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A modified SLIM-IM-QTOF for high resolution collision induced unfolding and native protein analysis

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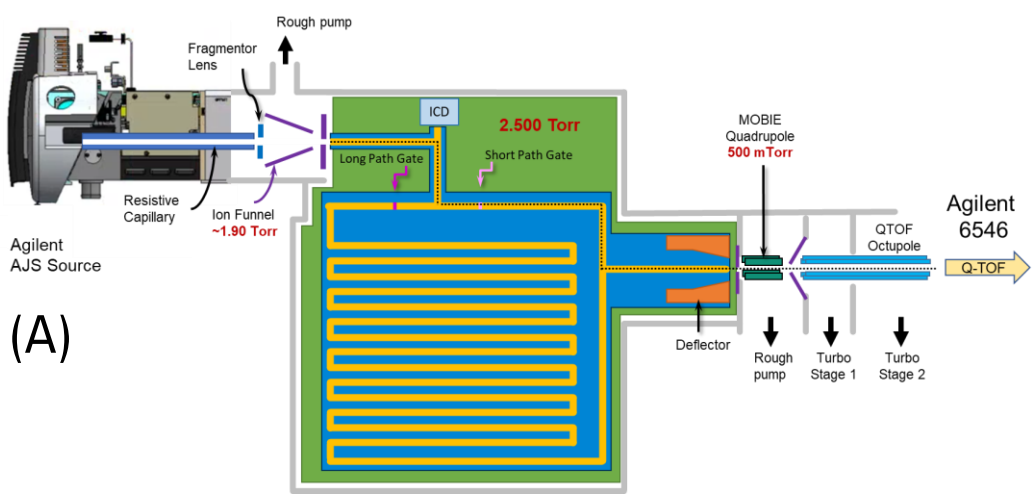
Introduction

Ion mobility-mass spectrometry techniques can be used to perform structural characterization of intact proteins. Coupling collision induced unfolding (CIU) to IM-MS techniques allows for screening of antibodies and antibody variants. This technique also provides structure stability information for protein complexes and proteins bound with ligands. This study employs an ion mobility instrument based on structures for lossless ion manipulation (SLIM) technology. MOBIE® 1.0 HRIM-QTOF (MOBILion Systems) is a high-resolution ion mobility instrument which allows very high-resolution ion mobility separation on a 13-meter path length SLIM device. We have modified this instrument with in-source ion activation hardware, placed before the SLIM device to allow for high-resolution CIU experiments. BSA, HSA, IgG, Bispecific and Trispecific antibody proteins were used to characterize this modified instrument.



