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Automated sample introduction method for high-throughput intact native protein analysis using collision induced unfolding coupled with drift-tube ion mobility-mass spectrometry

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Introduction

Ion mobility-mass spectrometry has become a valuable analytical tool in native protein analysis. In protein structure studies, ion mobility spectrometry provides rotationally averaged collision cross-section values that correlates to size and shape of the biomolecule. For proteins, ground state CCS and accurate mass is not specific enough to identify different proteins. Therefore, introduction of gas phase unfolding followed by ion mobility measurements provide unique fingerprints for native protein analysis. This collision induced unfolding (CIU) technique can be utilized to identify proteins and protein complexes as well as to obtain a qualitative description of the structural changes.[1,2,3] Typical CIU experiments utilize static nano-ESI or standard ESI using a syringe pump for sample introduction, however both methods are either difficult or impossible to automate. In this study, we have developed a new automated sample introduction method for high-throughput CIU experiments.

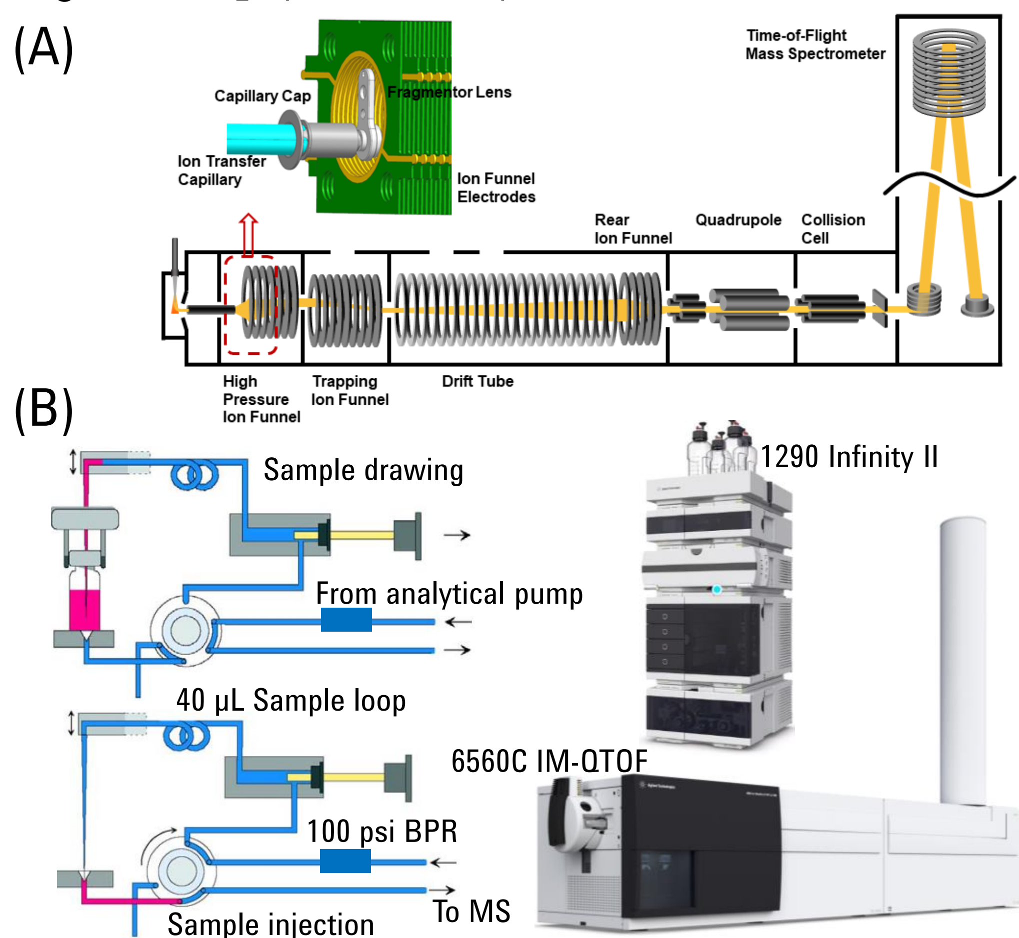
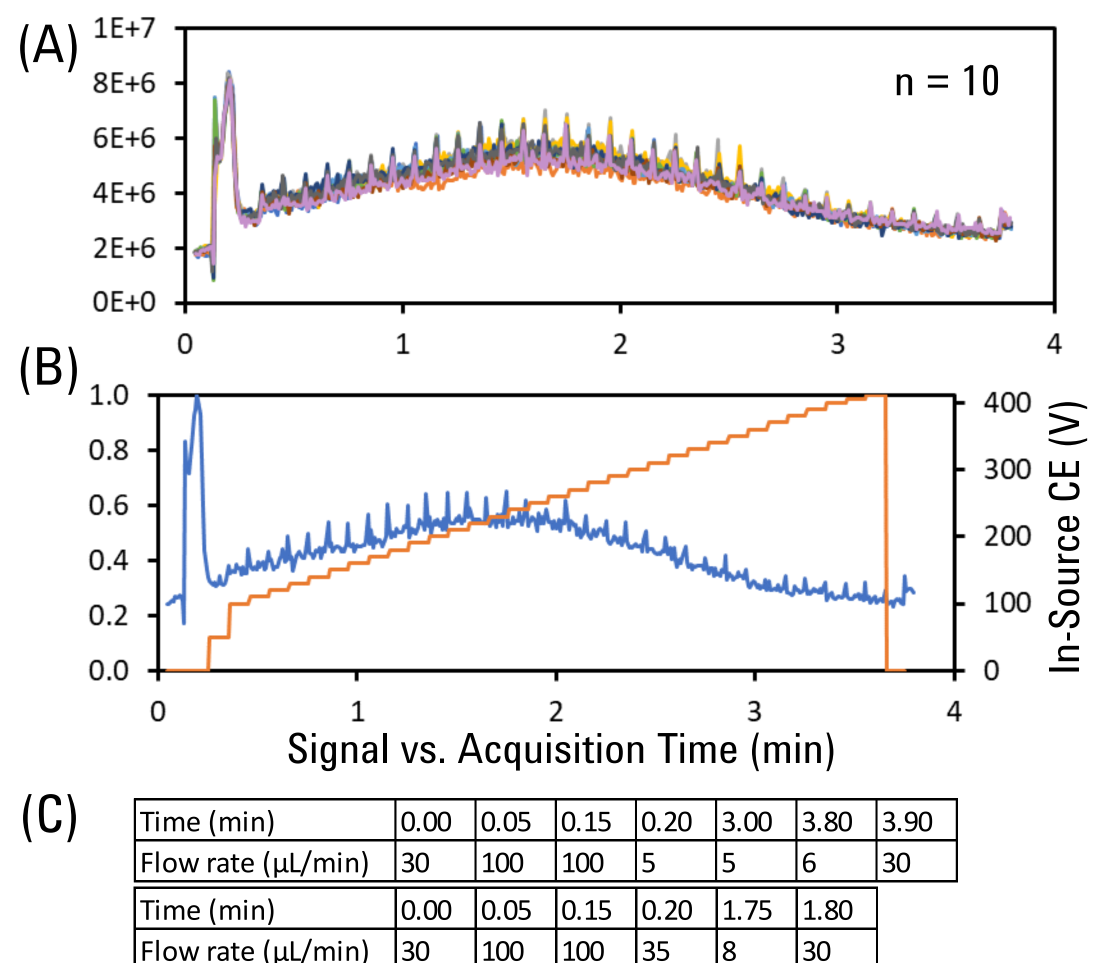


Figure 1: Schematic diagram of Agilent 6560C IM-QTOF instrument with In-Source ion activation hardware (A) and schematic diagram of the flow injection method with images of 1290 Infinity II LC system and 6560C IM-QTOF instrument (B).

Experimental

BSA and HSA proteins were dissolved in 100 mM ammonium acetate buffer and mAb proteins were dissolved in 200 mM ammonium acetate buffer at 1 µg/µL protein concentration prior to desalting using BioRad Bio-spin (P-6) columns. CIU experiments were carried out using an Agilent 6560C IM-QTOF instrument with the new In-Source ion activation hardware. Agilent 1290 Infinity II series LC system was updated with a 40 µL metering device and a 40 µL sample loop. A 100 psi back pressure regulator (IDEX Health and Sciences LLC, Oak Harbor, WA) was installed between the pump and the switching valve for flow injection method. 1.5 to 15 µL sample volumes were used with flow gradients in the range of 5 to 35 µL/min and 5 mM ammonium acetate in DI water as mobile phase. A time segment method was used for CIU experiments and CIUSuite software[3] was used for data analysis.



