Supporting Information

Gas Phase Stability of Protein Ions in a Cyclic Ion Mobility Spectrometry Travelling Wave Device

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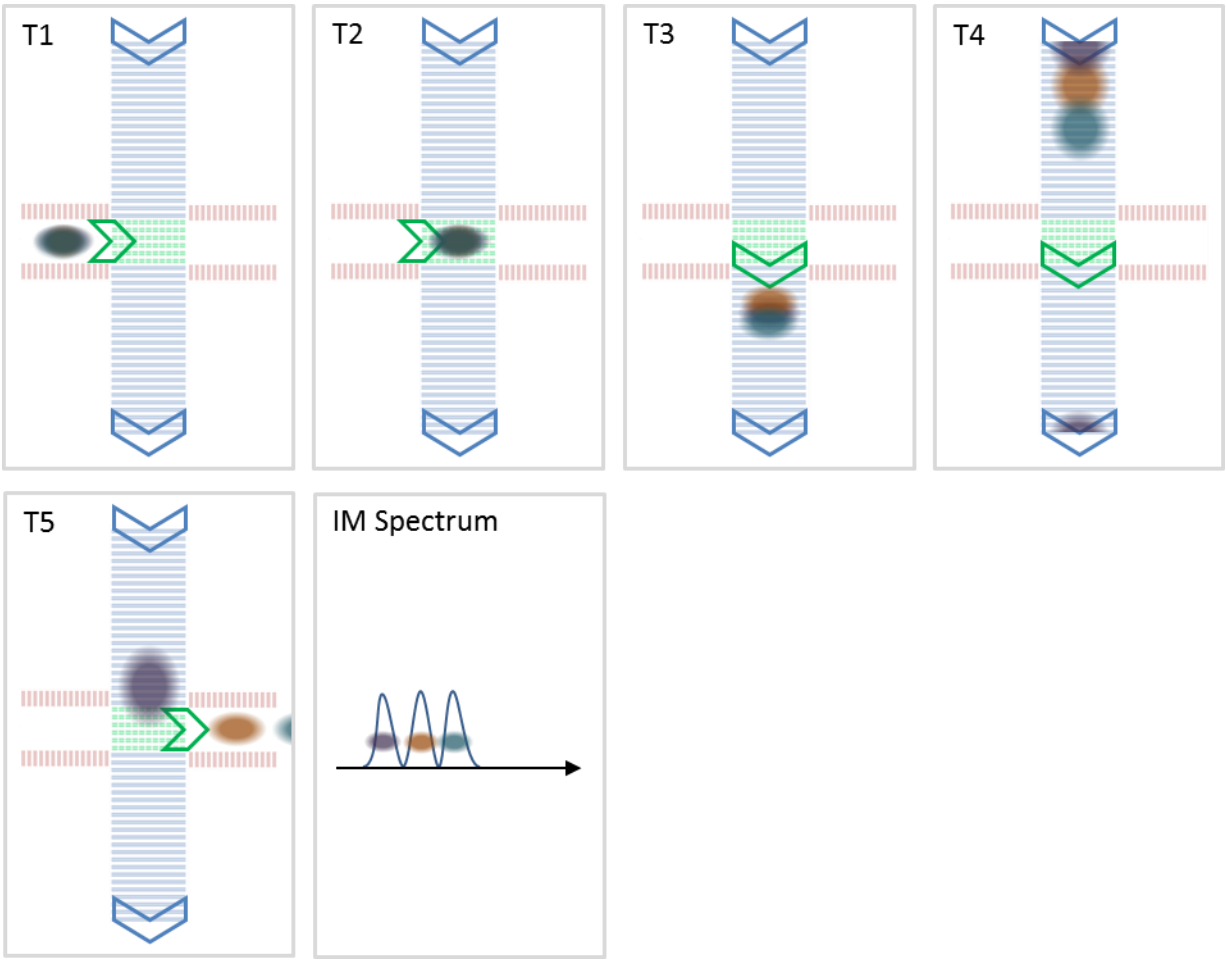

Figure S1. Events in single/multipass IMS operation. Time points 1 and 2 (T1 and T2): ions are injected from store (red) into the array (green). T3: direction of T-waves in the array (green arrow) changes to match those in cIM region (blue arrows); ions begin to drift around the cIM device. T4: after a certain number of passes three species appear separated. T5: Ions are ejected to the ToF and IM-MS spectrum is recorded.