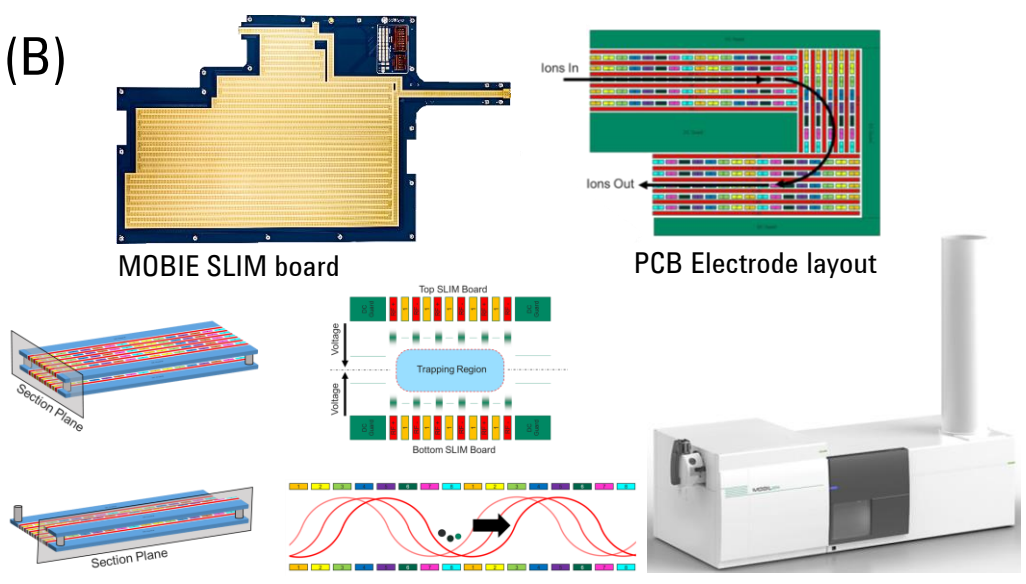
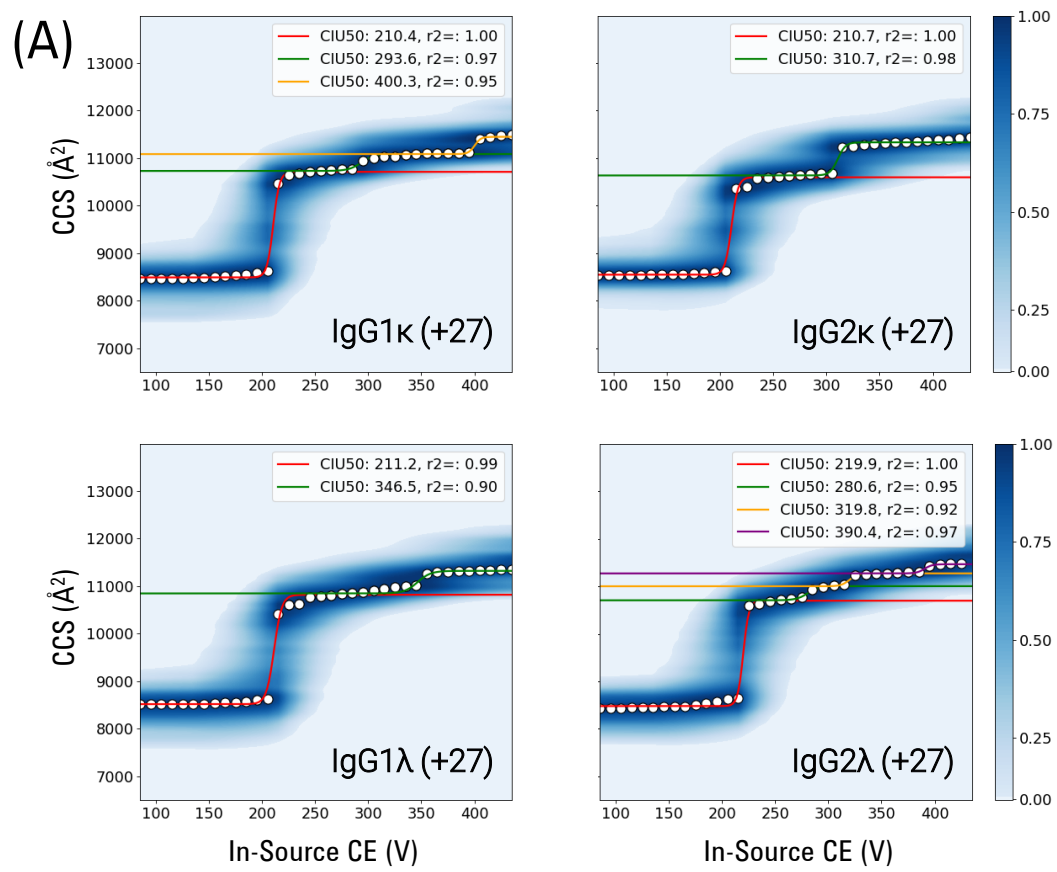


Figure 1: (A) Schematic diagram of HRIM-QTOF instrument with In-Source ion activation hardware and (B) SLIM technology and mechanism of ion transmission.