Time (min)	0.00	0.05	0.15	0.20	3.00	3.80	3.90
Flow rate (µL/min)	30	100	100	5	5	6	30

Time (min)	0.00	0.05	0.15	0.20	1.75	1.80
Flow rate (µL/min)	30	100	100	35	8	30

Figure 2. TIC plots for replicate HSA CIU experiments (A) showing the repeatability of the flow injection method and TIC plot overlapped with In-Source CE voltage ramp for CIU experiments (B). Flow injection methods used for 3.8-minute (BSA/HSA) and 1.75-minute (IgG) CIU experiments (C).

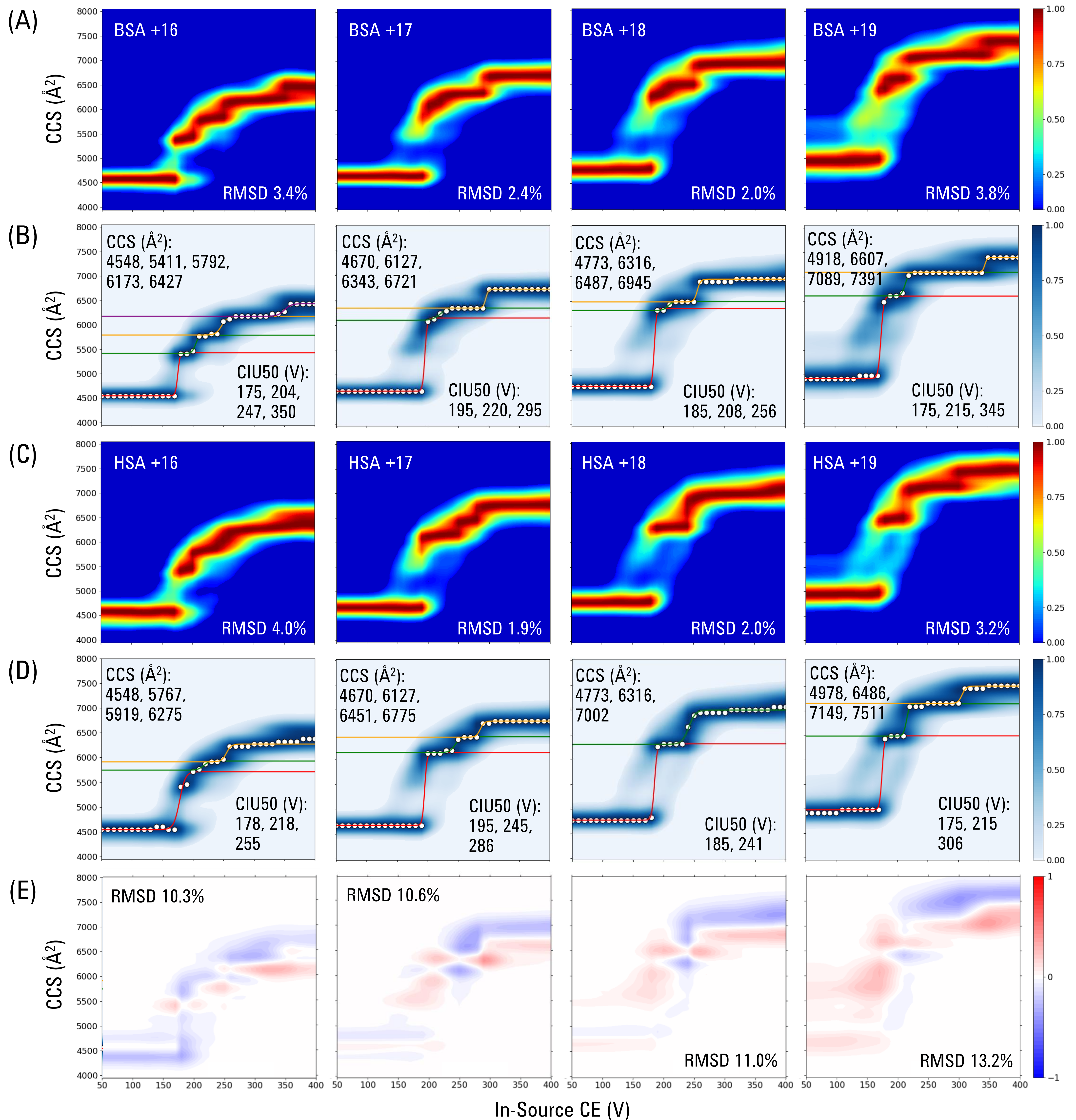
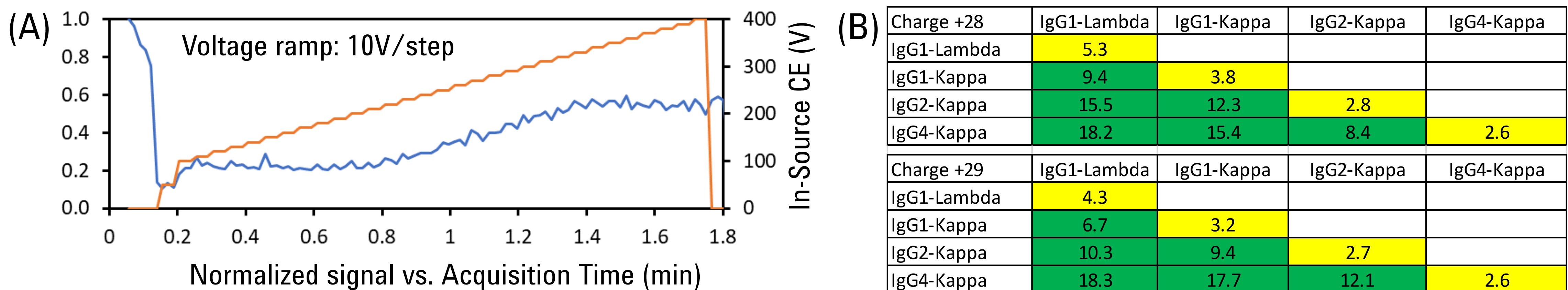