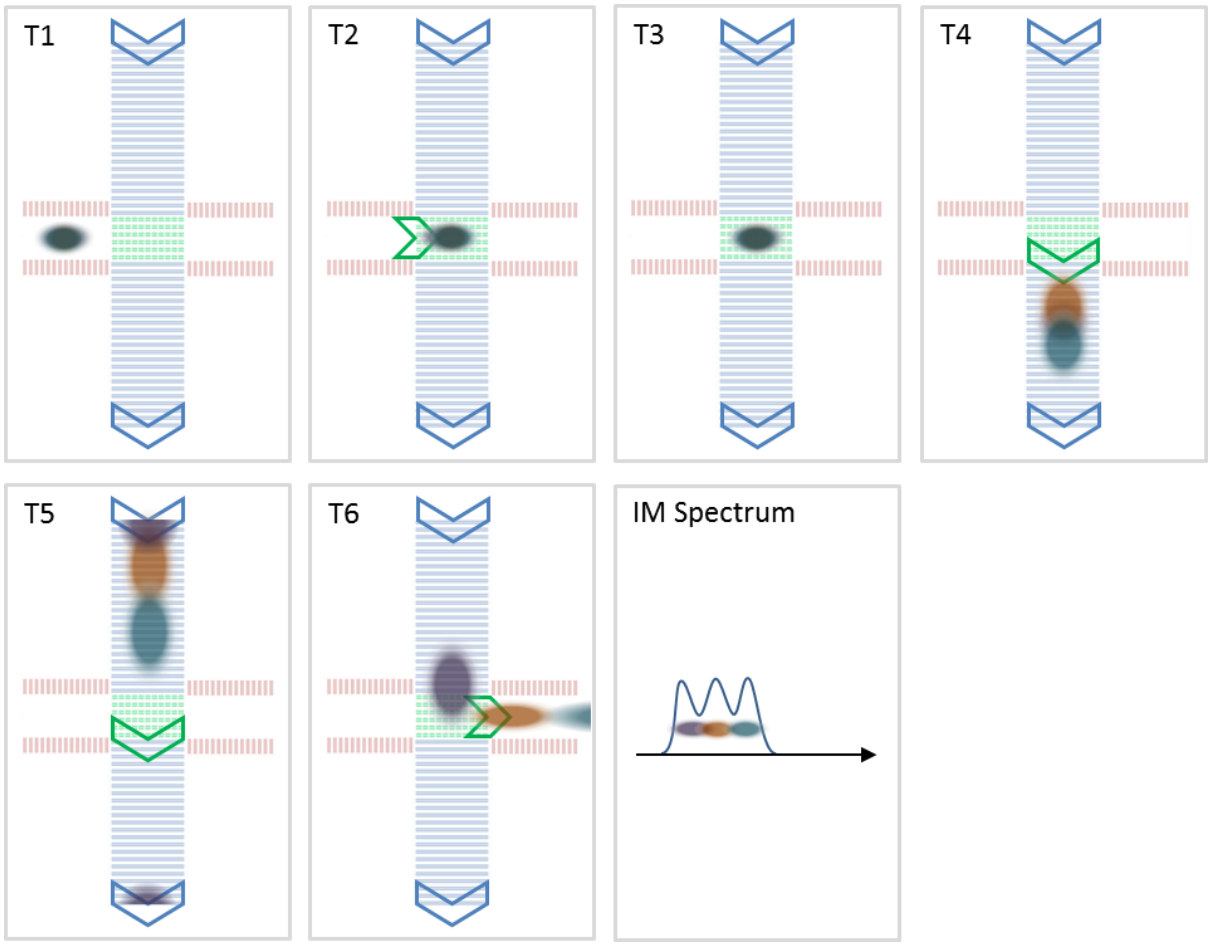

Figure S2. Events in "trapping" mode of operation. Time points 1 and 2 (T1 and T2): ions are injected from store (red) into the array (green). Before IM separation takes place, ions are trapped for a prolonged period of time in the array (T3). Steps T4-T6 correspond to T3-T5 steps in the Figure S1. The obtained IM spectrum is directly comparable to that in the Figure S1.

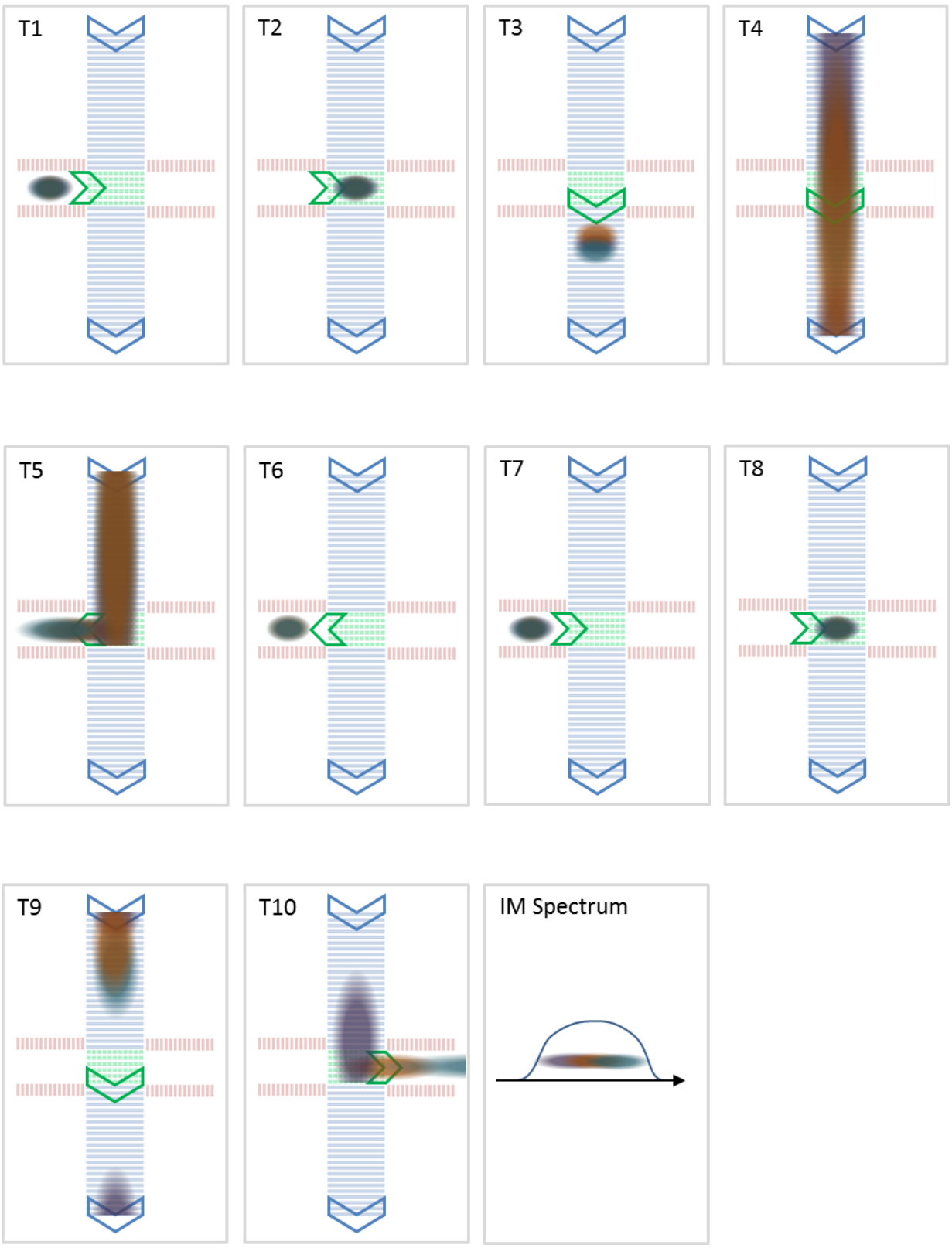

Figure S3. Events in “spinning” mode of operation. Time points T1-T3: ions are injected from store (red) into the array and IM separation takes place. T4: ions are passed around beyond the wrap around limit (i.e. time when fastest ions overtake the slowest ones). T5: ions are recollected in pre array store. T6-T10: ions are re-injected into the array and IM separation of ions that have been “spun” for prolonged period of time is performed. The obtained IM spectrum is directly comparable to that in the Figure S1.

