Rapid Online Buffer Exchange with the **DynaChip X1** Platform for Complex Biological Analysis

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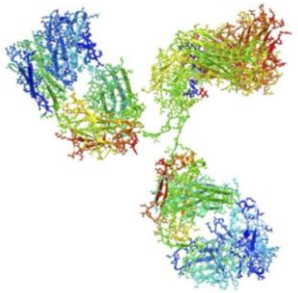
Overview

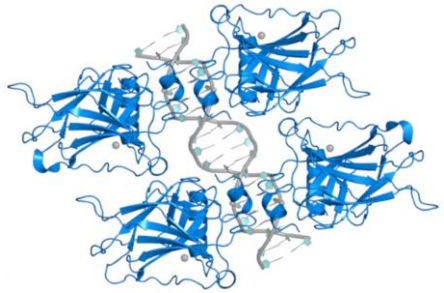
- Background
- DynaChip X1 Platform
- Results and Applications

Structural analysis is the key to biological insight for therapeutics

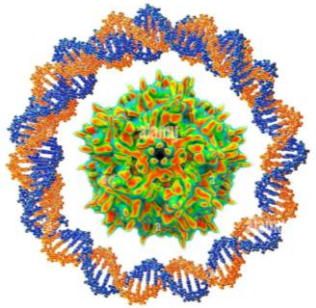
Native Mass Spec & CDMS are emerging as a powerful tools for structural analysis of complex biomolecules



mAbs



Disordered Proteins (IDPs)

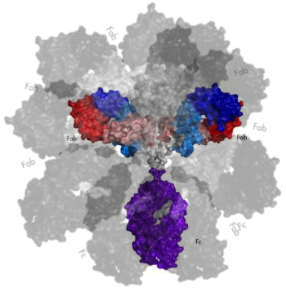


AAVs

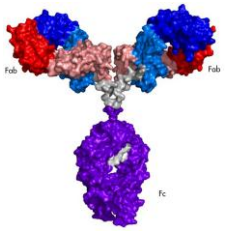
Mass Spec Challenges

1) Sample Prep—Cleaning the sample

Hours per sample to remove buffers, salts, detergents, etc.



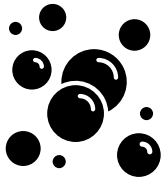
Dirty



Clean

2) Sample Introduction—Injection into the mass spec

Days to weeks fixing “plumbing issues”



Bubbles

Clogs

Leaks

DynaChip X1 enables fast and simple mass spec

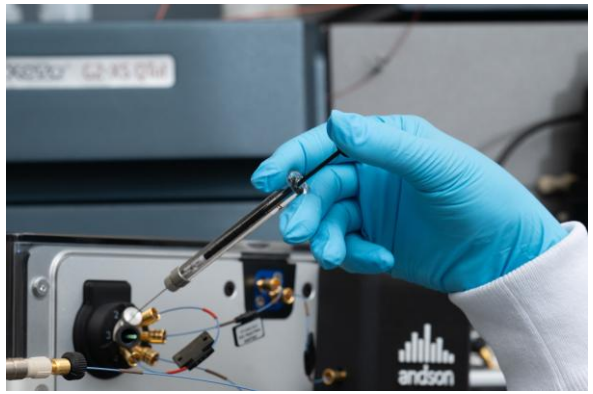
- **Sample cleaning** – High efficiency; Non-destructive
- **Sample injection** – Push-button operation; Results in minutes

DynaChip X1 Workflow

Enables both on-demand and automated analysis

1 Load Chip

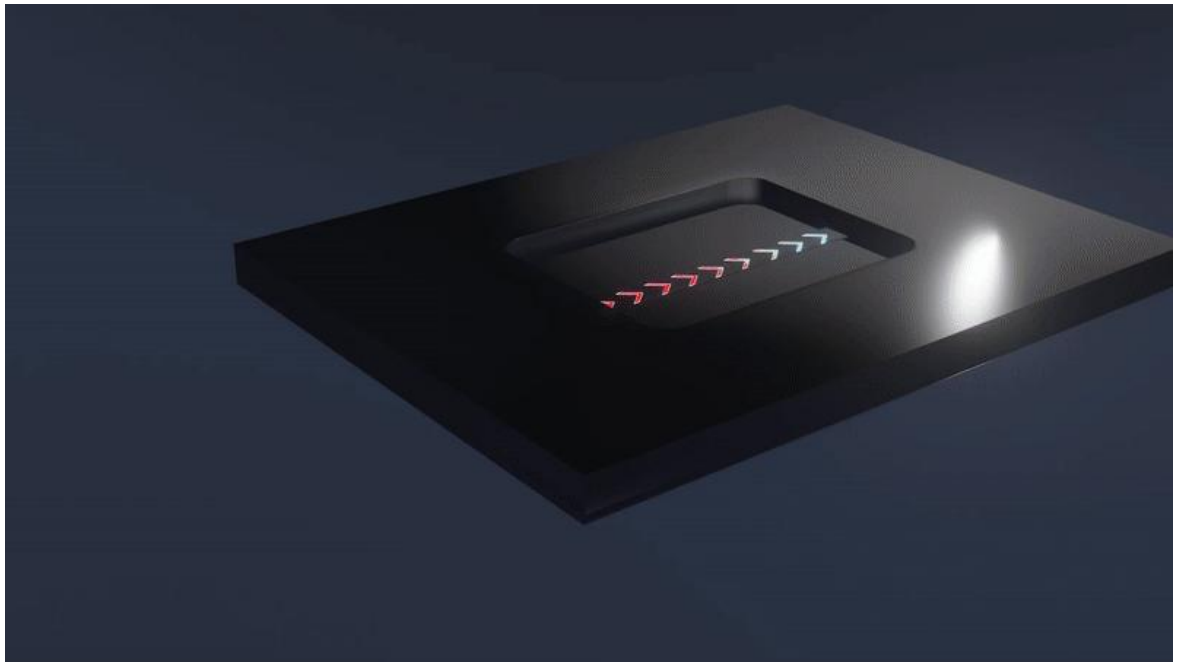
2 Inject Sample



On-Demand

Automated

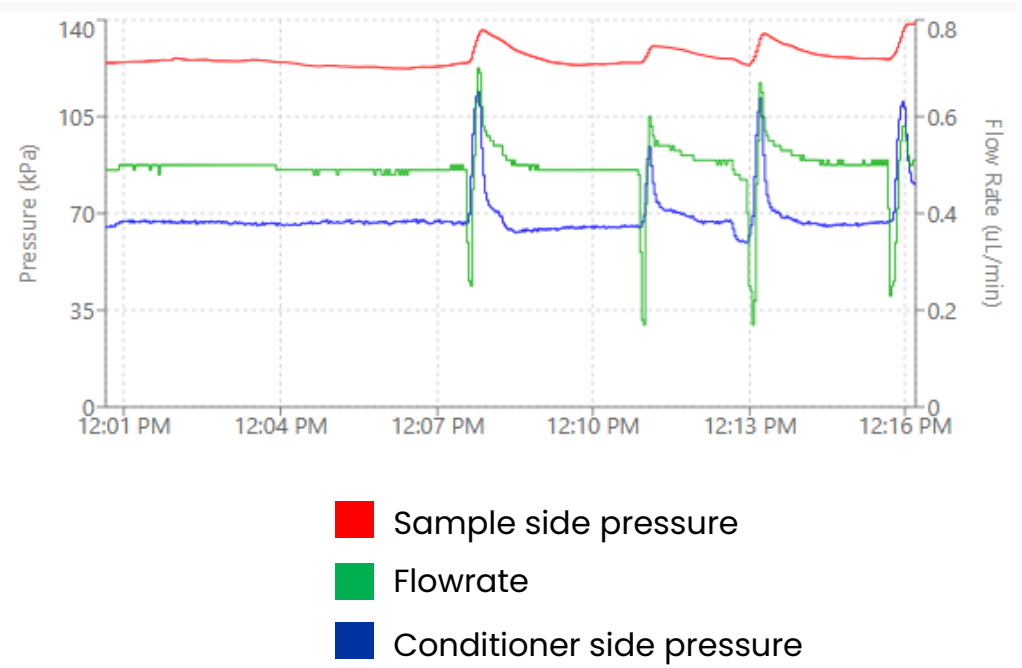
3 Results in Minutes



Fighting Bubbles, Clogs, and Leaks

- Real-time readouts: pressure/flow rate
- Automated alerts of system instability
- Identify “where” the issue is
  - Emitter
  - DynaChip Cartridge
  - Tubing and/or fitting
  - Other

Real-Time Readout –
Fused silica clog in union



Universal Compatibility

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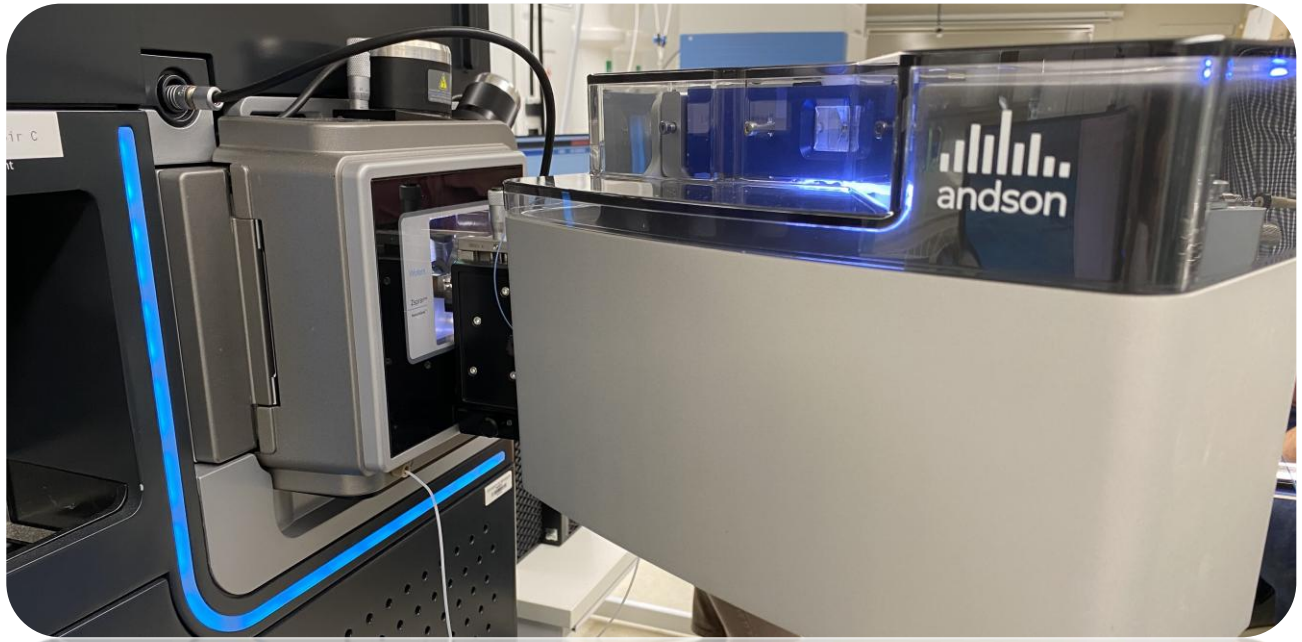

