Collision Induced Unfolding:

Rapid, Sensitive, and Information-Rich Protein Stability Measurements

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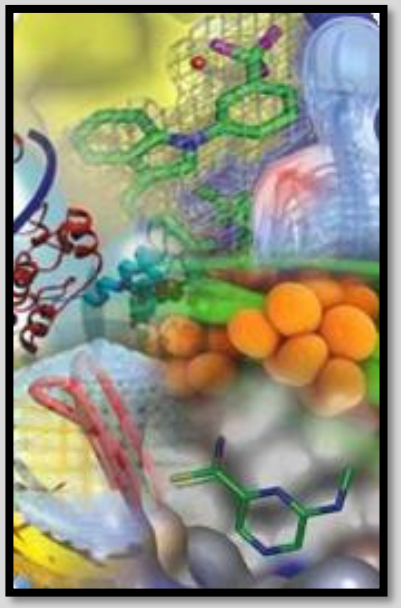
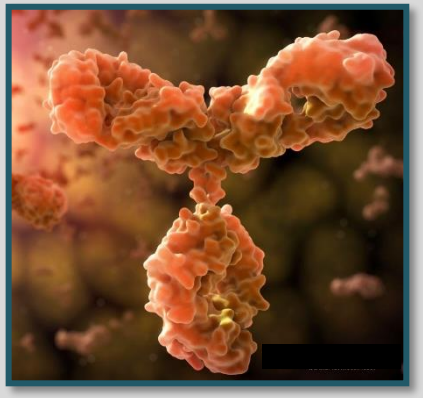
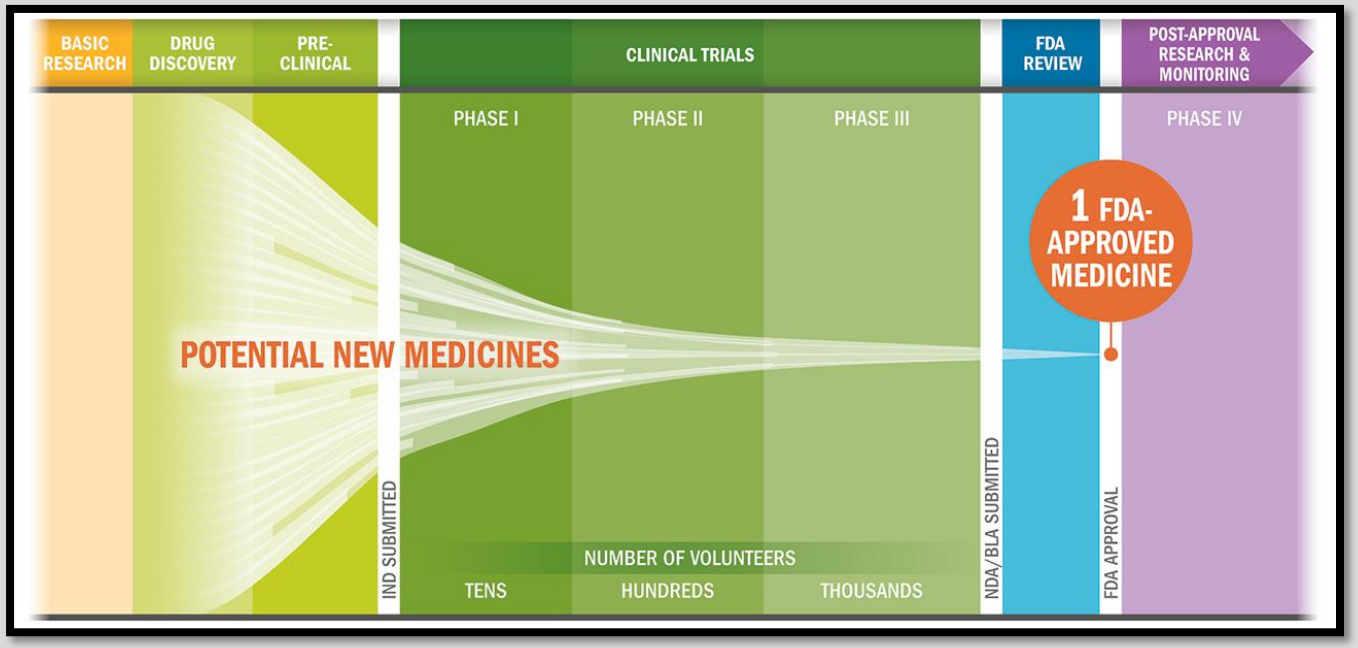
Drug Discovery: A Complex Problem



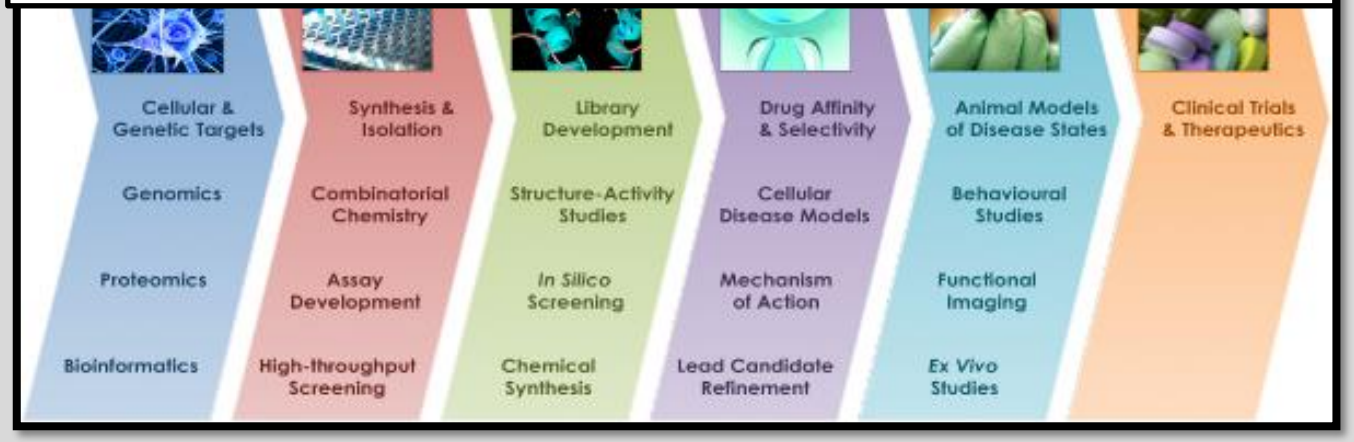
The task of drug discovery is exceedingly complex

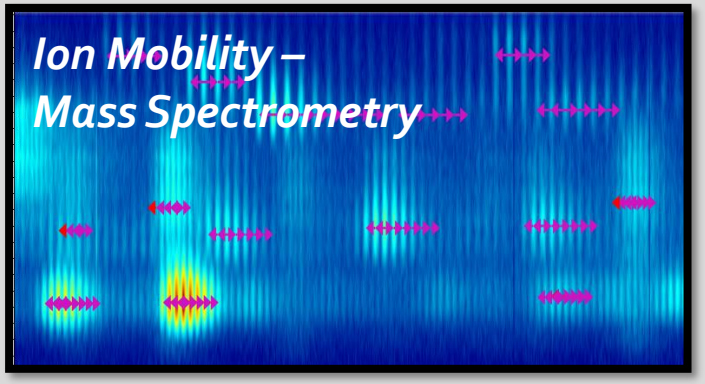


The complexity of these tasks are magnified for biotherapeutics



*With an available **small molecule chemical space of 10^{20}**, and many criteria that new drugs must satisfy, hundreds of thousands of candidates are often screened in order to find a single approved pharmaceutical, costing on **average $2.6B and taking 10 years to complete**.*

