Ion Mobility-Mass Spectrometry and Collision Induced Unfolding of Bispecific Antibody Therapeutics

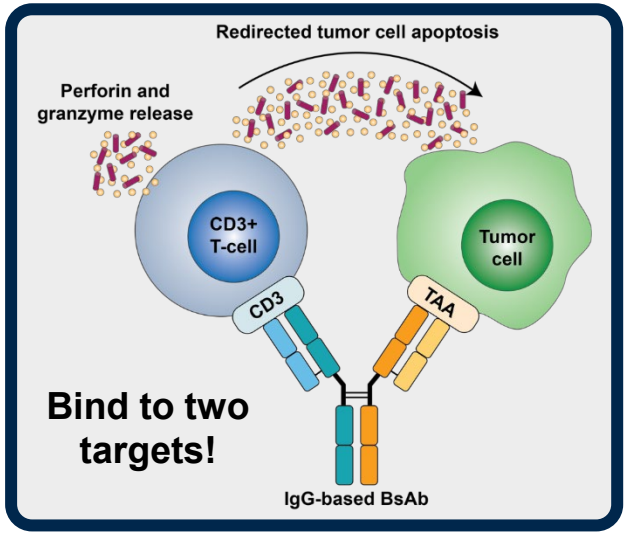
Rosendo C. Villafuerte-Vega[1], Henry Li[1], Thomas Slaney[2], Naresh Chennamsetty[2], Guodong Chen[2], Li Tao[2], and Brandon T. Ruotolo[1]

[1]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109

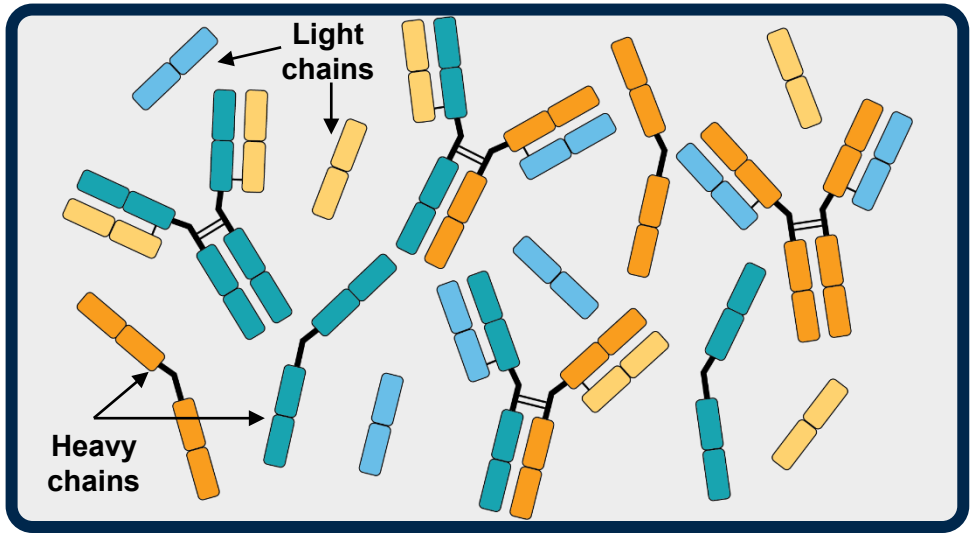
[2]Bristol Myers Squibb, New Brunswick, NJ 0890

Higher Order Structure: Next Generation Investigators
April 5, 2022

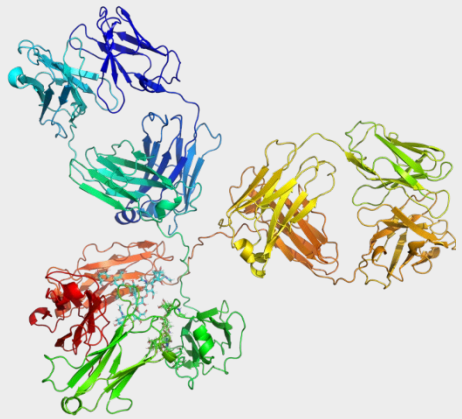
Bispecific Antibodies (BsAbs): Impact and Challenge



- Proper heterodimerization important for **specificity and functionality**
- Different chain combinations leads to **chain association issue**



- Antibody (mAb) therapeutics are **structurally complex** and **highly dynamic**
- Susceptible to **PTMs** and **degradation products**

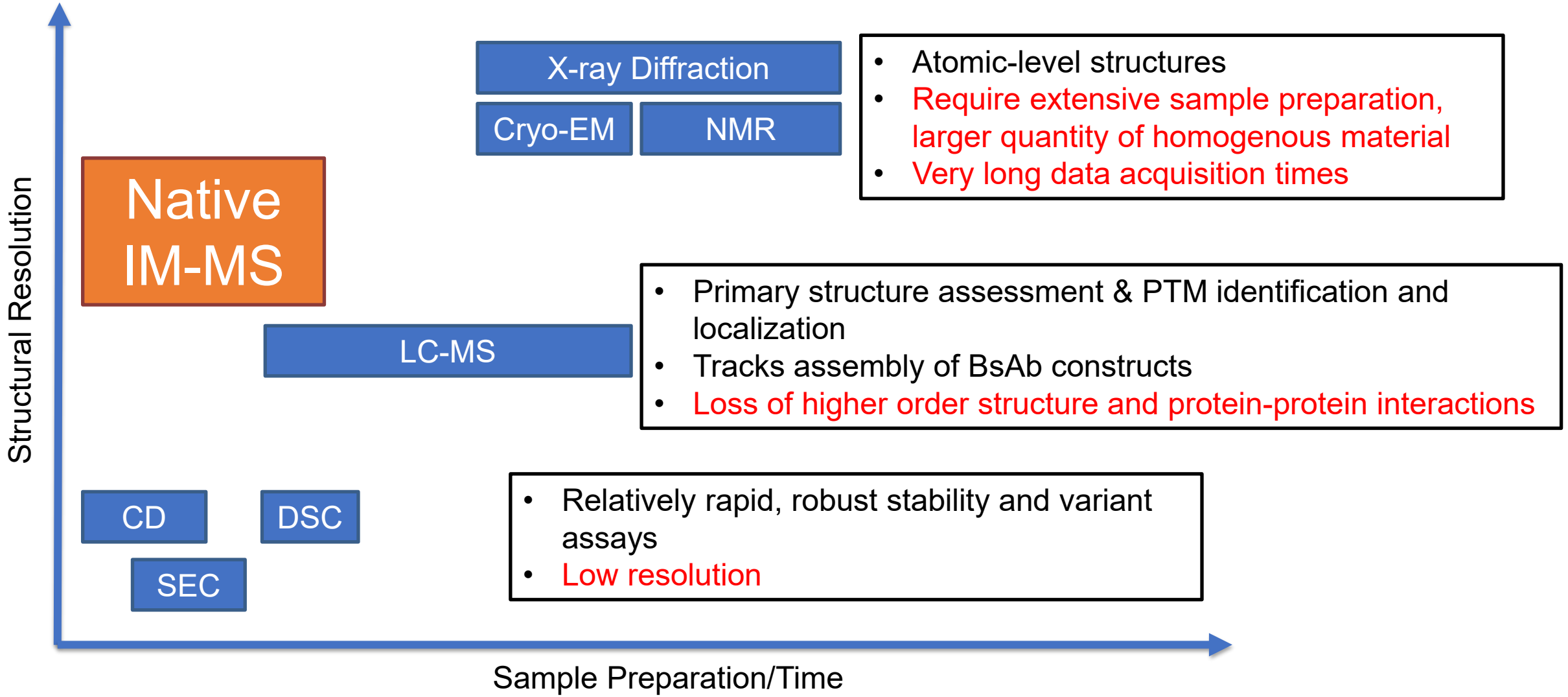


~ 150 kDa, PDB 1IGY

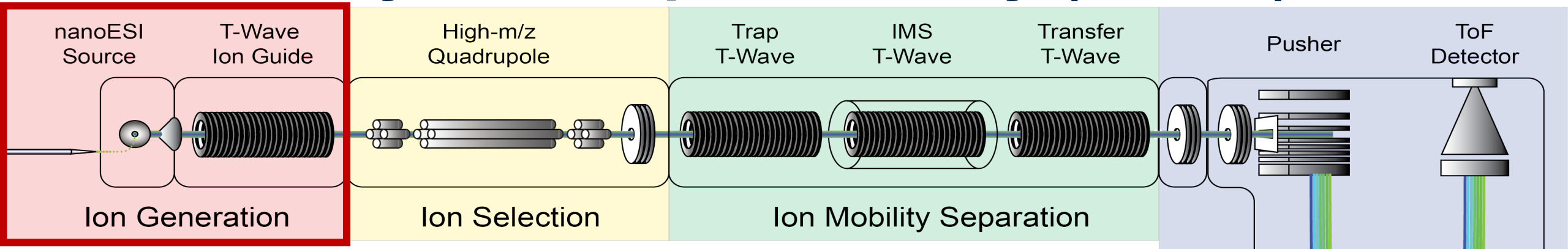
- Increase in **heterogeneity** and impact **higher order structure (HOS)**
- Small structural changes impact product **safety**, **functionality**, and **efficacy**
- Comprehensive characterization of HOS needed for **candidate assessment and selection**

Klein, C. et al. ***mAbs***, 2012, *4*(6), 653 – 663.
Labrijn, A. F. et al. ***Nature Reviews Drug Discovery***, 2019, 18, 585 – 608.

Analytical Tools for mAb Characterization

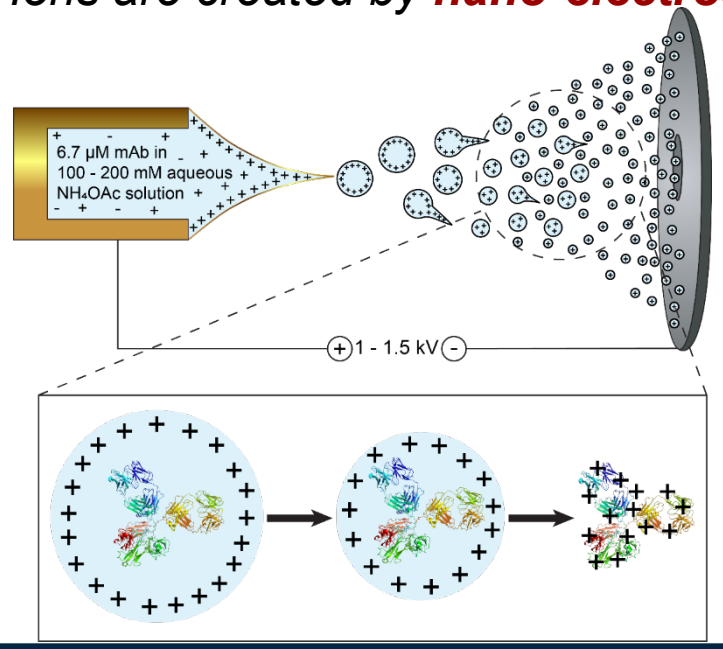


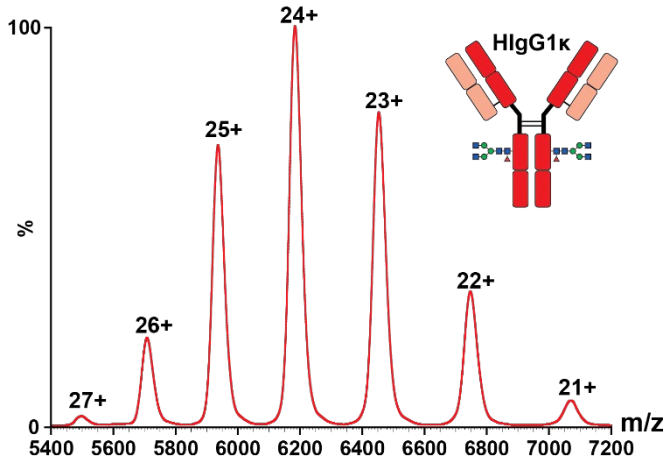
Ion Mobility-Mass Spectrometry (IM-MS)



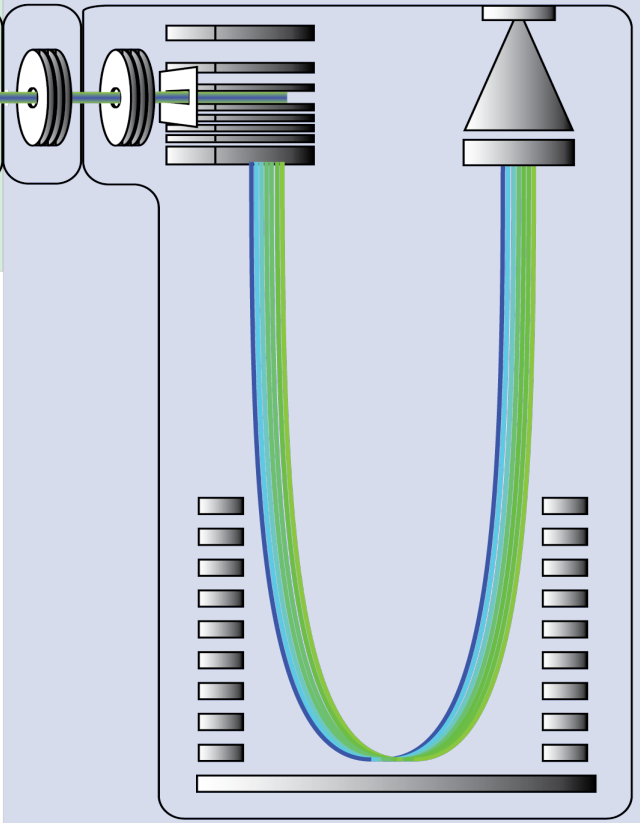
Waters Synapt G2: Quadrupole-IM-MS instrumentation

Ions are created by ***nano-electrospray ionization***



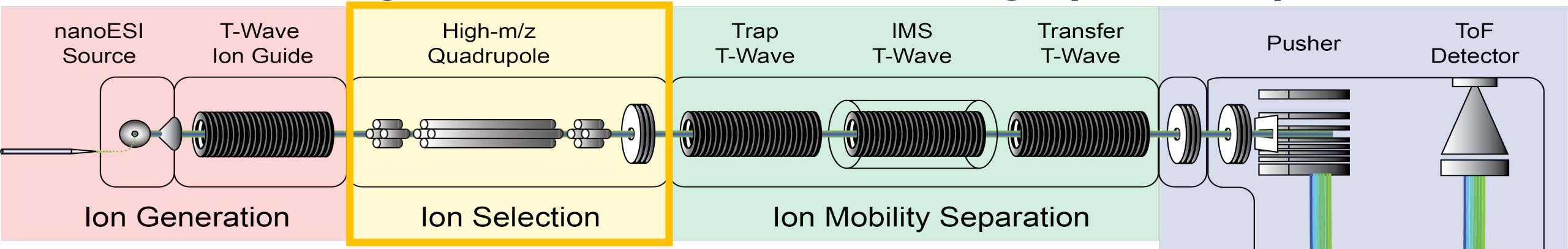


"Native-like"