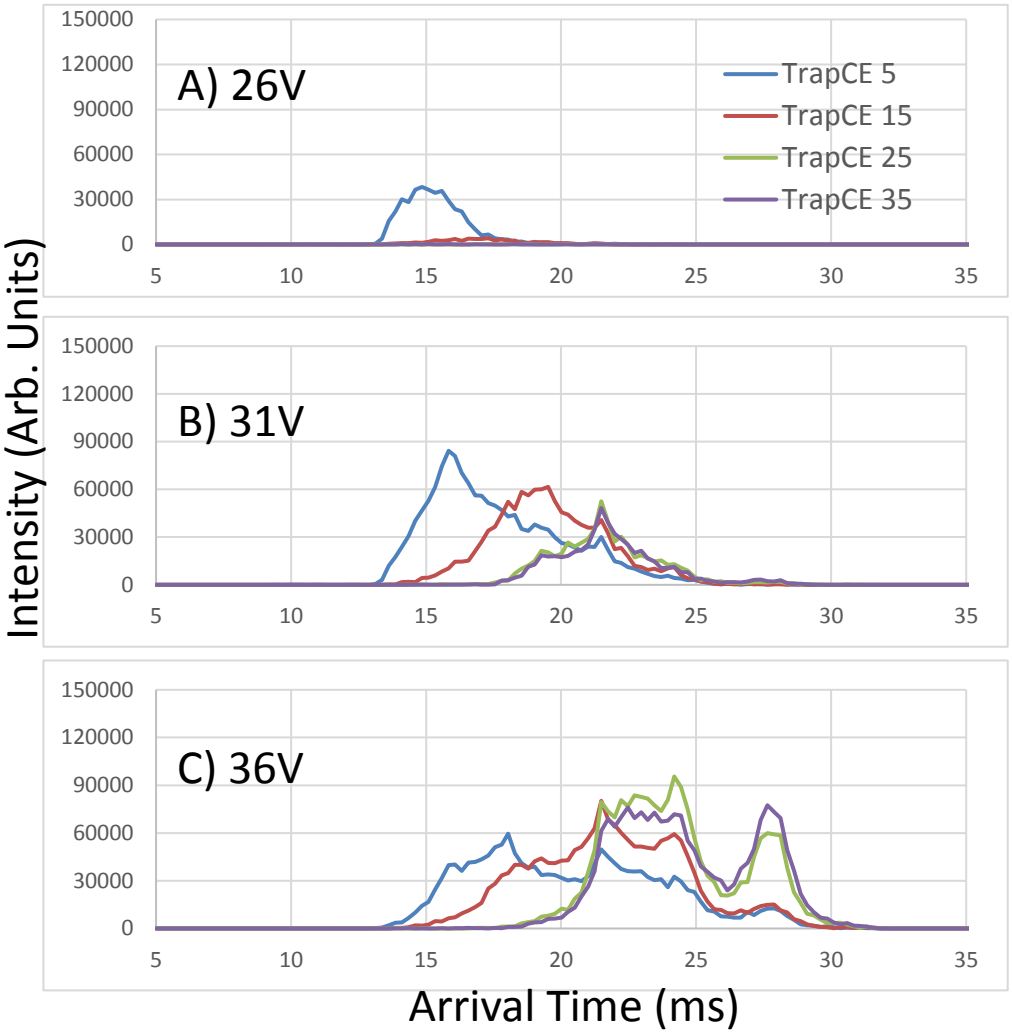

Figure S4. IM spectra from Injection of CytC +7 ions into the cIM as a function of He cell injection voltage; A) 26V, B) 31V, C) 36V and Trap collision energy (CE). At low injection voltage (A) and low Trap CE the more compact form is transmitted to the cIM. At higher trap CE the signal is significantly attenuated and may be a reflection of discrimination against any unfolded, lower mobility species, entering the He cell at this low injection voltage. At higher injection voltages, the expected unfolding of the protein is observed, but also, the signal at higher Trap CE is less attenuated, presumably due to less discrimination on entry to the He cell.

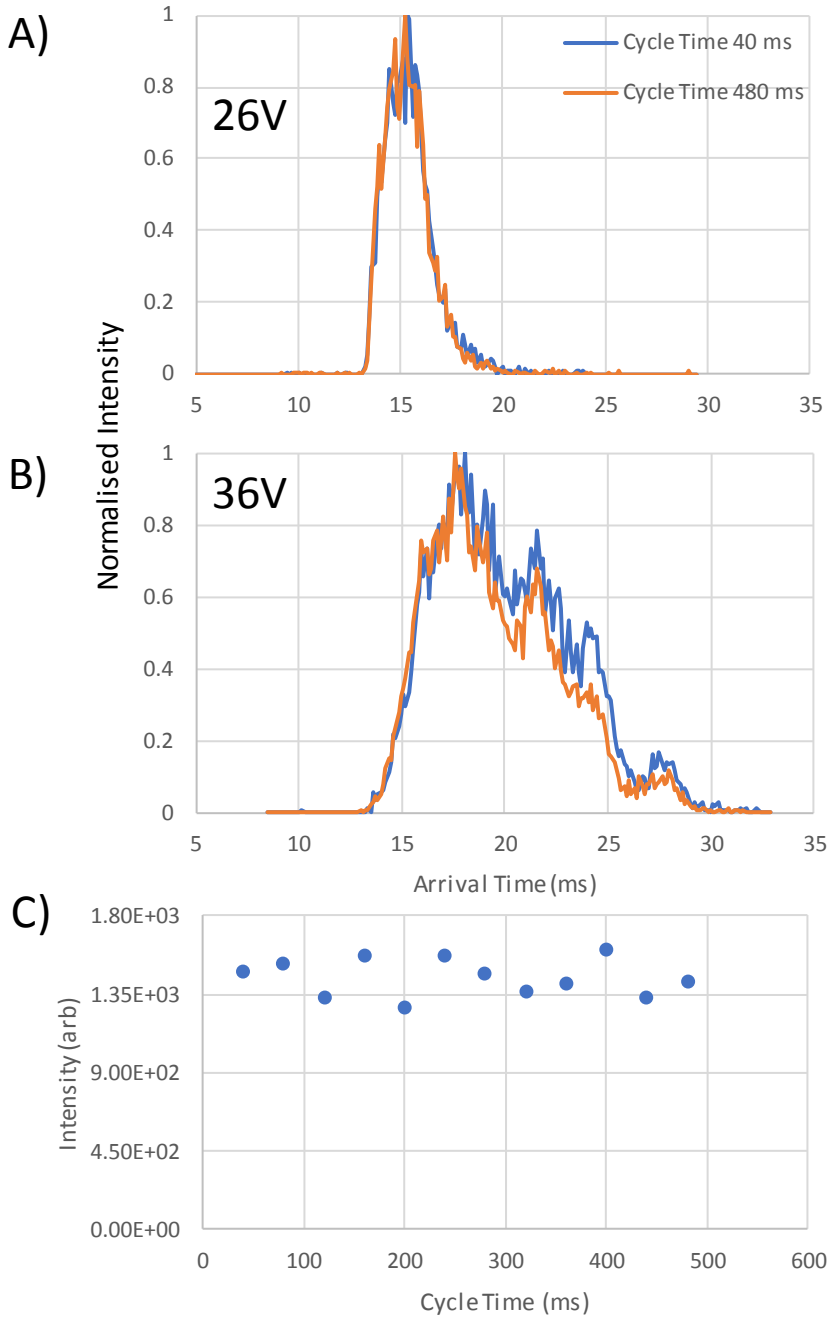

Figure S5. IM spectra obtained for CytC +7 at A) 26V and B) 36V injection voltage into the He cell at two different cIM experiment cycle times. These data indicate no significant difference in the populations of ions in the trap at the two different cycle times, suggesting no significant unfolding is taking place. The plot in C) is the area under the ATD of the ions injected at 26V into the He cell as a function of cycle time, indicating no significant loss. These data add further weight to the argument that little or no conformational change occurs in the Trap cell.