Thermo SCIENTIFIC

Waters™

Agilent Technologies

Megadalton Solutions

BRUKER

SCIEX
Coming soon

TrueMass Spectrometry

Roll-up integration with most commercial nanoESI interfaces

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DynaChip X1 Platform

Fundamentals & Capabilities

Selected List of Reagents Removed

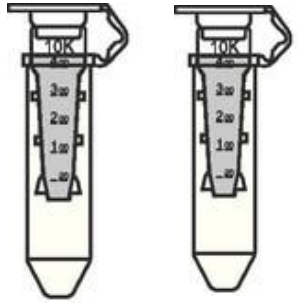
Reagent	Concentration Tested
Ammonium sulfate	500mM
★ NaCl	3M
KCl	2.7mM
Sodium Phosphate	20mM
★ Urea	8M
Glycine	250mM
Histidine-HCl	20mM
Imidazole	17.5mM
★ PBS	1X
Tris	80mM
HEPES	10mM
Sucrose	25mM

Reagent	Concentration Tested
Hexylene glycol	1%
Formamide	70%
Pluronic F-68	0.1%
ASB-14	300uM
C8E4 Detergent	2X CMC
MEGA-8	1mM
LADO	0.05%
Lauryl Maltose Neopentyl glycol	0.01%
Cholesteryl Hemisuccinate	0.001%
And More	

★ = Data shown in this presentation

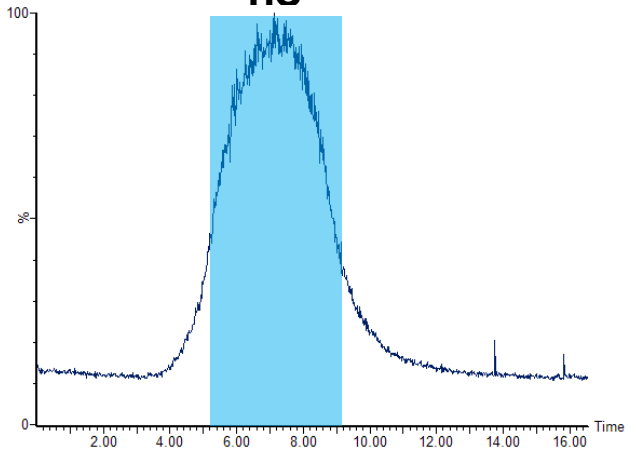
DynaChip X1 vs. Spin Filter

*Online clean up of **1x PBS** —NIST mAb*

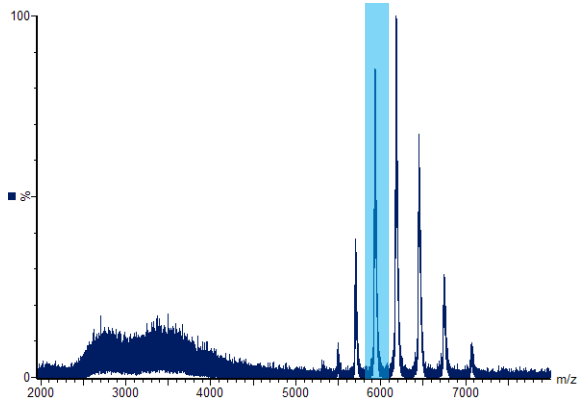


2X Spin Filter (30kDa):
60 min total

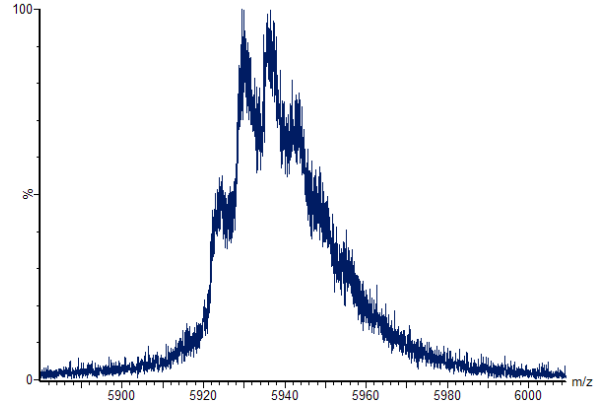
TIC



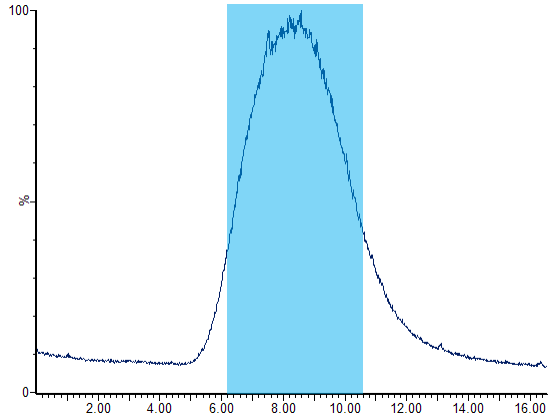
Charge State Distribution

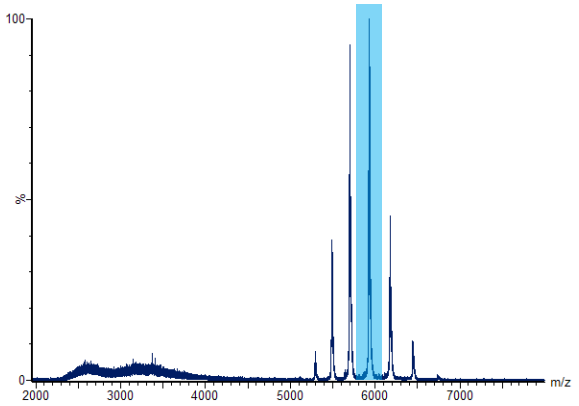


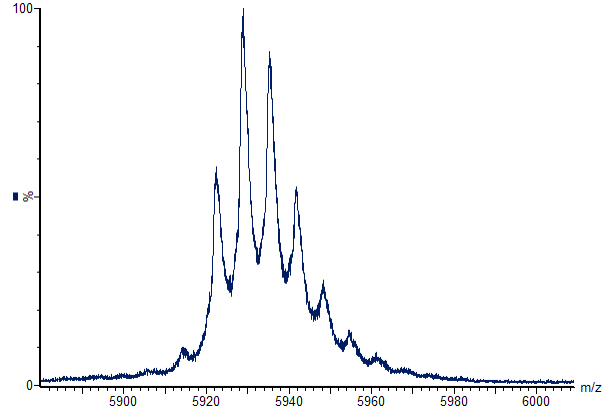
Glycoform Resolution



DynaChip X1:
15 min total run time







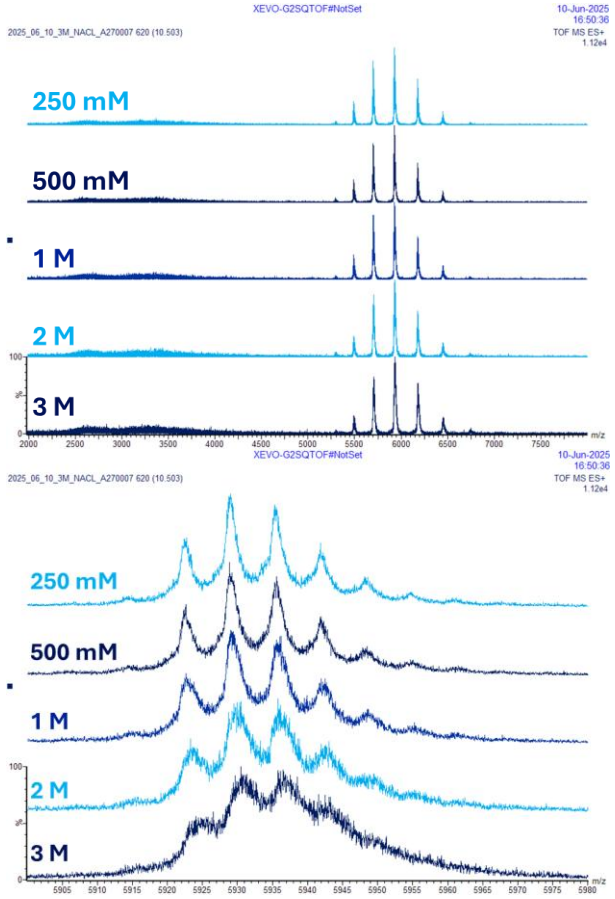
Data Acquired on: Waters Xevo G2-S Q-TOF

Exploring DynaChip Buffer Exchange Limits

Removal of 250 mM – 3 M *NaCl for Glycoform Resolved NIST mAb*

1.286g NaCl in 7.33 mL water

49% saturated solution



Full Spectrum

Single charge state zoom

- 0.1 mg/ml NIST mAb exchanged out of NaCl solutions

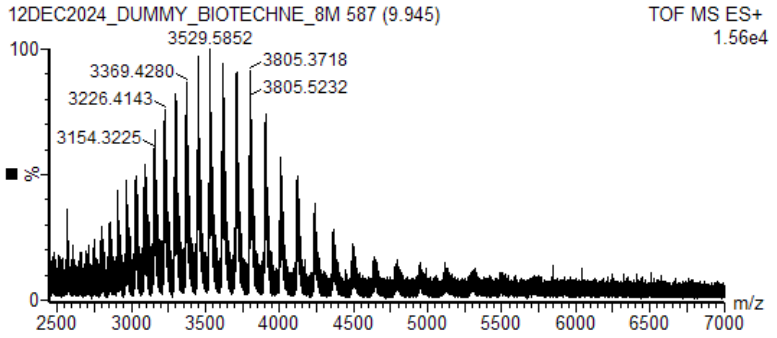
Data Acquired on: Waters Xevo G2-S Q-TOF