Experimental

A commercially available MOBIE instrument was modified with in-source ion activation hardware (Figure 1A). CIU experiments were carried out using an AJS source with a micro-nebulizer spray and syringe pump for sample delivery. Intact proteins were dissolved in DI water and buffer exchanged into 200 mM ammonium acetate using Bio-Rad Bio-spin P-6 columns. Sample concentrations were ~ 5-10 µM. A CIU fingerprint for each sample was obtained by ramping in-source CE voltage from 0 V to 435 V. All experiments were run in triplicate. CIUSuite 2 software was used for data analysis.



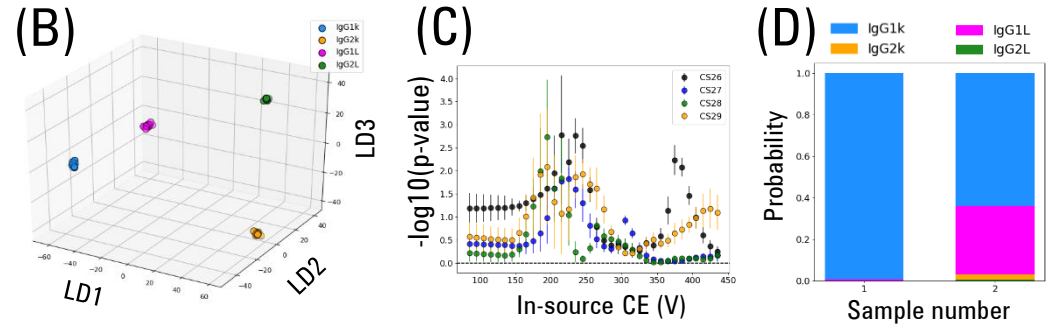


Figure 2: (A) CIU fingerprints for charge state +27 ion with CIU50 values for IgG1κ, IgG2κ, IgG1λ and IgG2λ antibodies from Sigma-Aldrich. (B and C) Linear discriminant (LD) analysis for the four antibodies using CIU fingerprints for charge states +26, +27, +28 and +29. (D) Accurate identification of Herceptin (Sample#1) and NIST mAb (Sample#2) as IgG1κ using this classification system. CIU fingerprint cross comparison RMSD values for all antibodies studied here are shown in Table 1.