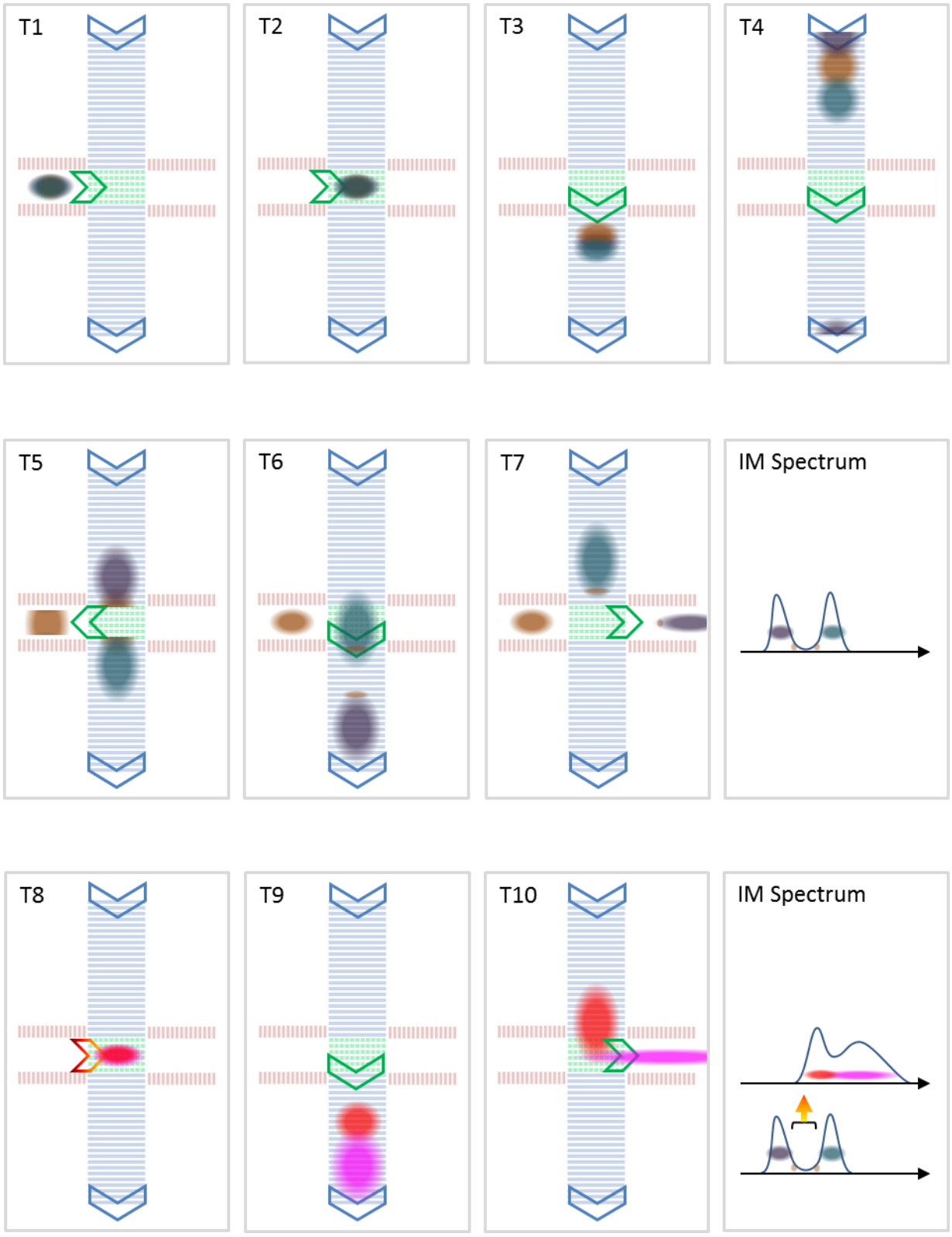

Figure S6. Events in IMS^2 mode of operation. Time points 1 and 2 (T1 and T2): ions are injected from store (red) into the array (green). T3: direction of T-waves in the array (green arrow) changes to match those in cIM region (blue arrows); ions begin to drift around cIM device. T4: after certain number of passes three species appear separated. T5: IM selection; as brown population of ions drifts through the array region, the array T-wave direction changes and the selected ion population is ejected into the store. T6, T7: ions remaining in the cIM travel for an additional pass around cIM before being ejected out. T8: ions from the store are re-injected into the array at high energy and form product ions. T9: Product ions (originating from brown precursor population) are mobility separated. T10: Ions are ejected to the ToF and IM-MS spectrum is recorded.

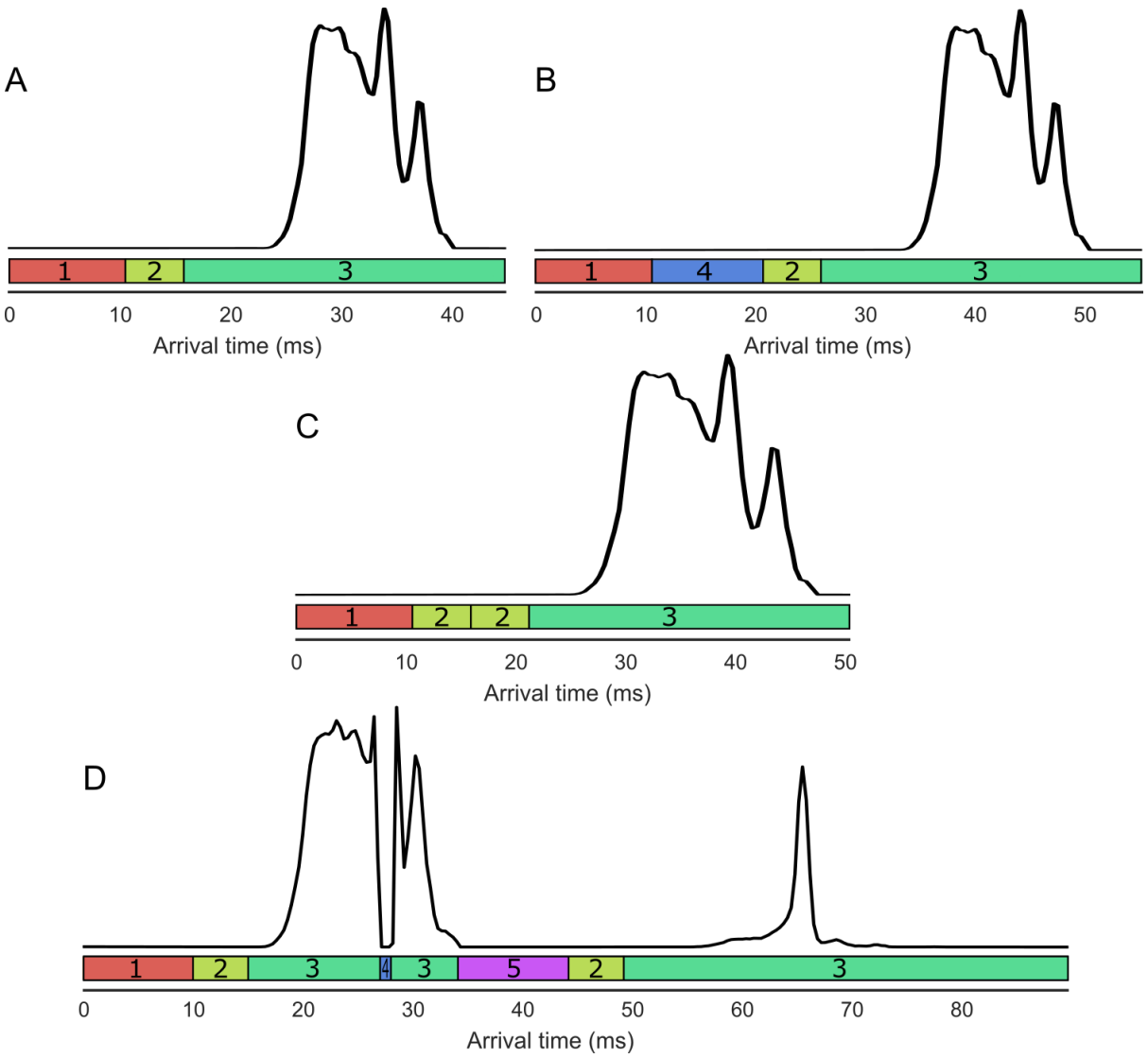

Figure S7 Representative arrival time distributions with timings from possible ion mobility experiments on the cIM. The numbers are related to a specific action 1: injection of ion packet, 2: mobility separation in racetrack, 3: eject from racetrack to ToF, 4: eject to array store, 5: inject from array store to racetrack (A) basic mobility experiment (B) trapping experiment (C) extended spinning experiment (D) IMS-CIU-IMS experiment.

