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Rapid Screening of Bispecific Antibodies and Antibody Impurities using In-source Collision Induced Unfolding coupled with IM-MS

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Introduction

The detection and quantitation of antibody impurities is typically performed by size-exclusion chromatography. However, this technique requires long run times and often has poor resolution. Ion mobility-mass spectrometry combined with Collision induced unfolding (CIU) technique can provide rapid identification of proteins and protein impurities. In this study, we have investigated the use of CIU and IM-MS for screening bispecific antibody samples.

Bispecific antibody samples bsAb1, bsAb2 and antibody counterparts with engineered cysteine: bsAb1.2, bsAb2.2, bsAb3.2, bsAb4.2 and SEC enriched dimers of bsAb1.2, bsAb3.2, and bsAb4.2 are tool molecules derived from culture supernatants from Janssen. Monomer molecular weight ~127 KDa. Herceptin (IgG1-κ) and NIST mAb (IgG1-κ) samples were used for initial method development (~148 KDa). IdeS enzyme was used to obtain $F(ab')_2$ fragments of standard proteins.

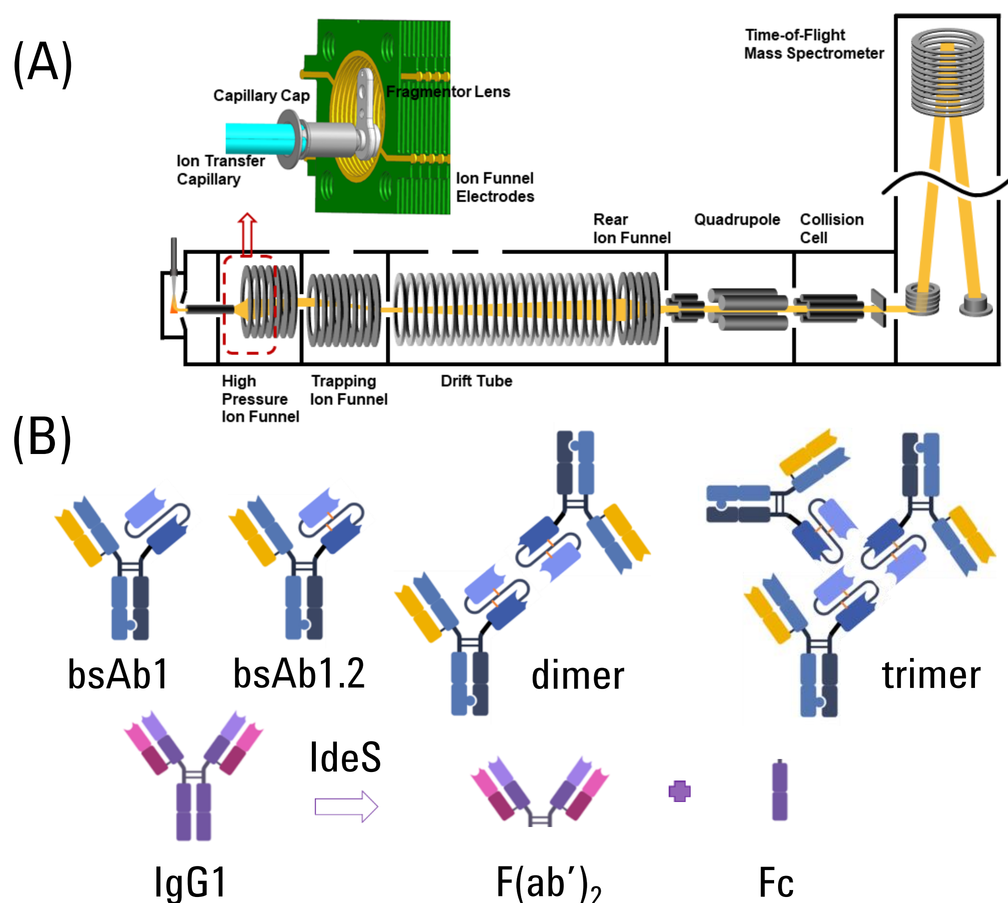

Figure 1: Schematic diagram of Agilent 6560C IM-QTOF instrument with In-Source ion activation hardware (A) and schematic renders of different types of antibodies used (B). bsAbx.2 version of the antibodies contain engineered disulfide bonds in Fv domain.