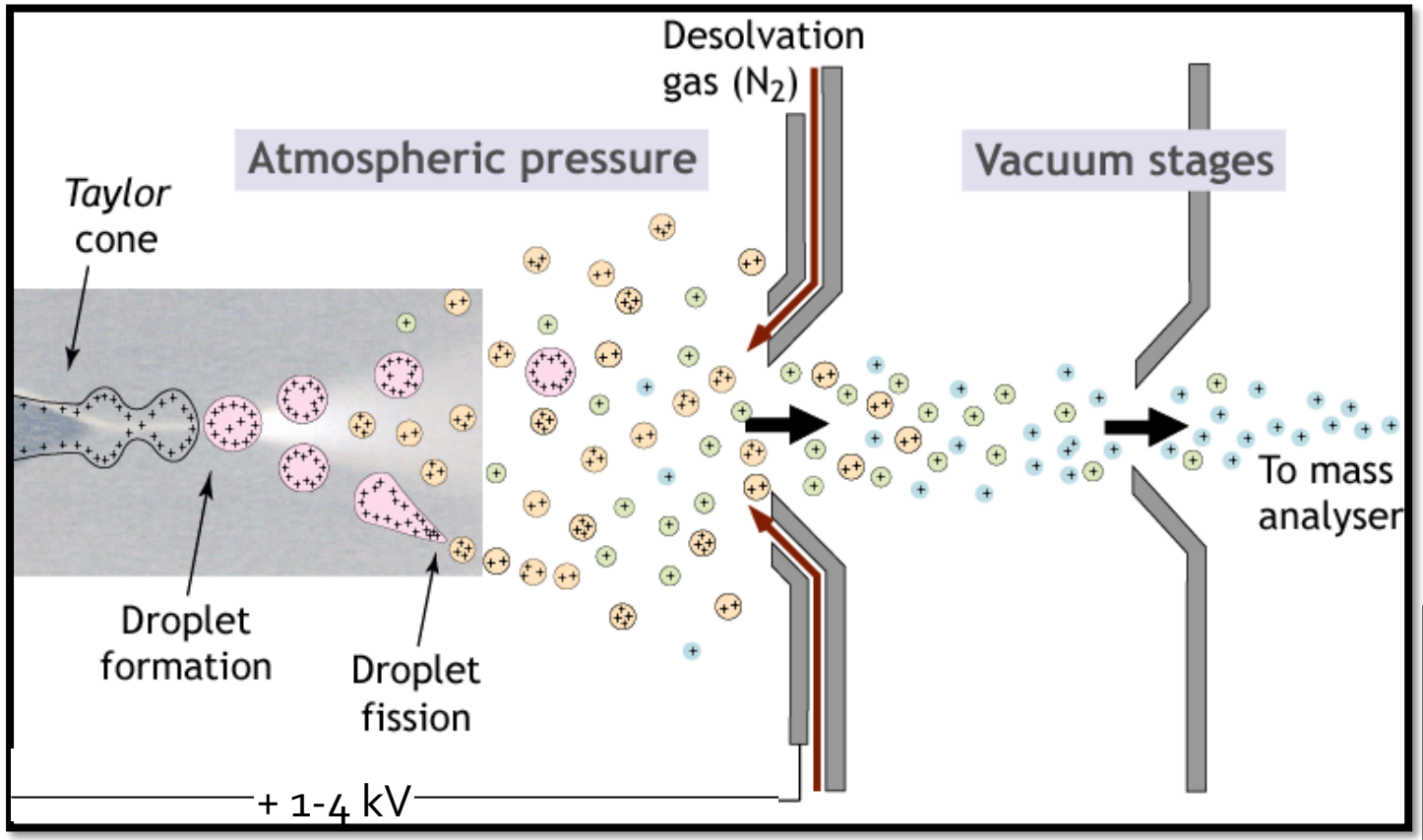


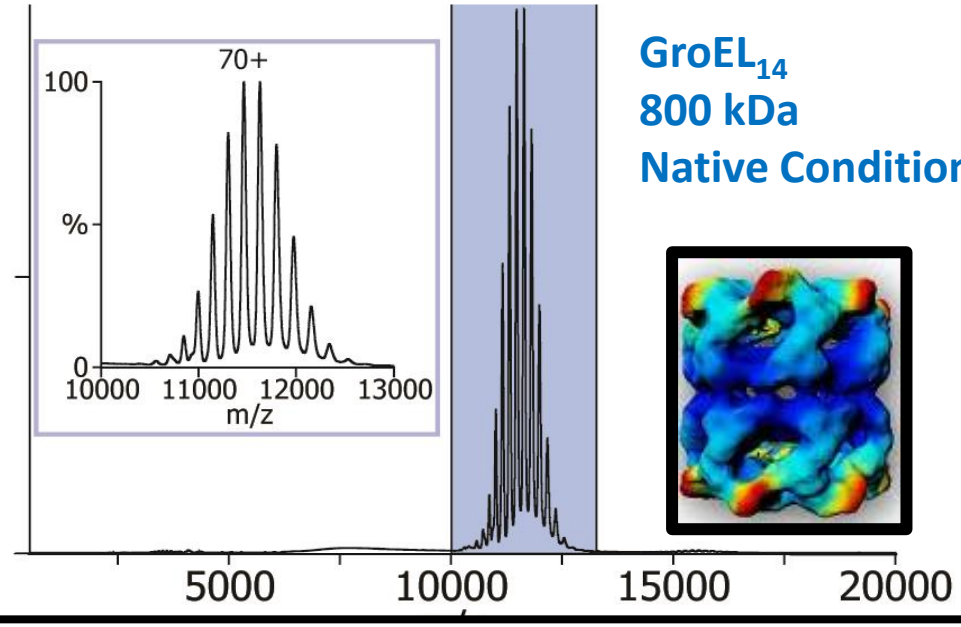


Structurally-sensitive forms of mass spectrometry (e.g. IM-MS) can perhaps fill these gaps, but faster, more information-rich methods must be developed and validated

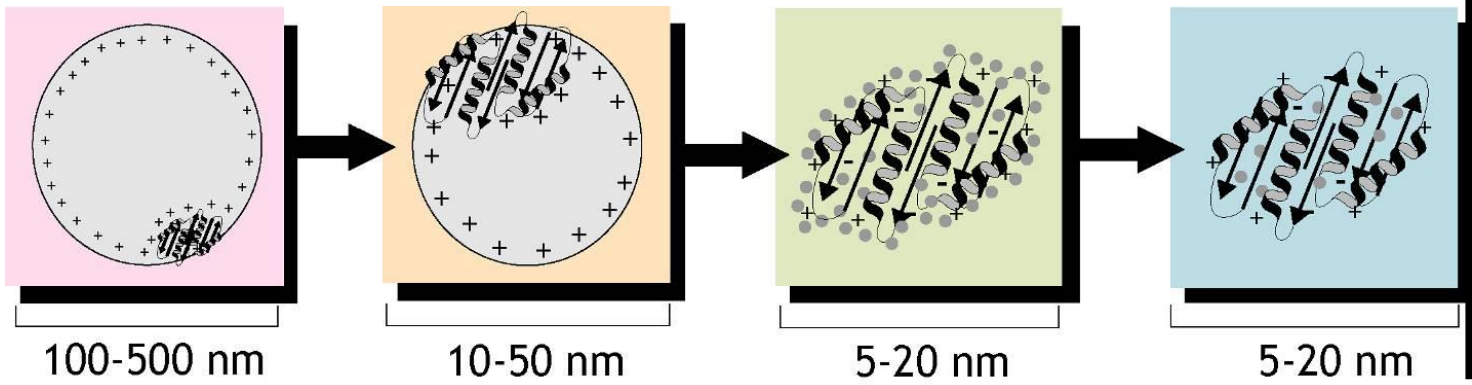
Many technologies are brought to bear on this process, but many gaps remain. Chief among these are the ability to ascertain the molecular mechanisms of active lead compounds quickly. This often requires structure/stability measurements to be taken on small amounts of unpurified protein – currently very challenging.

Native Mass Spectrometry: Basic Principles





$GroEL_{14}$
800 kDa
Native Conditions

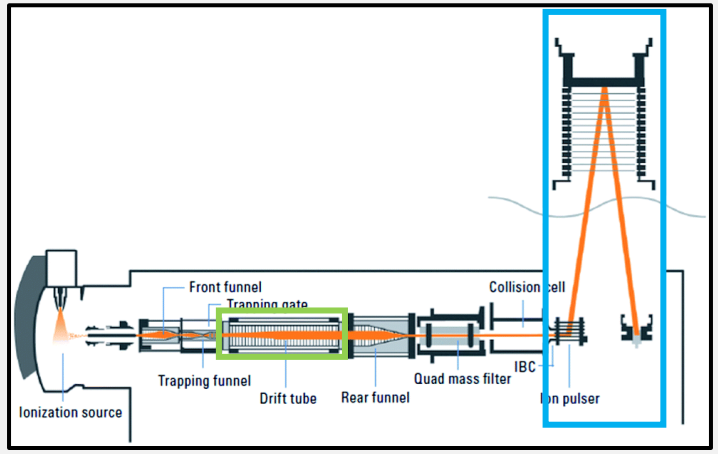


- Multiple classes of information can be extracted from Native MS experiments, for example:
  - Protein Oligomeric State(s)
  - Ligand Binding States / Stoichiometries
  - Binding Strength (K_D)

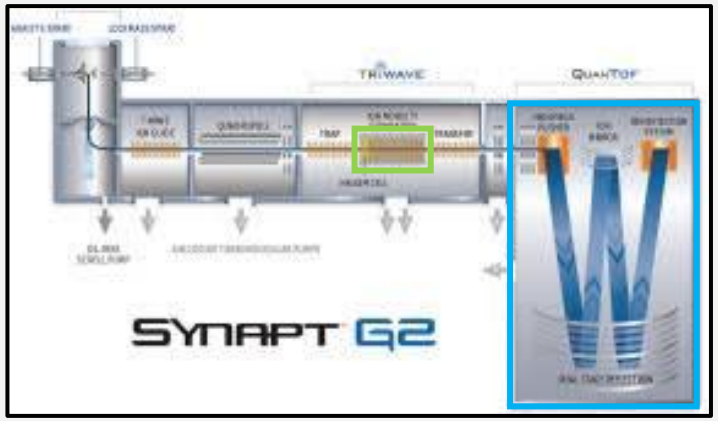
Ion Mobility-Mass Spectrometry (IM-MS)