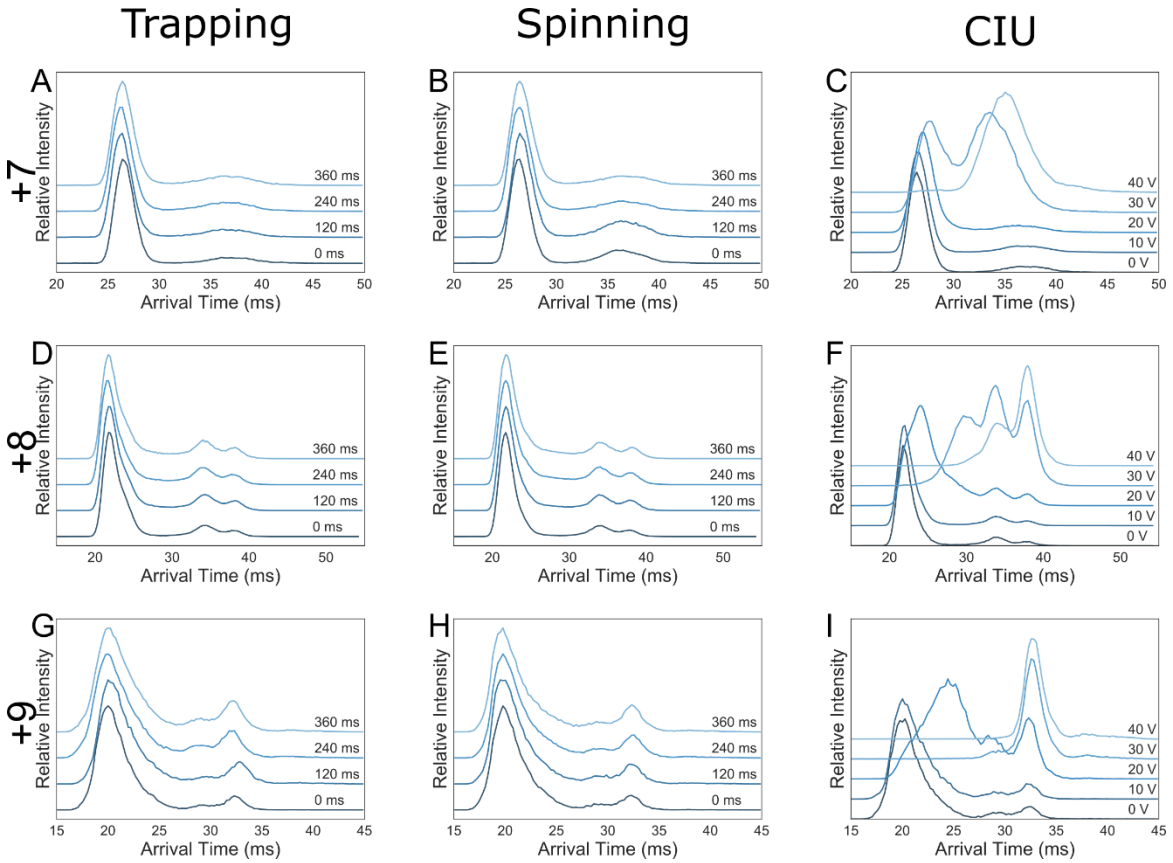

Figure S8 Trapping (A, D, G), spinning (B, E, H) and CIU (C, F, I) experiments for β-Lactoglobulin, from quadrupole isolated +7 (A - C), +8 (D - F) and +9 (G - I) charge states. Each figure is composed of ATD slices, arranged in increasing experimental increment, *ie*. trapping time (ms), from dark to light shading. Plotted is the drift time against relative intensity.

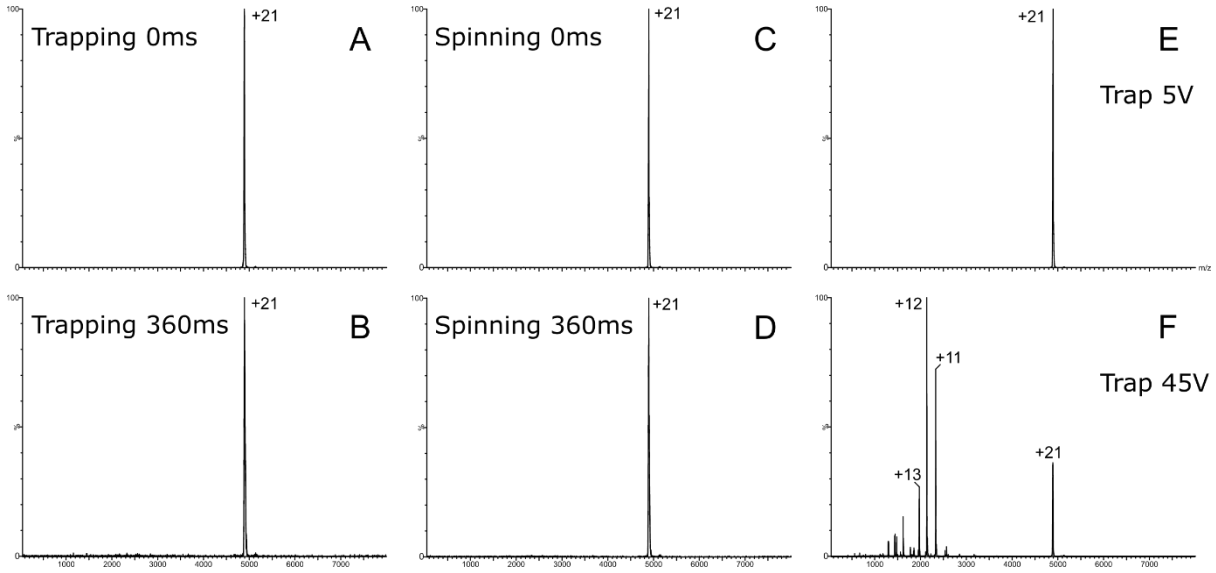

Figure S9 Mass spectra for the quadrupole isolated +21 charge state of Concanavalin A during initial (A, C, E) and final (B, D, F) increments of trapping (A – B), spinning (C – D) and CIU (E – F) experiments. Main peaks annotated. At high voltage during the CIU experiments the tetrameric +21 state decays dissociates into monomeric charge state distribution of +13 to +11. Spectra smoothed with a mean window of 5, twice.