Experimental/Results

Agilent 6560C IM-QTOF instrument with in-source ion activation capability was used for these experiments. Initial tests were carried out using AJS source with micro-nebulizer spray and syringe pump for sample delivery. Intact proteins were dissolved in PBS buffer and buffer exchanged into 200 mM ammonium acetate and desalted using Bio-Rad Bio-spin P-6 columns. Sample concentrations were ~ 4-10 μM. CIU fingerprint for each sample was obtained by ramping in-source CE voltage from 0 V to 430 V in 10 V increments. A time segment method with 12 seconds for each voltage step was used for CIU experiments and CIUSuite software was used for data analysis.

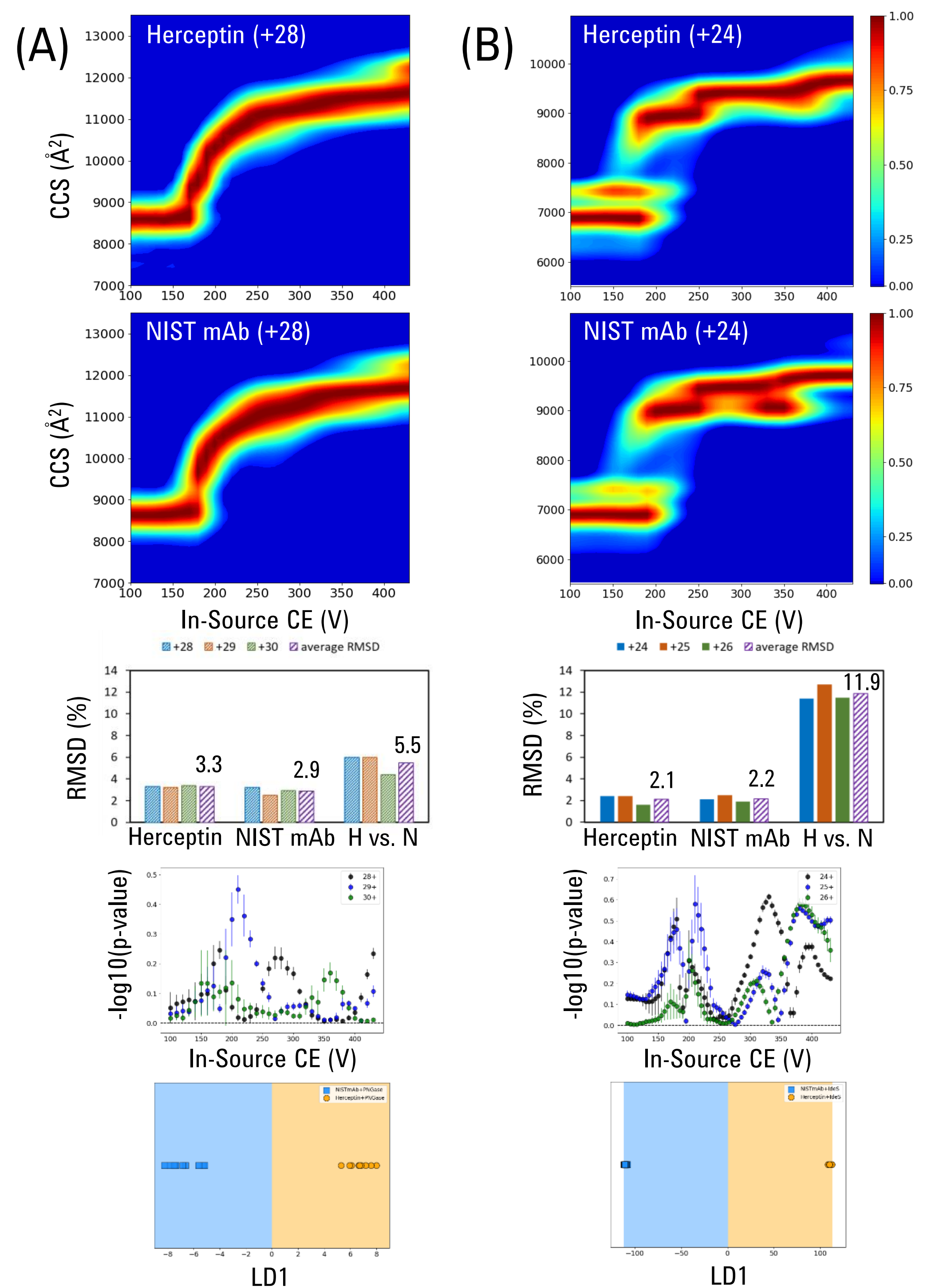

Figure 2: Herceptin and NIST mAb sample comparison with PNGase (A) and IdeS (B) digestion. IdeS digestion allowed better identification.