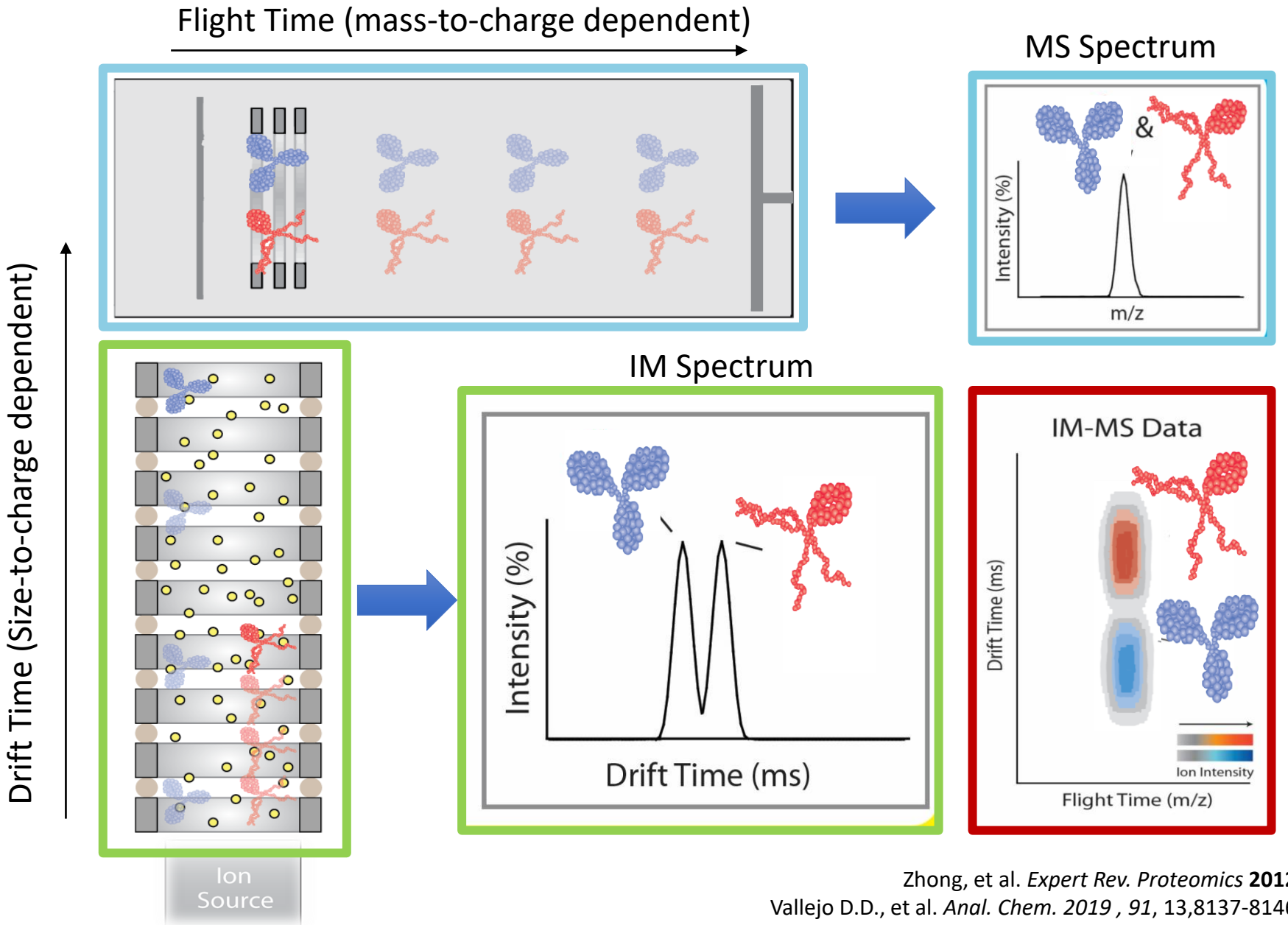
Two IM Platforms

Agilent 6560



Waters Synapt G2

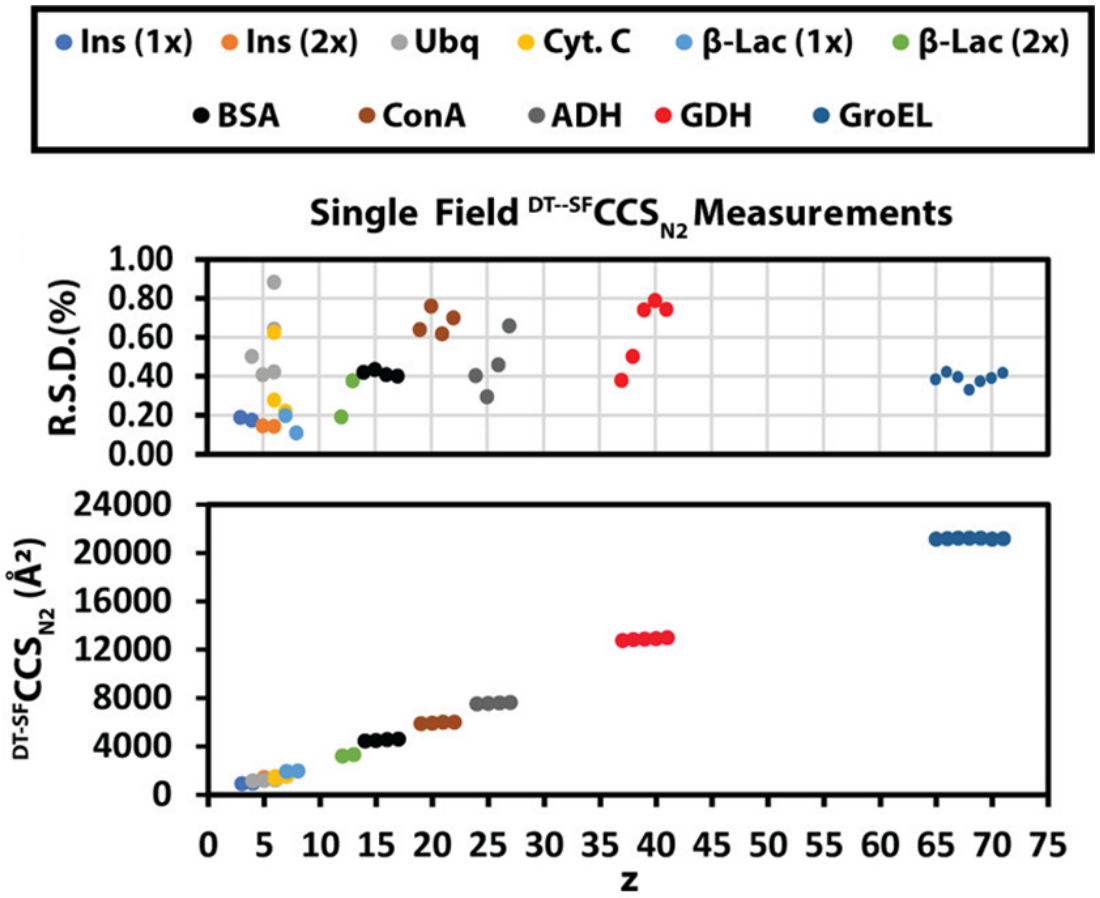


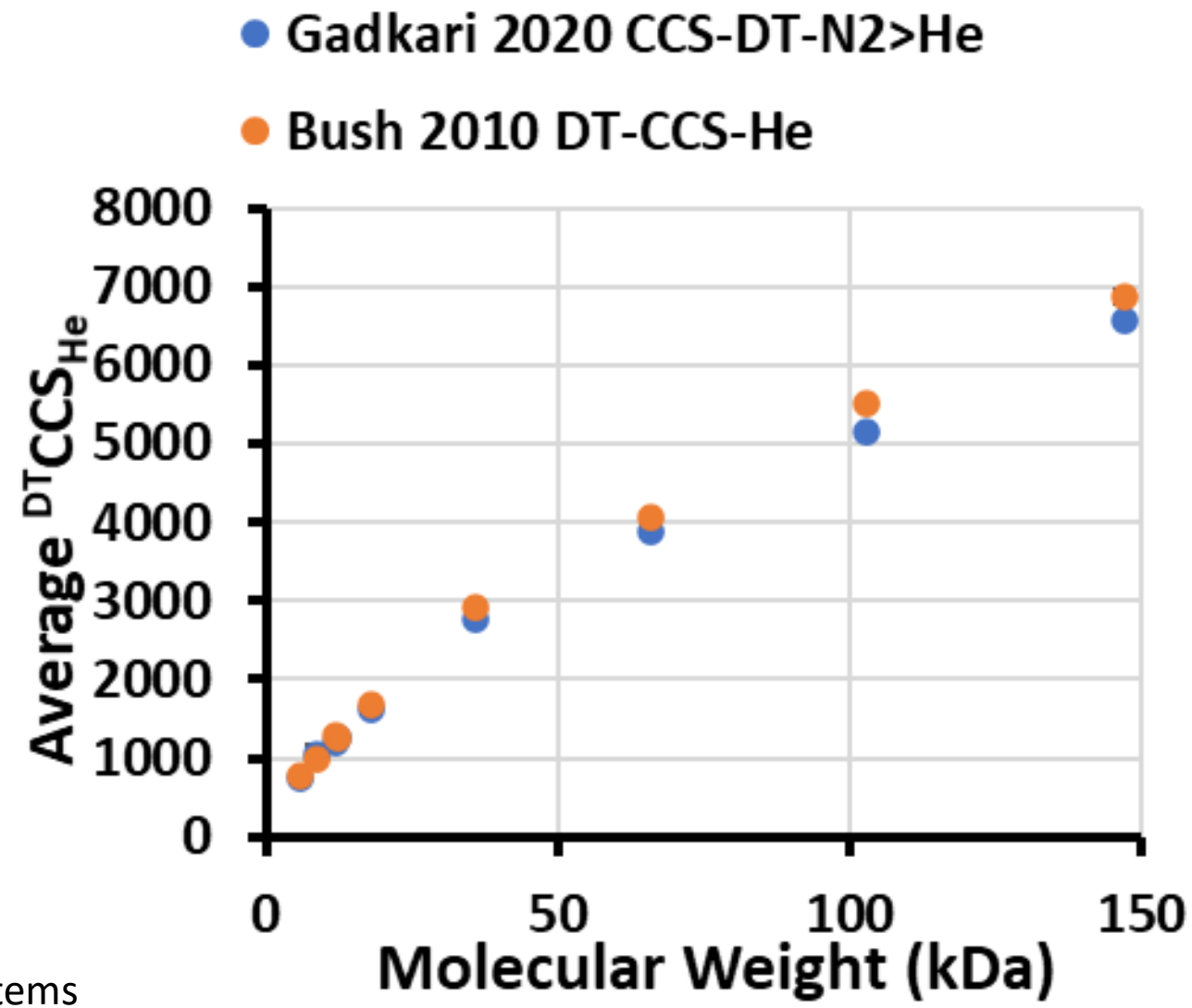


Zhong, et al. *Expert Rev. Proteomics* **2012**

Vallejo D.D., et al. *Anal. Chem. 2019 , 91*, 13,8137-8146

High Precision Collision Cross Section (CCS) Measurements



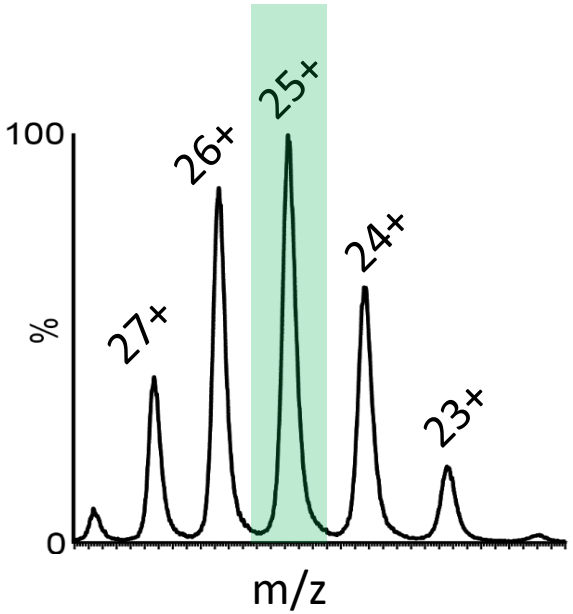


- <1% R.S.D. between replicate measurements on separate days