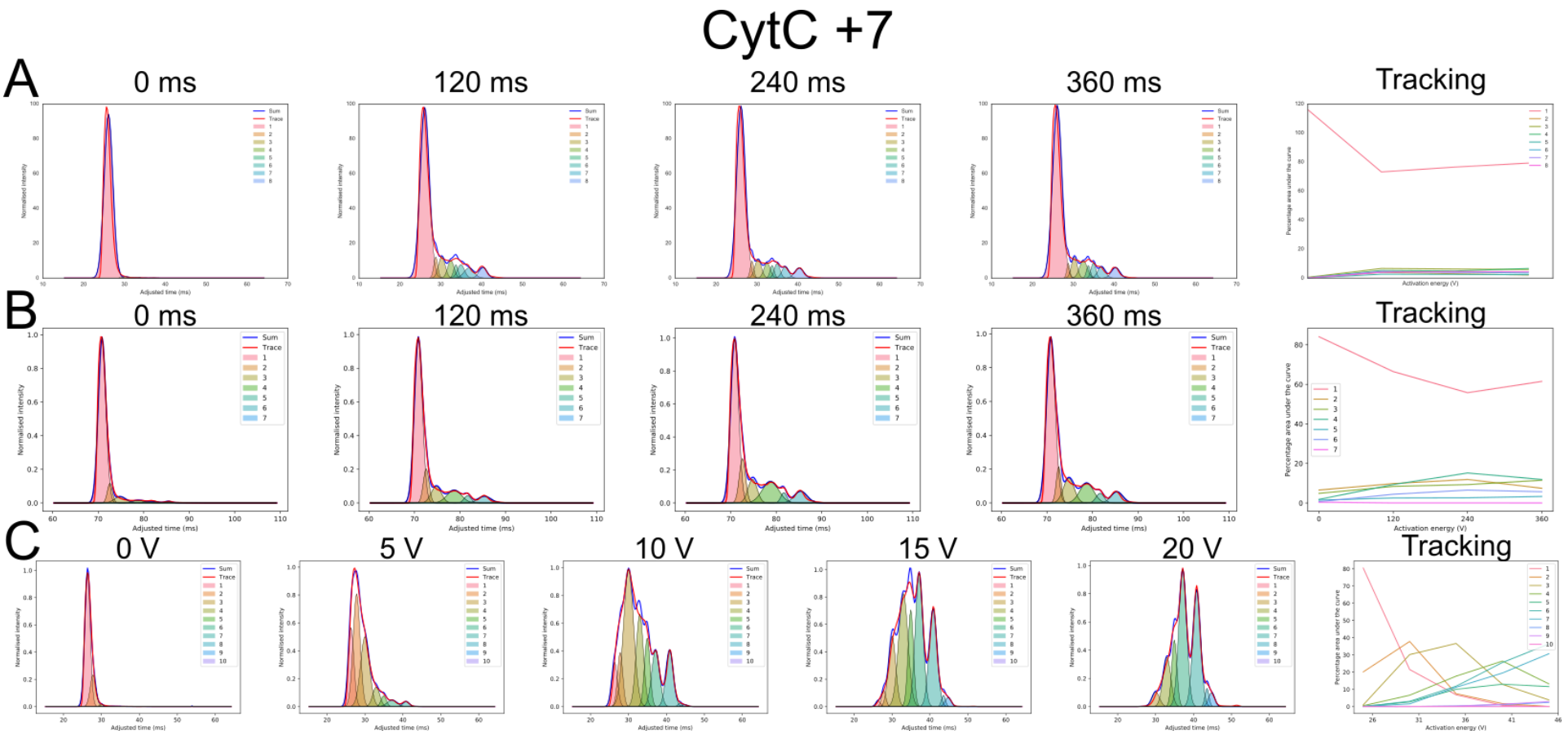

Figure S10 Population tracking for CytC +7 experiments A) tracking B) spinning C) CIU

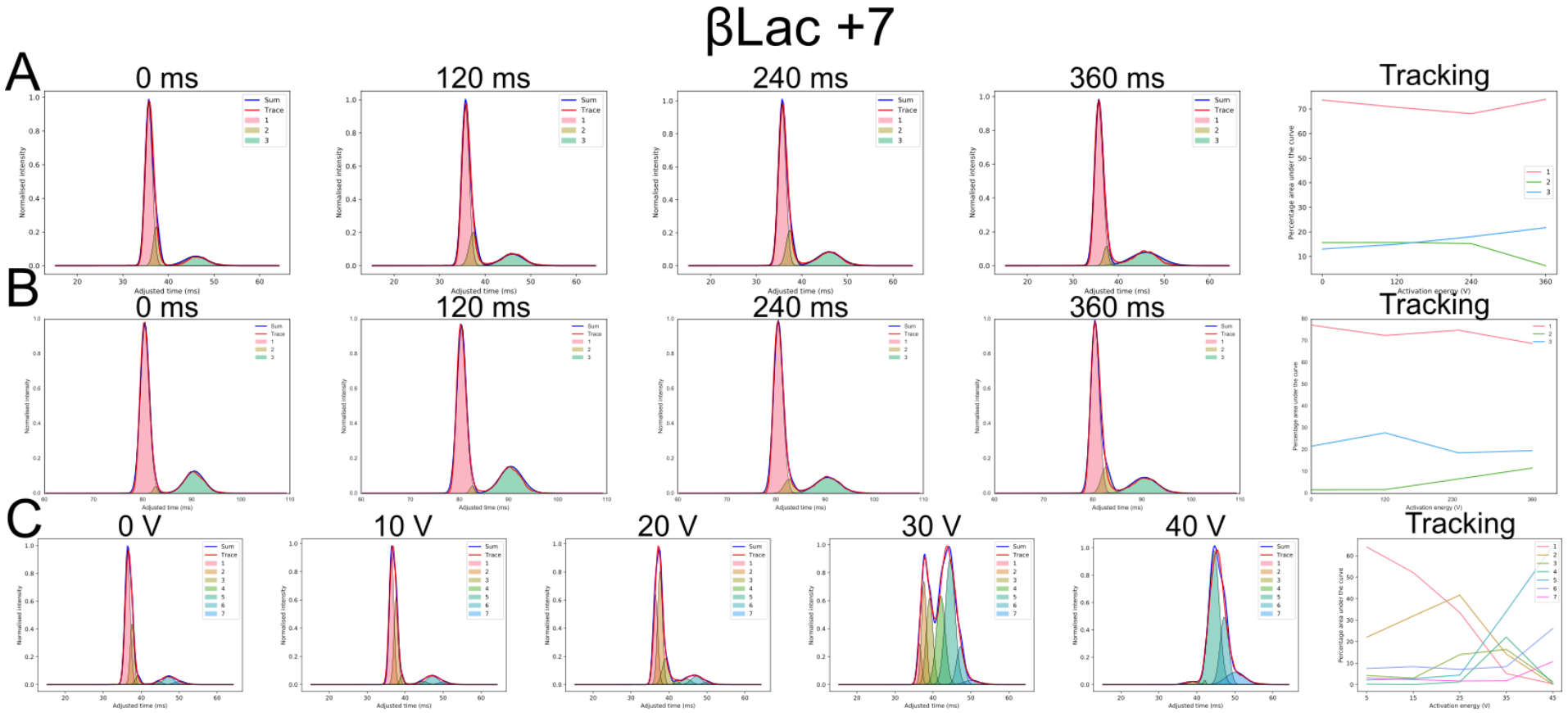

Figure S11 Population tracking for Blac +7 experiments A) tracking B) spinning C) CIU