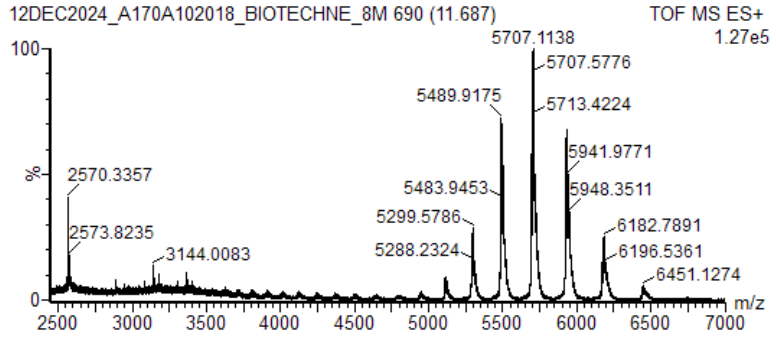
NIST mAb "Refolds" after Removal of 8M Urea

Direct Infusion

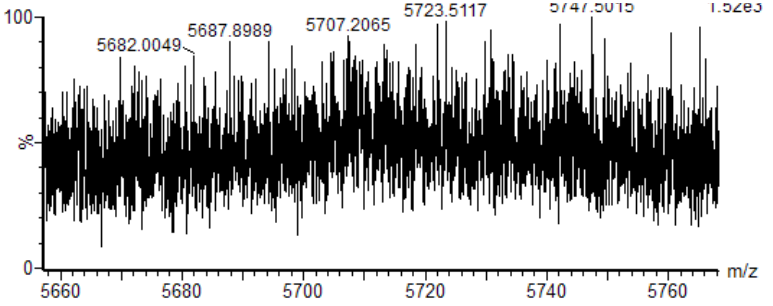
DynaChip X1

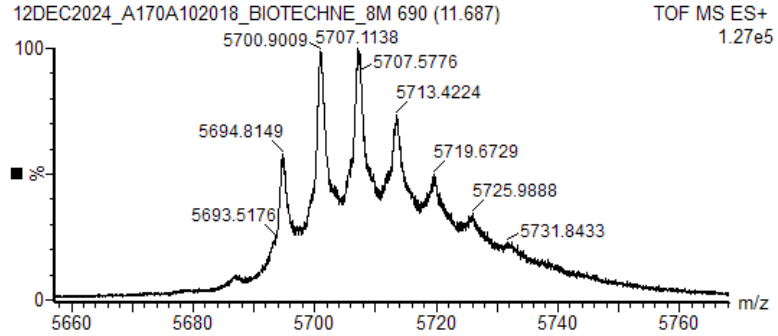
Spectra (avg. over elution)



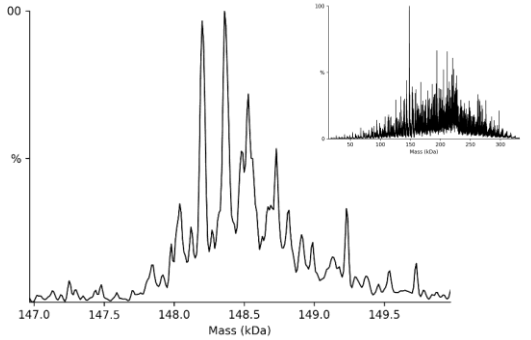


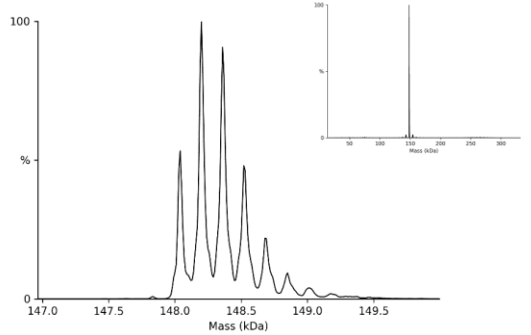
+27 Charge State





Deconvolution





- Glycoform resolution achieved following **DynaChip X1** buffer exchange
- Maintenance of **native confirmation** after sample cleanup
- Clean deconvolution following **DynaChip X1** sample treatment

Data Acquired on: Waters Xevo G2-S Q-TOF

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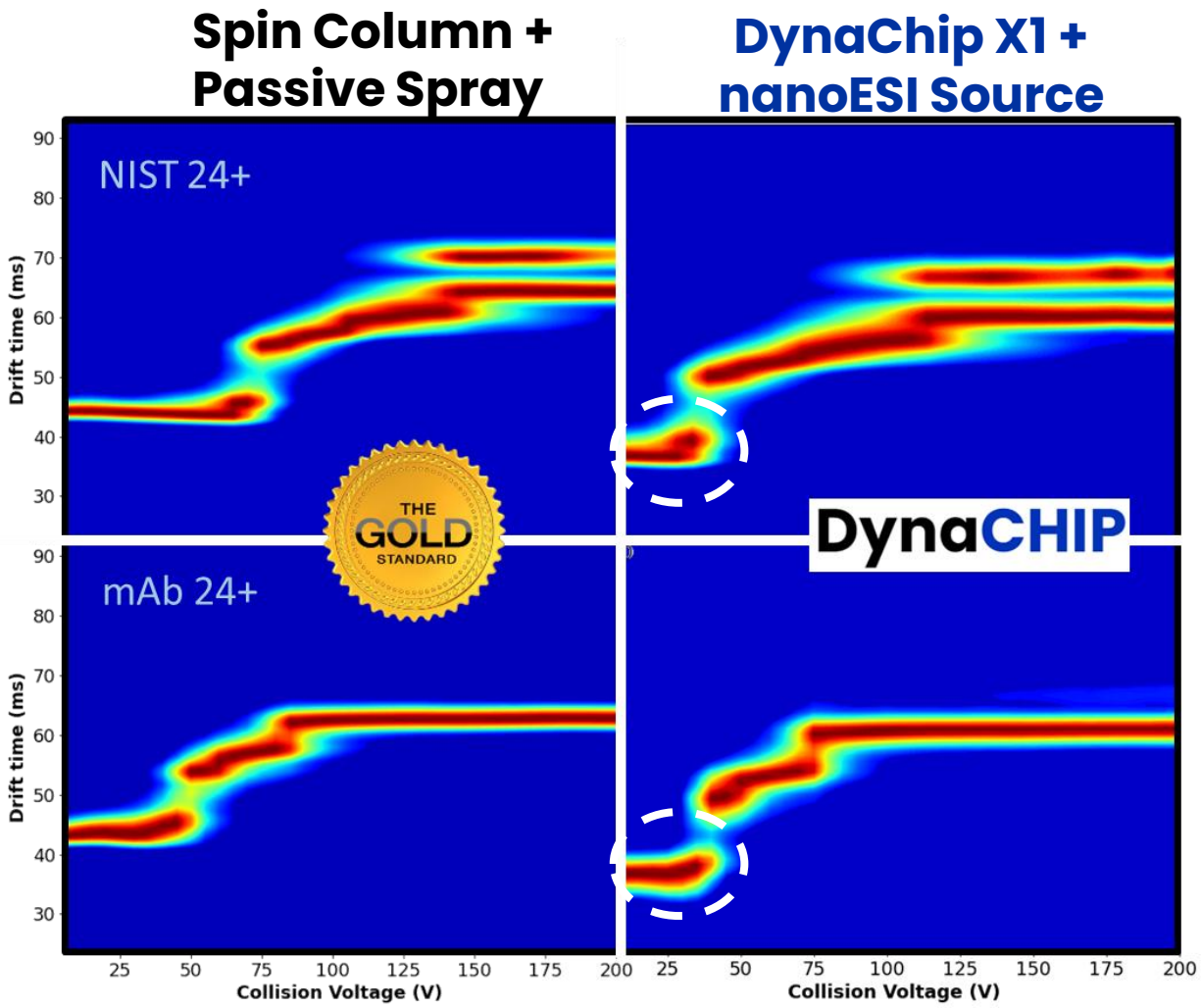
Results and Applications

