

# Structural Analysis of the Multimers of Bovine Serum Albumin by Ion Mobility Mass Spectrometry

## Authors

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## Abstract

This application note presents the use of the Agilent 6560 ion mobility LC/Q-TOF to analyze the formation of multimers of bovine serum albumin (BSA) in various mobile phases. Conversion from a native ammonium acetate mobile phase solution to more denaturing conditions correlates with a decrease in BSA multimers. Comparison of two-dimensional heat maps of these analyses provides impressions of how these multimer complexes are formed.

## Introduction

Proteins have dynamic structures that often adopt multiple conformational states depending on their function. Mass spectrometry (MS) is a powerful tool for the analytical characterization of biomolecules, providing sensitivity, speed, small sample consumption, and the ability to analyze complex mixtures. When coupled to ion mobility spectrometry (IMS), characterization of both the mass and molecular structure of these biomolecules can be obtained. This characterization provides information on the conformational states these proteins can adopt, and reveals information concerning subunit composition as well as the overall topology of the protein complex. Even the range of conformational states protein complexes can adopt can be examined, and the detection of protein assemblies in a single spectrum is made possible.

Different charge states fall along trendlines based on the increased force larger charge states experience as they travel through the drift tube. This enables an extra dimension of separation provided by the drift tube. These different trendlines allow increased selectivity in the detection of the various multimers of BSA. The resulting multidimensional distributions obtained from these three different solutions show the formation of different multimers.

## Experimental

### Sample preparation

BSA obtained from Sigma-Aldrich was buffer-exchanged with the use of Micro Bio-Spin P-6 Gel columns from Bio-Rad into 150 mM ammonium acetate. An Agilent nanospray source (G1992A) was coupled to an Agilent 6560 ion mobility LC/Q-TOF. BSA (20 mg/mL) was infused at a flow rate of 266 nL/min through a New Objective noncoated needle 360  $\mu$ m od, 75  $\mu$ m id, 30  $\mu$ m tip, 5 cm length from one of the following solution conditions: 150 mM ammonium acetate, 0.1% formic acid (by volume), and 50:50:0.1% water/acetonitrile/formic acid (by volume).

### Instrumental analysis

For sample analysis, the high pressure and trapping funnels of the Agilent 6560 ion mobility LC/Q-TOF (Figure 1) are operated at 200 V peak-to-peak, and the rear ion funnel is operated at 100 V peak-to-peak, with a fill time of 30,000  $\mu$ s and release time of 300  $\mu$ s applied to the trap funnel. In this instrument, a weak uniform field is applied across the ~80 cm long drift tube. The electric field applied across the drift tube was 12.12 V/cm, while the pressure was maintained at 3.94 Torr of nitrogen.

### Data analysis

Ion mobility heat maps were generated with an Agilent MassHunter IM-MS browser version 8.