- Average R.S.D.: 0.43 ± 0.20 %
- Some systematic differences (~3%) between IM-MS Systems

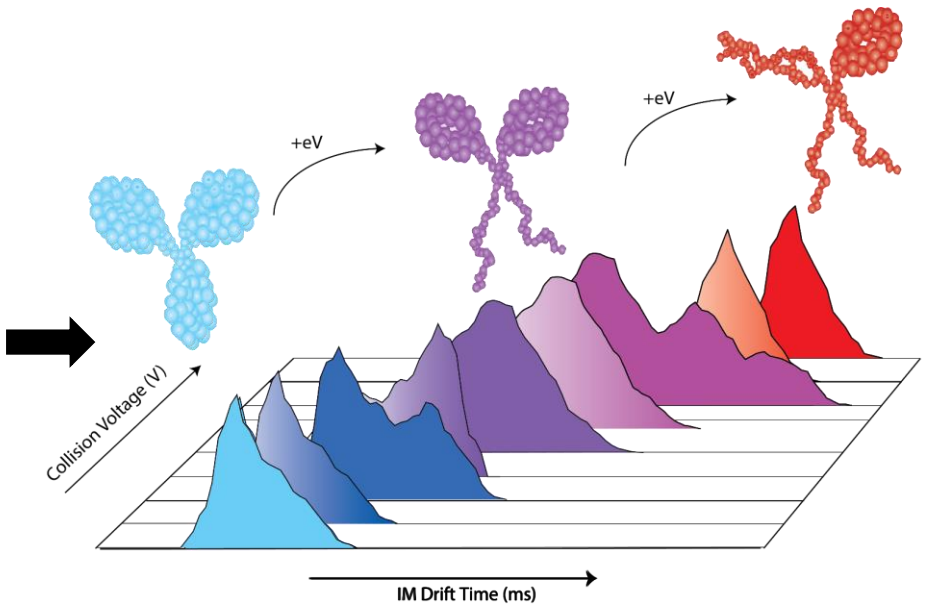
Gadkari V.V. et al. 2020. *Anal. Chem.* 92, 23, 15489–15496

Collision Induced Unfolding (CIU)

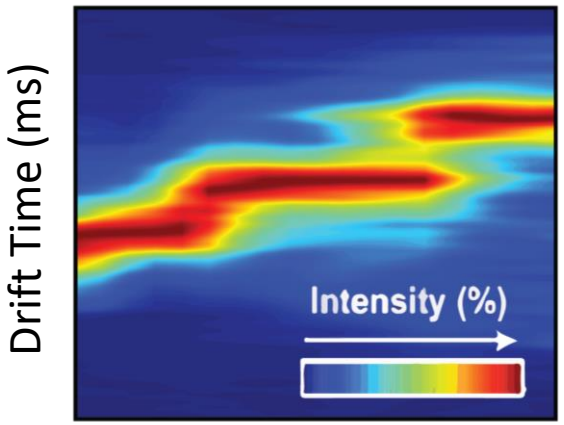
- CIU: Many IM measurements at varying energy



*Representative Native IgG Spectrum

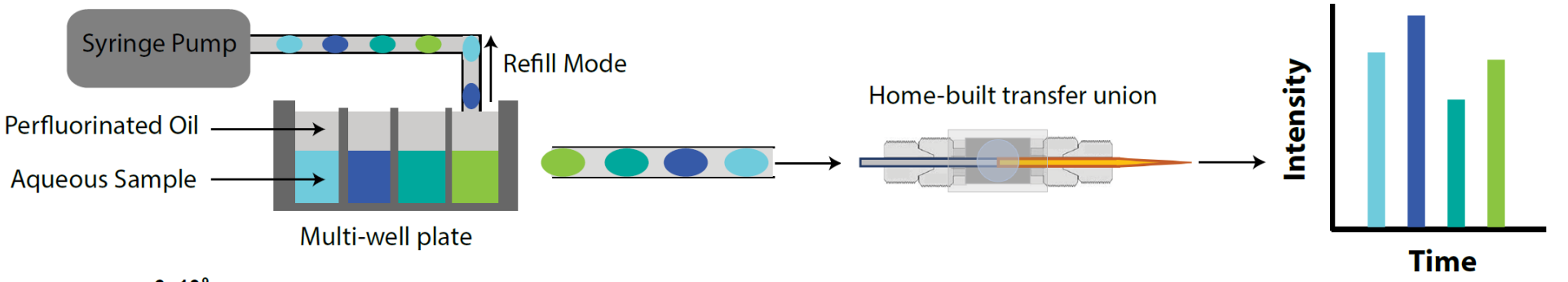


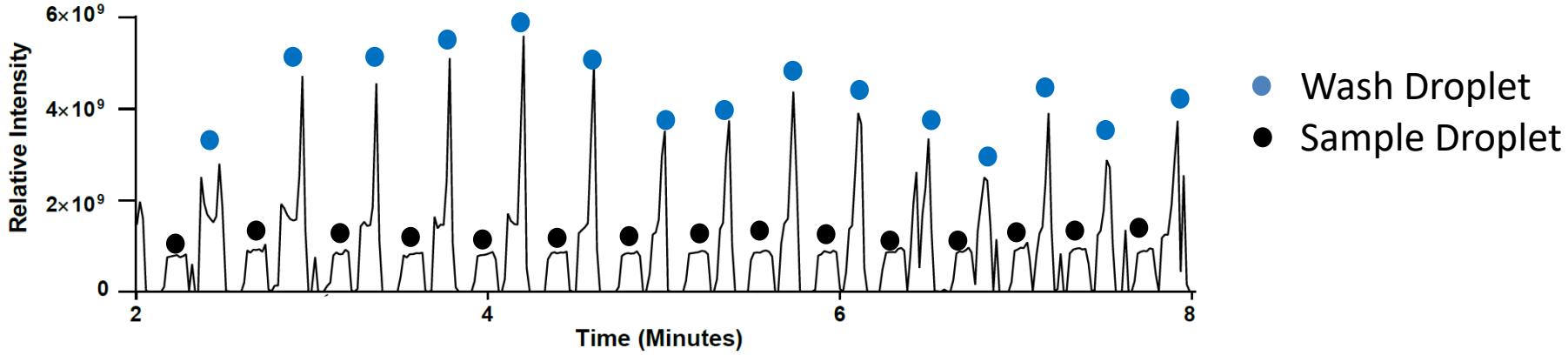
CIU "Fingerprint"