mAbs & ADCs

Native MS – Collision Induced Unfolding

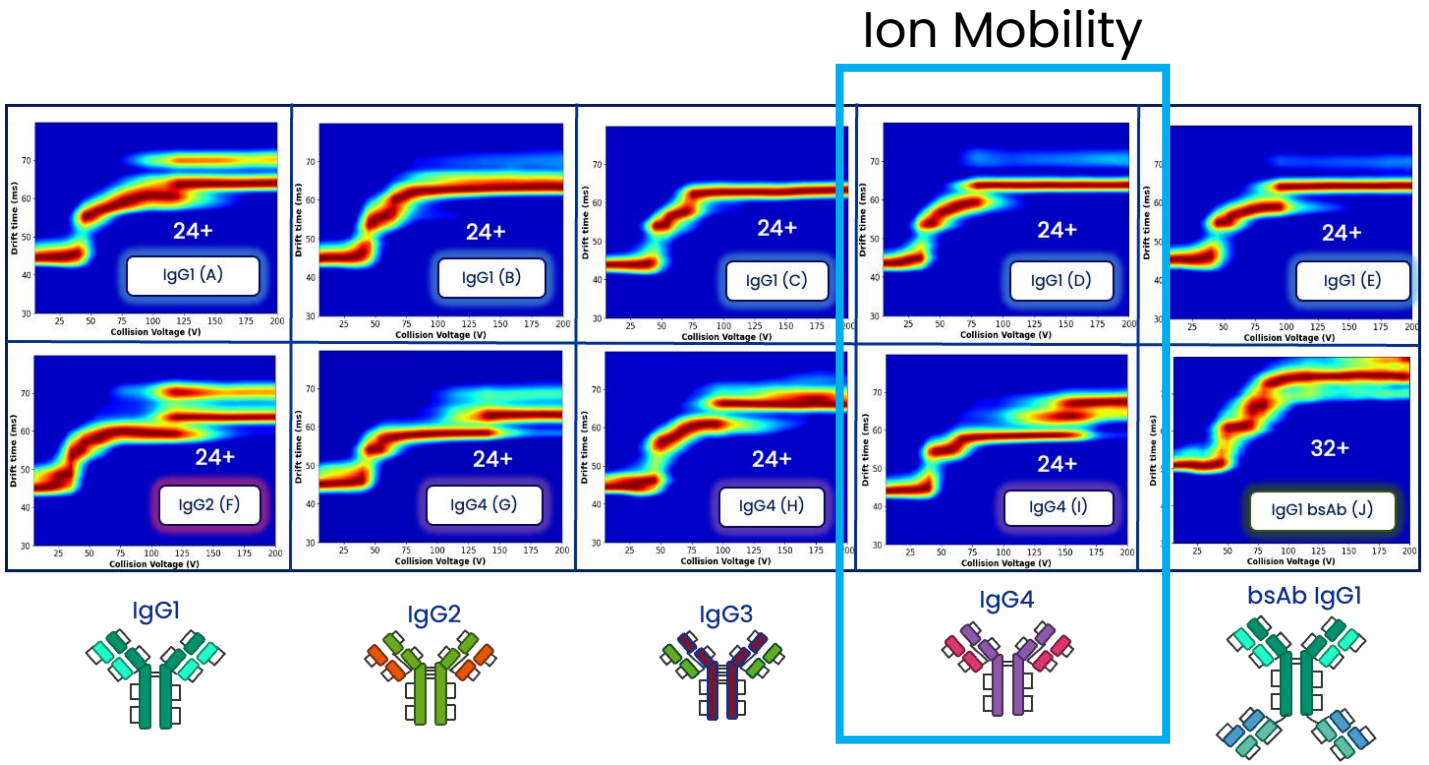


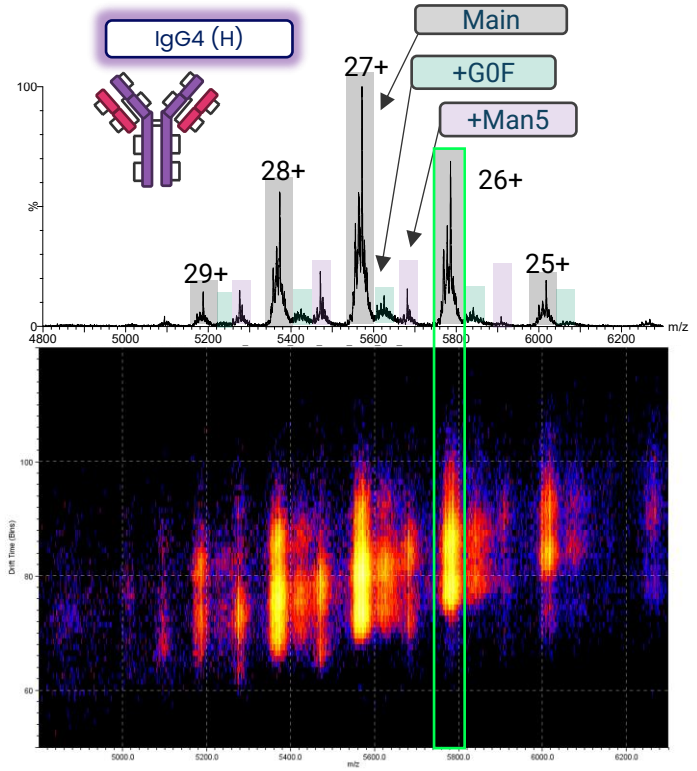
CIU Voltage Ramp: 5V steps @ 18 seconds (40 steps) = 12 min CIU collections

- Maintained **ground state transition** with DynaChip flow-through buffer exchange
- **Simplified native workflow:** commercial ion sources, no prior buffer exchange

Data Acquired on: Waters SELECT SERIES Cyclic IMS

Native MS – Collision Induced Unfolding & Ion Mobility





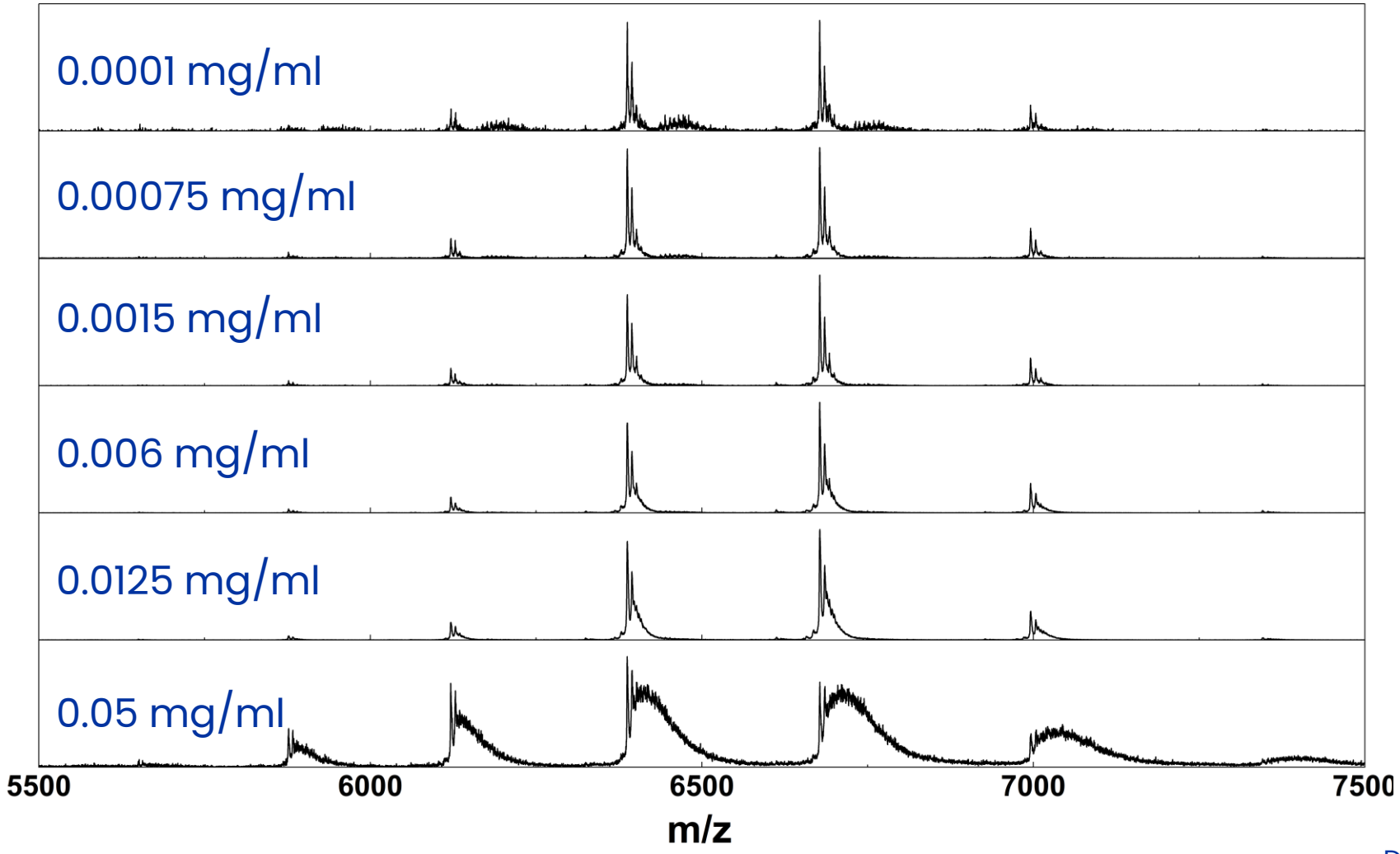
- CIU analysis of multiple mAb classes (above)
- CIU of forced degradation on Ig2 (*not shown*)

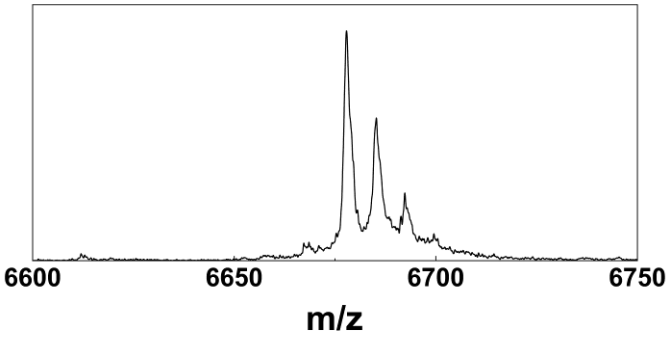
- CIU, IM-MS captured significant impact of Mannose-5 on stability
- Not captured as clearly in PTM analysis via LC-MS

Data Acquired on: Waters Cyclic IMS

Direct from Crude Cell-Culture Media mAb

*Highly sensitive analysis from day 5 culture media – **2.3 mg/mL***





- ✓ 3 bioreactor conditions
- ✓ 0-14 or 0-21 days
- ✓ Biological replicates
- ✓ **Over 100 samples run in ~ 2 days**

In collaboration with:

The University of Manchester

Data Acquired on: Waters Cyclic IMS

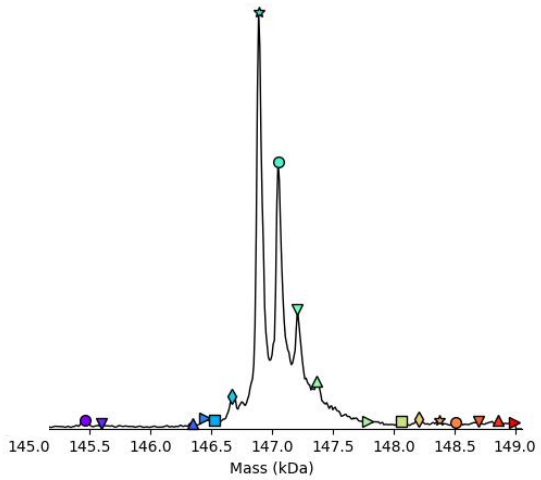
Direct from Crude Cell-Culture Media mAb

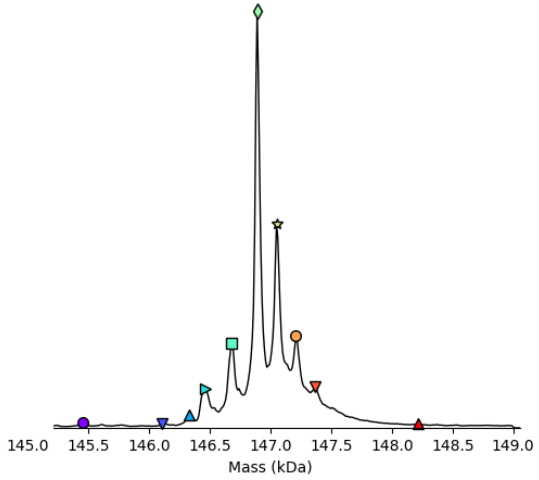
High-throughput mAb product monitoring – Ambr250 bioreactors