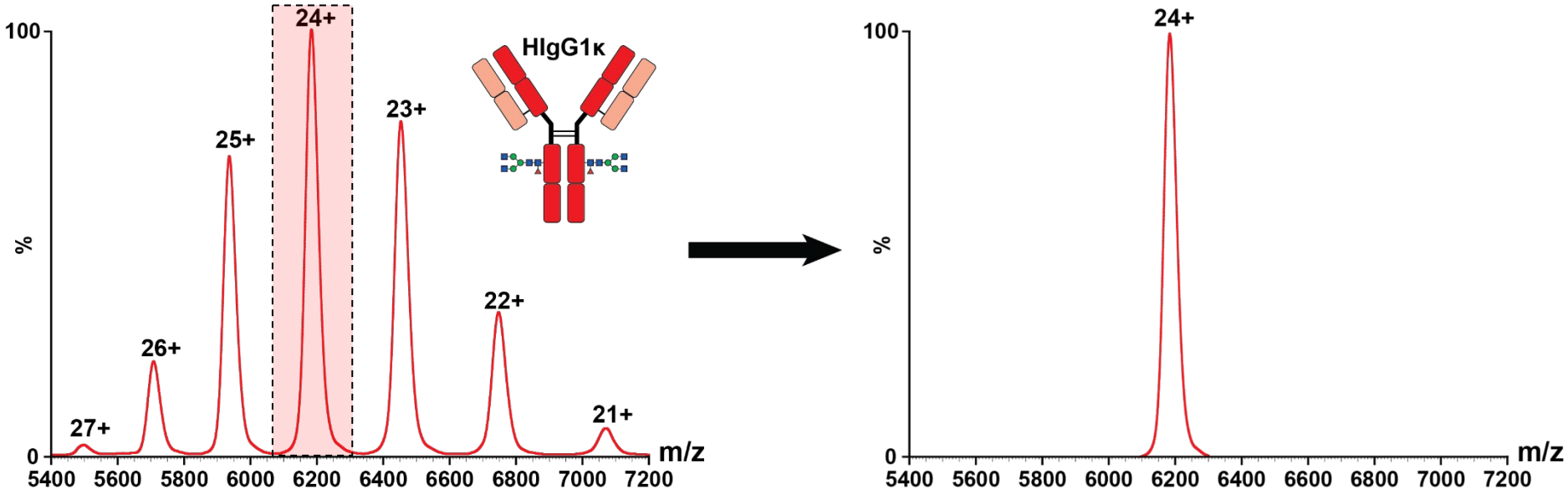
Ion Mass Analysis

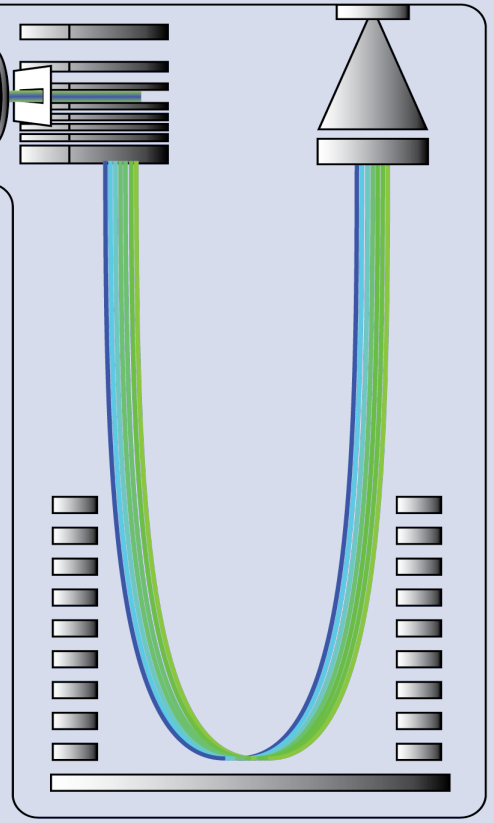
Ion Mobility-Mass Spectrometry (IM-MS)



Waters Synapt G2: Quadrupole-IM-MS instrumentation

*Ions are selected by a **quadrupole mass filter***

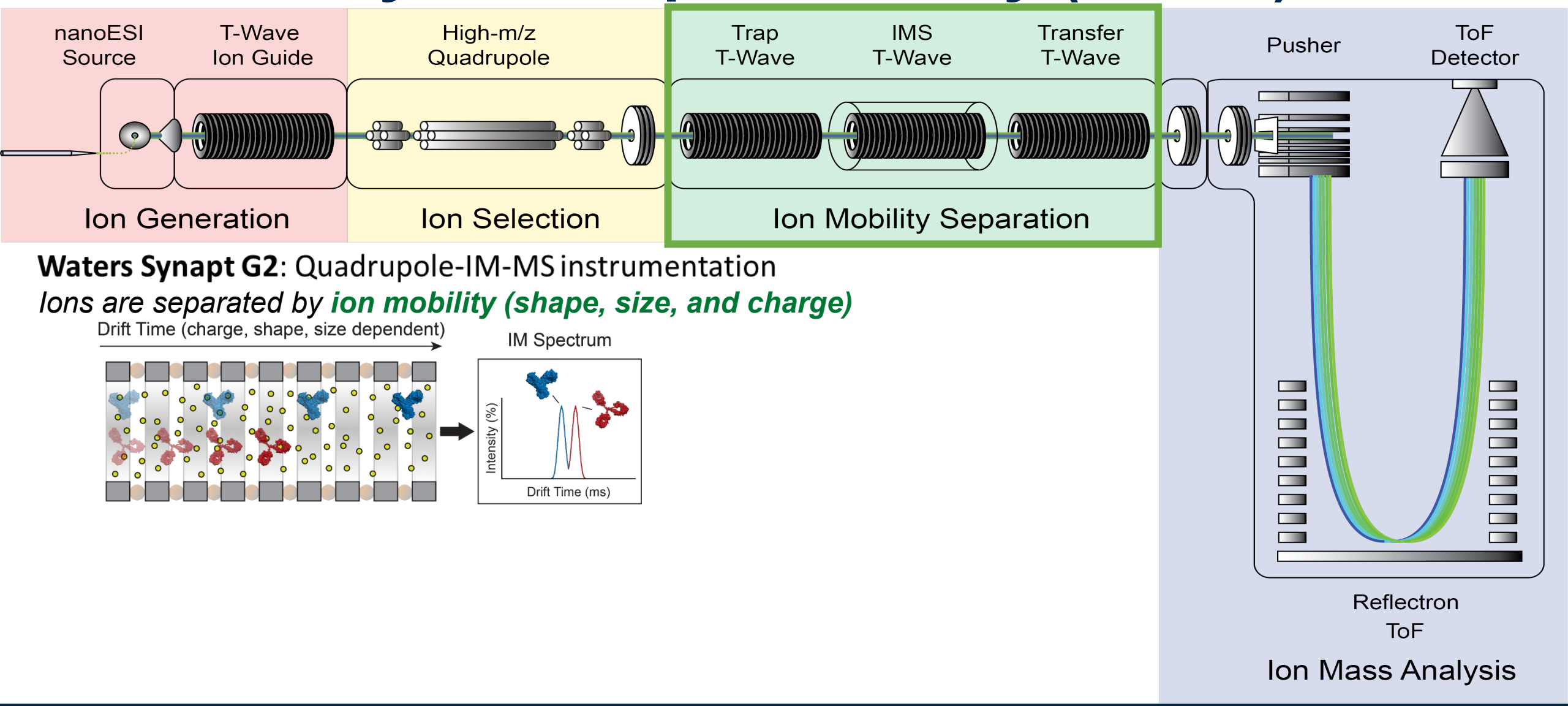




Reflectron ToF

Ion Mass Analysis

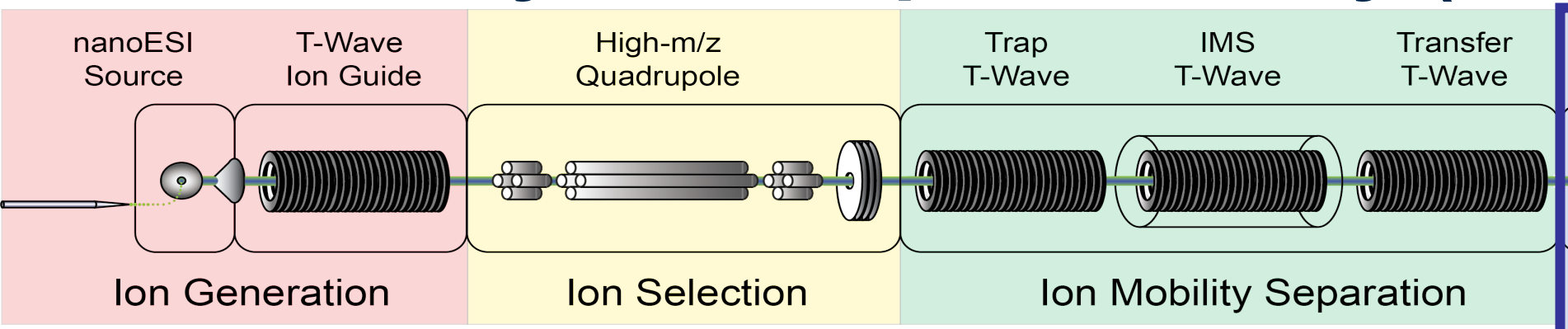
Ion Mobility-Mass Spectrometry (IM-MS)



Waters Synapt G2: Quadrupole-IM-MS instrumentation

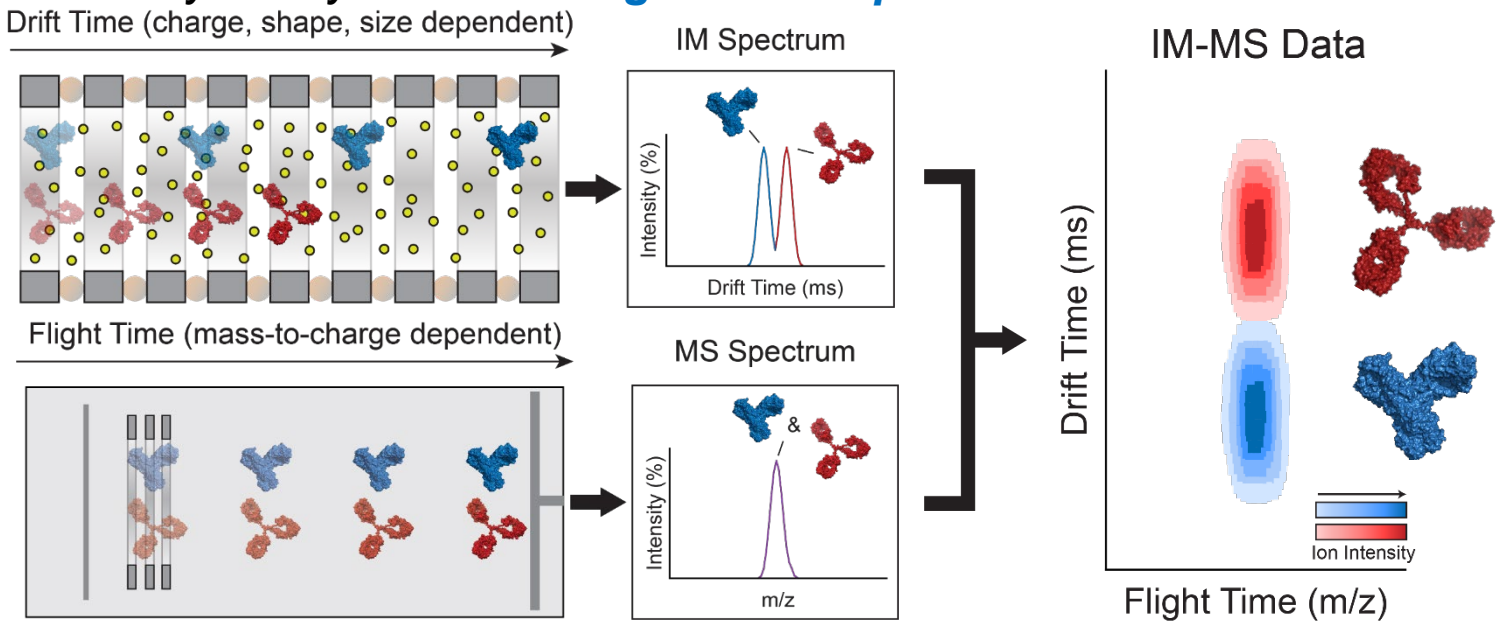
*Ions are separated by **ion mobility (shape, size, and charge)***

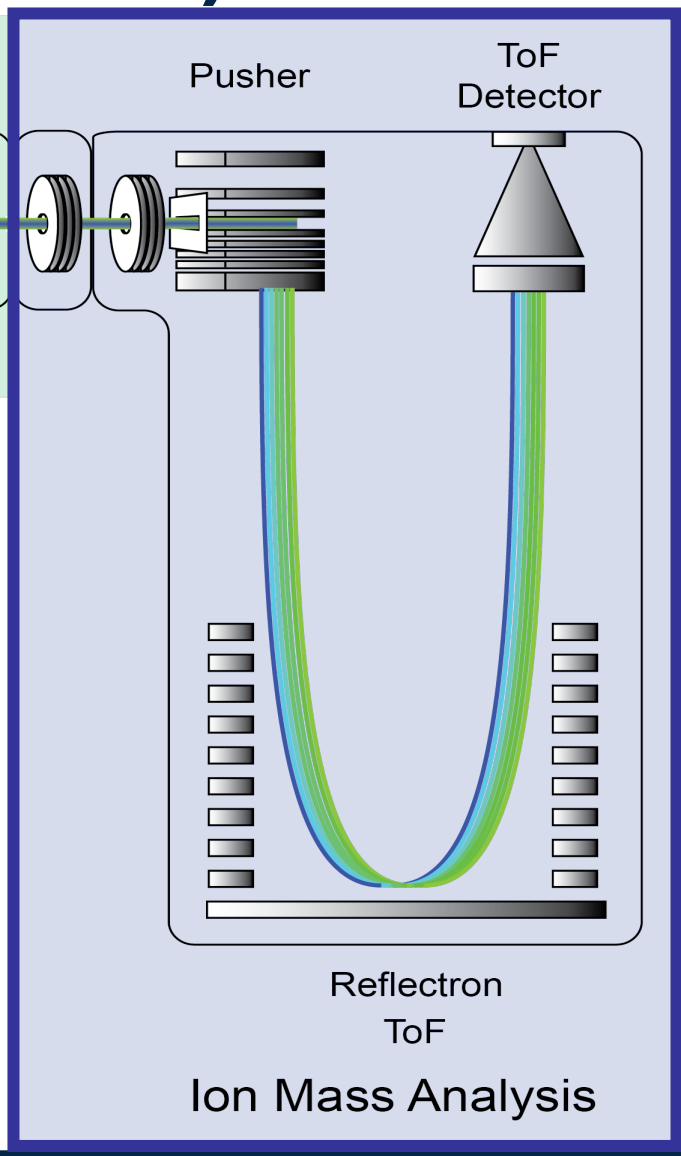
Ion Mobility-Mass Spectrometry (IM-MS)