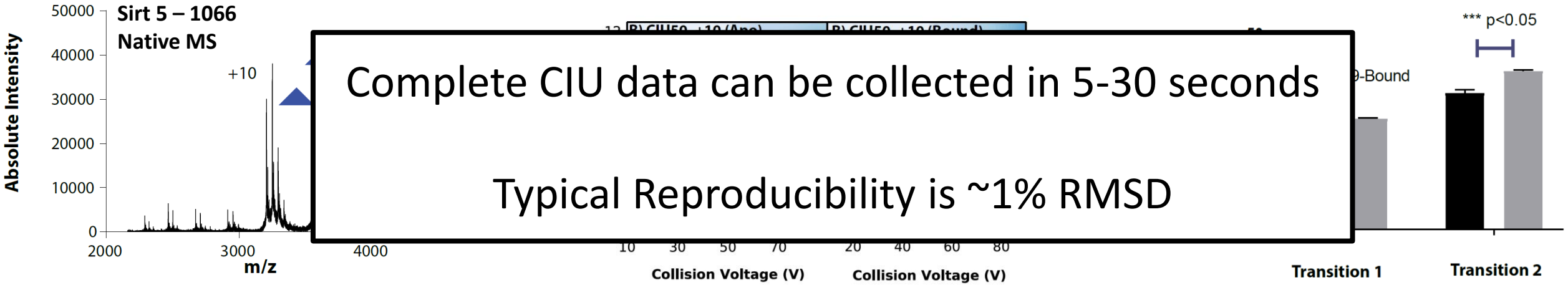


Adapted from Dixit S. M.*, *et al Chem. Biol.* 2018 42, 93-100.

CIU is Both Fast and Reproducible



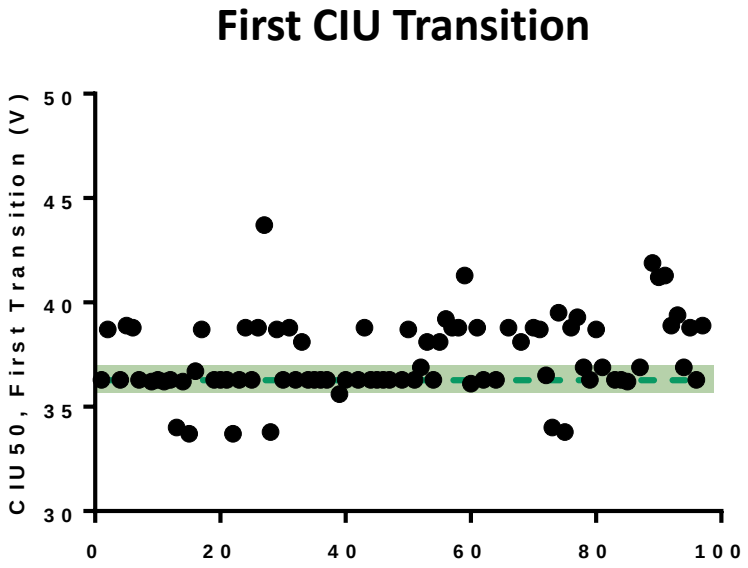


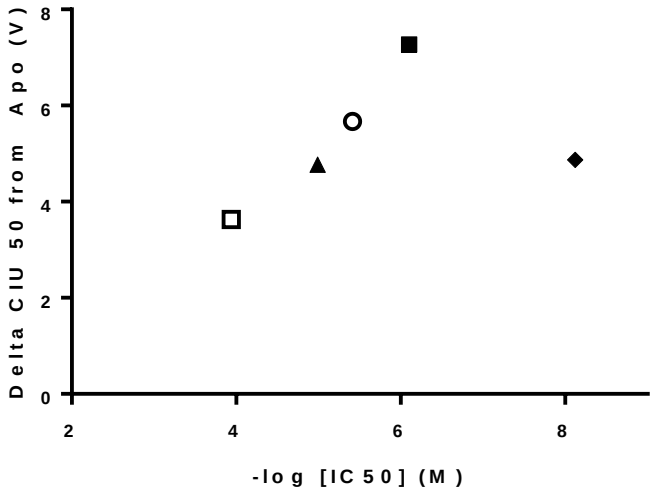


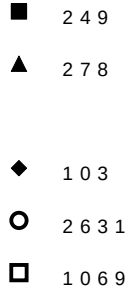
Complete CIU data can be collected in 5-30 seconds

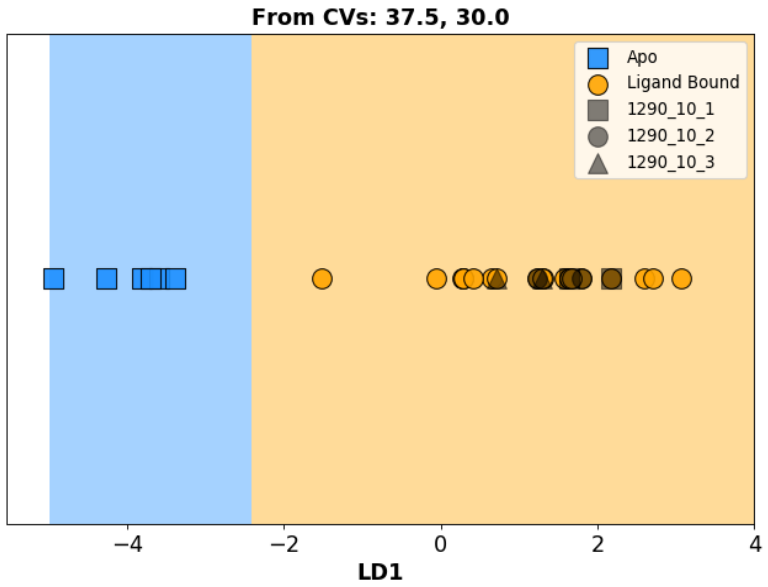
Typical Reproducibility is ~1% RMSD

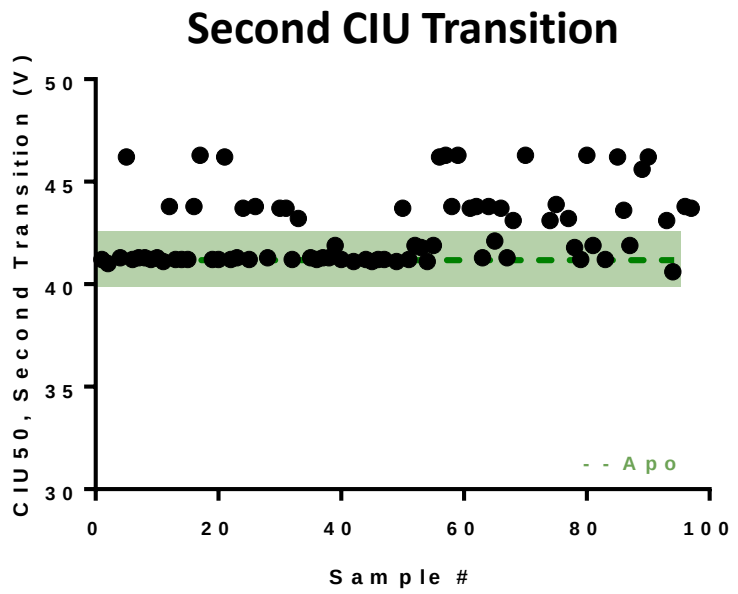
A 96 Compound Sirt5 Inhibitor Screen using CIU