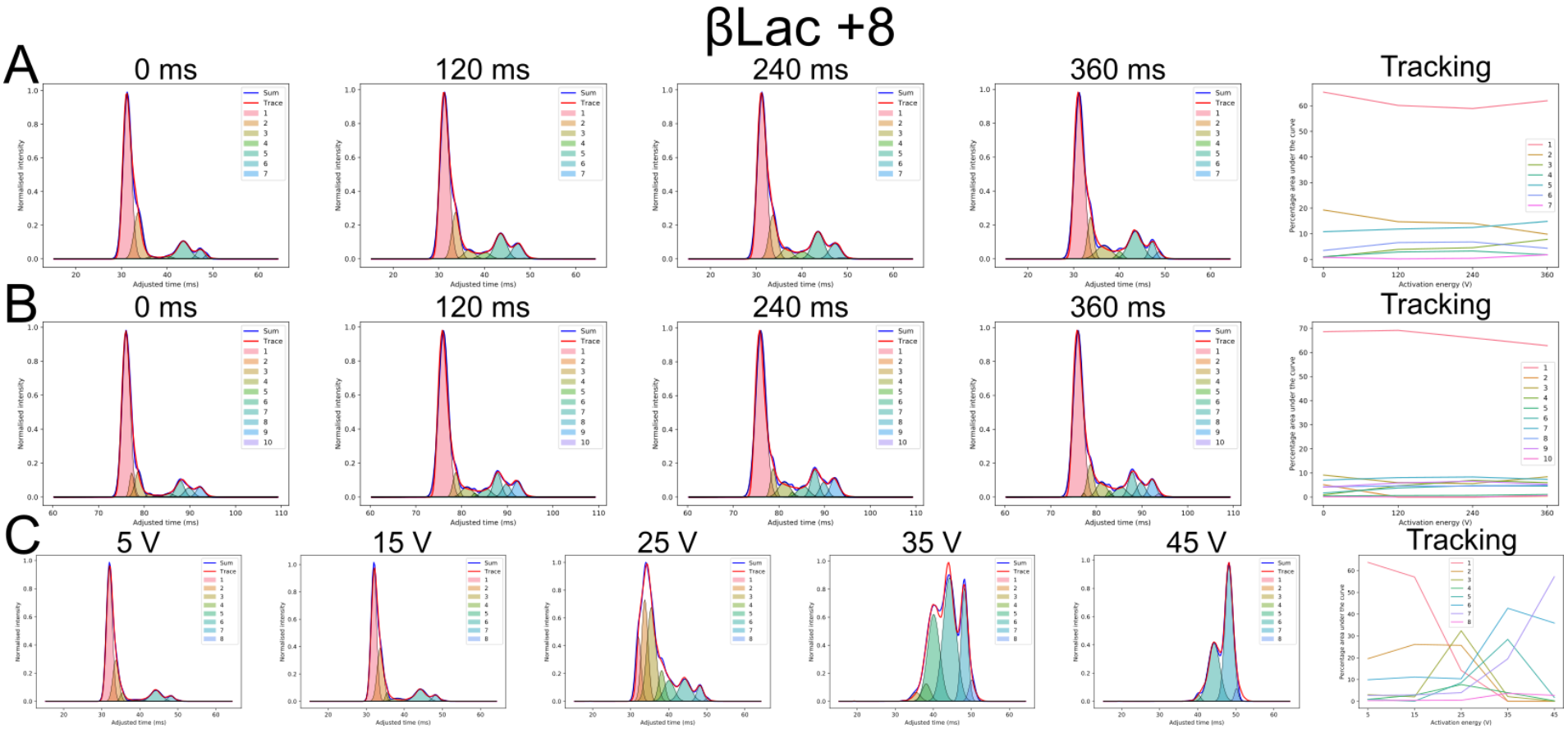

Figure S12 Population tracking for Blac +8 experiments A) tracking B) spinning C) CIU

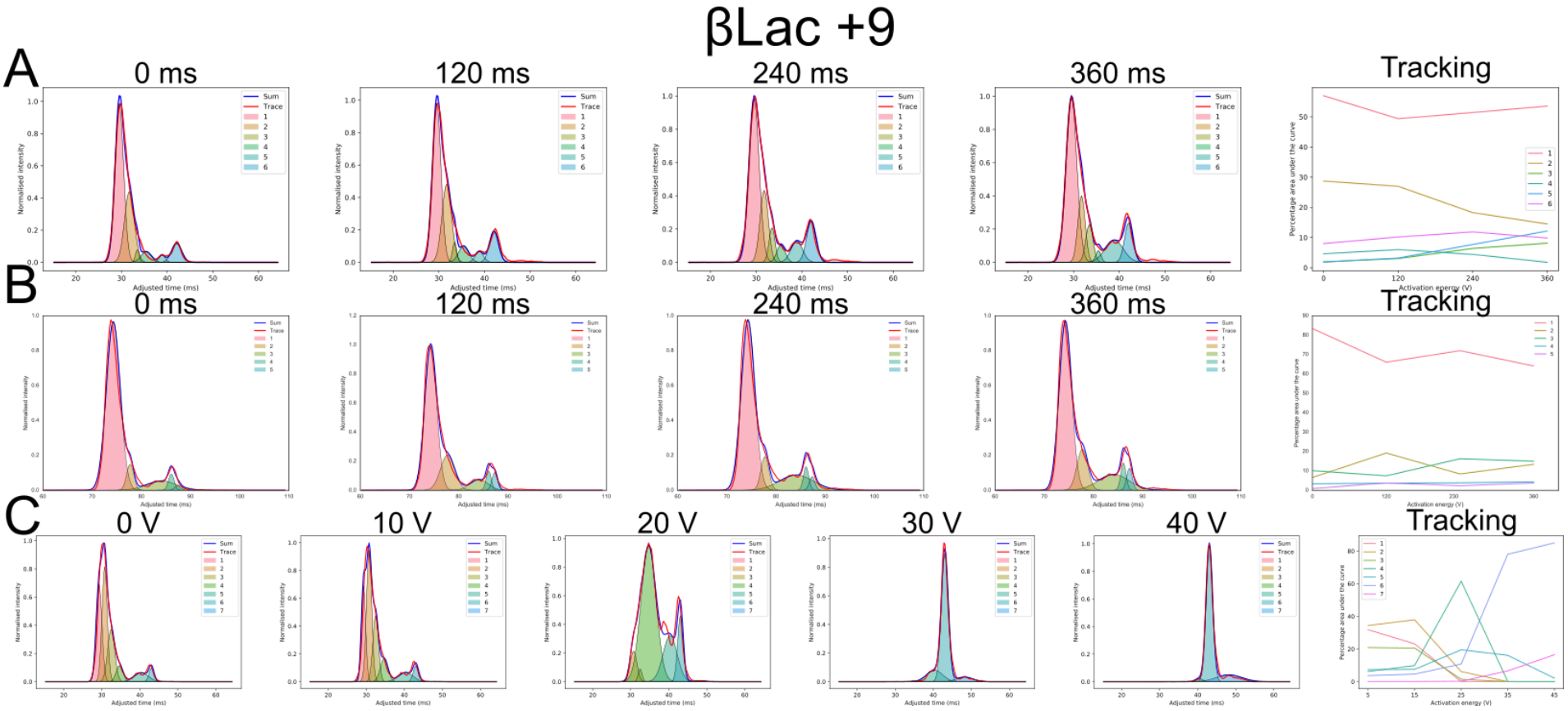

Figure S13 Population tracking for Blac +9 experiments A) tracking B) spinning C) CIU