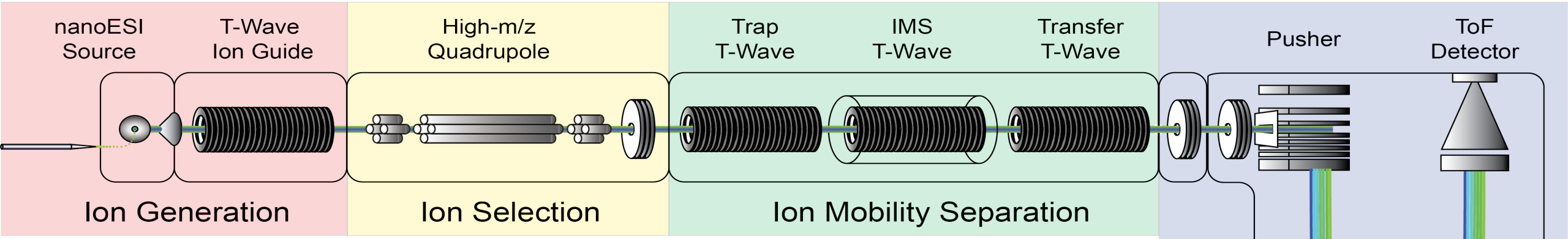
Waters Synapt G2: Quadrupole-IM-MS instrumentation

*Ions are analyzed by a **time-of-flight mass spectrometer***

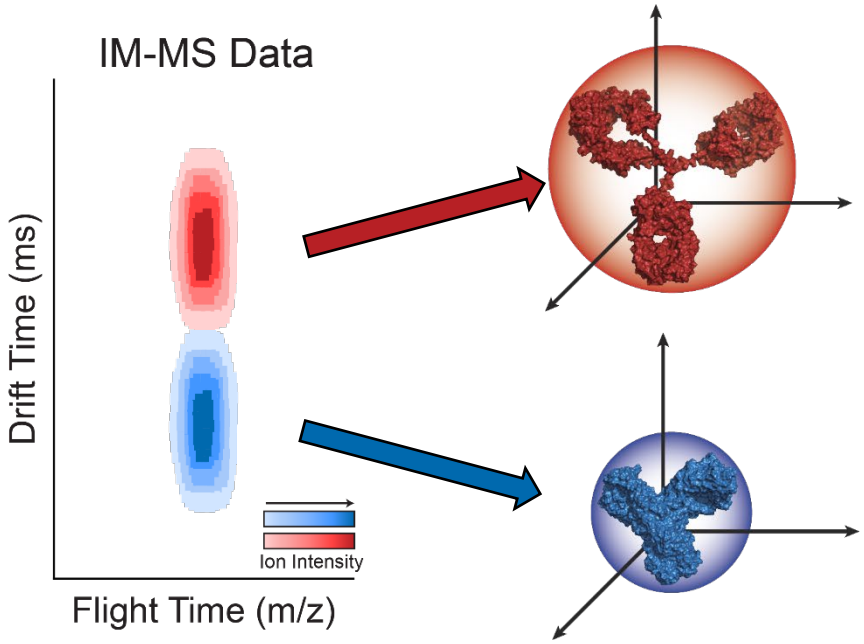




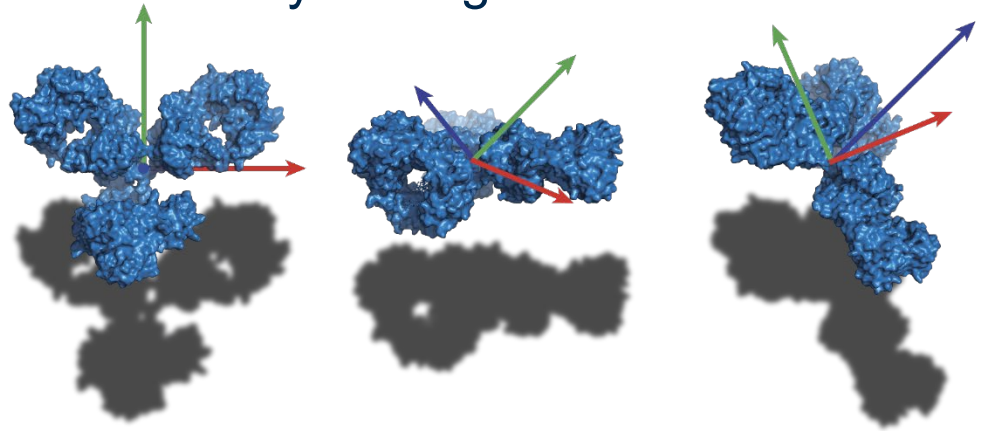
Ion Mobility-Mass Spectrometry (IM-MS)

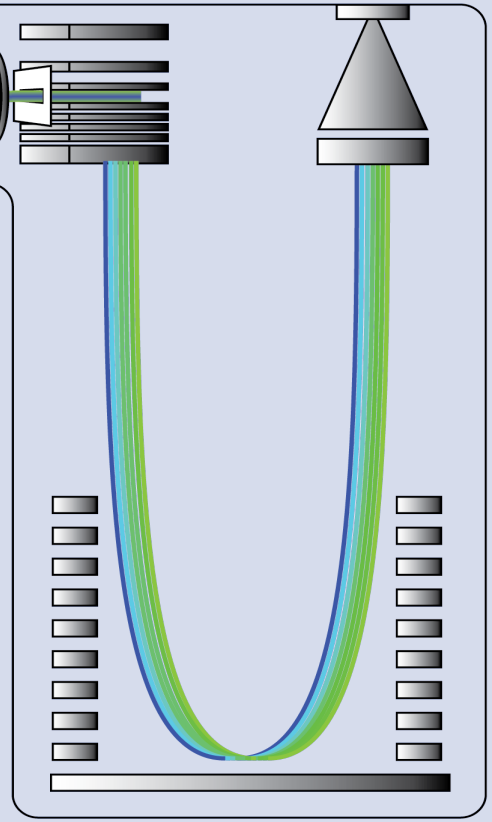


Waters Synapt G2: Quadrupole-IM-MS instrumentation



Collision **C**ross **S**ections (**CCS**):
rotationally averaged surface area



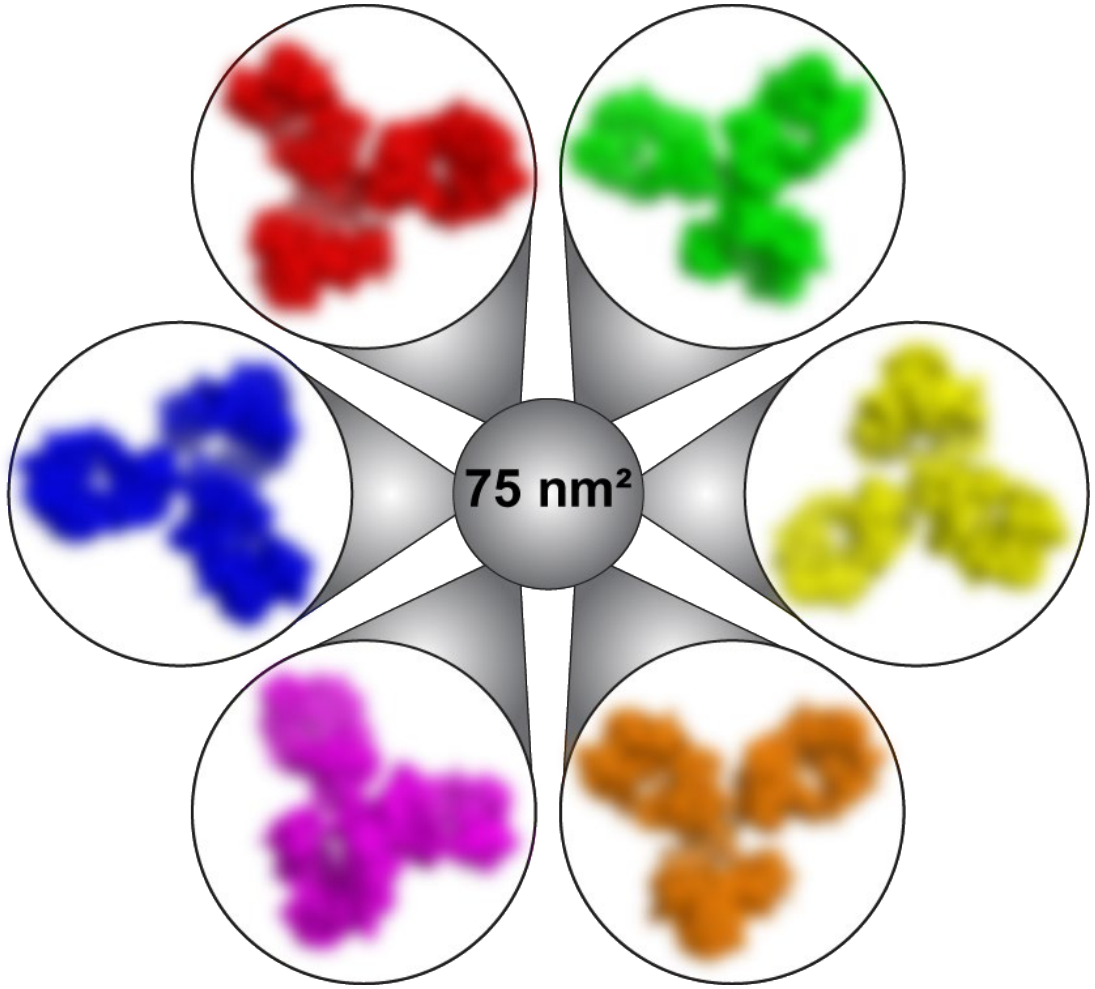


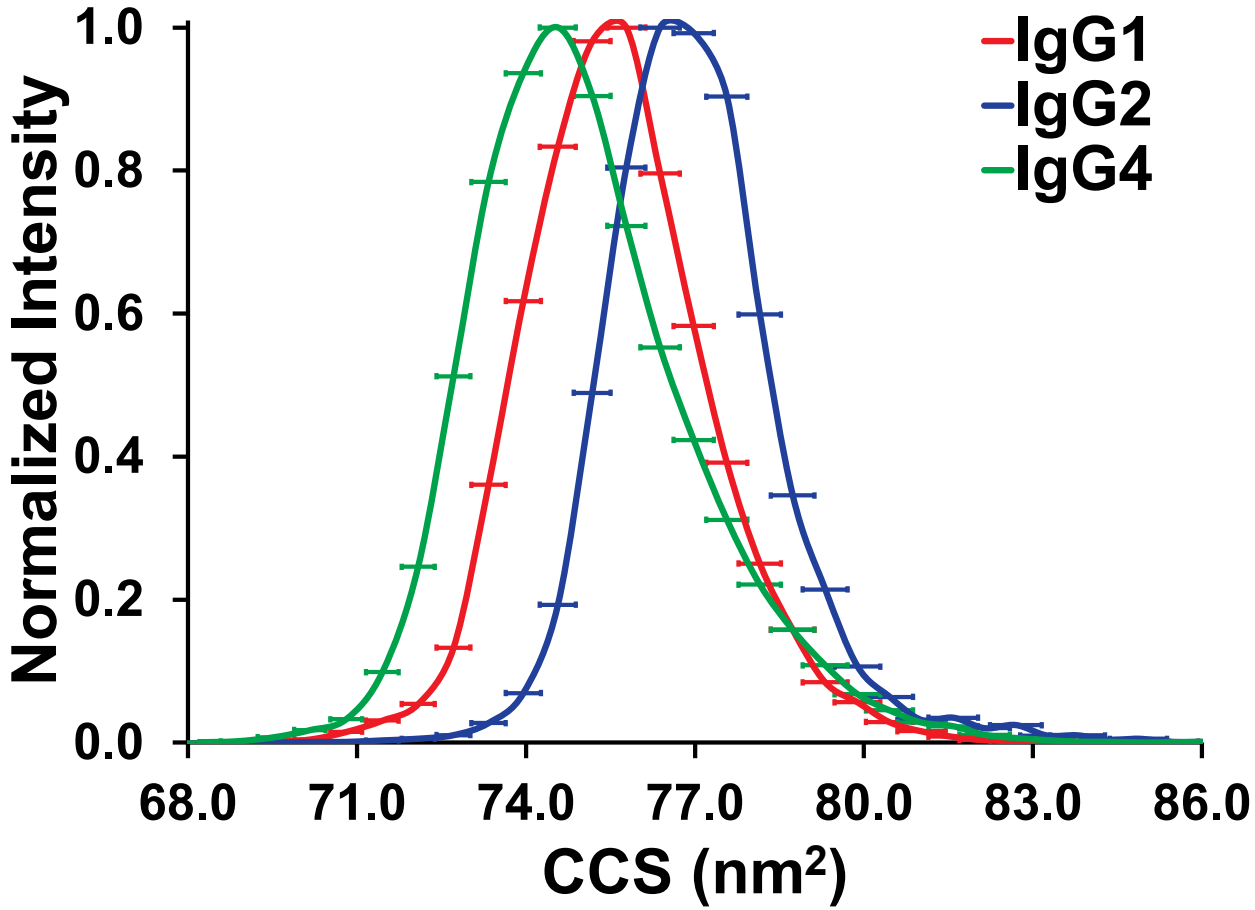
Ion Mass Analysis

Adapted from Marklund, E. G., et al. ***Structure,*** 2015, *23*(4), 791 – 799.

Limitations of CCS as structural probes

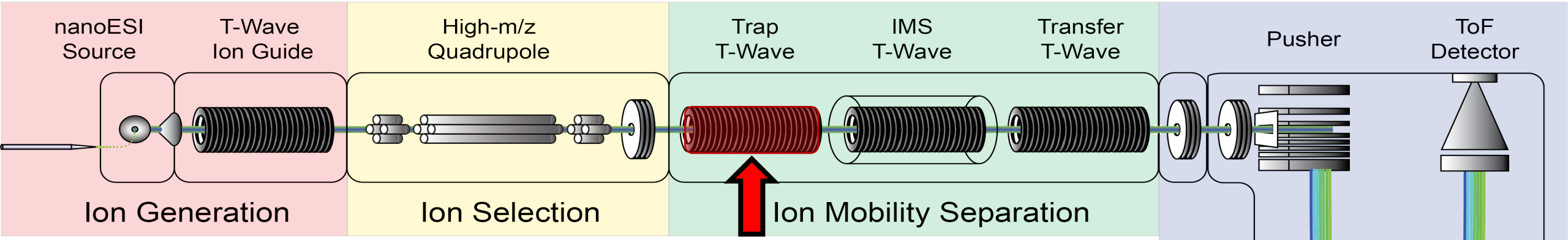
mAbs are very **dynamic** and **flexible**!