Day 5

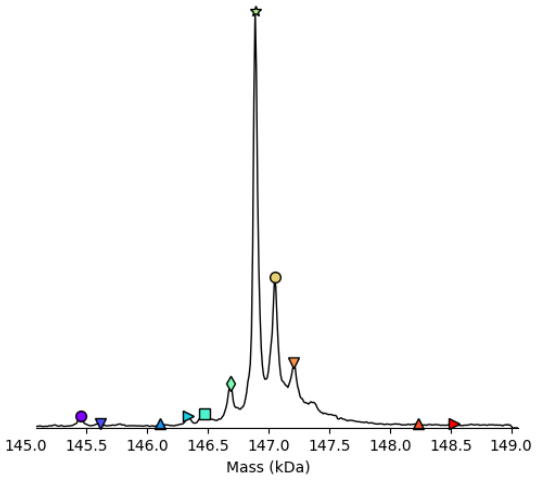
Day 14

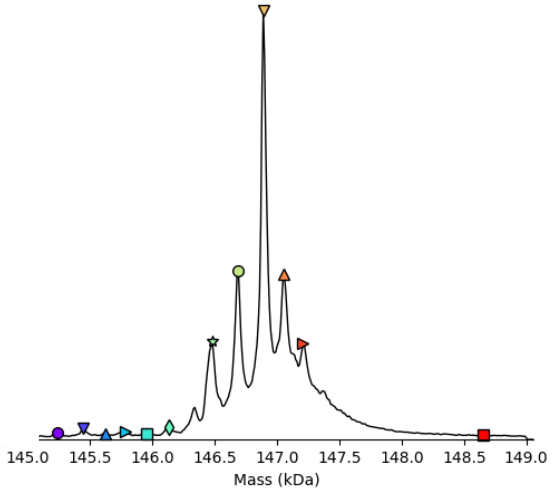
Control





Intensified Fed-Batch





- ✓ **Glycan Profile**
- ✓ Condition comparison
- ✓ 10 minute per sample

In collaboration with:

The University of Manchester

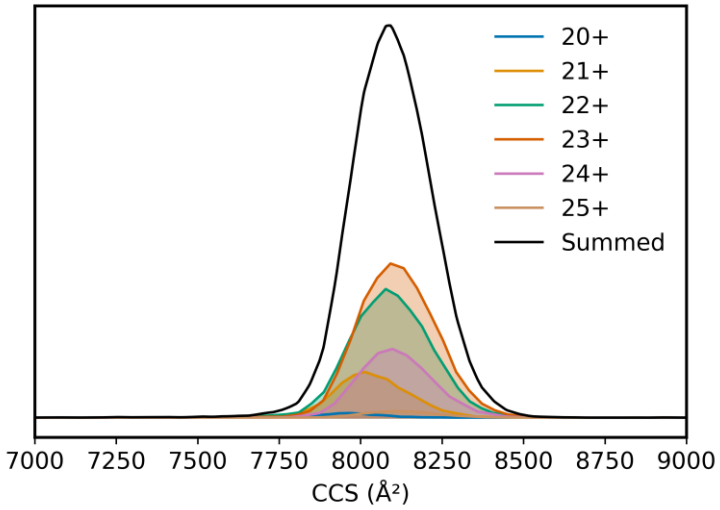
Data Acquired on: Waters Cyclic IMS

Direct from Crude Cell-Culture Media mAb

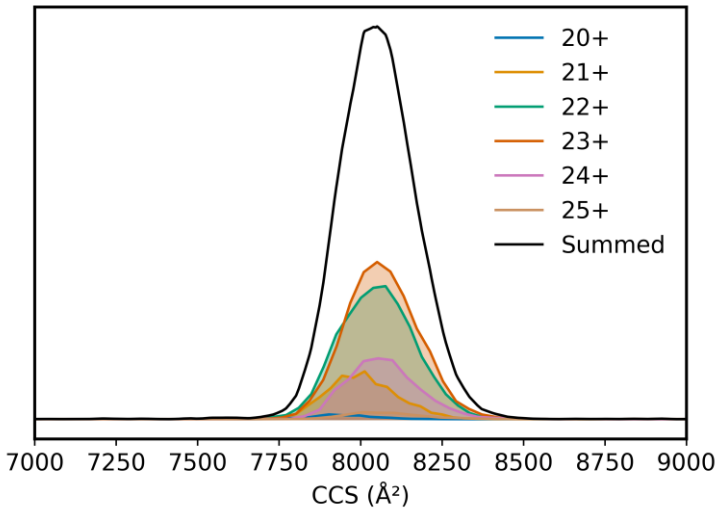
High-throughput mAb product monitoring – Ambr250 bioreactors

Collision Cross Section Distribution

Day 5



Day 14



CIU

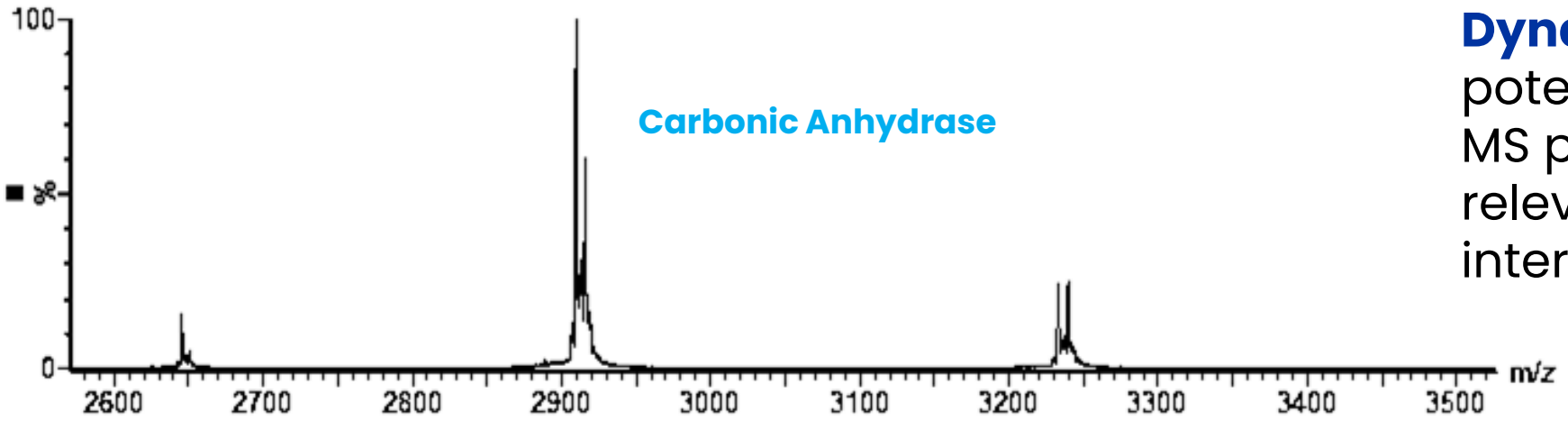
- ✓ **Collision Cross Section Distribution**
- ✓ **CIU**
- ✓ High level structural insights
- ✓ 10 minute per sample

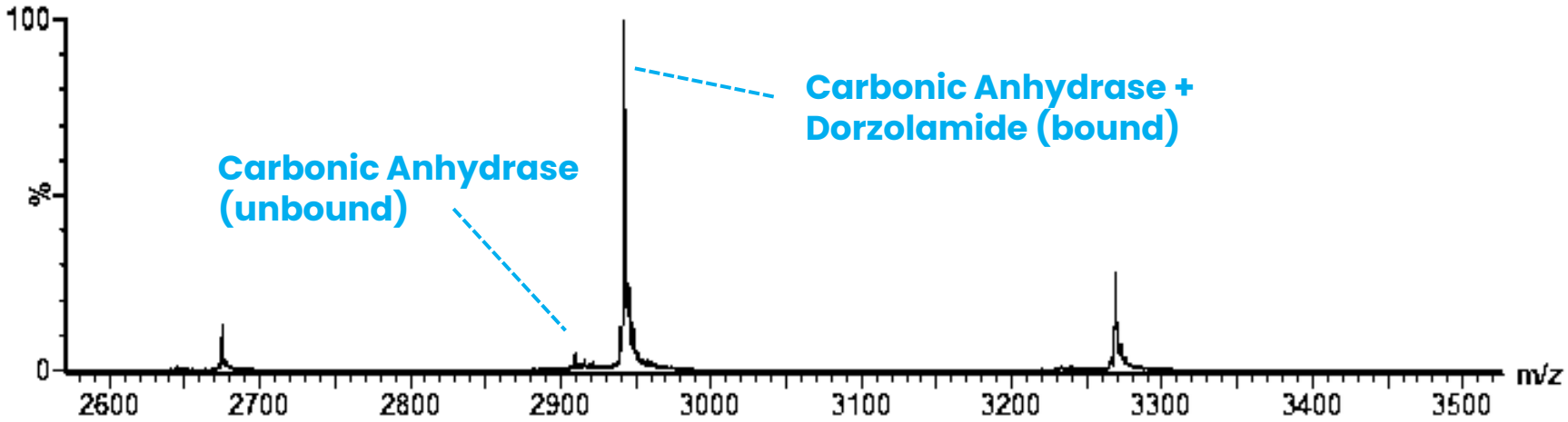
In collaboration with:

The University of Manchester

Data Acquired on: Waters Cyclic IMS

Protein-Ligand Interactions





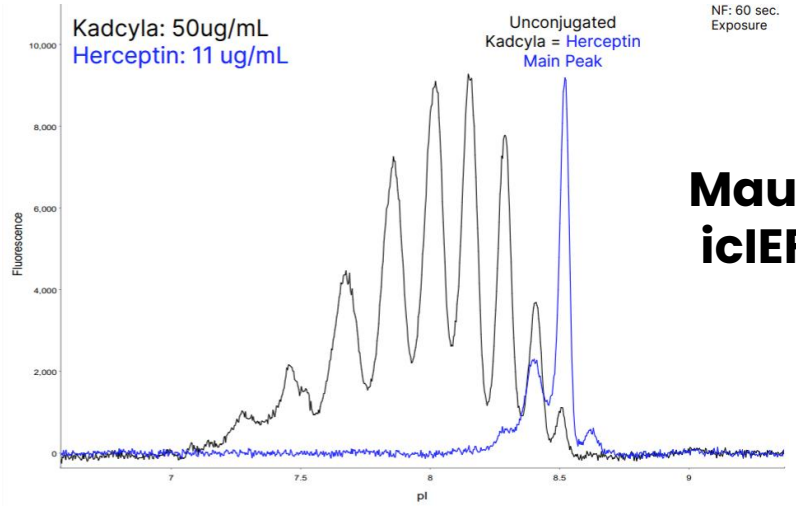
DynaChip X1 has the potential to provide native MS platform for biologically relevant protein-ligand interactions.