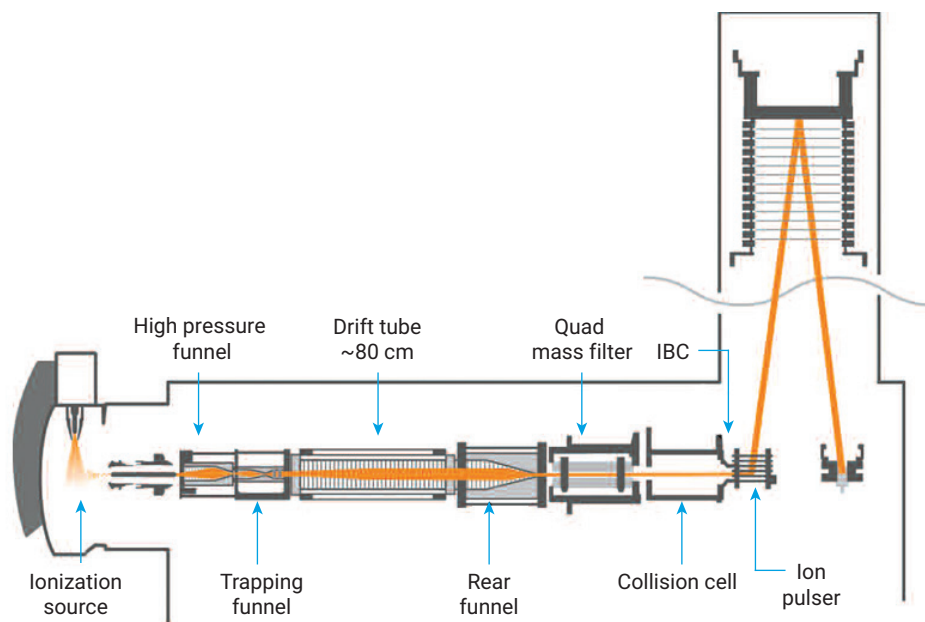


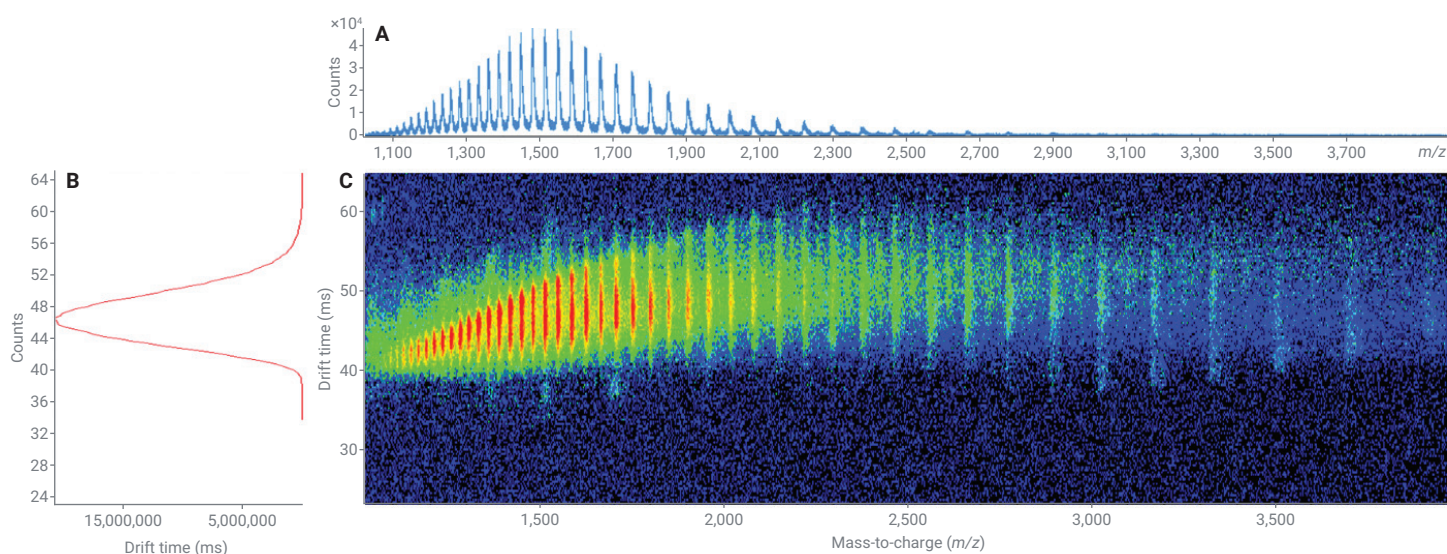
Figure 1. Schematic of the Agilent 6560 ion mobility LC/Q-TOF.