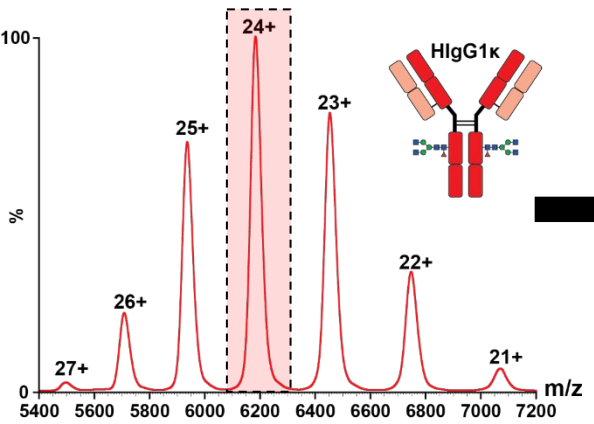


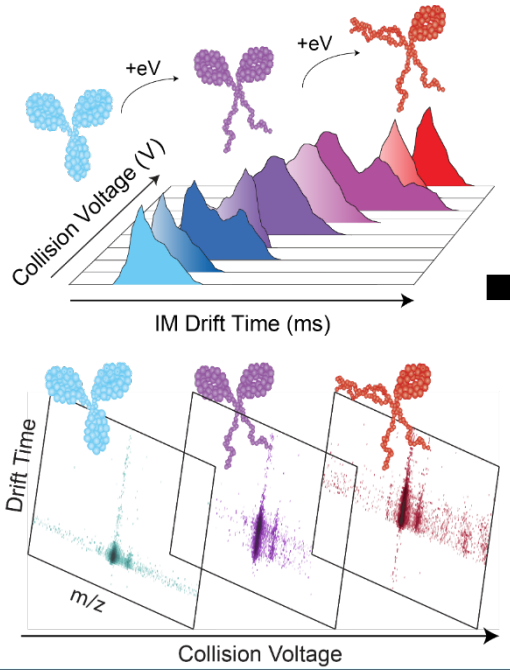
Limited structural information based on CCS alone!

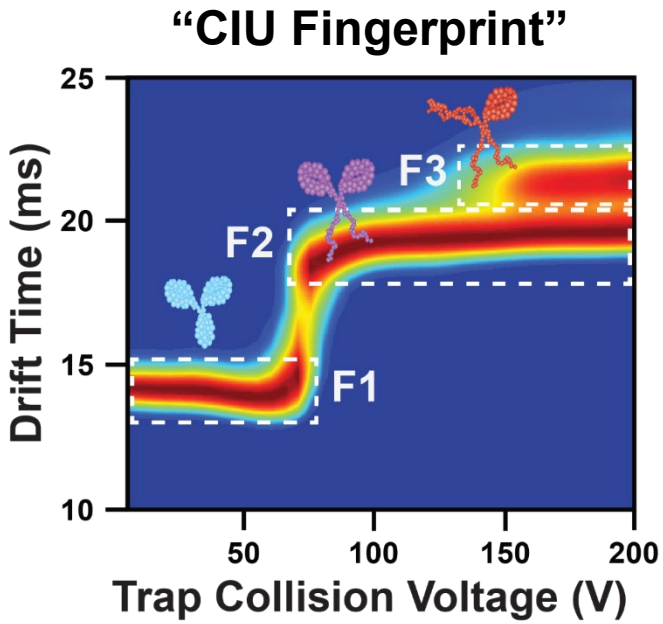
Collision Induced Unfolding (CIU)



Waters Synapt G2: Quadrupole-IM-MS instrumentation





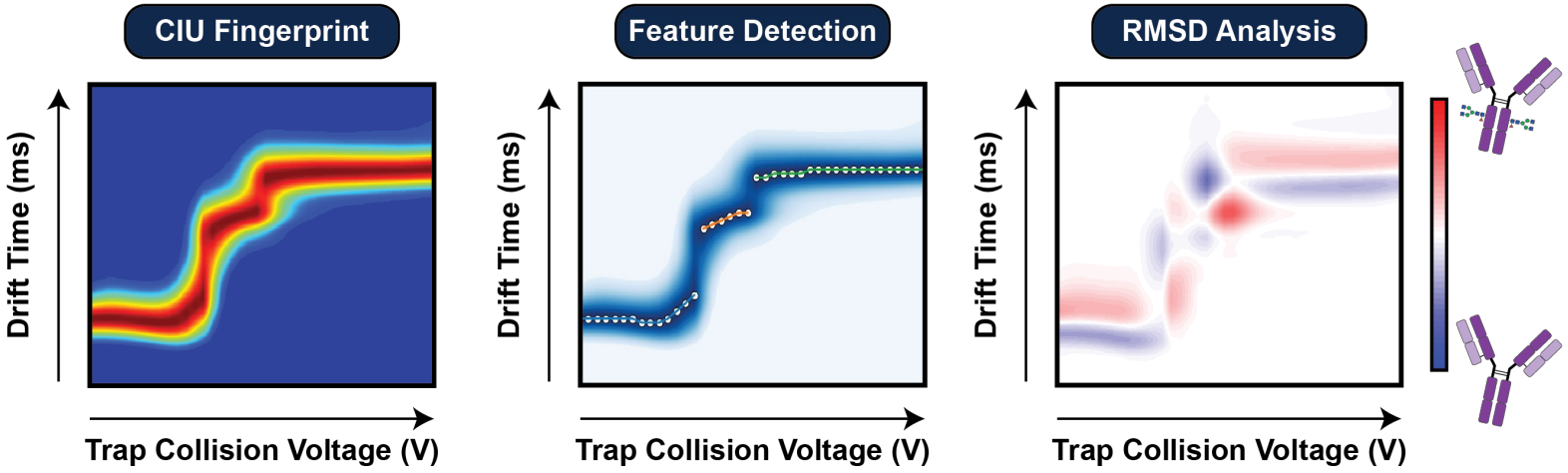


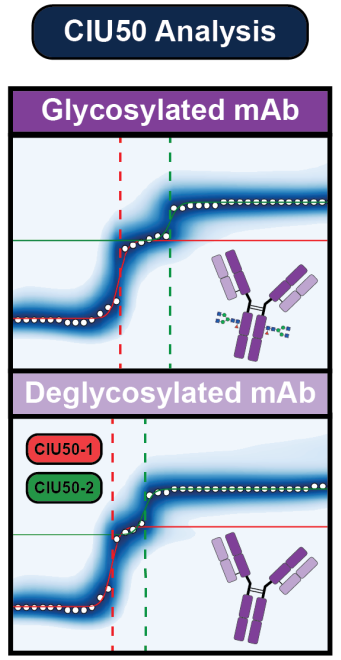
Reflectron ToF

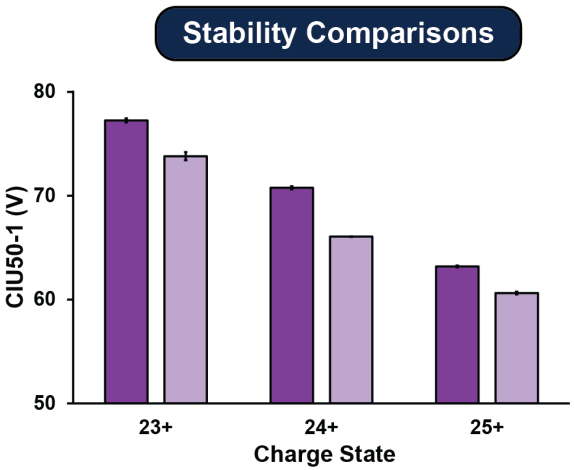
Ion Mass Analysis

Adapted from Dixit, S. M., et al., ***Curr. Opin. Chem. Biol***., 2018, 42, 93 – 100.

CIU Data Analysis Workflow





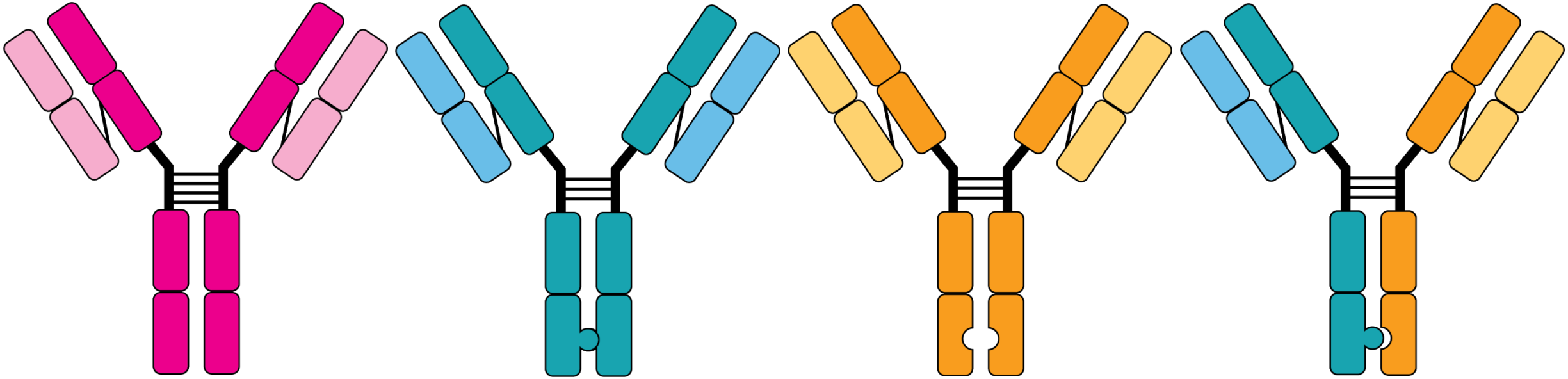


- **Feature detection** quantifies centroid drift times (or CCS) corresponding to a unique conformation present across multiple collision voltages
- **Root-mean-squared deviation (RMSD) analysis** calculates global differences between two CIU fingerprints
- **CIU50 analysis** models the transition region between features as a logistic function
  - Quantifies midpoint voltage between adjacent features – termed “CIU50” value

- Comparisons of CIU50 values between different samples can provide insights on **protein stability** and **susceptibility to gas-phase unfolding**

Polasky, D. A., et al. ***Anal. Chem.***, 2019, *91*, 3147 – 3155.