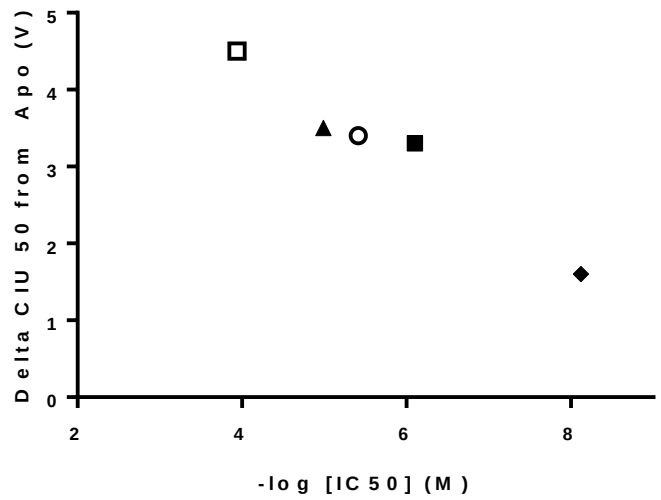


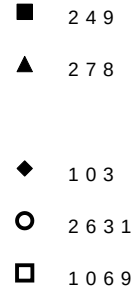












- **~60min** for 96 compound screen (10s per sample, performed in triplicate, and including wash steps between samples)
- Green area represents apo Sirt5 CIU response +/- 3σ
- All known inhibitors, and inhibitor analogues, **increased Sirt5 stability in a manner highly correlated with IC50 values**
- Semi-automated data interpretation possible with **CIUSuite2**, including ML classification

Collaboration with Kennedy Lab (UM)

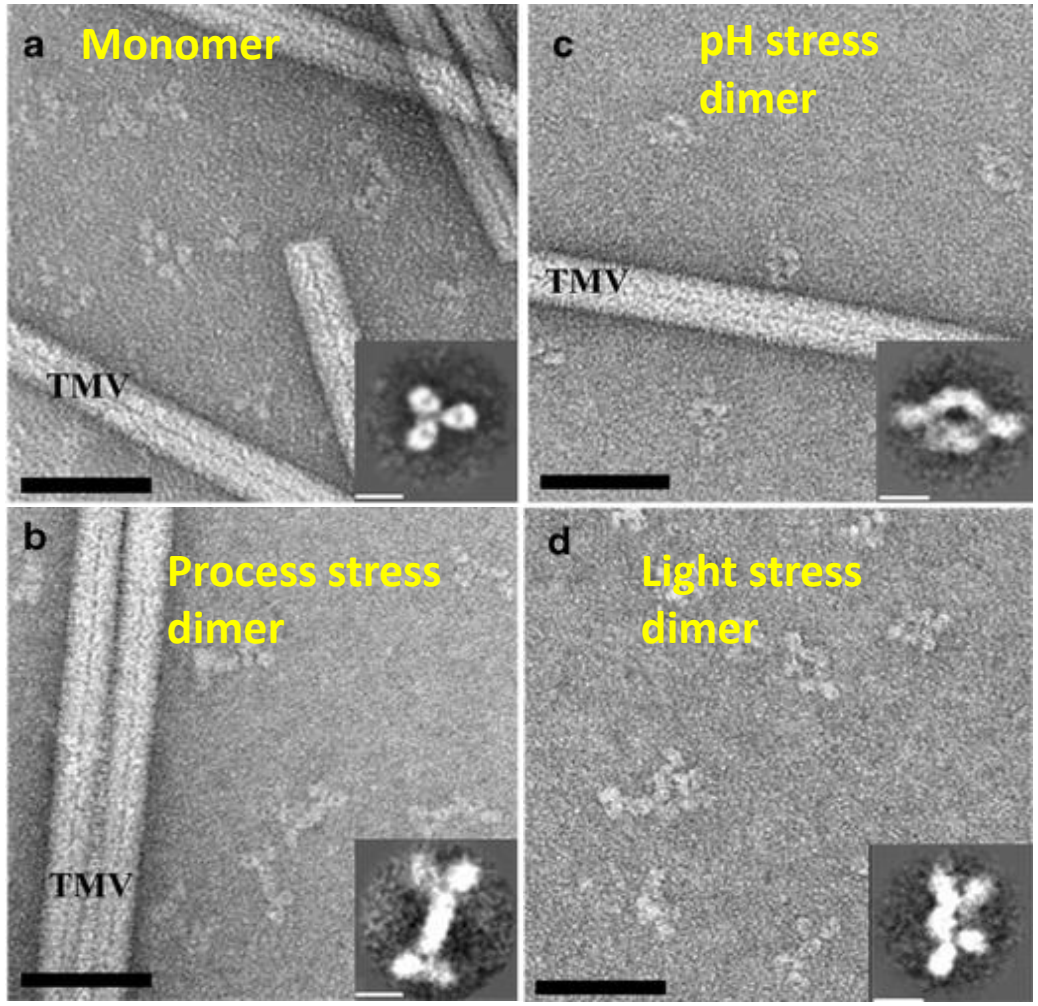
Stress Induced Aggregation

- Several stress factors throughout the production process may induce antibody aggregation:
  - Heating
  - Mechanical
  - pH change
  - UV light

Impact Drug Efficacy & Safety

Goal: Understand Mechanism of Aggregation by Stress

Negative stain TEM images

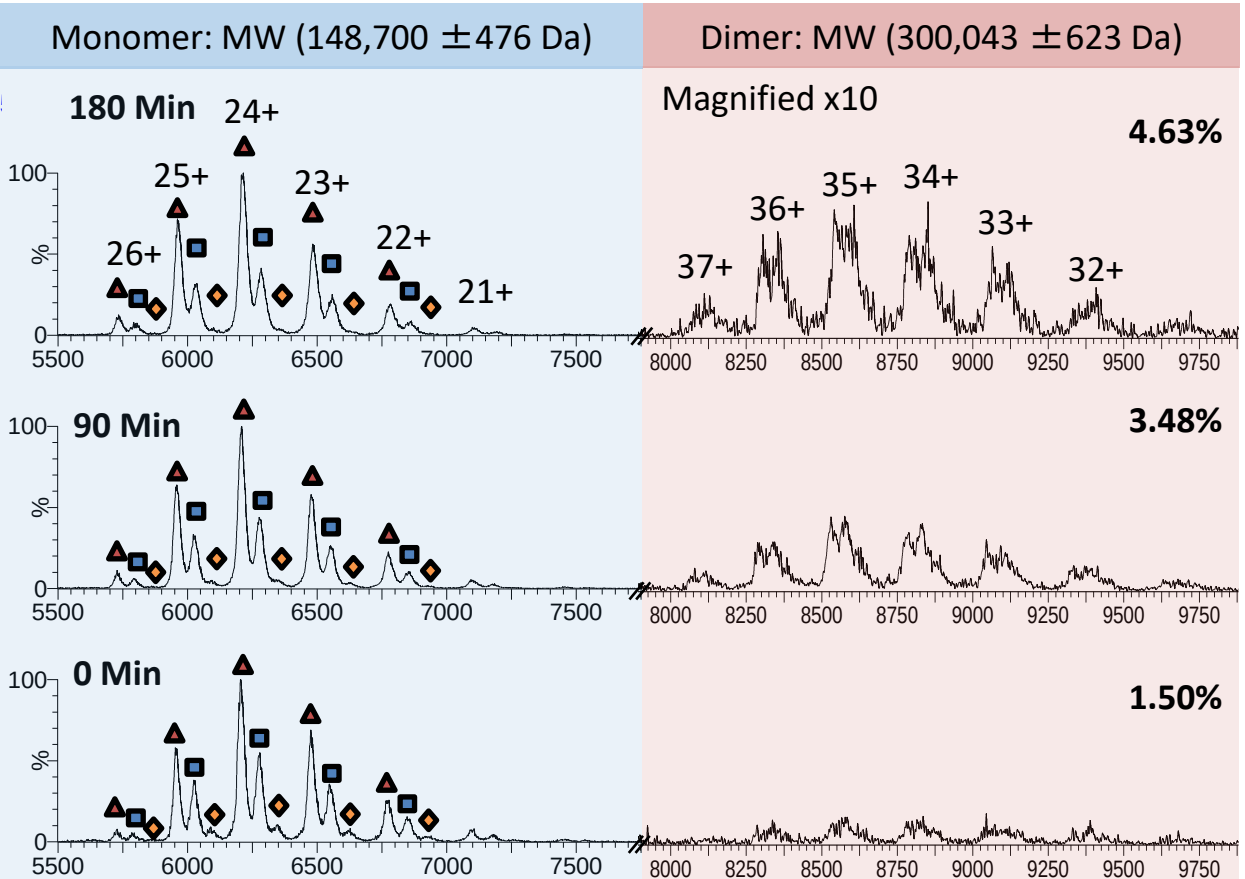


Paul, R. *et al. Pharm. Res.* **29,** 2047–59 (2012).