## Results and discussion

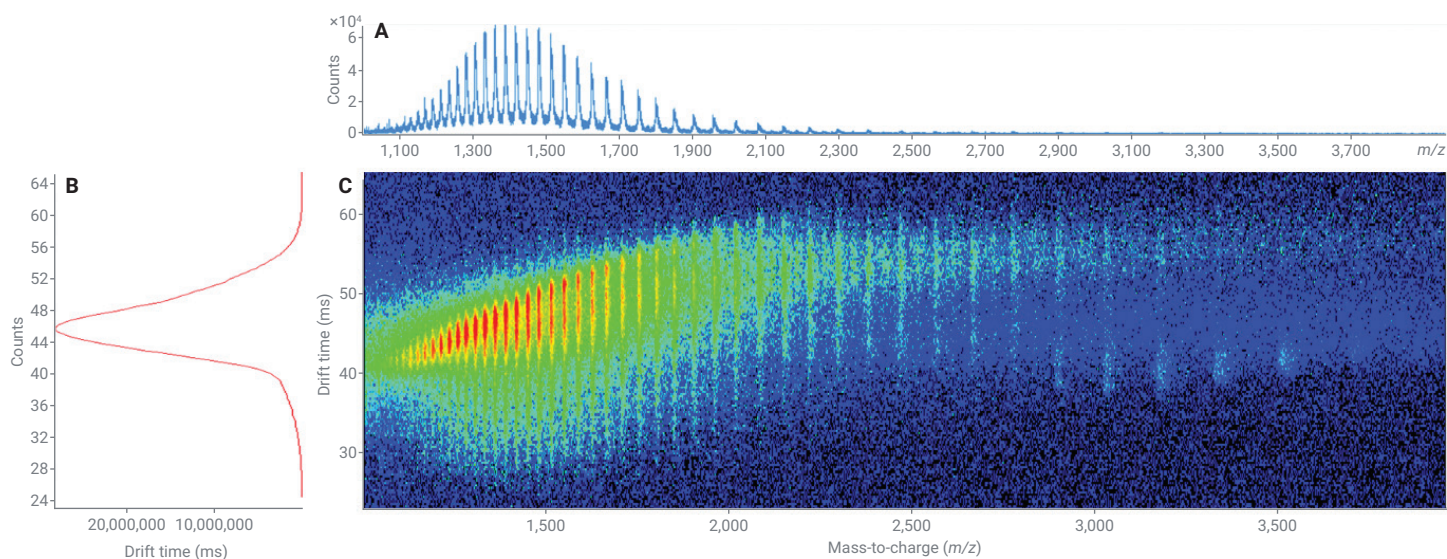
For the high pressure and trapping funnels, 200 V peak-to-peak was found to optimize transmission of native BSA, similar to those used by Gabelica *et al.*<sup>1</sup> Comparison of the mass spectra obtained with 50:50:0.1% water/acetonitrile/formic acid

to 0.1% formic acid shows similar  $m/z$  species. A slightly different abundance distribution of the charge states results (Figures 2A and 3A). However, their corresponding heat maps (drift time versus  $m/z$ ) show larger differences that are not accounted for by the mass spectra alone.

Higher charged species are known to experience higher force, and to travel faster through the drift tube with correspondingly faster drift times (lower drift time values). If we consider a monomer versus a dimer, they will have overlapping MS signals if the dimer is double the charge of the monomer, difficult to differentiate with mass spectrometry alone. The corresponding trend line of the dimer in these heat maps appears below the monomer.



**Figure 2.** Mass spectrum of  $m/z$  1,000 to 4,000 (A); overall drift spectrum (B); heat map of drift time (ms) versus  $m/z$  for BSA in 50:50:0.1% water/acetonitrile/formic acid (C).



**Figure 3.** Mass spectrum of  $m/z$  1,000 to 4,000 (A); overall drift spectrum (B); heat map of drift time (ms) versus  $m/z$  for BSA in 0.1% formic acid (C).



Also, the dimer trendline will have additional (lower in abundance and less resolved at lower  $m/z$ ) signal between the overlapping  $m/z$  signals, corresponding to nonoverlapping charge states (Table 1).