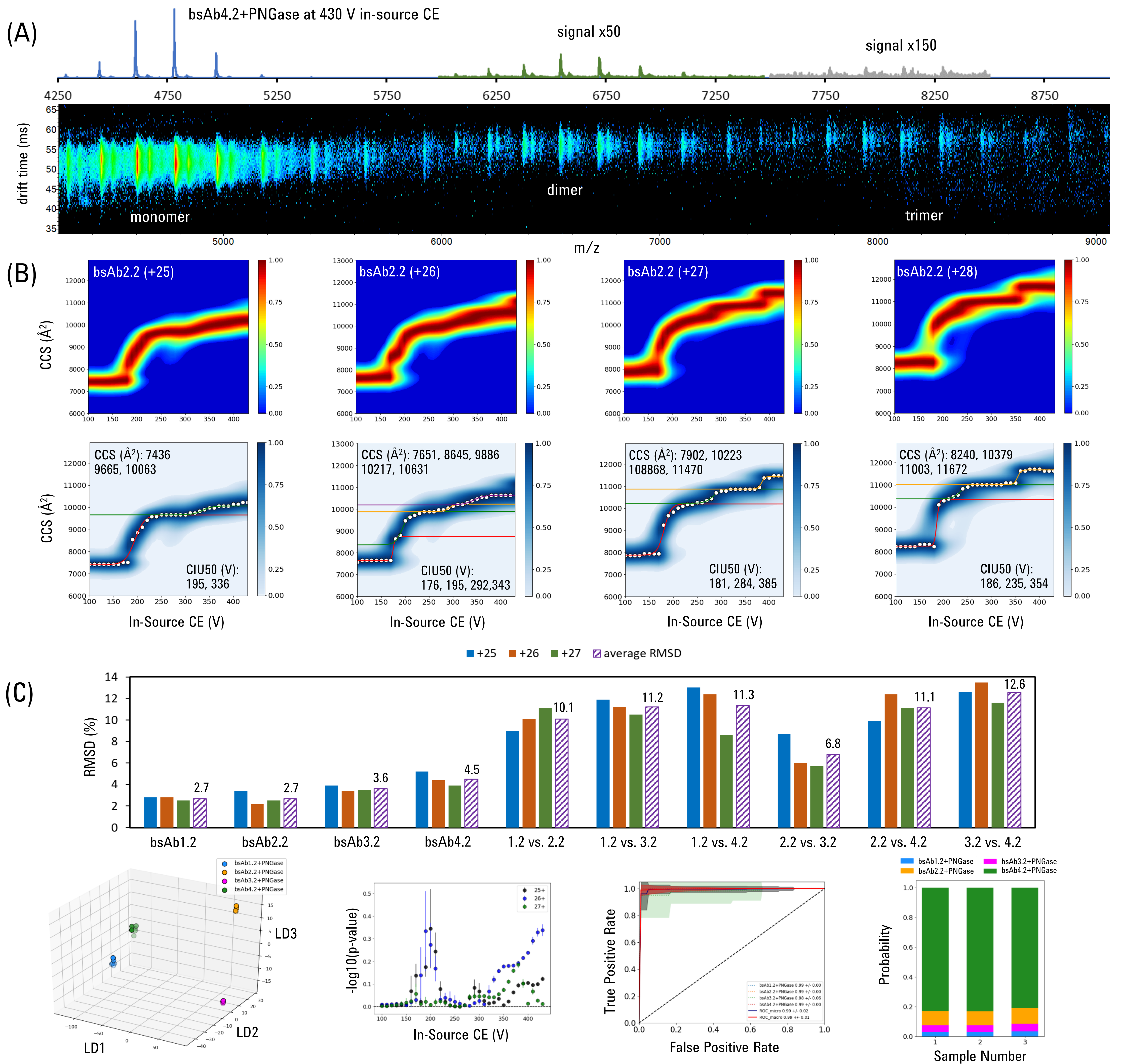

Figure 3: Mass spectrum and 2D heat map for bsAb4.2 at 430 V in-source CE (A). CIU fingerprints for bsAb2.2 charge states +25, +26 and +27 (B). RMSD comparison and protein classification based on CIU fingerprints (C). Average RMSD for charge states +25, +26 and +27 for triplicate runs were 2.7%, 2,7%, 3.6% and 4.5% for bsAb1.2, bsAb2.2, bsAb3.2 and bsAb4.2 respectively. Cross comparison RMSD vales were in the range of 10.1% to 12.6% indicating considerable structural differences. However, for bsAb2.2 and bsAb3.2, cross comparison RMSD is 6.8% indicating relatively