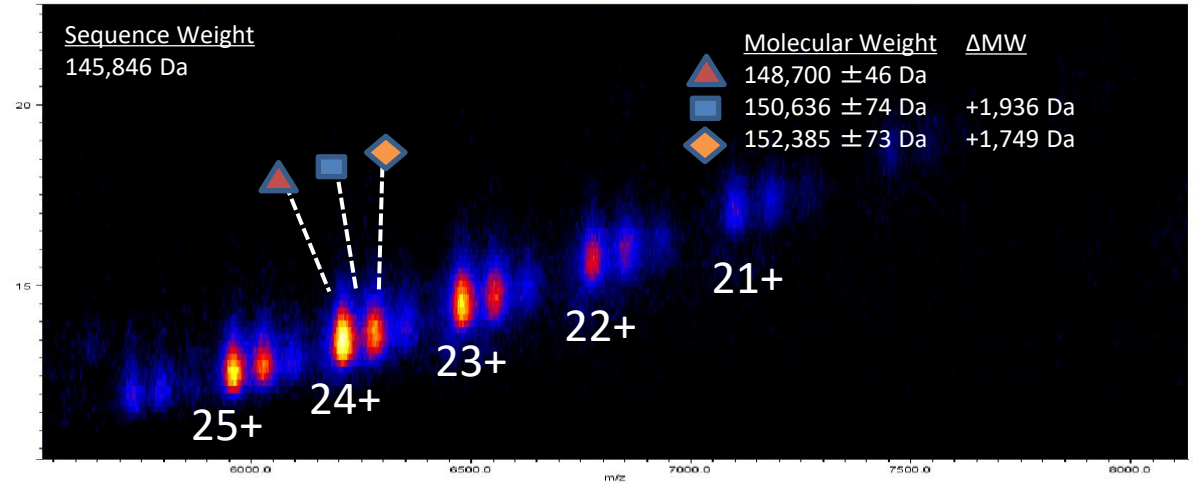
In collaboration with Joe Eschweiler, Abbvie

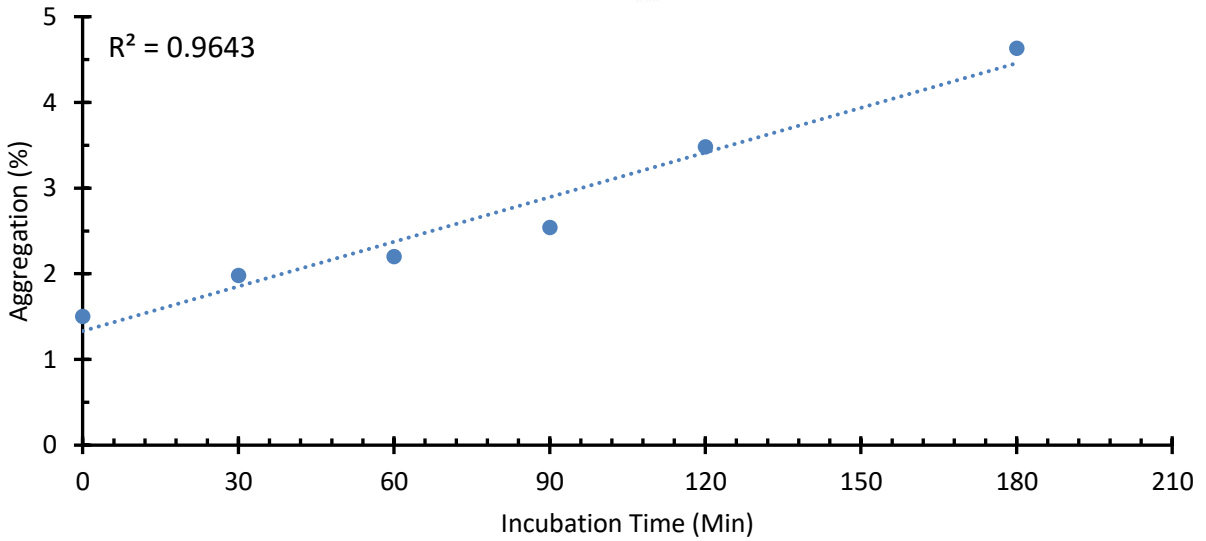
Antibody Aggregation Probed by IM-MS

- In L-histidine formulation buffer
- Dimer aggregation from 50°C Incubation can be quantified through mass spectrum
- Aggregation time provides a good linear correlation
- Intact mass calculation reveals glycoforms



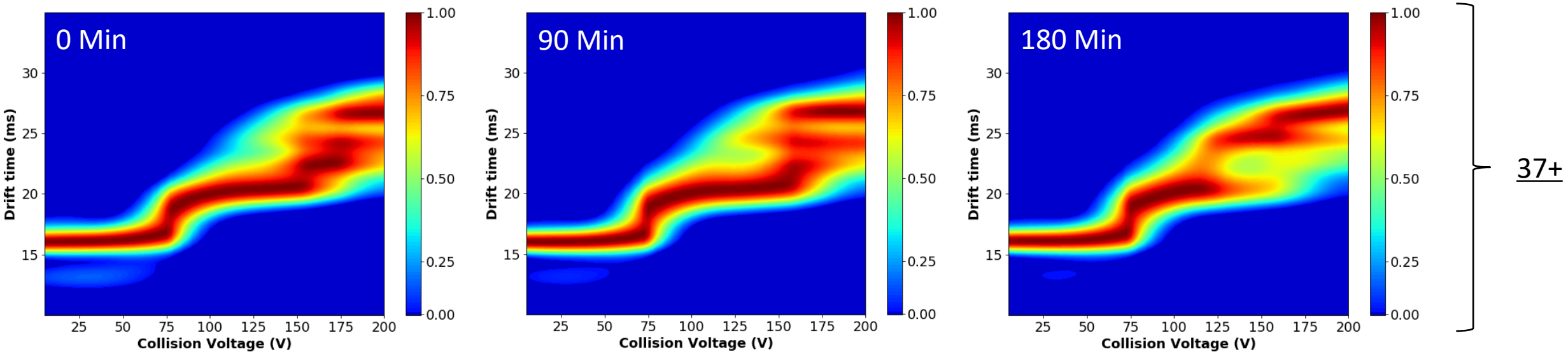
Monomer Series

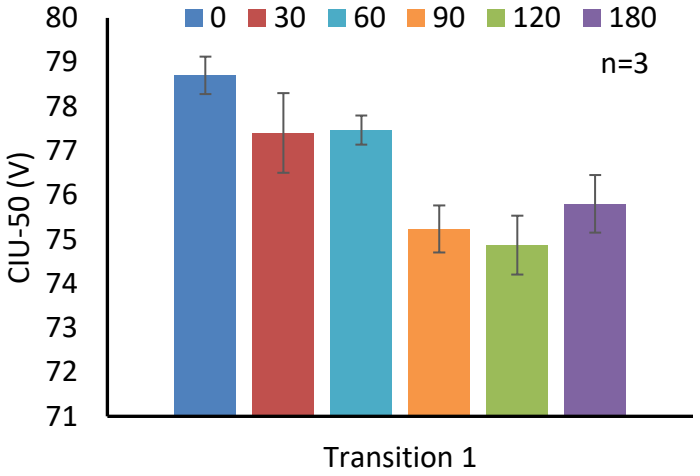


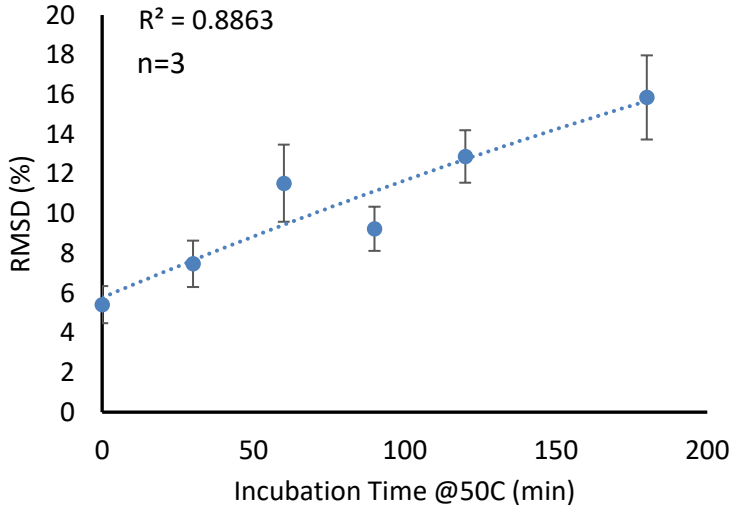


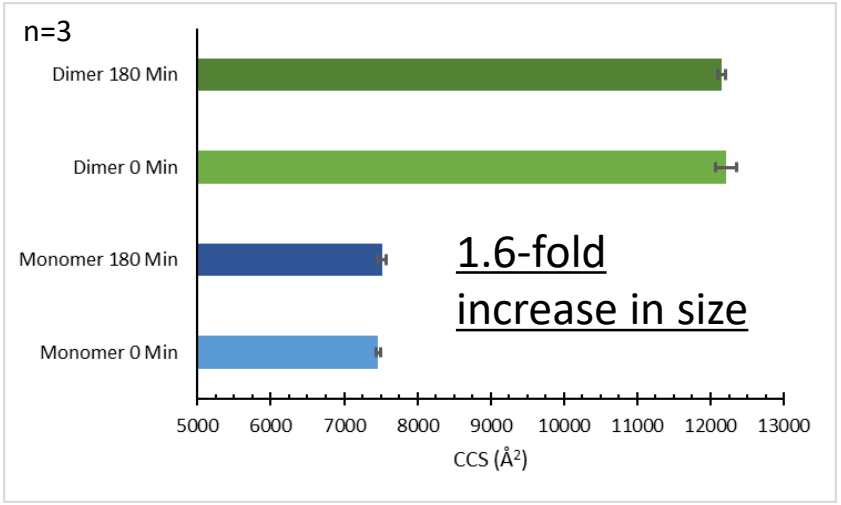
IgG1 Model Antibody: Dimer and Quantitative Changes

- Qualitative changes in dimer fingerprints over heat stress time course
- CIU50 shows tractable destabilization, and RMSD provides correlation with incubation times.