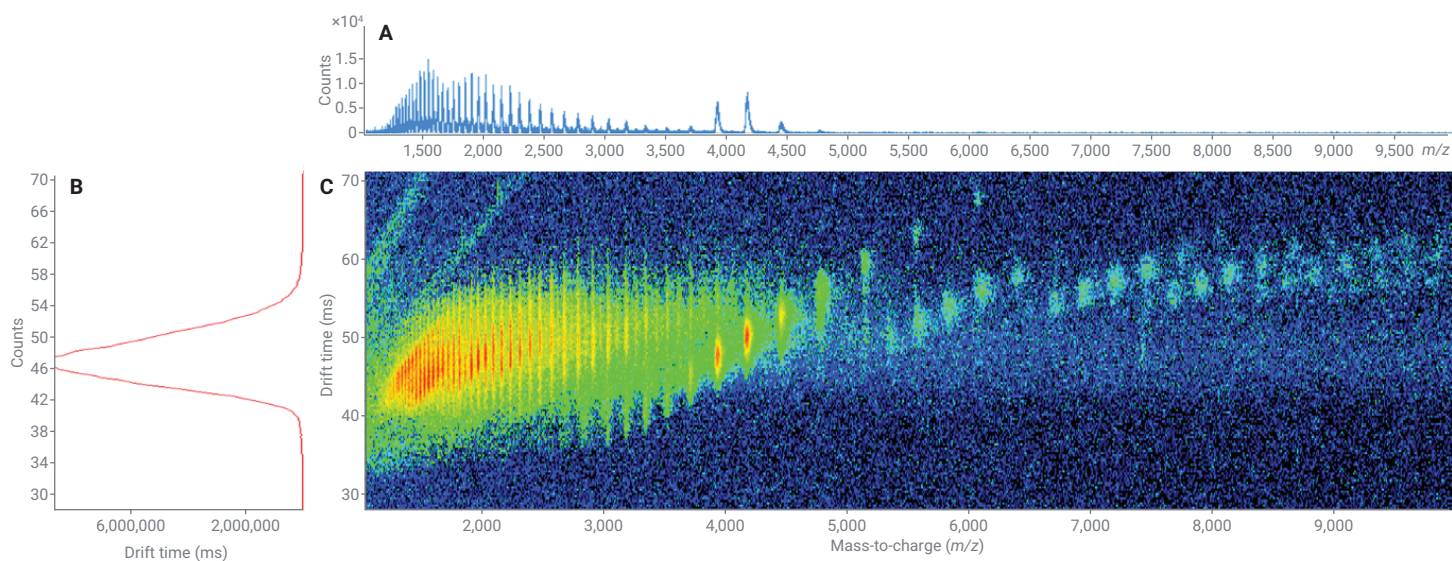
Comparison of the corresponding heat maps (Figures 2 and 3) show that both contain a potential multimer. However, the 0.1% formic acid solution favors the multimeric species slightly more, as evidenced by the larger spread of drift times to shorter times for a given  $m/z$ . This can also be seen in the overall drift spectra, with the use of the 0.1% formic acid solution giving a more "fronted peak".

Comparison of the mass spectrum from the 150 mM ammonium acetate solution (native conditions) to those of either the 50:50:0.1% water/acetonitrile/formic acid or the 0.1% formic acid (denaturing conditions) mobile phases show

**Table 1.** Example of approximate theoretical masses of BSA monomer, dimer, trimer, and tetramer for charge states 1 to 8. Matching colors indicate how overlapping  $m/z$  peaks with different charge states occur between multimers.

Charge	Monomer ( $m/z$ )	Dimer ( $m/z$ )	Trimer ( $m/z$ )	Tetramer ( $m/z$ )
1	66,435	132,869	199,303	265,737
2	33,218	66,435	99,651	132,869
3	22,146	44,290	66,435	88,580
4	16,610	33,218	49,827	66,435
5	13,288	26,575	39,861	53,148
6	11,073	22,146	33,218	44,290
7	9,492	18,982	28,473	37,963
8	8,305	16,610	24,914	33,218

much larger differences. These native conditions result in a shift to a higher  $m/z$  signal, indicative of lower charge states and multimeric species (see Figure 4A) compared to the denaturing conditions. The resulting spectra observed in Figures 2 to 4 resemble those obtained via other laboratories with similar instrumentation.<sup>2-4</sup>



**Figure 4.** Mass spectra  $m/z$  1,000 to 10,000 (A). Overall drift spectrum (B) and heat map (C) of drift time (ms) versus  $m/z$  for BSA in 150 mM ammonium acetate.

Inspection of the corresponding heat map confirms multimeric species, given the spread of drift times at each corresponding  $m/z$  and the resulting trendlines. The heat map has two interesting regions indicative of

higher-order multimers that are not present in the denaturing conditions (Figure 5). The overall drift spectra for the expanded view from  $m/z$  3,250 to 8,250 indicates multiple drift peaks starting to separate.