Figure 3: BSA (A,B) and HSA (C,D) CIU fingerprints and comparison plots (E) for charge states +16 to +19. Data shown for the 3.8-minute experiment with 4 µL sample injection. In-source CE (V) ramped from 0 V to 400 V. Data acquisition time for each time segment/voltage step was 6 seconds. For each sample, 10 experiments were performed. Average RMSD values for all replicate runs are reported on the CIU plots. Data acquisition rate for these CIU experiments were 2 Hz. A one-minute-long blank run (15 µL DI water) was performed after each sample run to clean the LC system and prevent accumulation of ammonium acetate salt used for sample preparation.

Results and Discussion



Charge +28	IgG1-Lambda	IgG1-Kappa	IgG2-Kappa	IgG4-Kappa
IgG1-Lambda	5.3			
IgG1-Kappa	9.4	3.8		
IgG2-Kappa	15.5	12.3	2.8	
IgG4-Kappa	18.2	15.4	8.4	2.6

Charge +29	IgG1-Lambda	IgG1-Kappa	IgG2-Kappa	IgG4-Kappa
IgG1-Lambda	4.3			
IgG1-Kappa	6.7	3.2		
IgG2-Kappa	10.3	9.4	2.7	
IgG4-Kappa	18.3	17.7	12.1	2.6

Figure 4: TIC plot for IgG1-κ (15 µL sample) CIU experiment with In-Source CE ramp overlay (A) and table of RMSD values for IgG CIU experiments (B). Data acquisition time for each time segment/voltage step was 3 seconds.