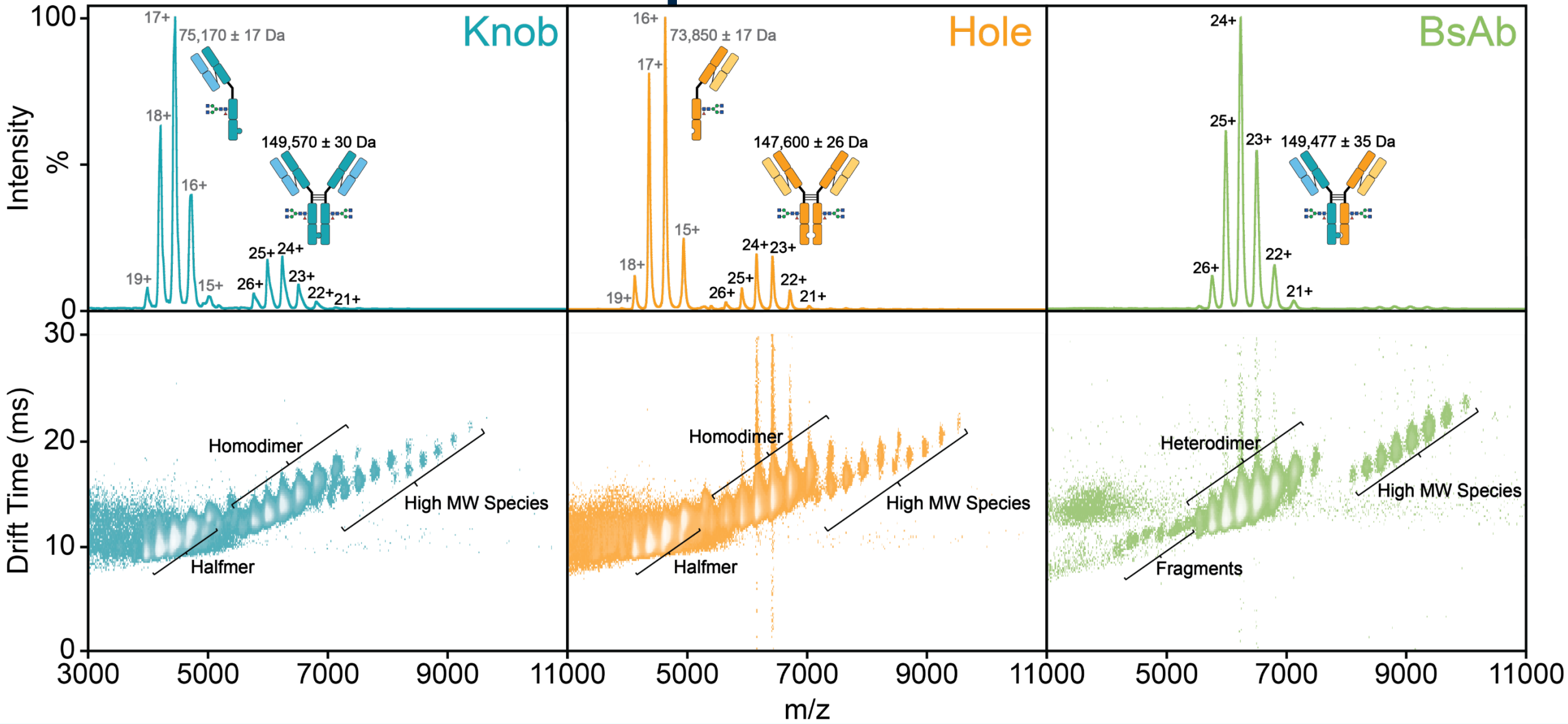
Characterization of a model BsAb



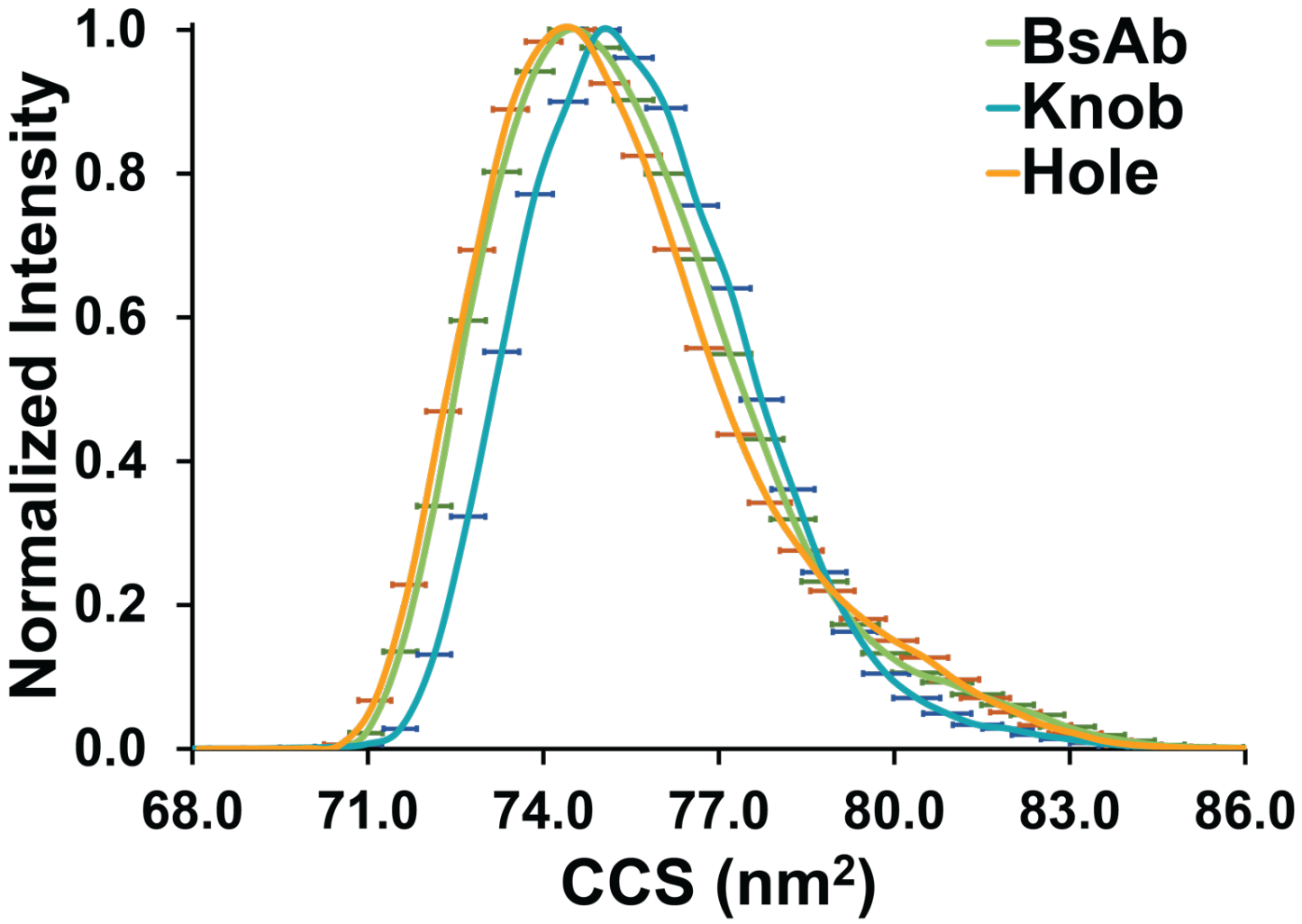
IgG2-based, knob-into-hole (KiH) bispecific → one disulfide bond mutated out

- **Knob**: T366W
- **Hole**: T366S, L368A, Y407V

IM-MS of BsAb and parental constructs

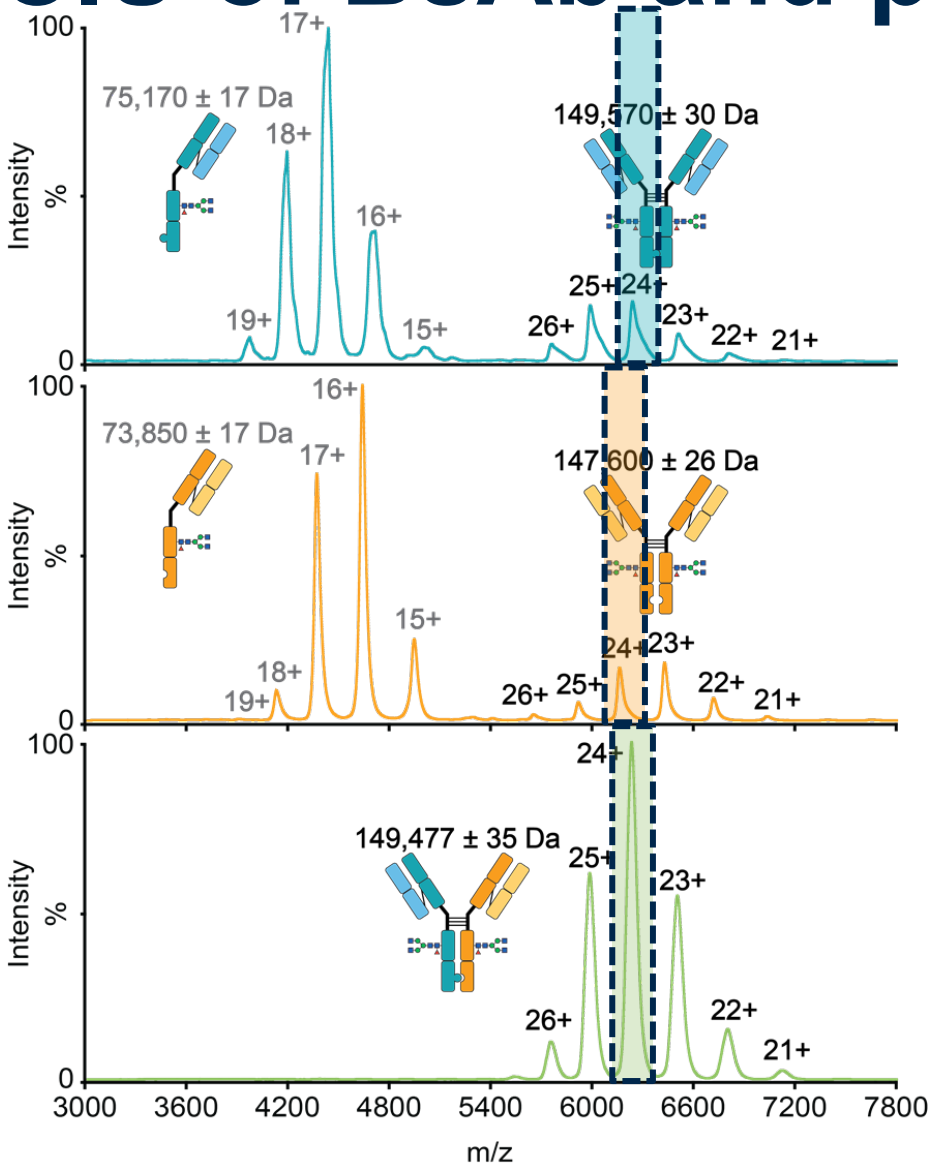


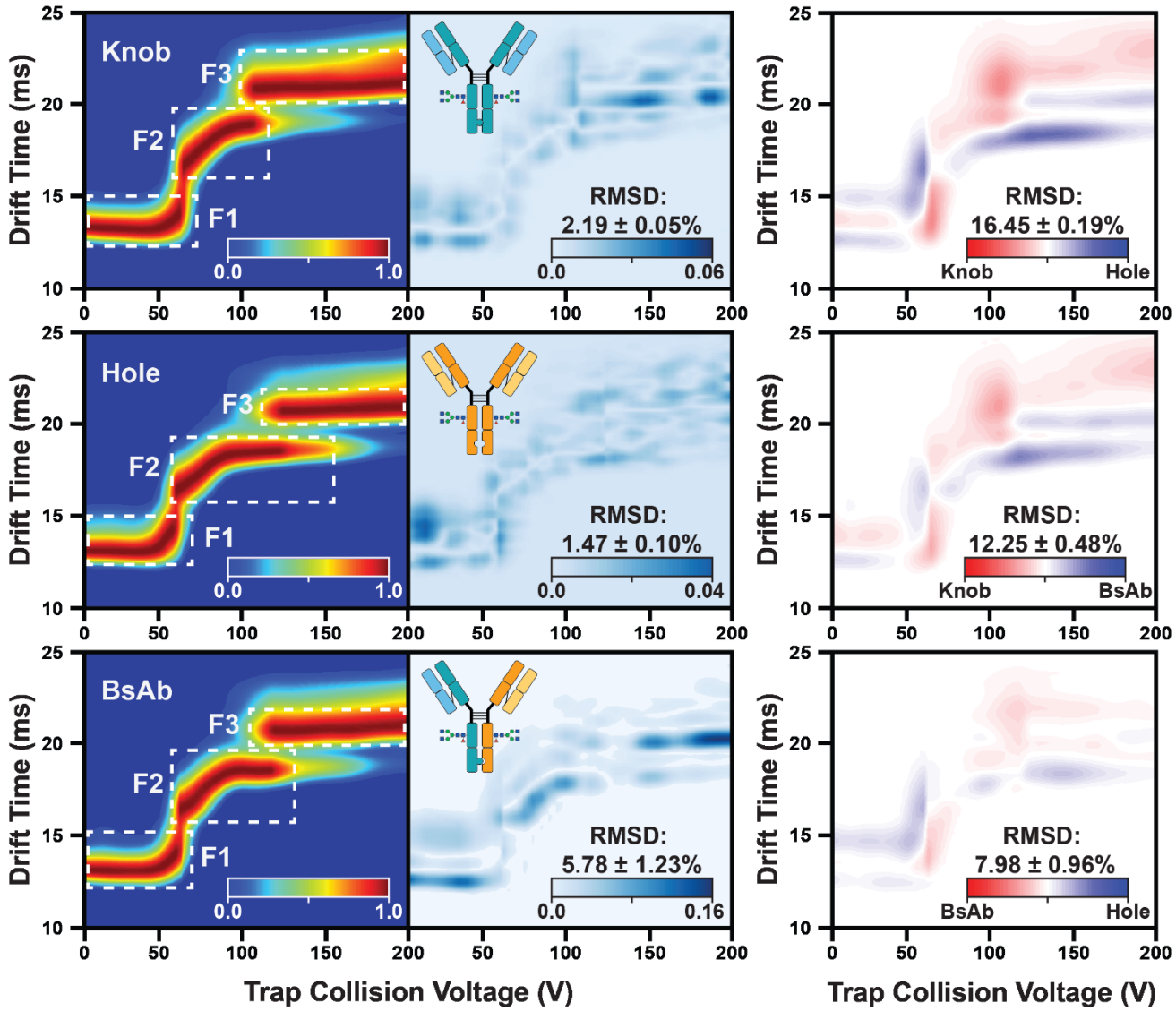
BsAb and parental construct CCS



- CCSs overlap substantially
  - Antibodies are dynamic and occupy a wide range of structural forms!
  - Slight differences attributed to variability in molecular weight
- **Limited HOS information based on CCS alone!**

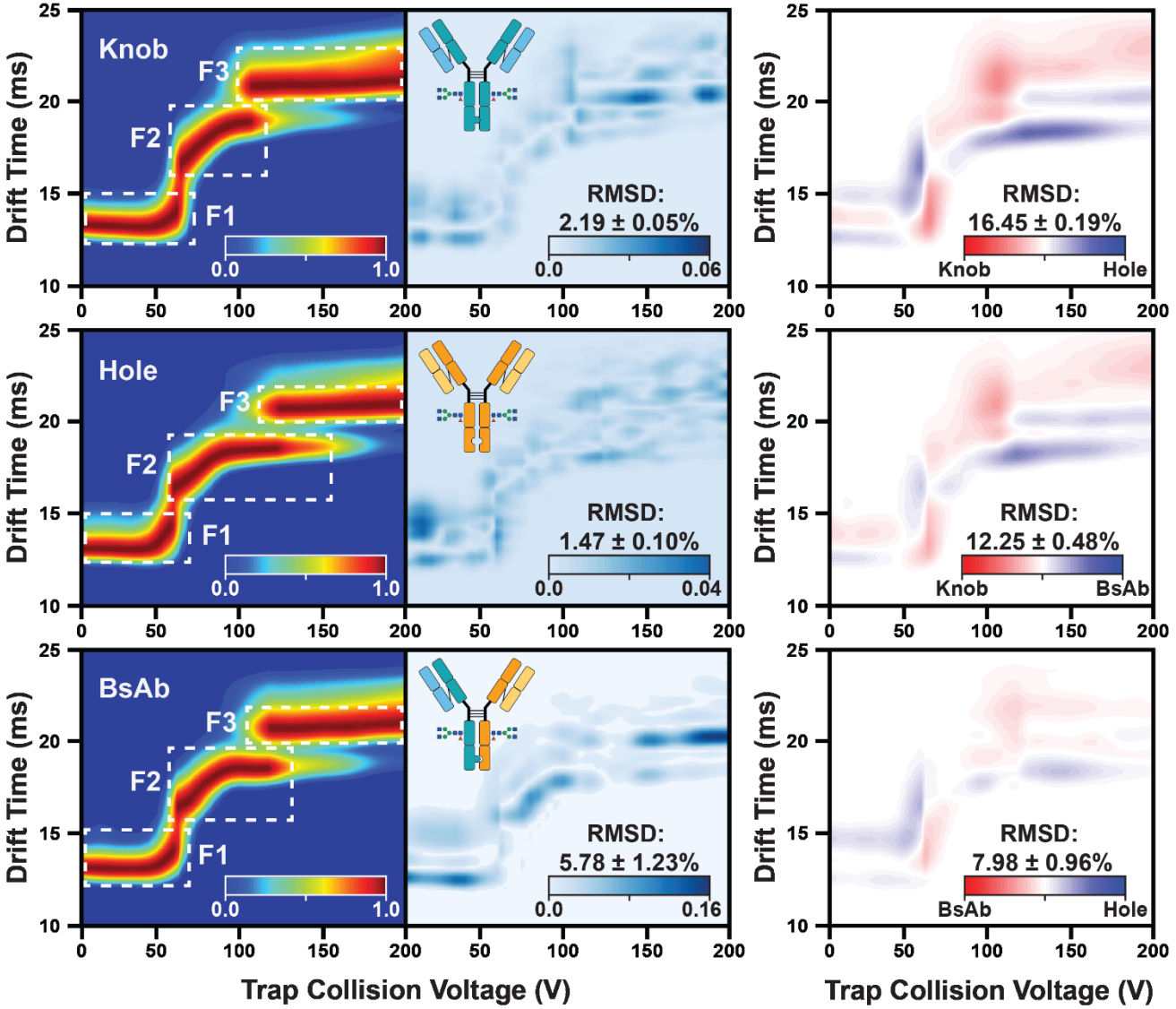
CIU of BsAb and parental constructs



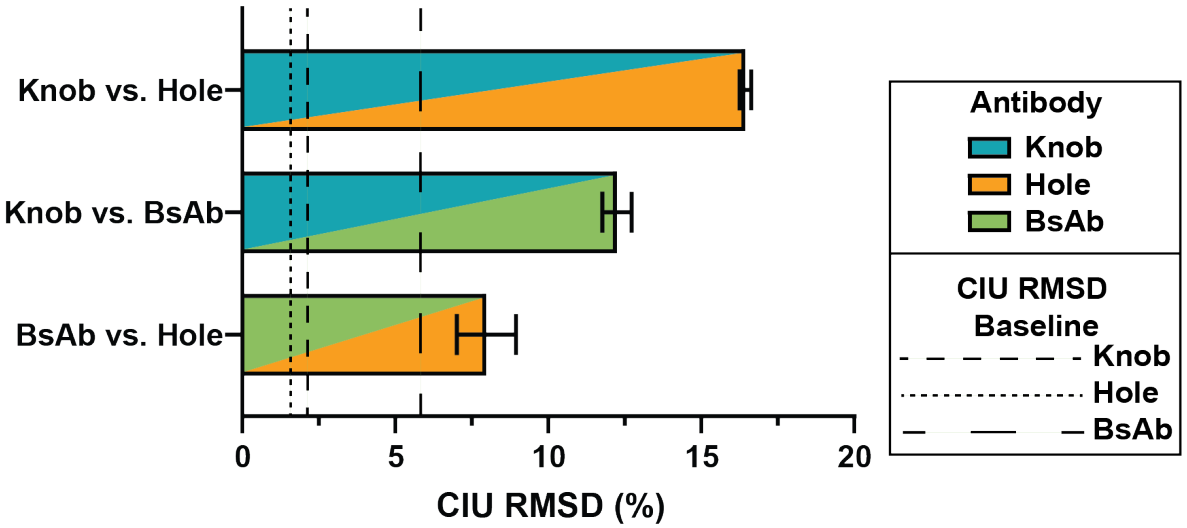


Average fingerprints for charge state 24+ (*n* = 3)