Results and Discussion

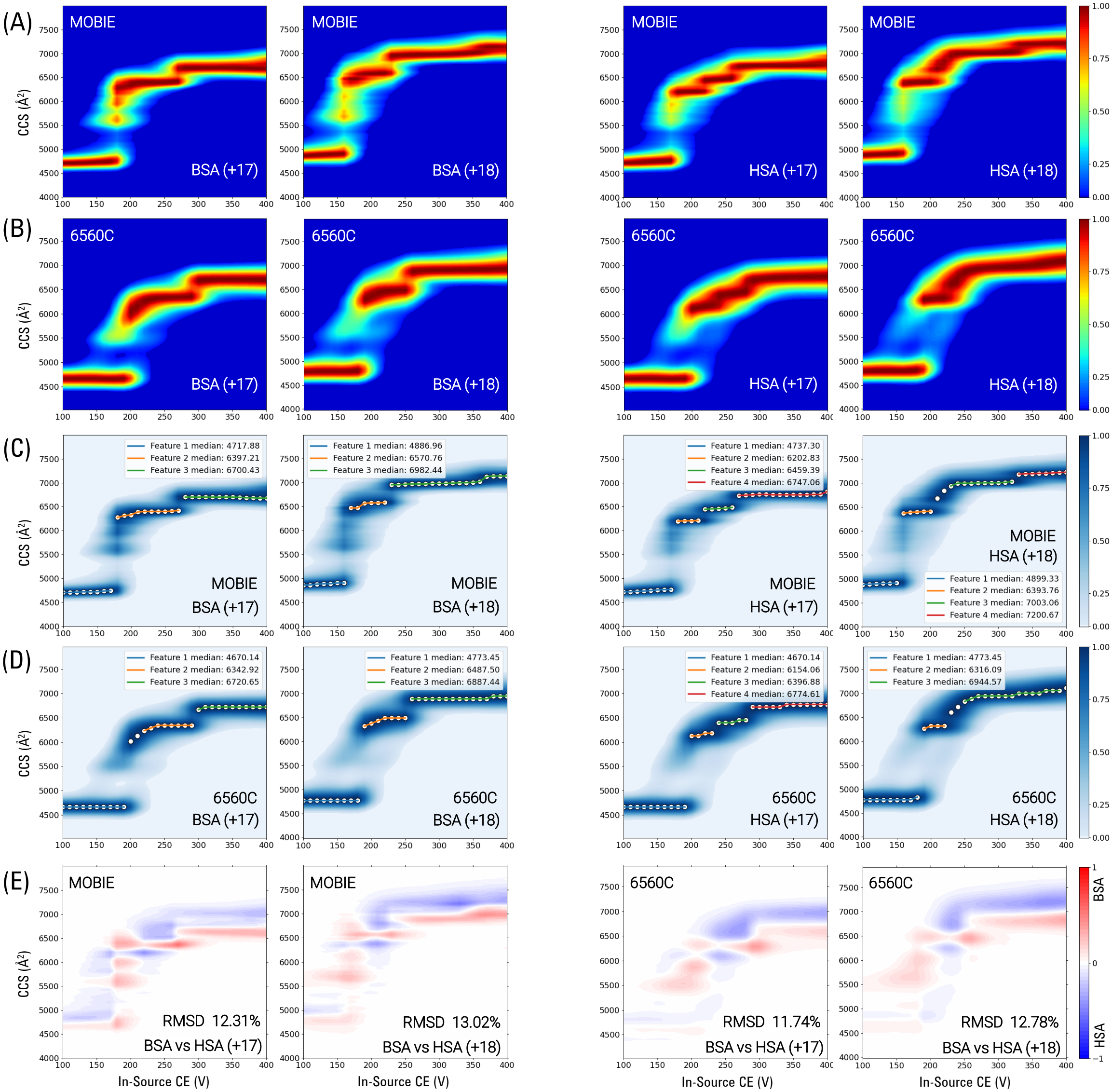


Figure 3: (A) CIU fingerprints for BSA and HSA charge states +17 and +18 for modified HRIM-QTOF and (B) Agilent 6560 IM-QTOF. (C) Feature detection for BSA and HSA charge states +17 and +18 for modified HRIM-QTOF and (D) Agilent 6560 IM-QTOF. CIU fingerprint comparison for BSA versus HSA charge states +17 and +18 for modified HRIM-QTOF (E-left) and Agilent 6560 IM-QTOF (E-right). Both instruments provided similar results indicating the reproducibility of the CIU experiments. For some CIU fingerprints, HRIM-QTOF data showed more defined transitions (HSA +17) and additional features (HSA +18).