Data Acquired on: Waters Xevo G2-S Q-TOF

ADCs—Drug Antibody Ratio (DAR) Analysis

Compatibility with 3rd Party Systems like MauriceFlex

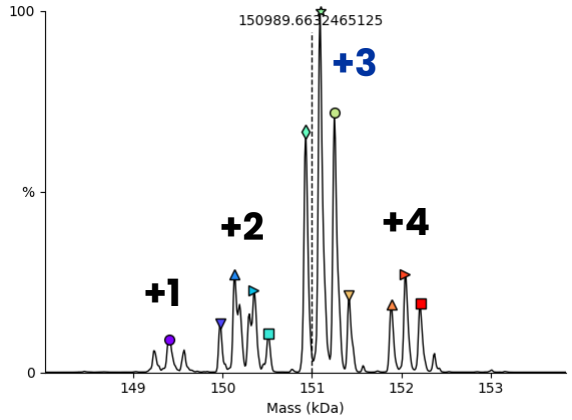
Acidic peaks have increasing lysine conjugation or increasing drug to antibody ratio (DAR)



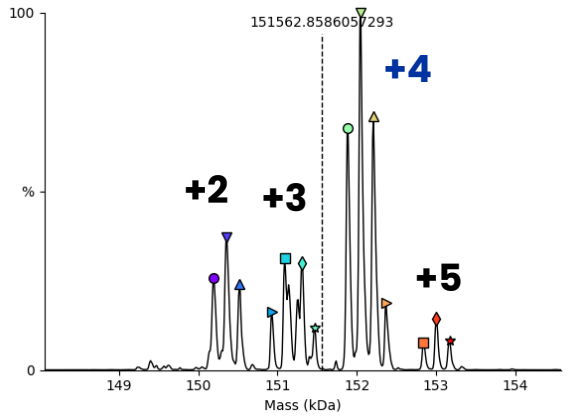
MauriceFlex icIEF Profile

Representative Deconvolutions

Fraction C12



Fraction C11



pI

Example Fraction	Average DAR
Herceptin (Unloaded)	0.00
B12	2.52
C12	2.88
C11	3.48
C9	4.35
C7	4.70
C6	5.07

Data Acquired on: Waters Xevo G2-S Q-TOF

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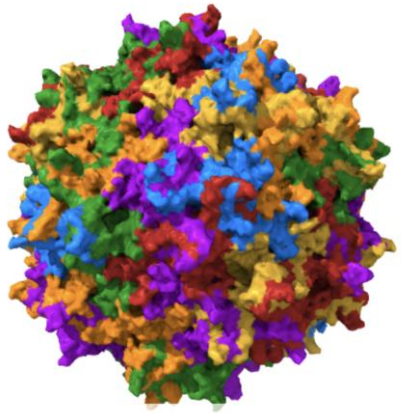
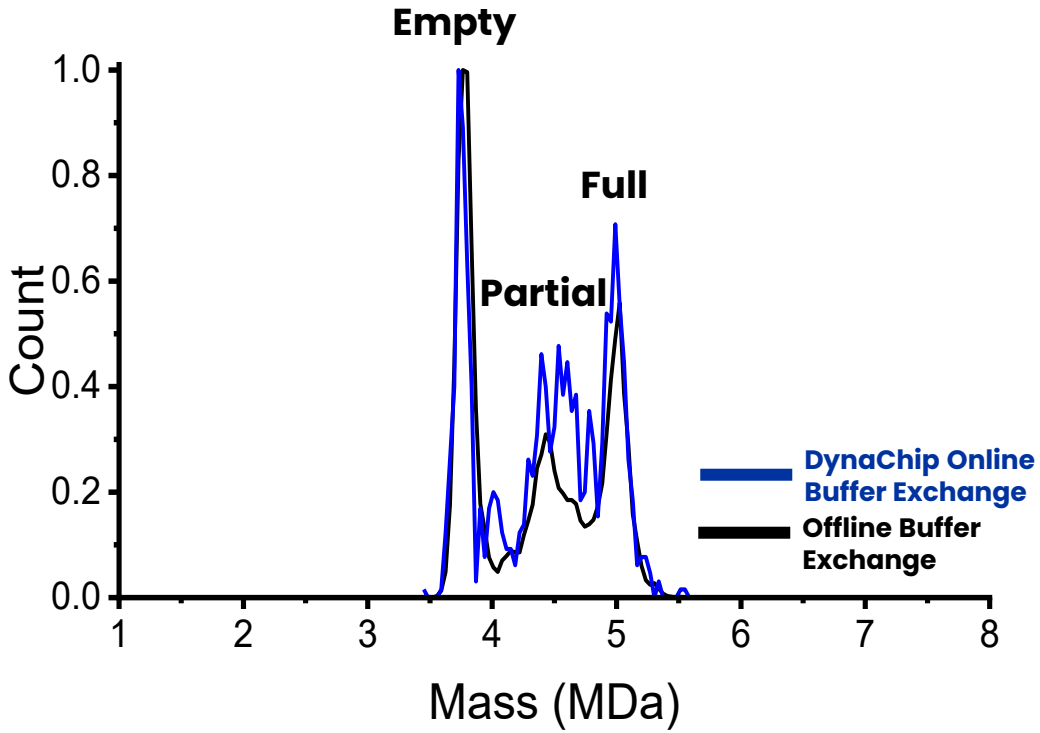
Results and Applications

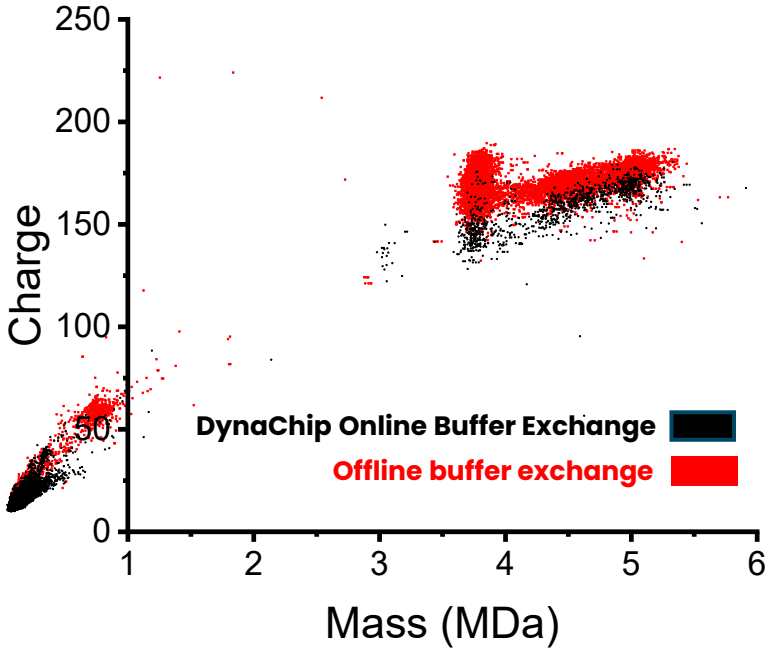
Large Molecules & CDMS

Full/Empty AAV Capsid Ratio with Online DynaChip X1



AAV8
scDNA HEK production





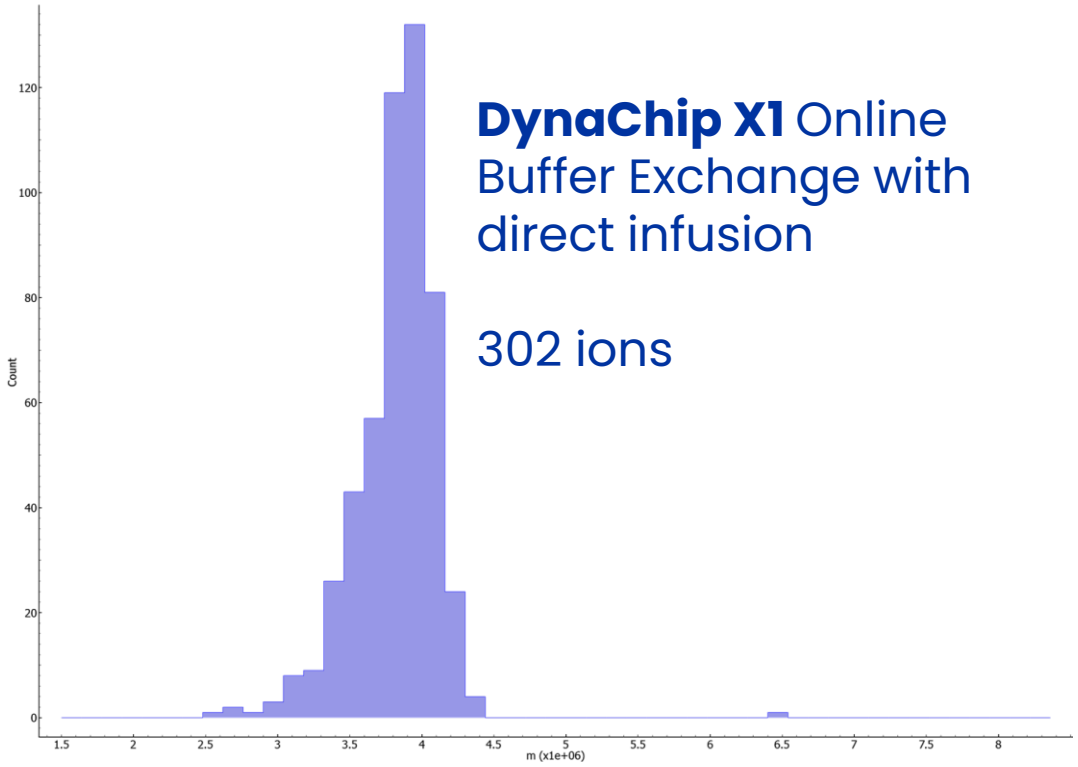
Prep	Structure	Mass (MDa)	FWHM (MDa)
Offline (2x spin column)	Empty	3.777	0.163
	Partial	4.481	0.135
	Full	5.008	0.101
DynaChip	**Empty**	**3.754**	**0.122**
	Partial	**4.537**	**0.220**
	Full	**4.996**	**0.100**