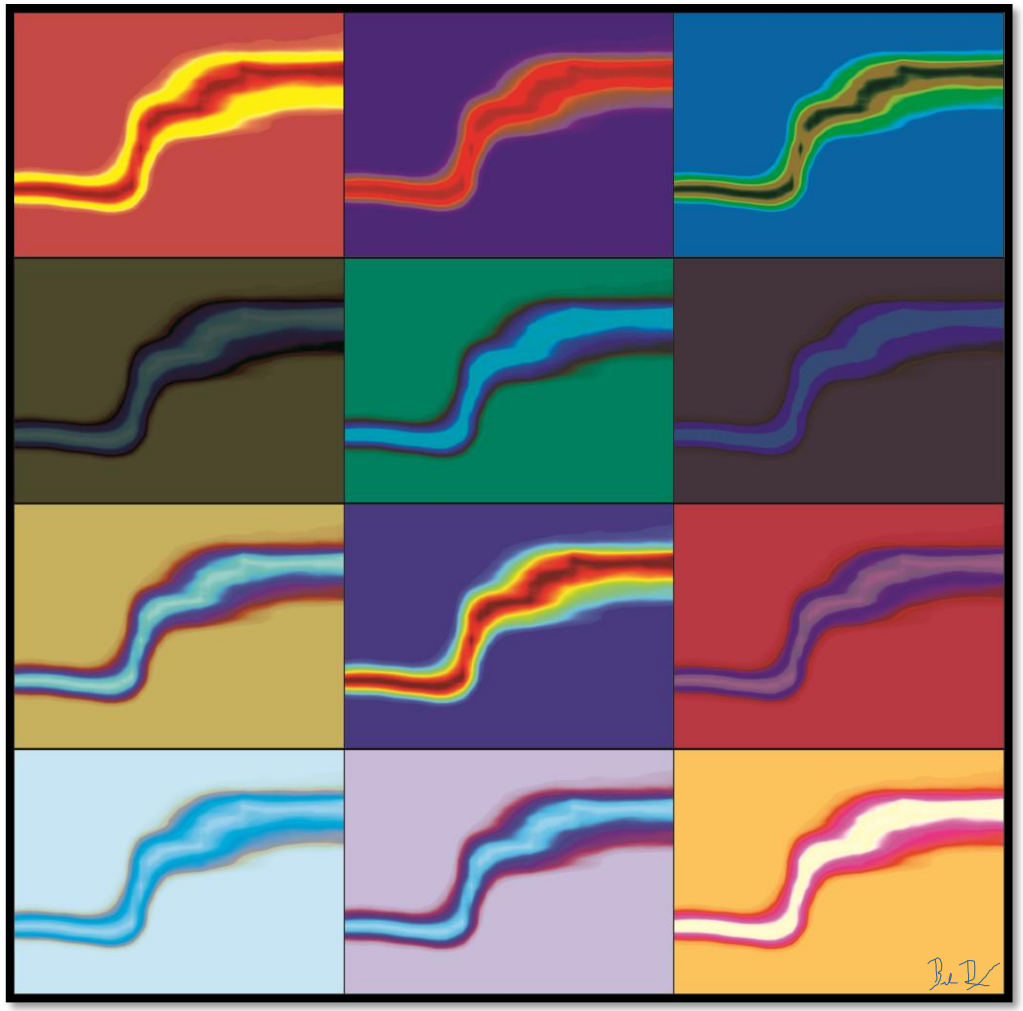


Conclusions

- Structural Mass Spectrometry tools are growing rapidly in terms their utility for rapidly analyzing complex protein mixtures and extracting valuable structural/biophysical information

- CIU is a rapid, potentially transformative tool for measuring protein stabilities within mixtures, requiring small amounts (5 μL of sample at ~1μM), without the need for labeling.

- Our efforts with both biotherapeutics and small molecule drug targets highlight the molecular-level mechanistic detail that can be revealed through quantitative CIU analysis.



Acknowledgments

Ruotolo Group

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- Joe Keating

Abbvie

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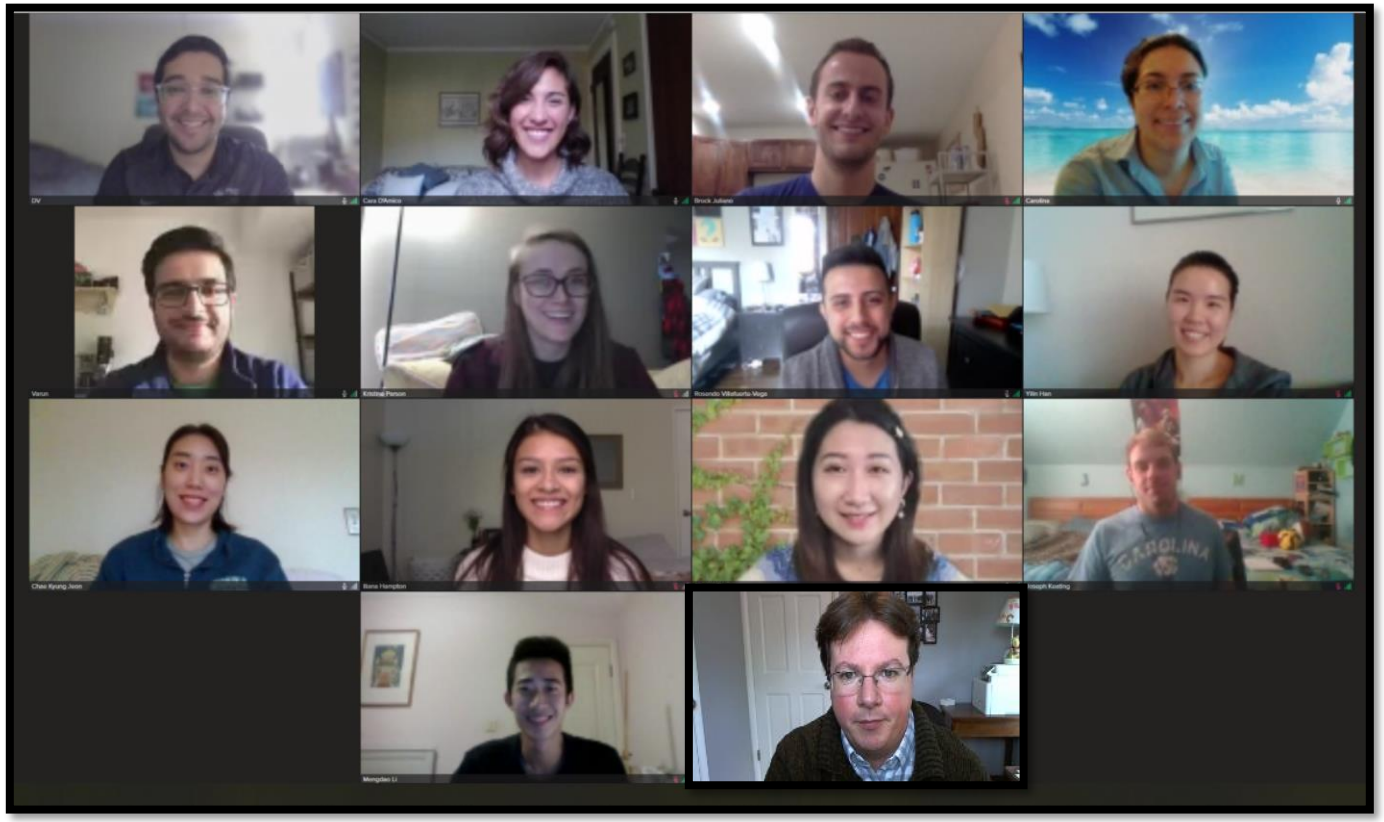
Robert Kennedy Group (UM)



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CENTER FOR RESEARCH ON COMPLEX GENERICS at the University of Maryland, Baltimore and the University of Michigan