Results and Discussion

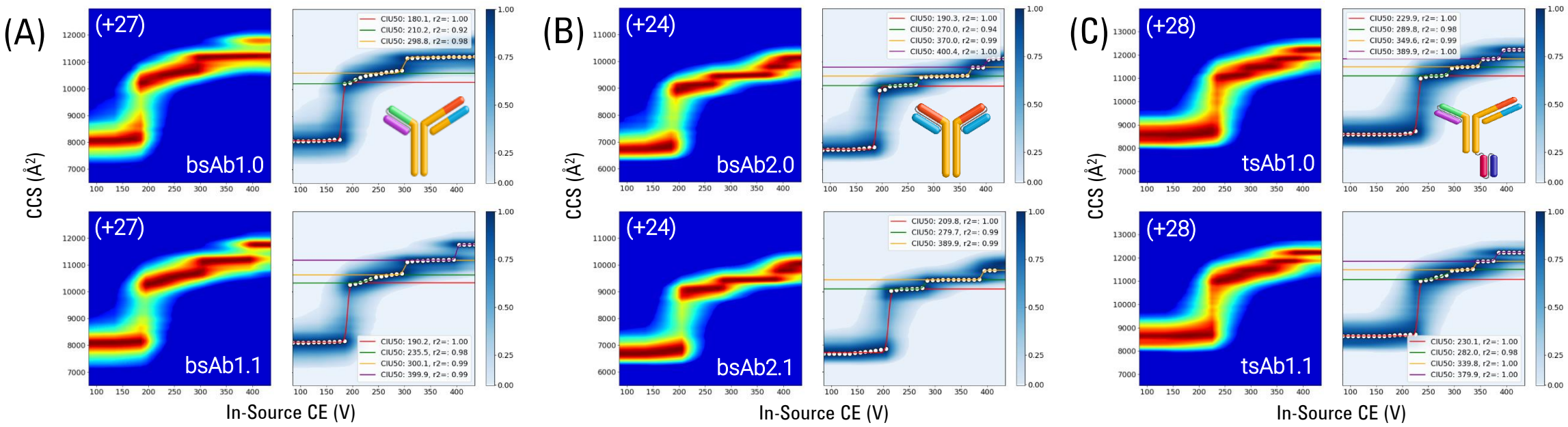


Figure 4: CIU fingerprint comparison for (A and B) two pairs of bispecific antibodies and (C) one pair of trispecific antibody. The two bsAbs and tsAb were designed by paring commercially available RSV, Her2 and TNFα binders by Johnson and Johnson. The antibodies denoted as bsAbx.1/tsAbx.1 have sequence modifications to prevent enzymatic cleavage at the hinge region. Cross comparison RMSD values for bsAb1 and tsAb1 are 7.85% (triplicate RMSD 3.28%) and 8.76% (triplicate RMSD 4.15%) respectively, indicating slight structural differences within the pairs. However, for the bsAb2 pair, the RMSD value is 14.34% (triplicate RMSD 3.56%) indicating significant structural and stability differences.