DynaChip X1 streamlines CDMS workflow with online buffer exchange and direct nanoESI integration

Data Acquired on: Megadalton CDMS System

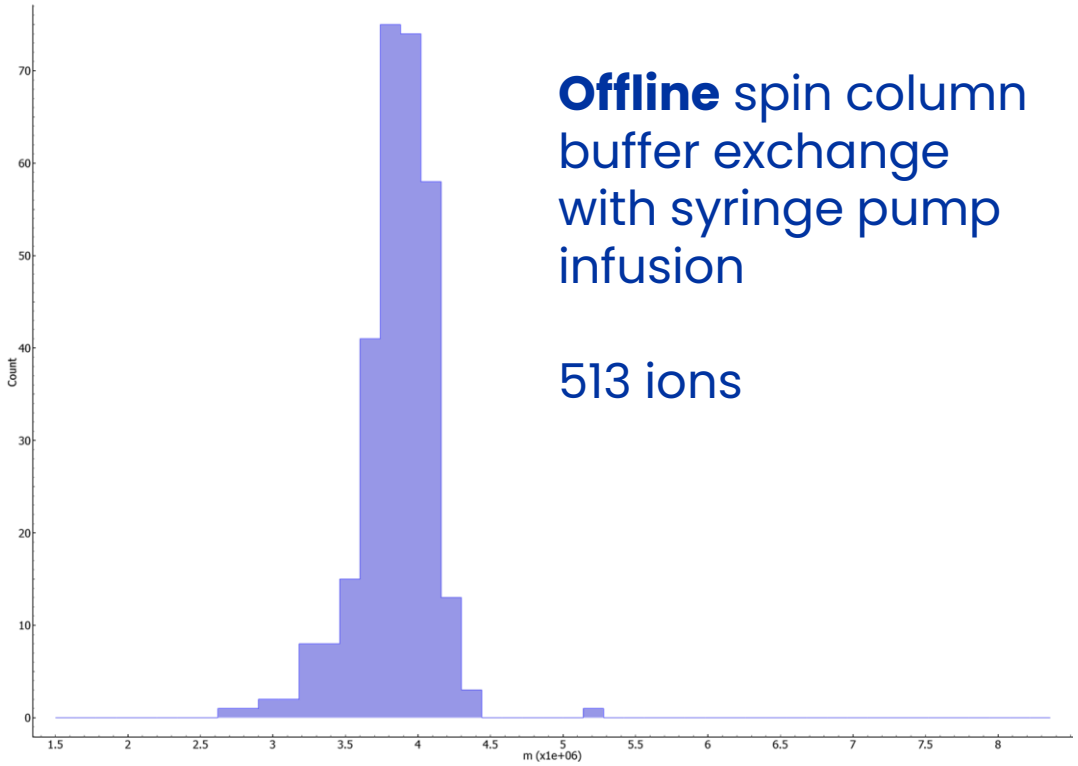
CDMS – AAV 5

Enabling online AAV analysis from small sample volumes



DynaChip X1 Online Buffer Exchange with direct infusion

302 ions



Offline spin column buffer exchange with syringe pump infusion

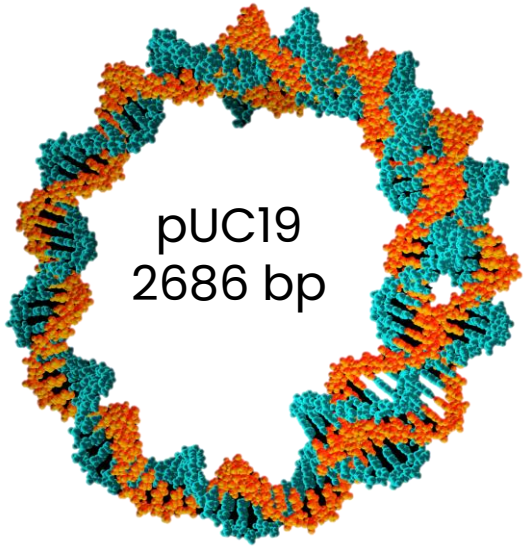
513 ions

In collaboration with:

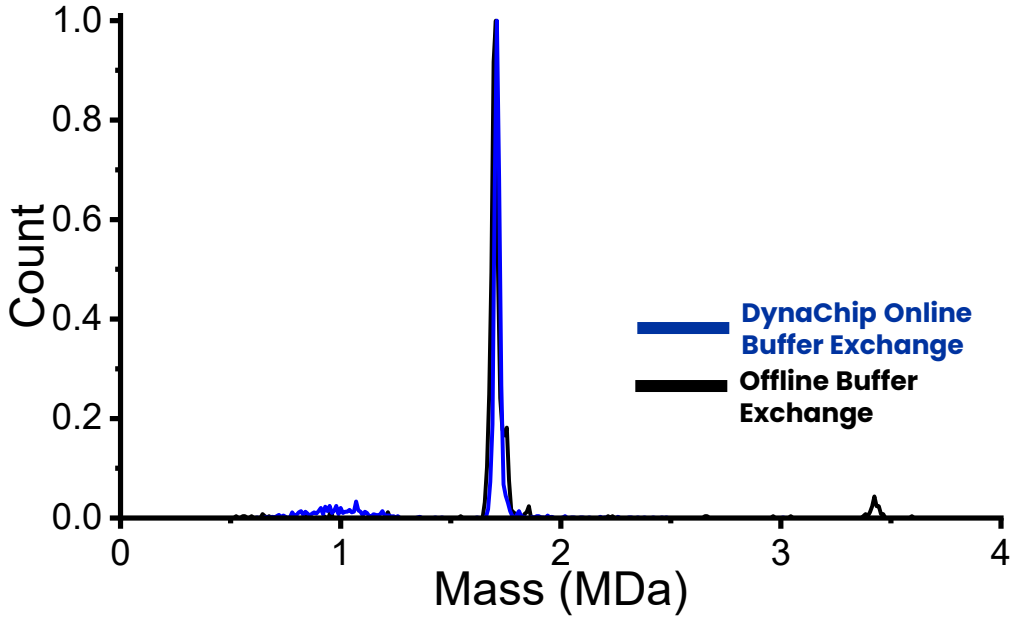
Data Acquired on: TrueMass CDMS

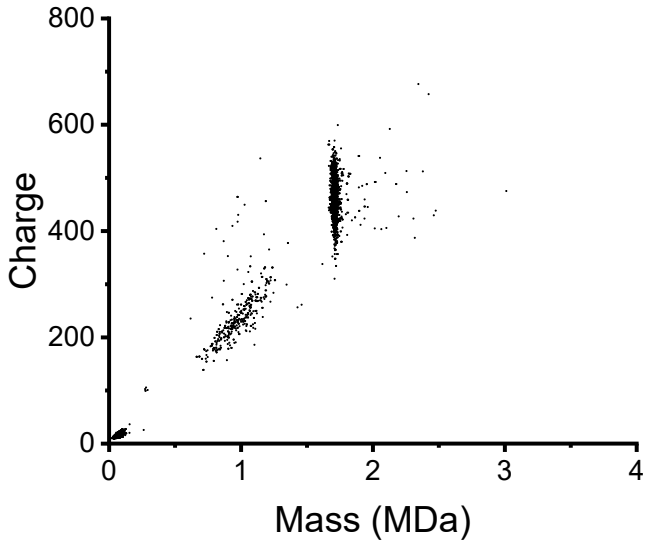
Customization of Buffer for "Gentle" Ionization

*DNA Plasmid (pUC19) Exchanged into **20 mM Ammonium Acetate***



DNA Plasmid (pUC19)
20 mM ammonium acetate

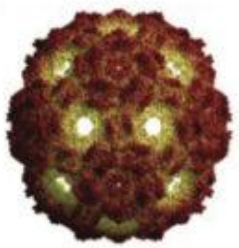




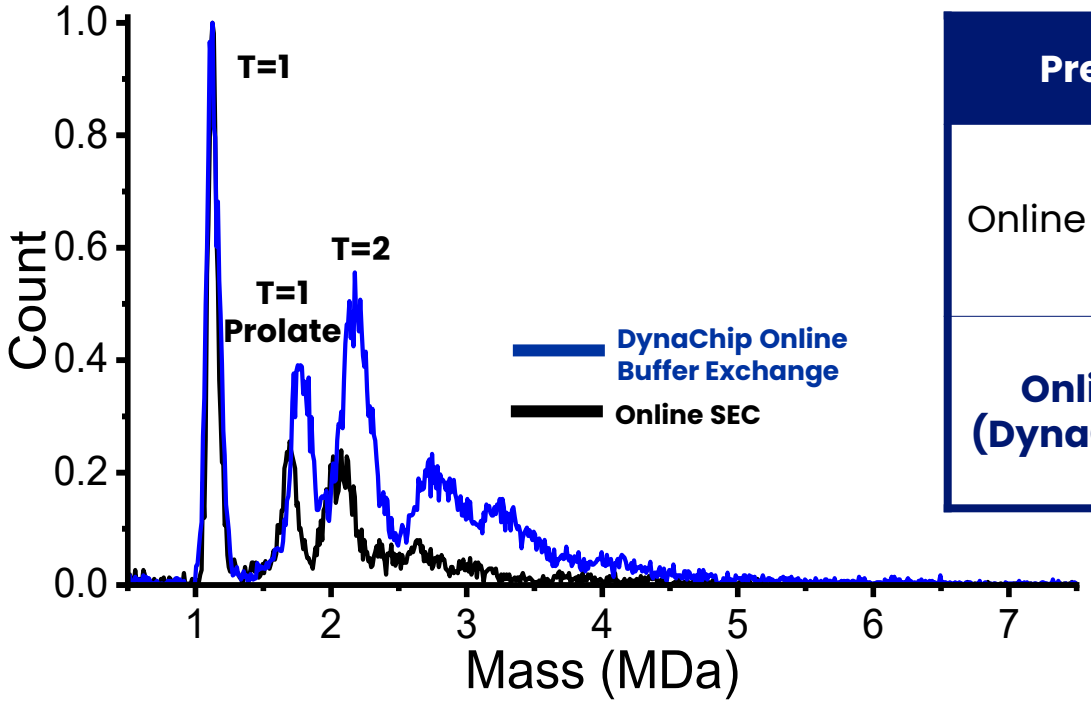
Prep	Mass (MDa)	FWHM (MDa)
Offline (2x spin column)	1.700	0.036
DynaChip	**1.711**	**0.028**