similar structures for those two proteins. Linear discriminant (LD) analysis for these four proteins indicated that they can be identified using CIU fingerprints. For LD analysis, charge state +25, +26 and +27 CIU curves were used. Probability vs. sample number plot shows the correct identification of bsAb4.2 sample using classification function in CIUSuite2 software.

Results and Discussion

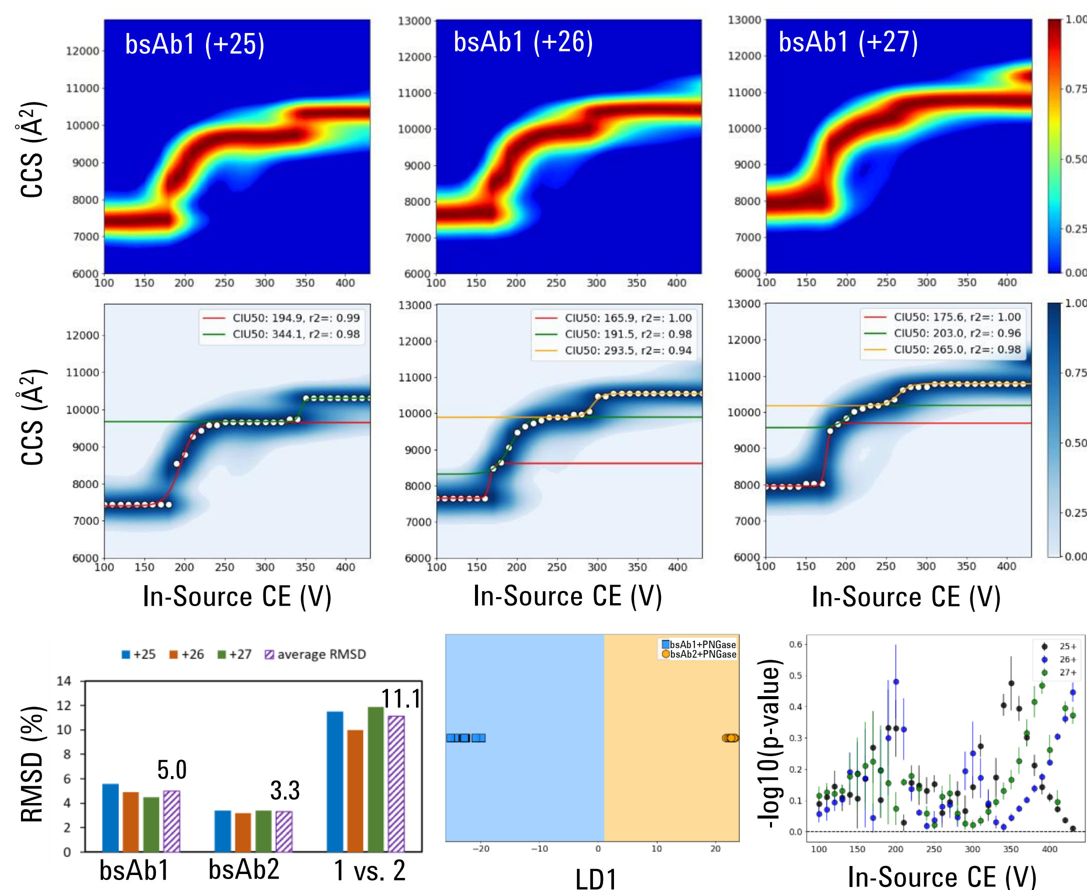

Figure 4. bsAb1 and bsAb2 sample comparison. Average RMSD value for charge states +25, +26 and +27 is 11.1% indicating these two antibodies have relatively different structures.