CIU of BsAb and parental constructs

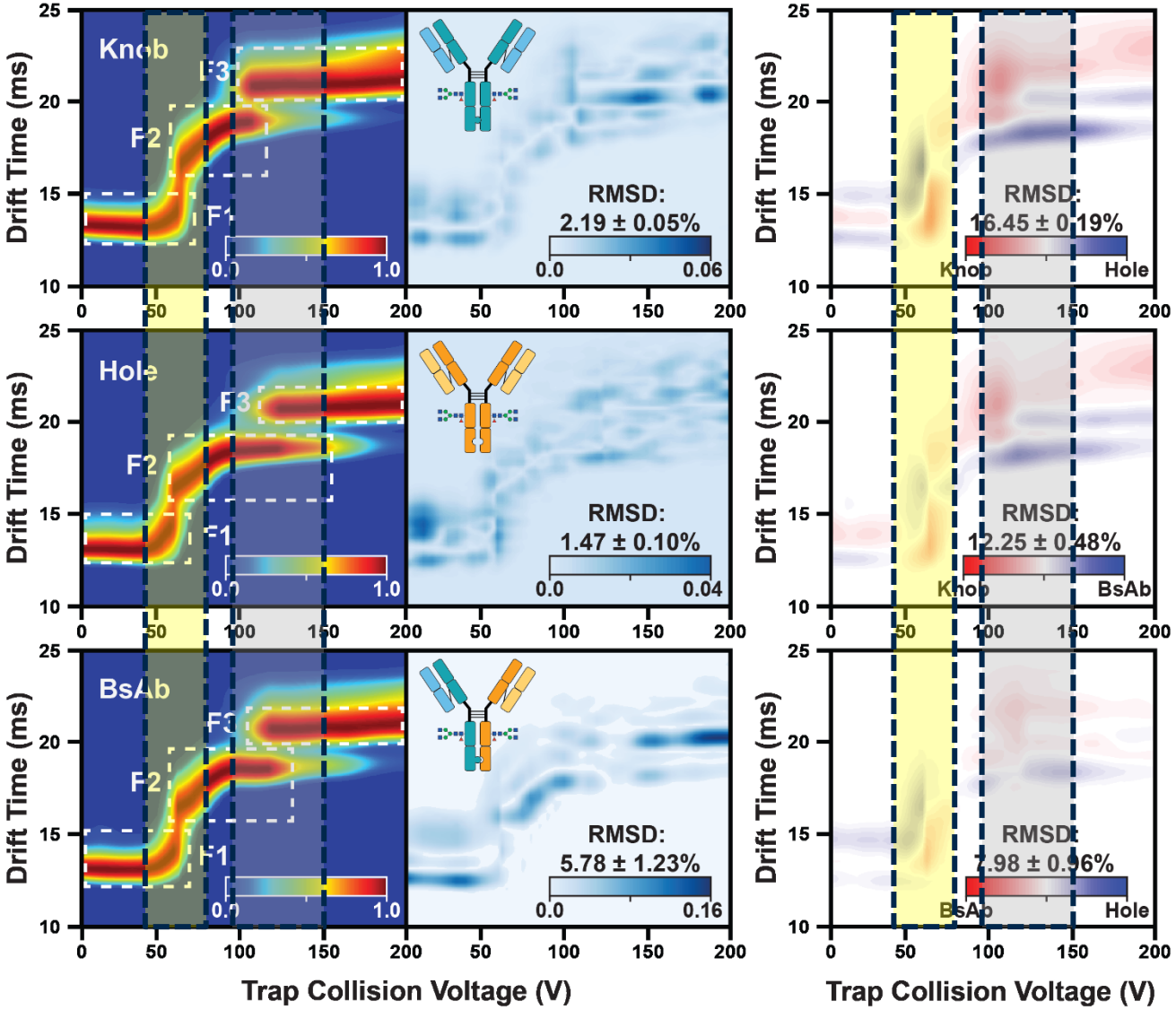


RMSD analysis reveals BsAb closely resembles Hole homodimer in conformational landscape

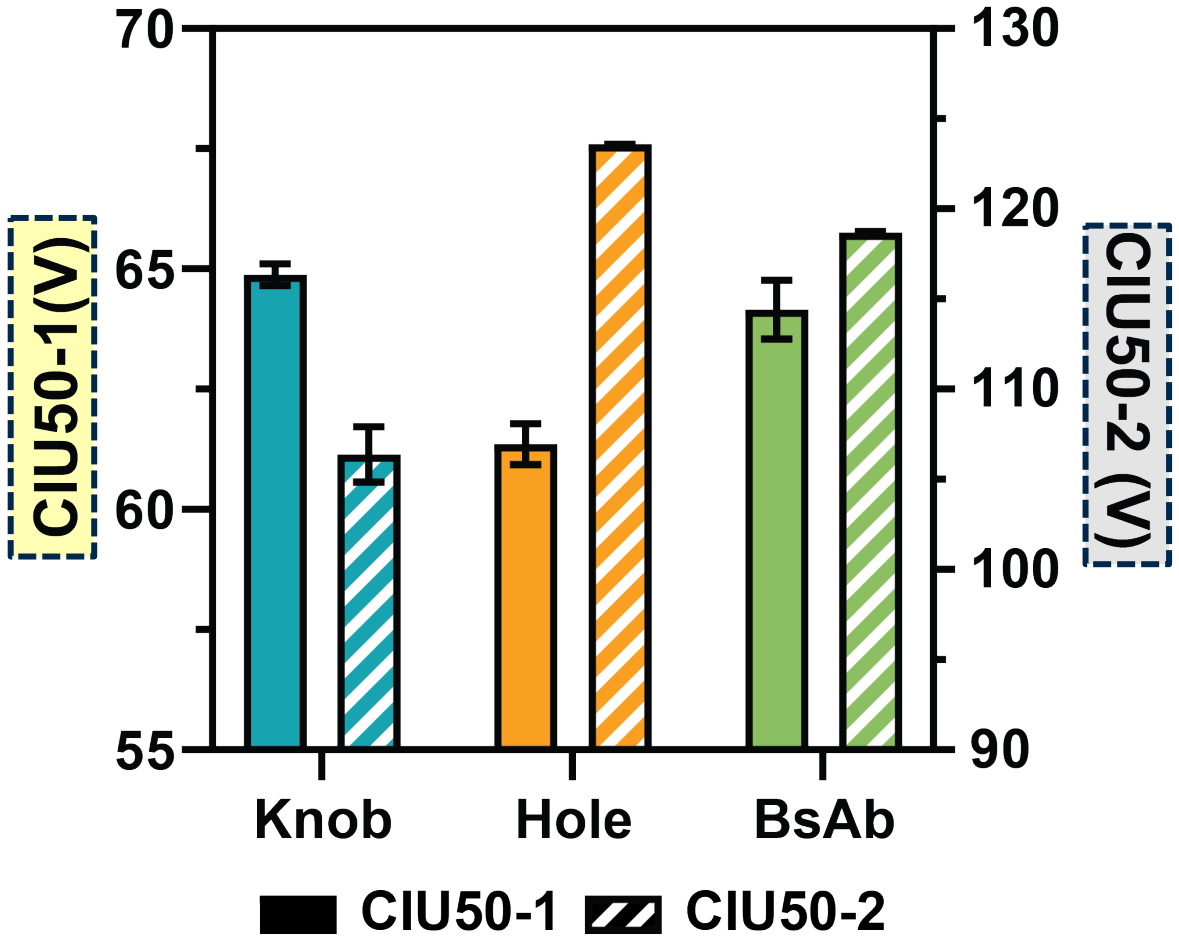


Average fingerprints for charge state 24+ (*n* = 3)

CIU of BsAb and parental constructs



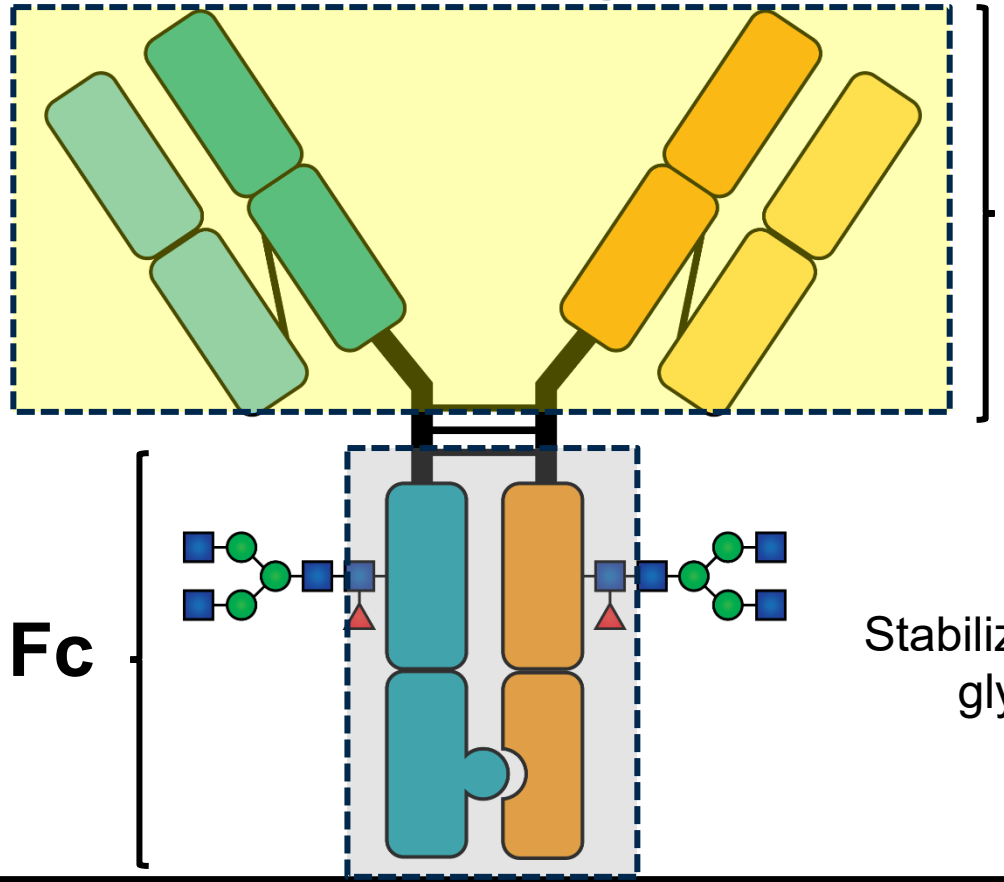
BsAb is an intermediate of Knob and Hole homodimers in conformational stability



Average fingerprints for charge state 24+ (*n* = 3)

What is causing these features and transitions to be different across constructs? How is BsAb unfolding?

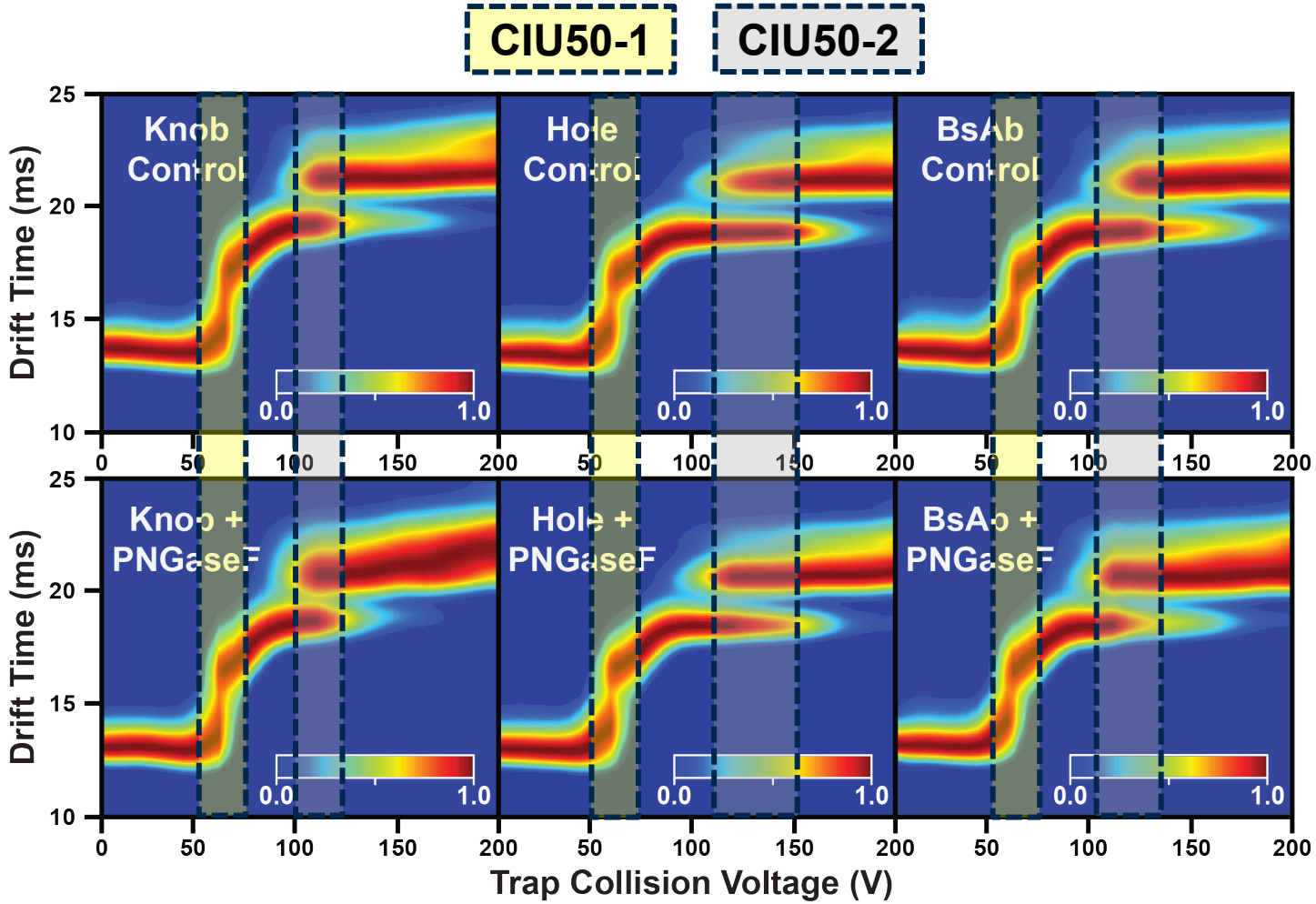
Sequence variations in antigen binding domains?