Data Acquired on: Megadalton CDMS System

Comparing SEC-CDMS & DynaChip-CDMS



Bacteriophage Qbeta



Prep	Structure	Mass (MDa)	FWHM (MDa)
Online (SEC)	T=1	1.127	0.085
	T=1 Prolate	1.693	0.033
	T=2	2.068	0.057
Online (DynaChip)	**T=1**	**1.127**	**0.081**
	T=1 Prolate	**1.781**	**0.050**
	T=2	**2.181**	**0.102**

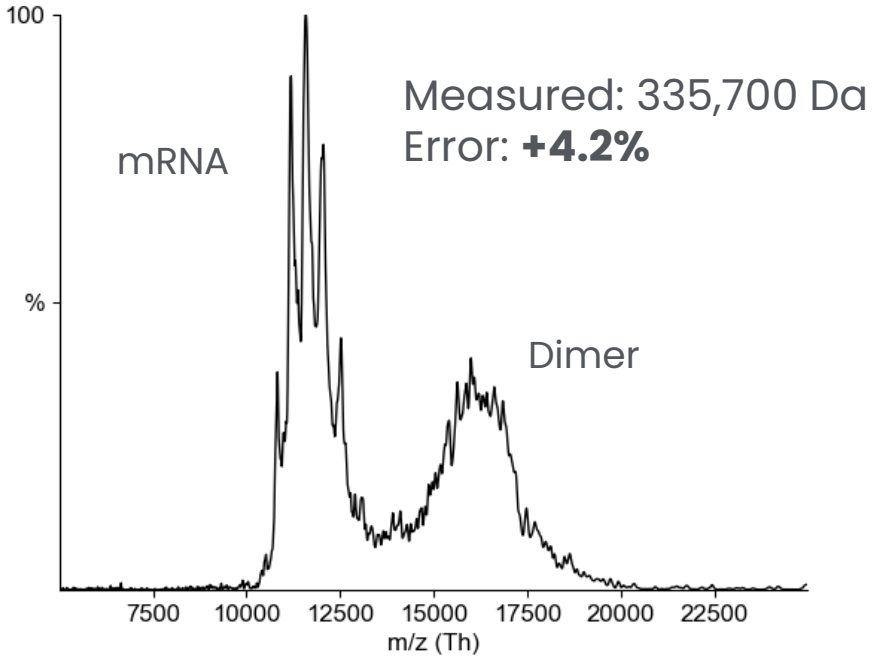
DynaChip X1 comparable results to SEC at higher throughput
while preserving sensitive biomolecular structures.

Data Acquired on: Megadalton CDMS System

Efficient Desalting and Native mRNA Analysis

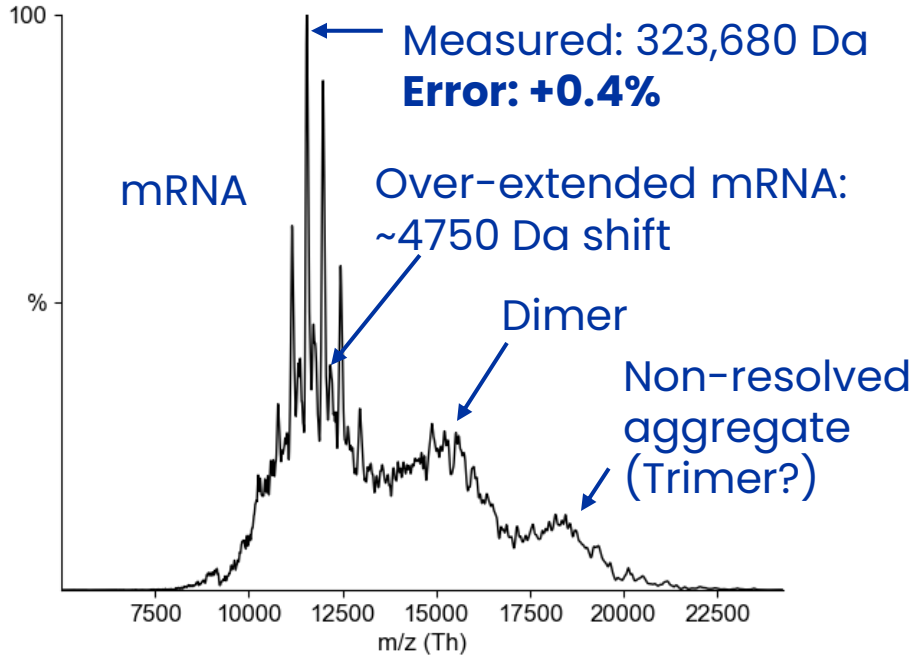
Higher Spectral Quality and Richer Information

Dilute & Shoot:
30 x dilution, long acquisition



1 mg/ml EGFP mRNA in 1mM Sodium Citrate
Sequence Mass: 322,316 Da
Length: 997 nt

DynaChip X1: 5 uL injection



In collaboration with:

University of Minnesota

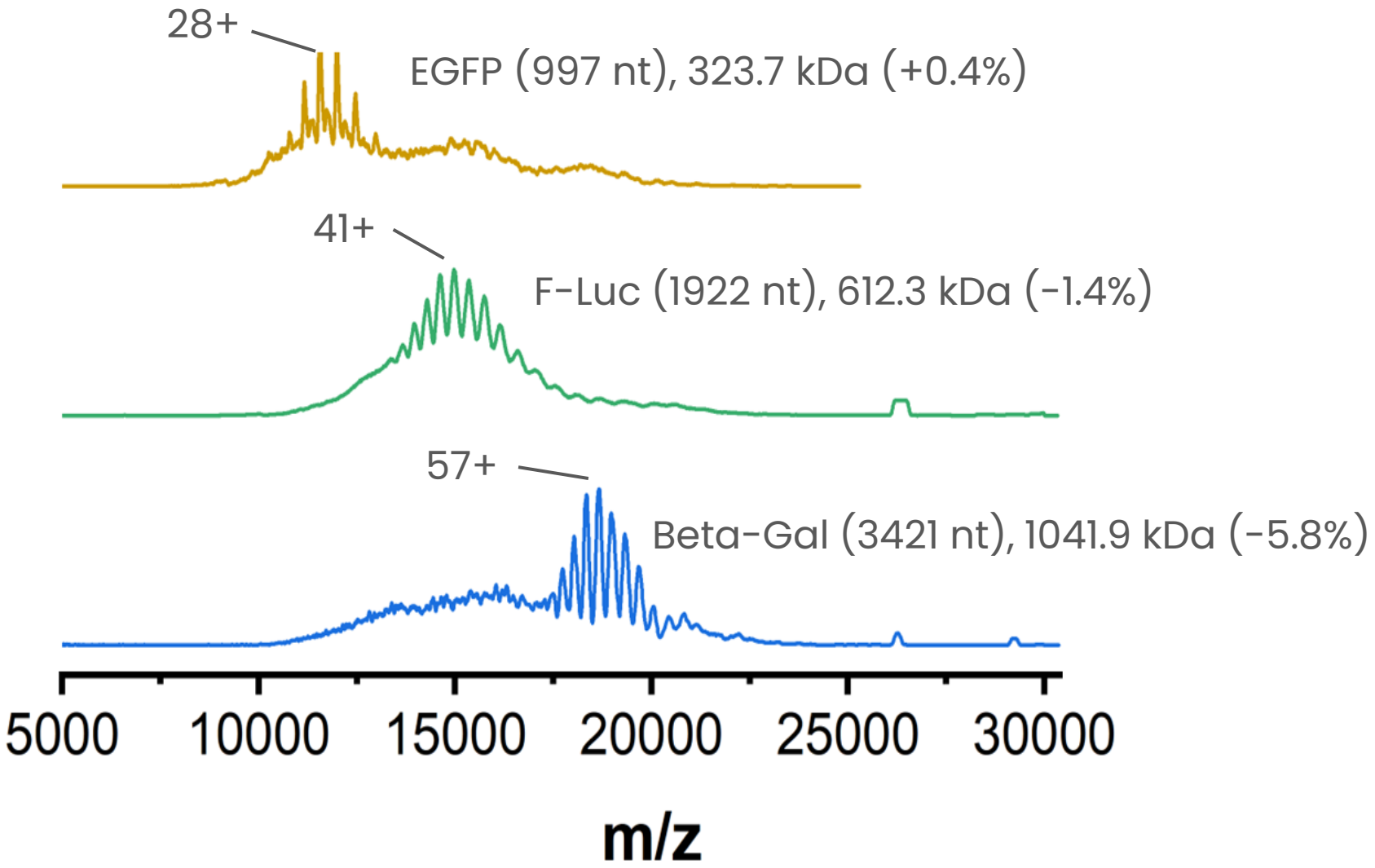
Gadkari Research Group

Data Acquired on: Thermo Q Exactive UHMR MS

- ✓ Significant improvement in spectral quality and ESI intensity
- ✓ Significant removal of adducts as demonstrated +0.4% error in mass
- ✓ **Higher resolution revealing overextended population**

Rapid and Efficient Online Cleanup of mRNA

Enabling Native Charge State Resolution of Megadalton Species



- ✓ DynaChip enables resolution on RNAs in the 1000nt + range
- ✓ F-Luc and Beta-Gal measured mass were lower than predicted sequence mass.
- ✓ Lowered mass could be due to degradation—only possible tell that with sufficient clean-up and removal of adducts

In collaboration with:

University of Minnesota

Gadkari Research Group

Data Acquired on: Thermo Q Exactive UHMR MS

Thanks to CASSS MS, Collaborators, Teammates

All technical data available online at www.AndsonBiotech.com/Resources/

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