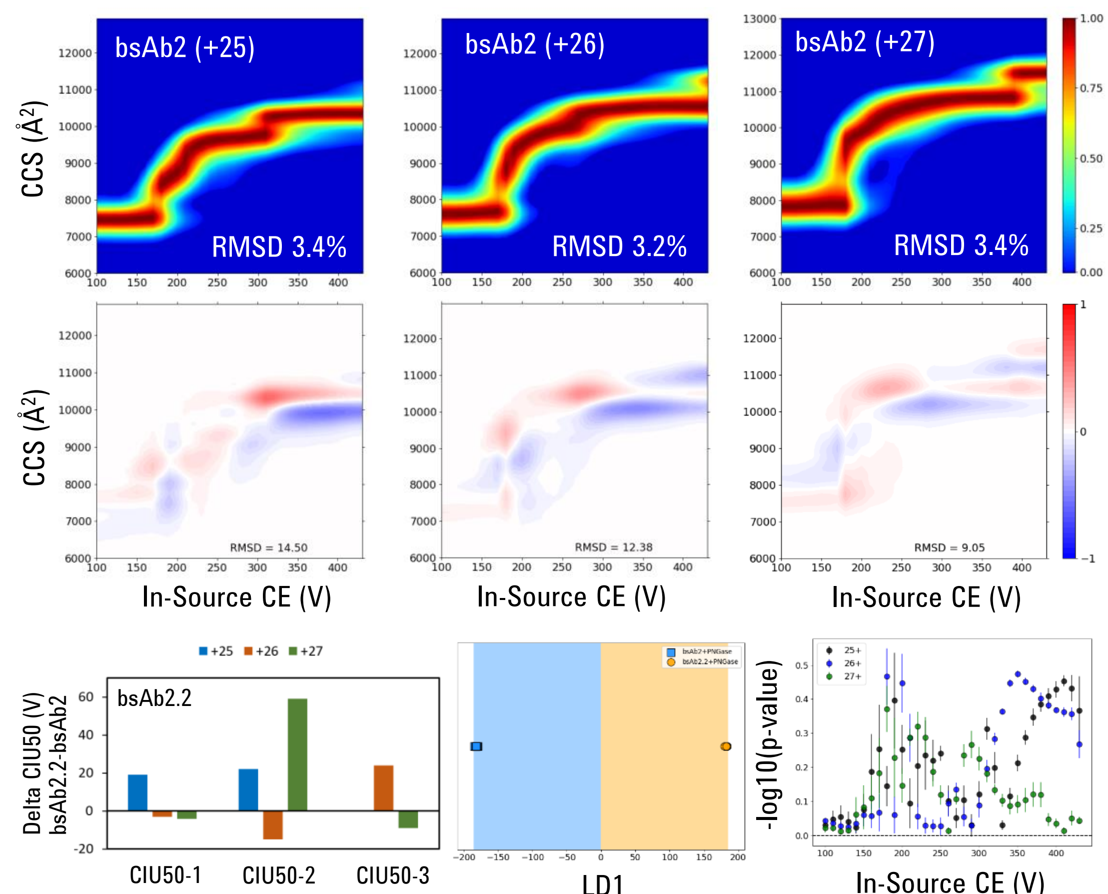

Figure 5. bsAb2 and bsAb2.2 comparison. These two antibodies have same primary sequence, however, bsAb2.2 has engineered disulfide bonds in the Fv domain. Based on CIU fingerprint comparisons and CIU50 values, bsAb2.2 has a relatively more stable structure.