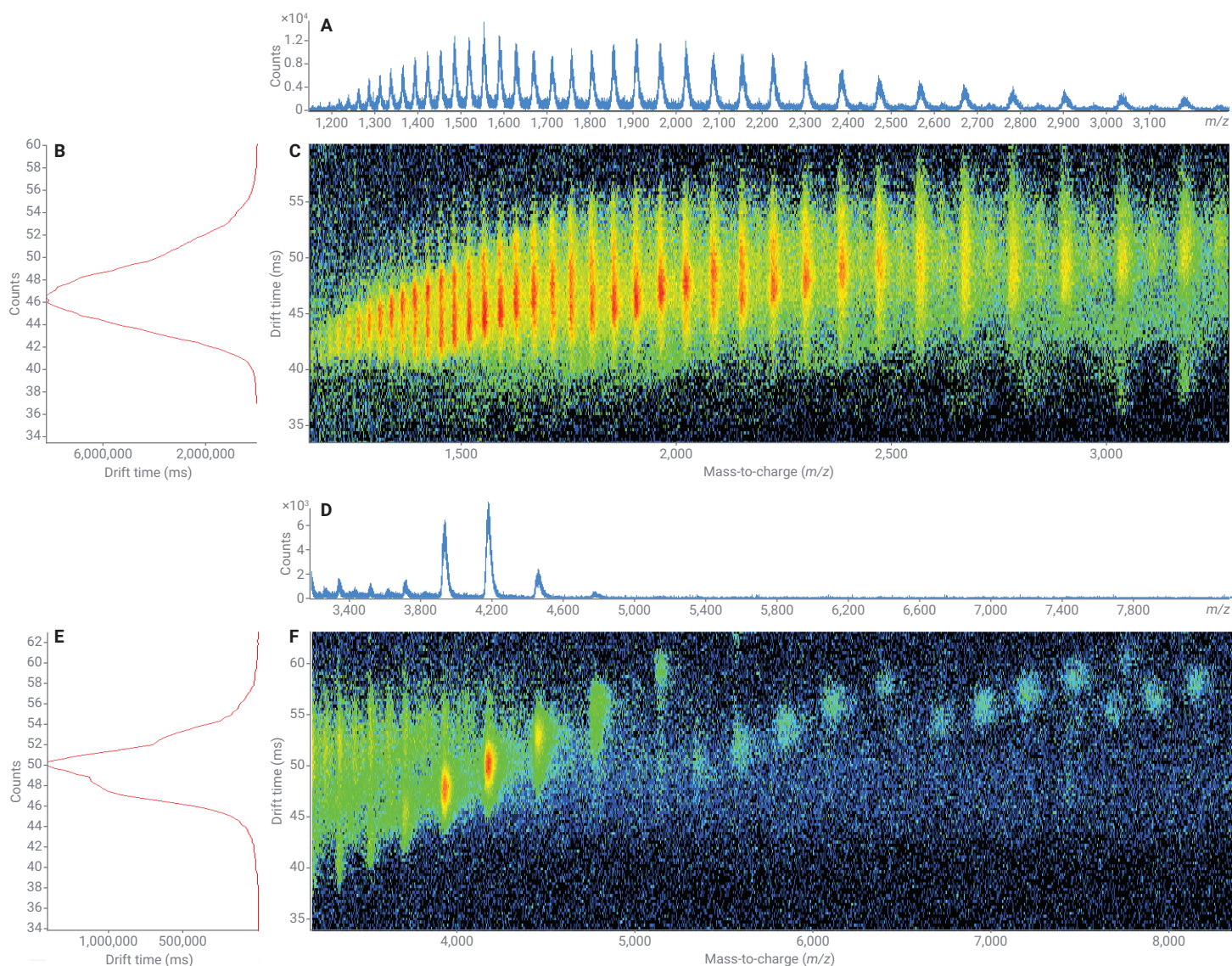


Figure 5. Expanded view of Figure 4 from  $m/z$  1,000 to 3,250 (A to C) and from  $m/z$  3,250 to 8,250 (D to F).

## Conclusion

The 150 mM ammonium acetate solution displayed the largest multimeric complexes compared to the other two solutions. As the solution conditions changed, the observed multimers decreased to predominantly monomer in the 50:50:0.1% water/acetonitrile/formic acid solution. Comparisons of the two-dimensional plots of drift time versus  $m/z$  give insight into how BSA monomers combine to form these multimeric species.

## References

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