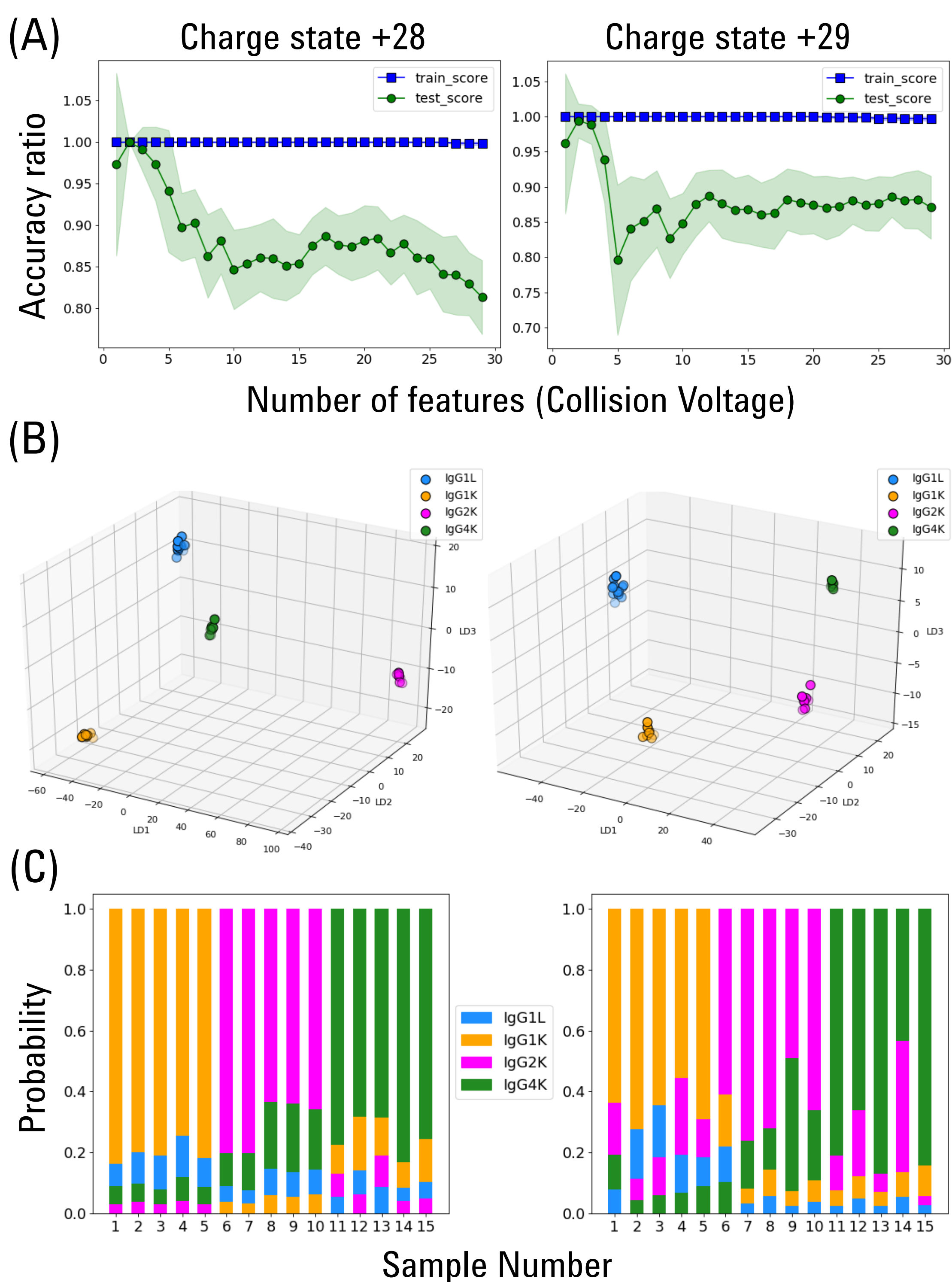


Figure 5. The use of classification function in CIUSuite 2 (version 2.3) software to identify IgG variants. For this analysis, IgG1-λ, IgG1-κ, IgG2-κ and IgG4-κ proteins were used as the training set. Six sample runs were used for IgG1-λ and four sample runs were used for other proteins. The classification analysis was done for charge state +28 and +29 separately. Figure 5(A) shows the accuracy ratio for each feature and (B) shows the LD plots for the four samples. Figure 5(C) shows the identification of IgG1-κ (run1-5), IgG2-κ (run6-10) and IgG4-κ (run11-15) proteins.

