure S6: Influence of the trap funnel RF amplitude on the CIU results obtained when varying the trap entrance grid delta voltage. When normalizing the arrival time distribution at each voltage, the CIU plots look strange at low trap funnel RF (120 V_{p-p}). In reality, the signal intensity is lost at some specific voltage ranges (see bottom row where the normalization is done over the entire experiment, not spectrum per spectrum). As the RF amplitude is increased, there is no more signal loss, but the activation at low voltage is becoming prominent. For ubiquitin7+, 160 V_{p-p} presents a good compromise to carry out CIU experiments.

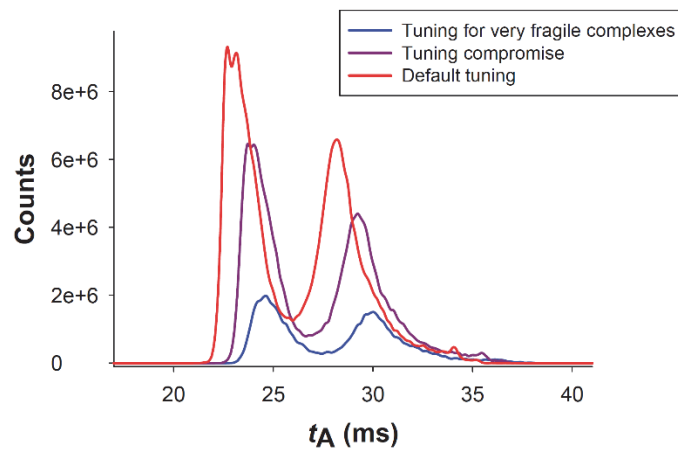


Figure S7: Effect of changing the voltage gradient behind the IM drift tube (parameters, see Table 2) on the arrival time distribution of ubiquitin⁷⁺ (produced from 99% H₂O, 1% acetic acid, drift tube in 3.89 Torr helium with $\Delta V = 390$ V). The measured collision cross section is unchanged, but the t_0 value changes (5.21 ms for the softest conditions, 4.83 ms for the compromise tuning, 3.70 ms for the default installation tuning).