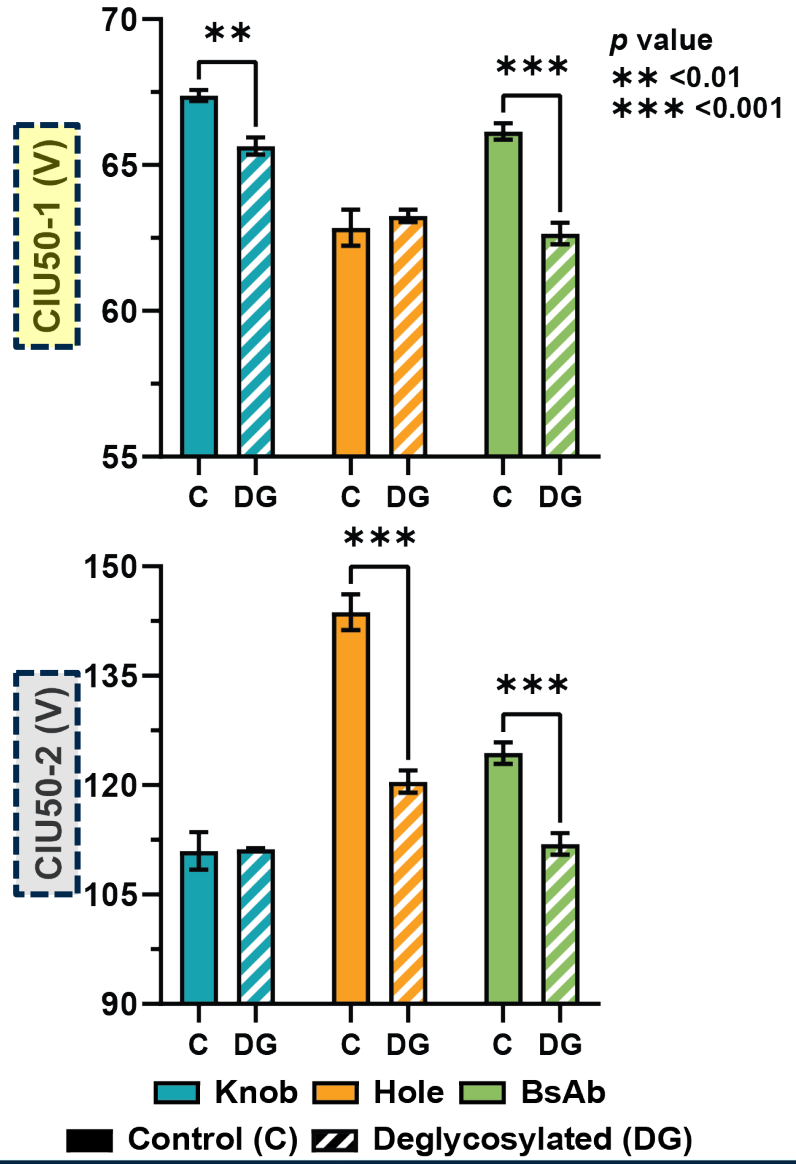


Stabilization of Fc from glycosylation?

Average fingerprints for charge state 24+ (*n* = 3)

Impact of deglycosylation on CIU transitions

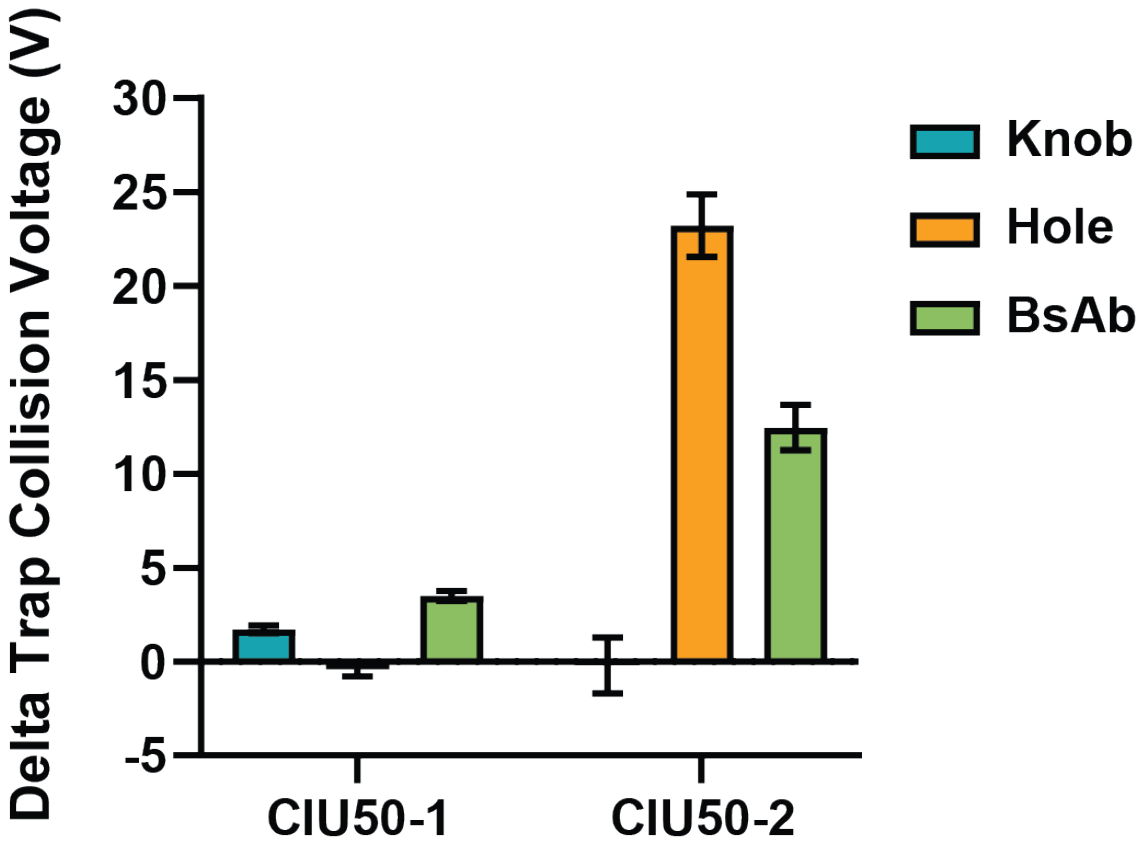


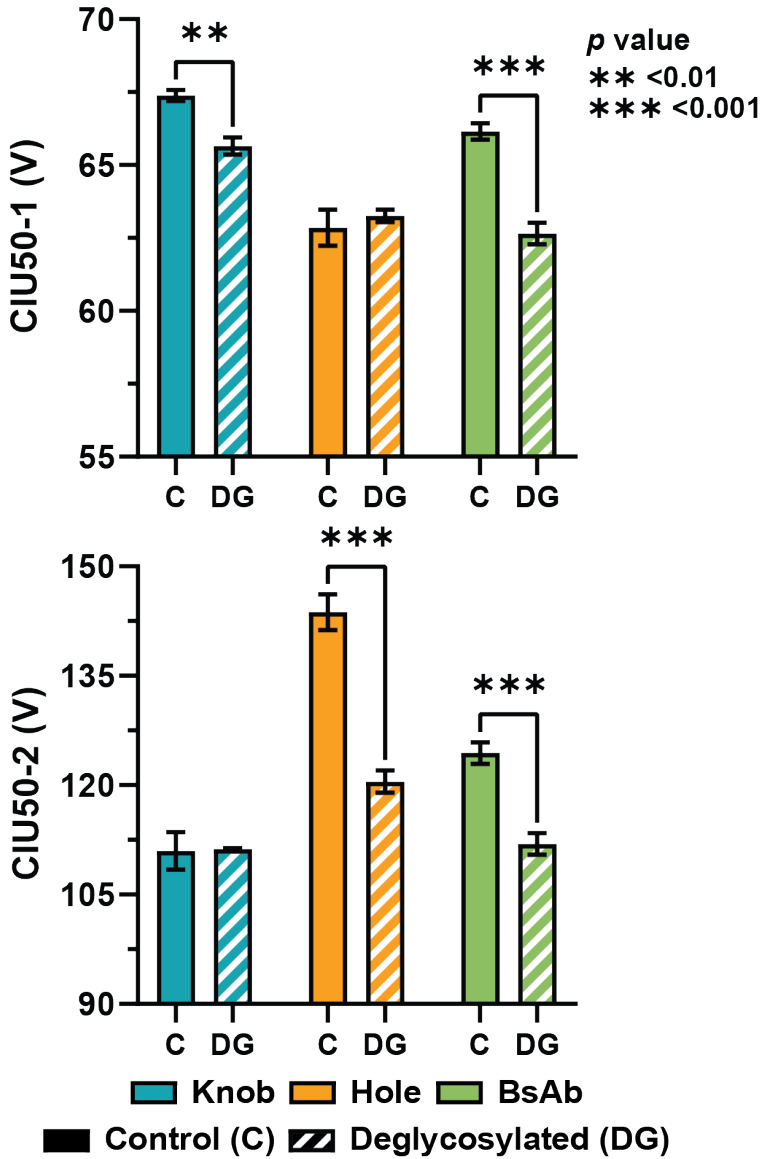


Average fingerprints for charge state 24+ (*n* = 3)

Impact of deglycosylation on CIU transitions

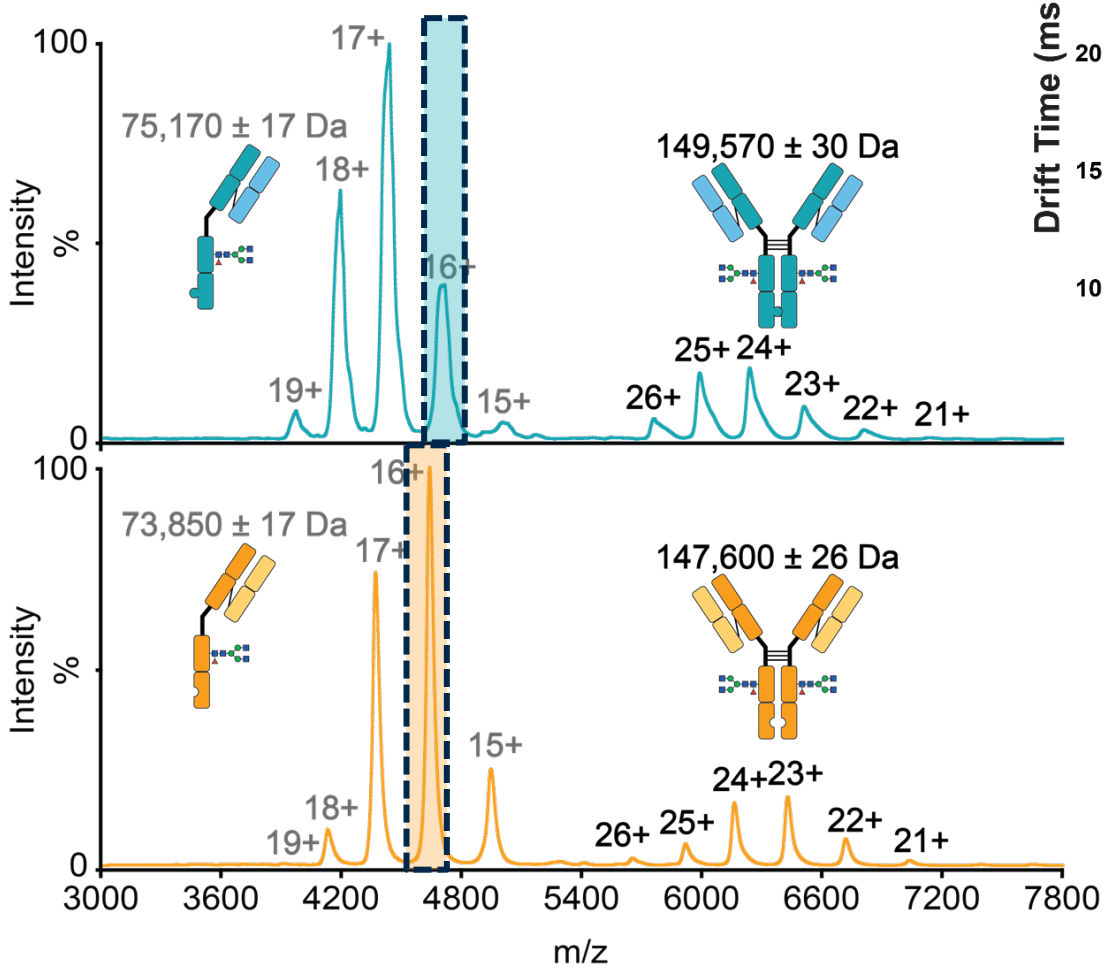
Removal of glycans known to destabilize CH_2 domain in Fc region