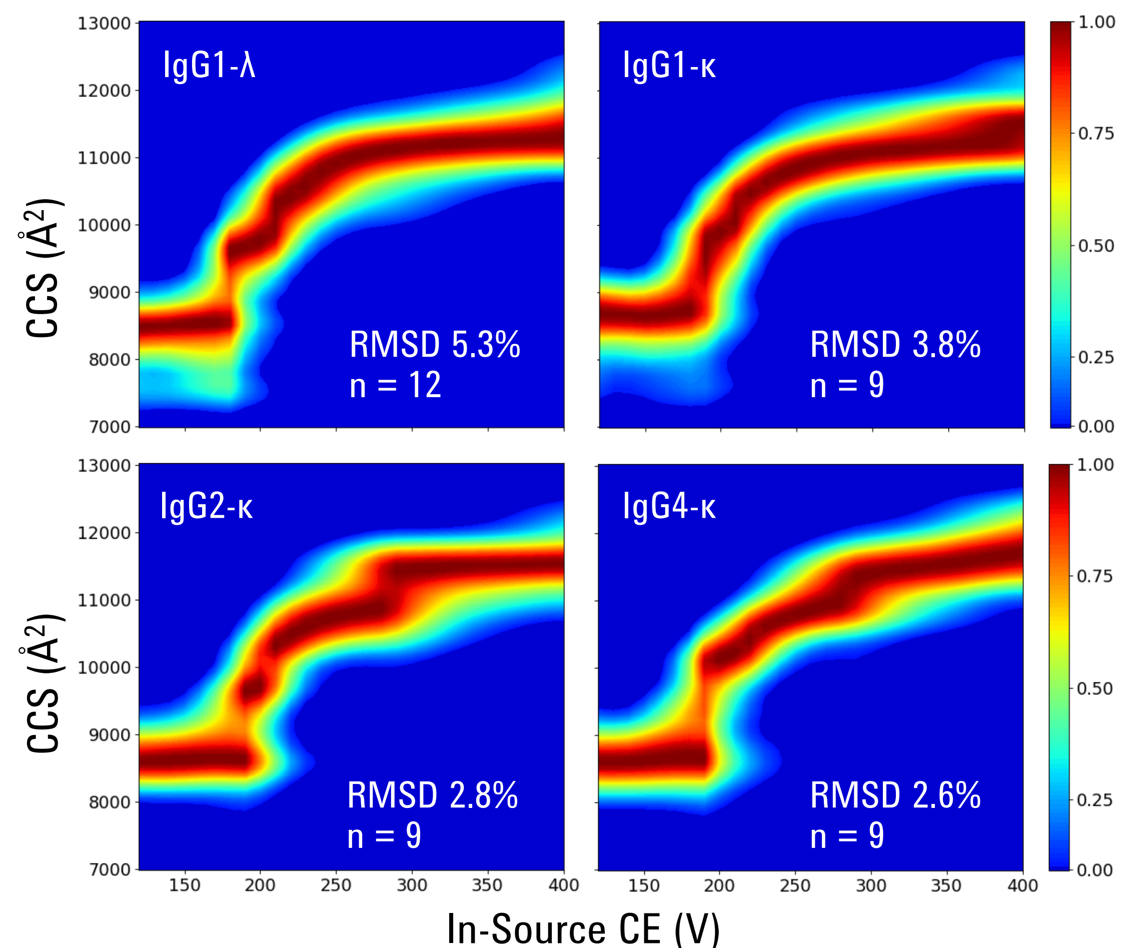


Figure 6. CIU fingerprints for IgG protein experiments (charge state +28). Average RMSD values for replicate runs are given on the CIU plots.

Conclusions

- Agilent 6560C IM-QTOF instrument allows CIU experiments of large native proteins and protein complexes under nitrogen drying gas conditions.
- The flow injection method developed in this study allows rapid and automated CIU experiments using micro-gram quantities of protein samples. AJS source with micro-nebulizer used for ESI.

References

[1]Vallejo, D. D. et al., *Anal. Chem*. 2019, 91, 8137-8146

[2]Gadkari, V. V. et al., *Anal. Chem*. 2020, 92, 15489-15496

[3]Eschweiler, J. D. et al., *Anal. Chem*. 2015, 87, 22, 11516-11522

https://sites.lsa.umich.edu/ruotolo/software/ciusuite-2/

https://explore.agilent.com/asms

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