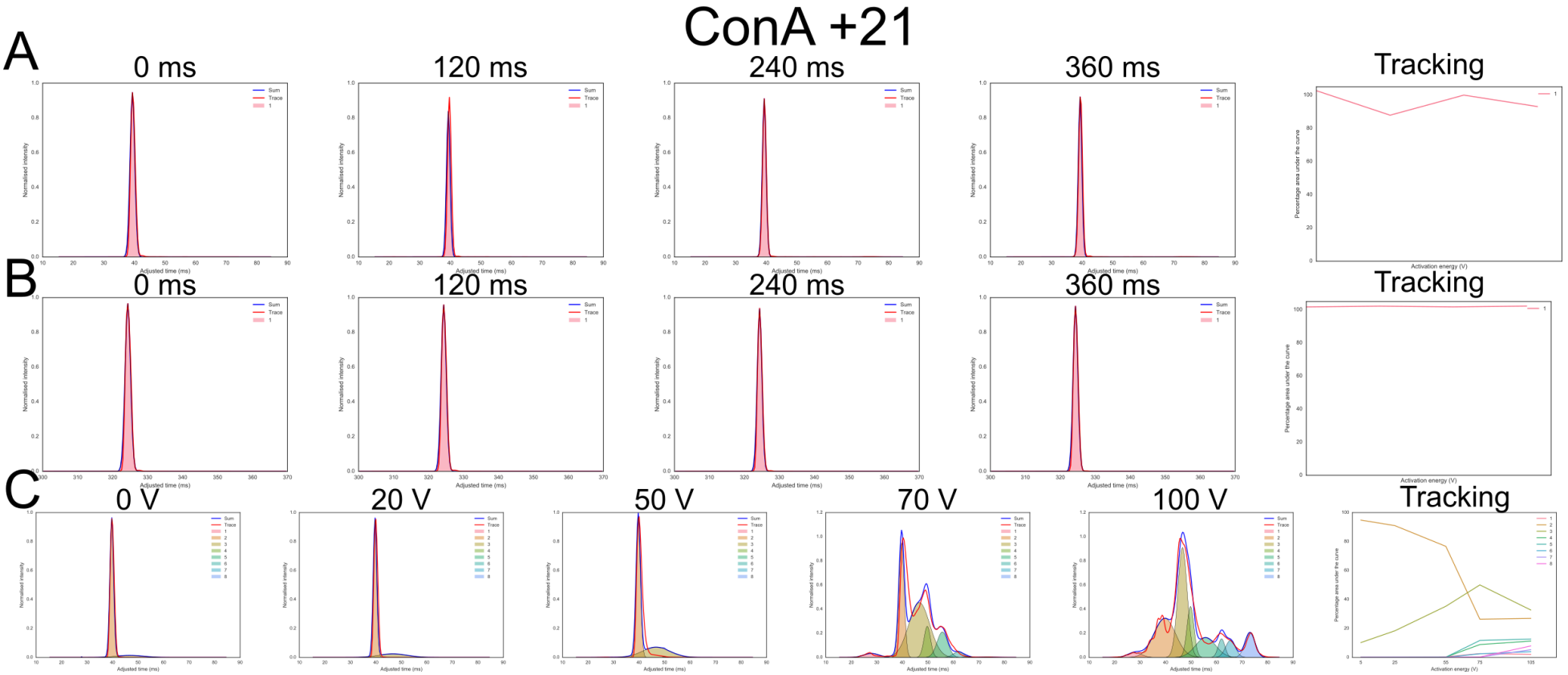

Figure S14 Population tracking for ConA+21 experiments A) tracking B) spinning C) CIU

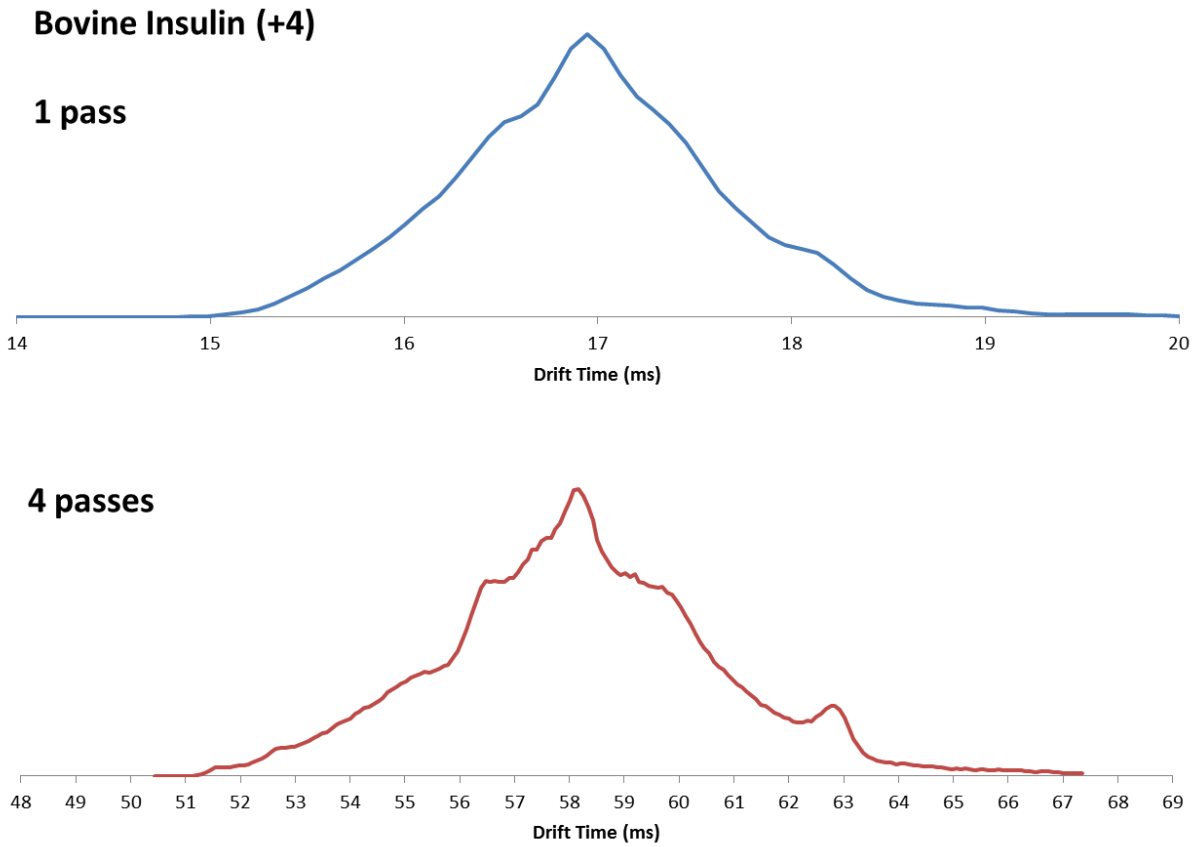

Figure S15 Bovine insulin after 1 pass and 4 passes (compressed) in the cIM. Conformations become more distinct after multiple passes.