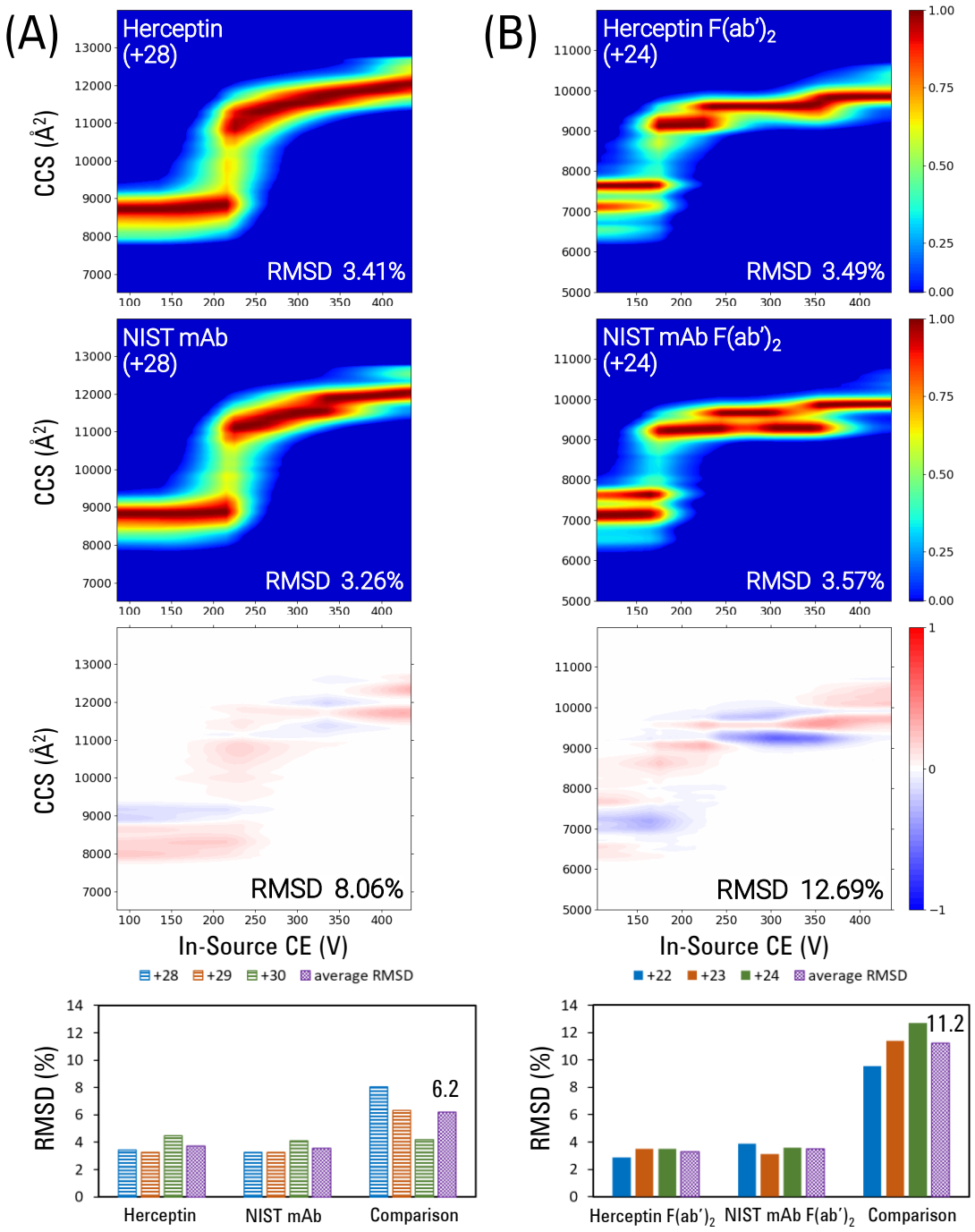


Figure 5. Herceptin and NIST mAb CIU fingerprint data with IdeS digestion. (A) CIU fingerprints for intact Herceptin and NIST mAb are similar and cannot be used to differentiate these two antibodies. (B) In contrast, $F(ab')_2$ fragment CIU fingerprints show significant differences for these two antibodies.

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Table 1: CIU fingerprint comparison RMSD% values

Charge +26	IgG1-Kappa	IgG2-Kappa	IgG1-Lambda	IgG2-Lambda
IgG1-Kappa	3.6			
IgG2-Kappa	16.8	3.1		
IgG1-Lambda	5.8	19.1	3.6	
IgG2-Lambda	10.7	17.1	10.8	4.3

Charge +27	IgG1-Kappa	IgG2-Kappa	IgG1-Lambda	IgG2-Lambda
IgG1-Kappa	3.3			
IgG2-Kappa	11.8	2.8		
IgG1-Lambda	6.1	12.6	4.0	
IgG2-Lambda	10.8	13.6	8.9	4.2

Low RMSD values for triplicate runs (2.8% to 4.3% for charge states +26 and +27) indicate the reproducibility of these CIU experiments. Cross comparison RMSD values in the range of 5.8% to 19.1% indicate some of these antibodies can be screened based on CIU fingerprints.

Conclusions

- Commercially available HRIM-QTOF (MOBIE® 1.0, MOBILion Systems) instrument was modified with in-source ion activation hardware (same hardware available in Agilent 6560C IM-QTOF)
- This study demonstrates the successful unfolding of intact BSA/HSA proteins and antibody proteins under native conditions
- Qualitatively, both Agilent 6560C and modified HRIM-QTOF instrument gave similar CIU data and cross comparison RMSD values.

References

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