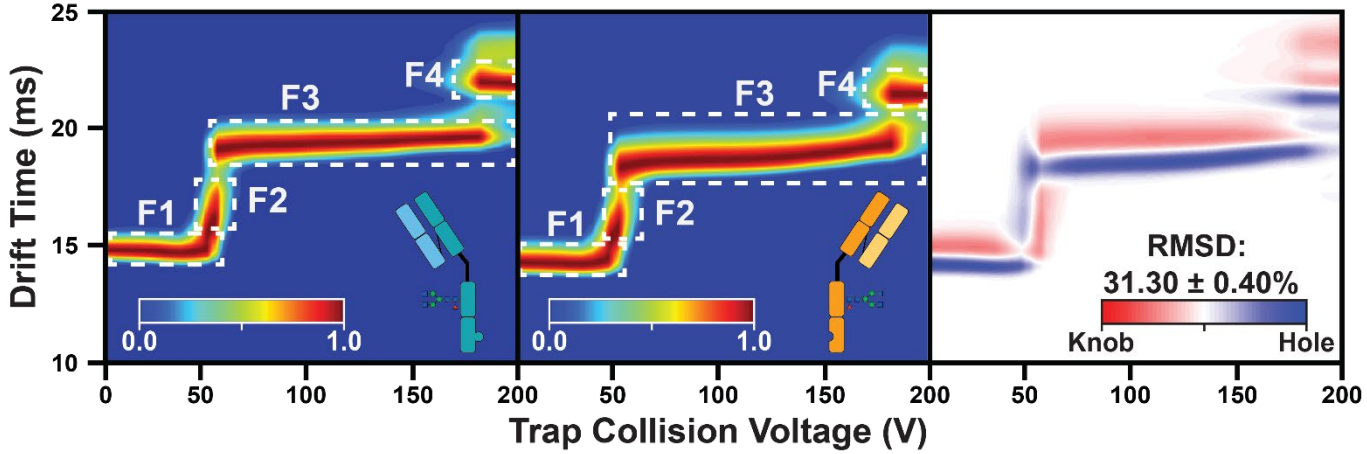


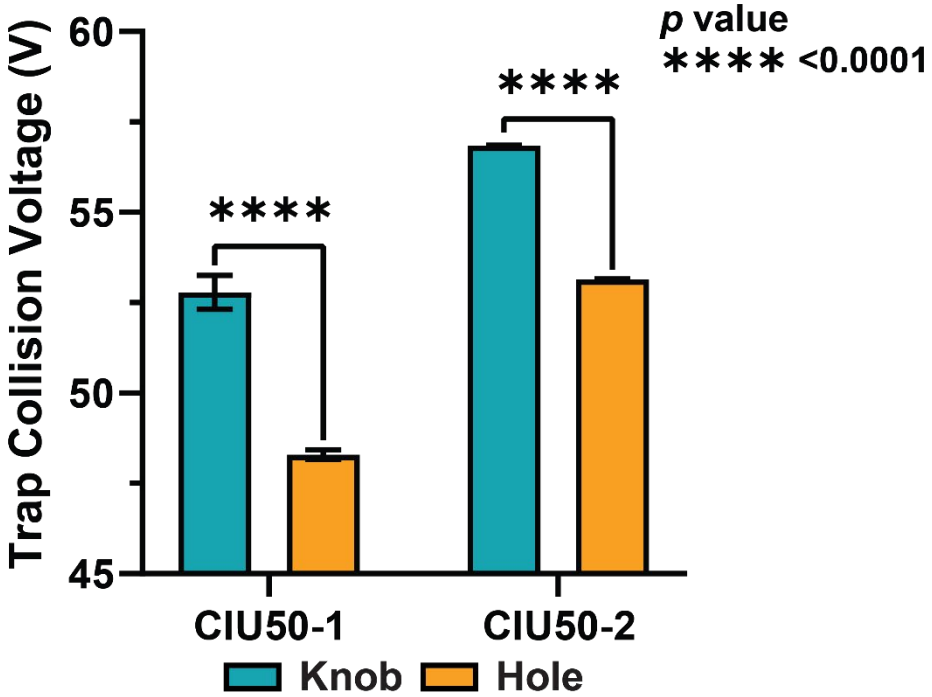


Average fingerprints for charge state 24+ (*n* = 3)

CIU of Knob and Hole "Halfmers"

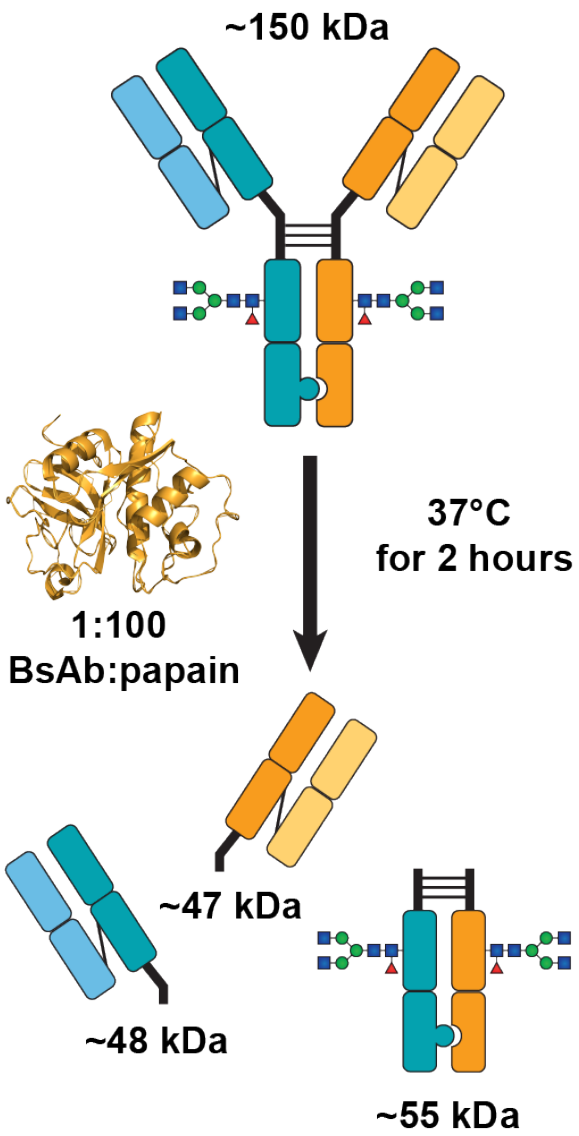


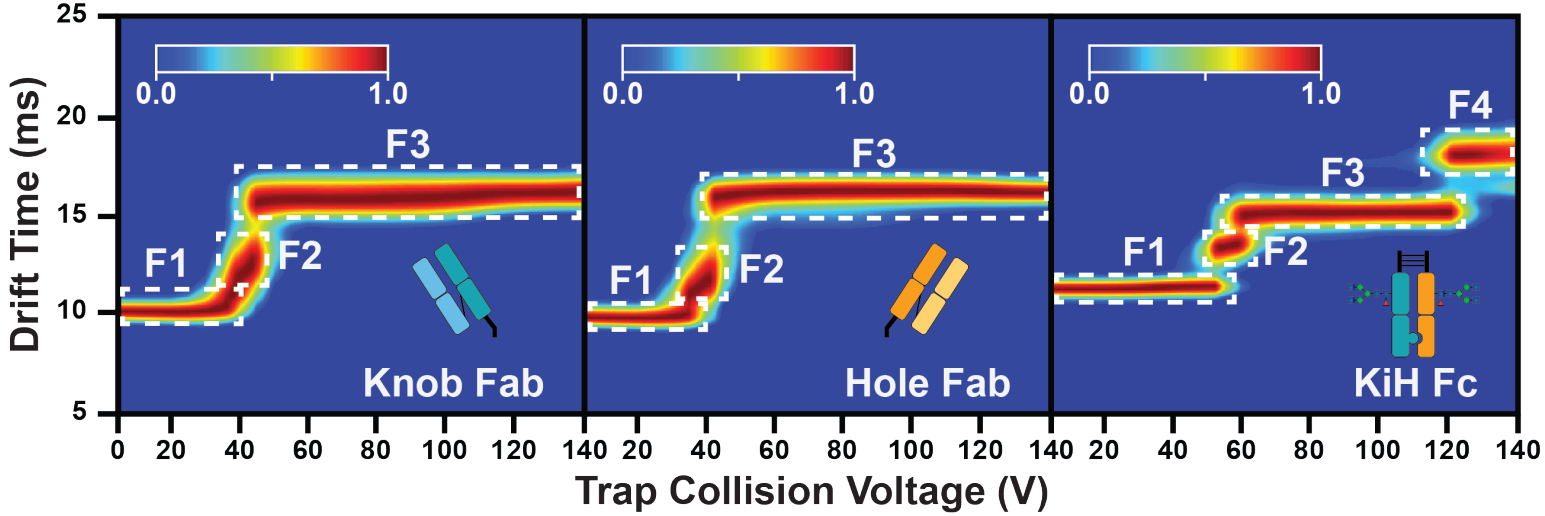


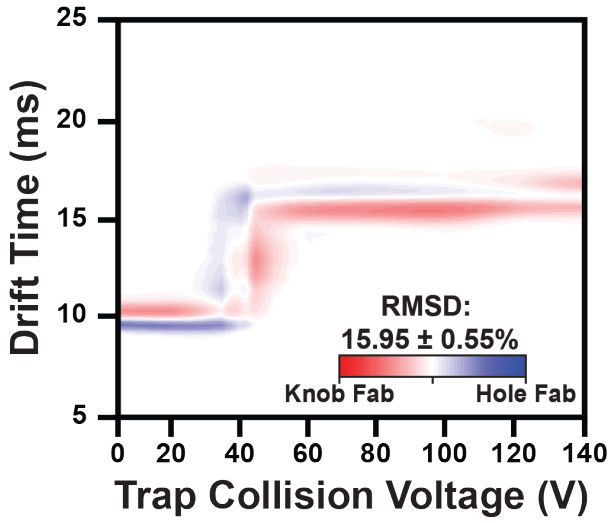


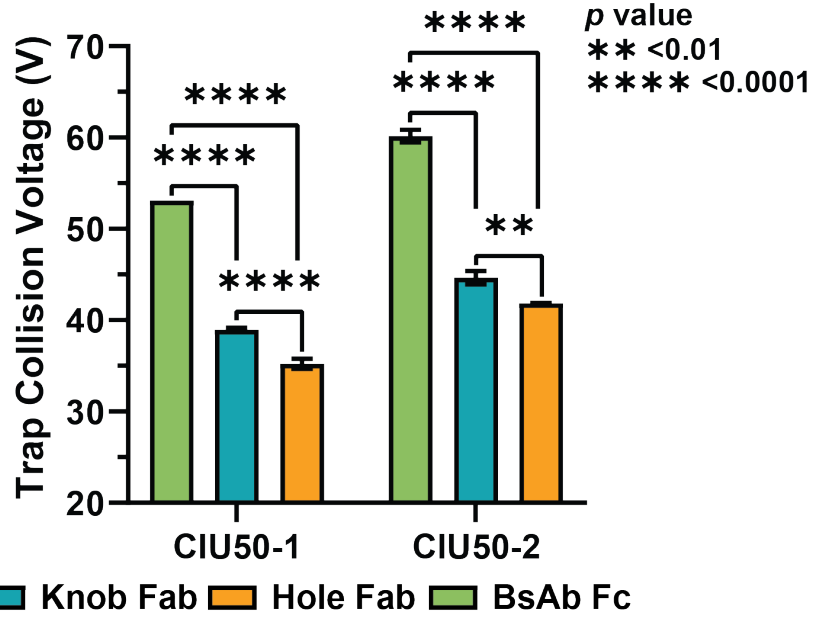
Average fingerprints for charge state 16+ shown (*n* = 3)

CIU of BsAb Fab and Fc







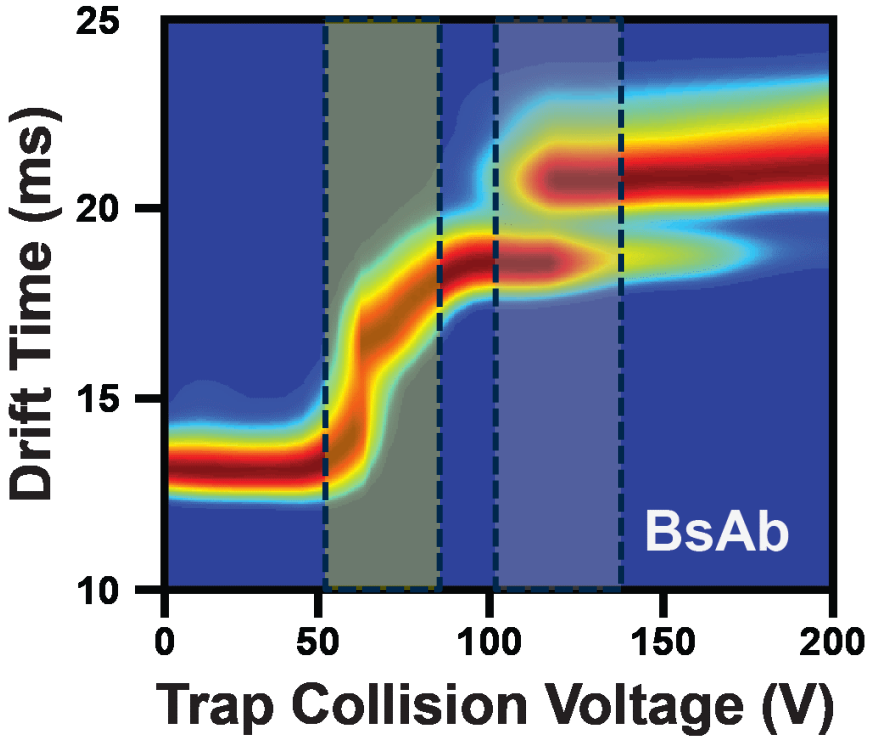


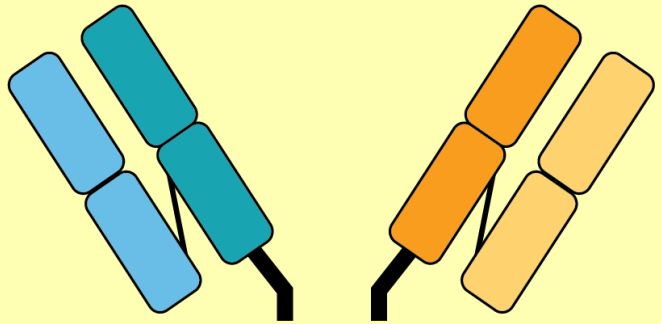
Average fingerprints for charge state 13+ shown (*n* = 3)

Putting it all together…

- Trends in global conformational stability consistent across intact, halfmer, and Fab fragment data
- Unmodified Knob component is less susceptible to unfolding per CIU50-1 analysis

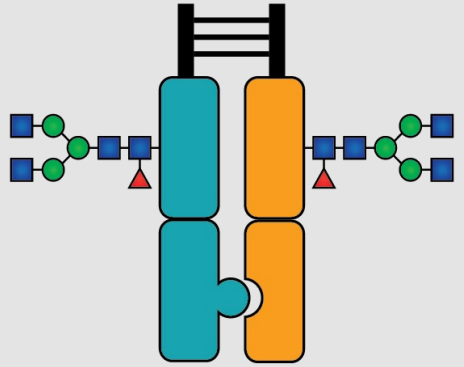
- Fc is globally more stable than individual Fab components
- Deglycosylation leads to greater destabilization in the second unfolding transition per CIU50-2 analysis
- Unmodified Hole component less susceptible to unfolding





- Unfolding of hole component dominates

Fab



- Unfolding of knob component dominates

Fc

Conclusions and Future Directions

- IM-MS combined with CIU permitted us to make structural connections between BsAb and its parental homodimer constructs
- Accomplished annotation of unfolding pathways using a middle level approach as well as patterns in deglycosylated samples
- Able to probe which component of the BsAb was unfolding
- Next step: expanding the IM-MS and CIU pipeline to BsAbs produced using other technologies

Acknowledgements

Ruotolo Group

- **Prof. Brandon T. Ruotolo**
- Dr. Varun Gadkari
- Dr. Cara D'amico
- Kristine Parson
- Carolina Rojas Ramirez
- Yilin Han
- Brock Juliano
- Chae Kyung Jeon
- Iliana Levesque
- Anna Anders
- Devin Makey
- Steven DeFiglia
- Victoria Tran
- **Henry Li**
- Joe Keating
- Mengdao Li

Bristol Myers Squibb

- Dr. Thomas Slaney
- Dr. Guodong Chen
- Dr. Li Tao
- Dr. Naresh Chennamsetty

CIUSuite 2 QR

NSF

CHEMISTRY
UNIVERSITY OF MICHIGAN

Bristol Myers Squibb

RACKHAM GRADUATE SCHOOL
UNIVERSITY OF MICHIGAN