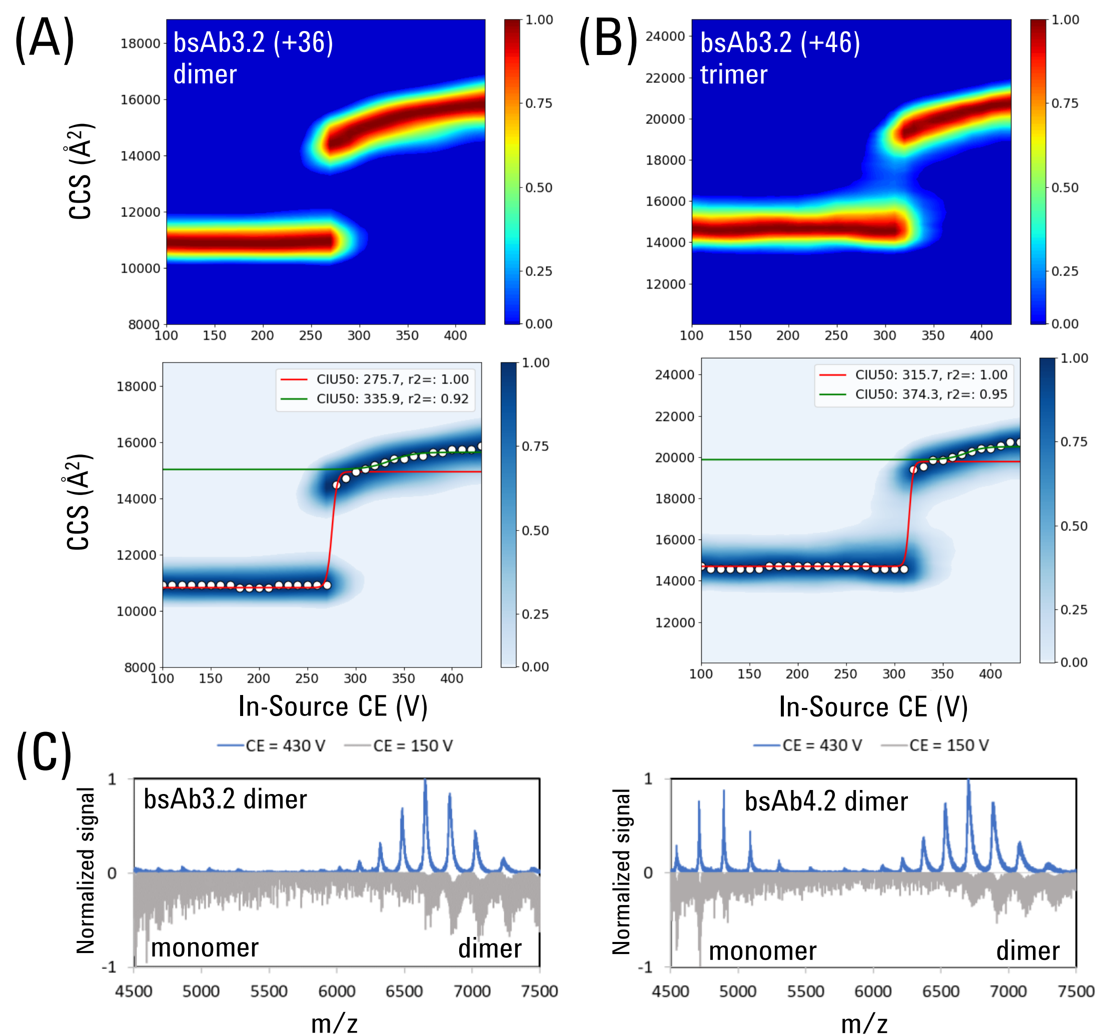

Figure 6. bsAb3.2-dimer (A) and bsAb3.2-trimer (B) CIU data. Mass spectra for dimer samples (C) at low in-source CE (150 V) and high in-source CE (430 V), showing the dissociation of non-covalently bound dimer into monomer. Based on dimer dissociation at higher in-source CE voltages, the bsAb3.2 sample has more covalently bound dimers.

	First feature CCS (Å²)			CIU50 (V)		
	charge +36	charge +37	charge +38	charge +36	charge +37	charge +38
bsAb1.2-dimer	10821	11122	11301	275	256	247
bsAb3.2-dimer	10821	11122	11180	276	256	236
bsAb4.2-dimer	10821	11004	11301	277	265	246

Collision cross section and CIU50 value comparison for dimers. The CCS and CIU50 values for dimerized proteins are very similar and CIU fingerprints are almost identical.

Conclusions

- This study demonstrates the use of Agilent 6560 IM-QTOF instrument for characterizing bispecific antibody samples and identifying impurities.
- A set of bsAbx.2 (with engineered disulfide bonds) antibodies were screened, and a classification method was developed using charge states +25, +26 and +27. This classification scheme was successfully used to identify a known sample (bsAb4.2) as a test evaluation.
- Noncovalent dimers can be dissociated to monomers at high in-source CE voltages.

References

[1]Boucher, L. E. et al., *MABS* 2023, 15, 1, 2195517

[2]Gadkari, V. V. et al., *Analyst* 2023, 148(2), 391-401

[3]Kurulugama, R. T. et al., *ASMS* 2022, TP 298

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