Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Developing the analytics and analytical workflows supporting the analysis of the next generation of biotherapeutic and gene therapies.

Scott J. Berger, Ph.D. and Ximo Zhang, Ph.D.

Waters Lunch Seminar

Tuesday January 28, 2020

WCBP 2020
Diamond
Program Partner

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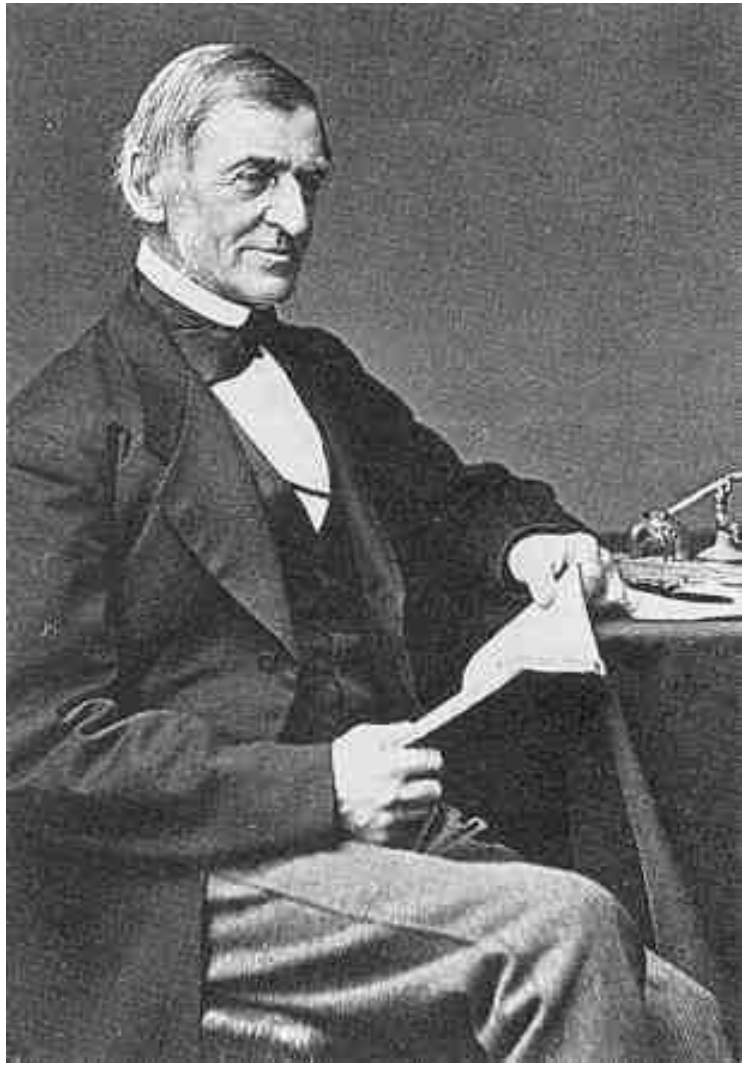
What has become clear to you since we last met?

Ralph Waldo Emerson
b. 1803 - d.1882



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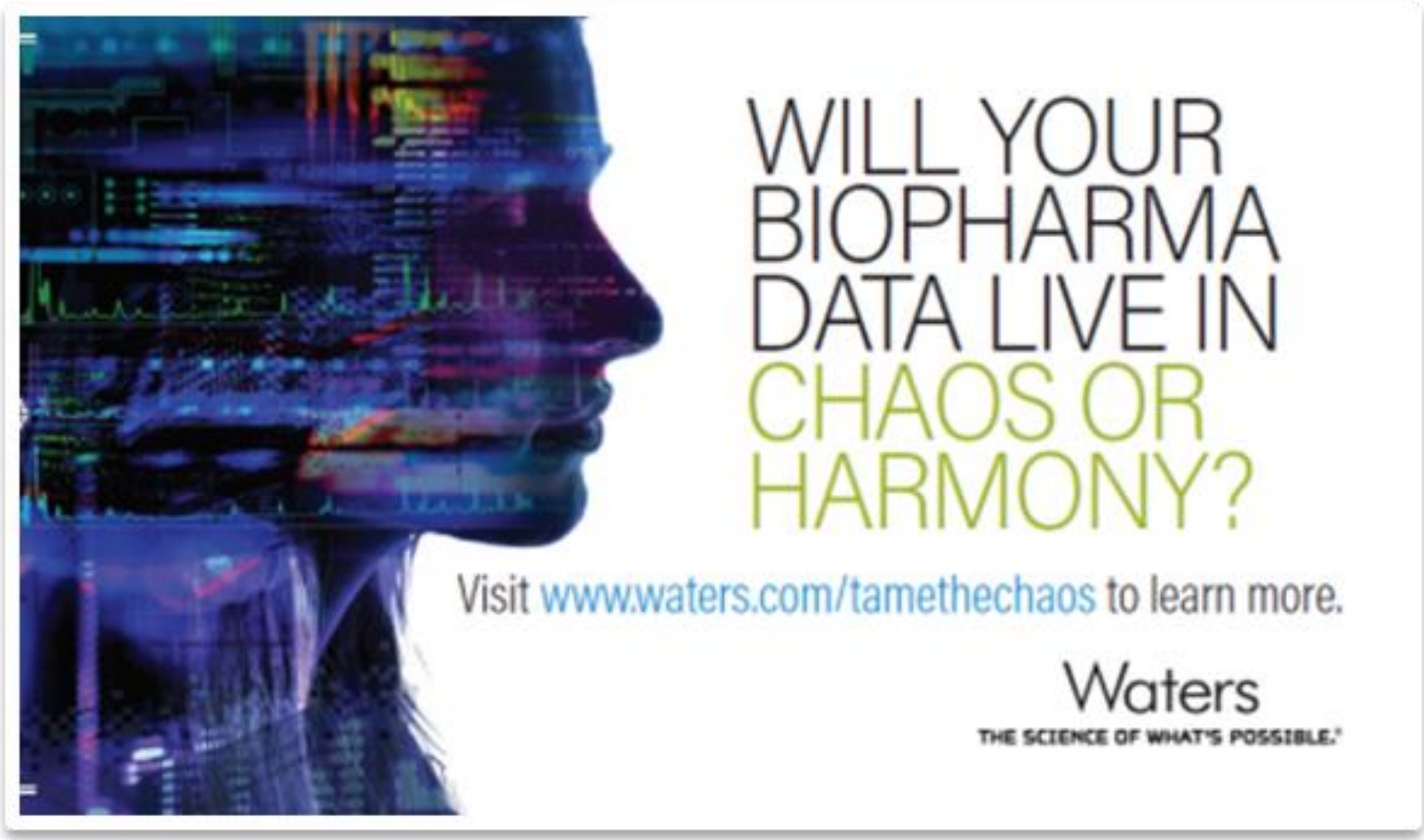
WCBP 2018: Setting the vision of biopharmaceutical harmonization



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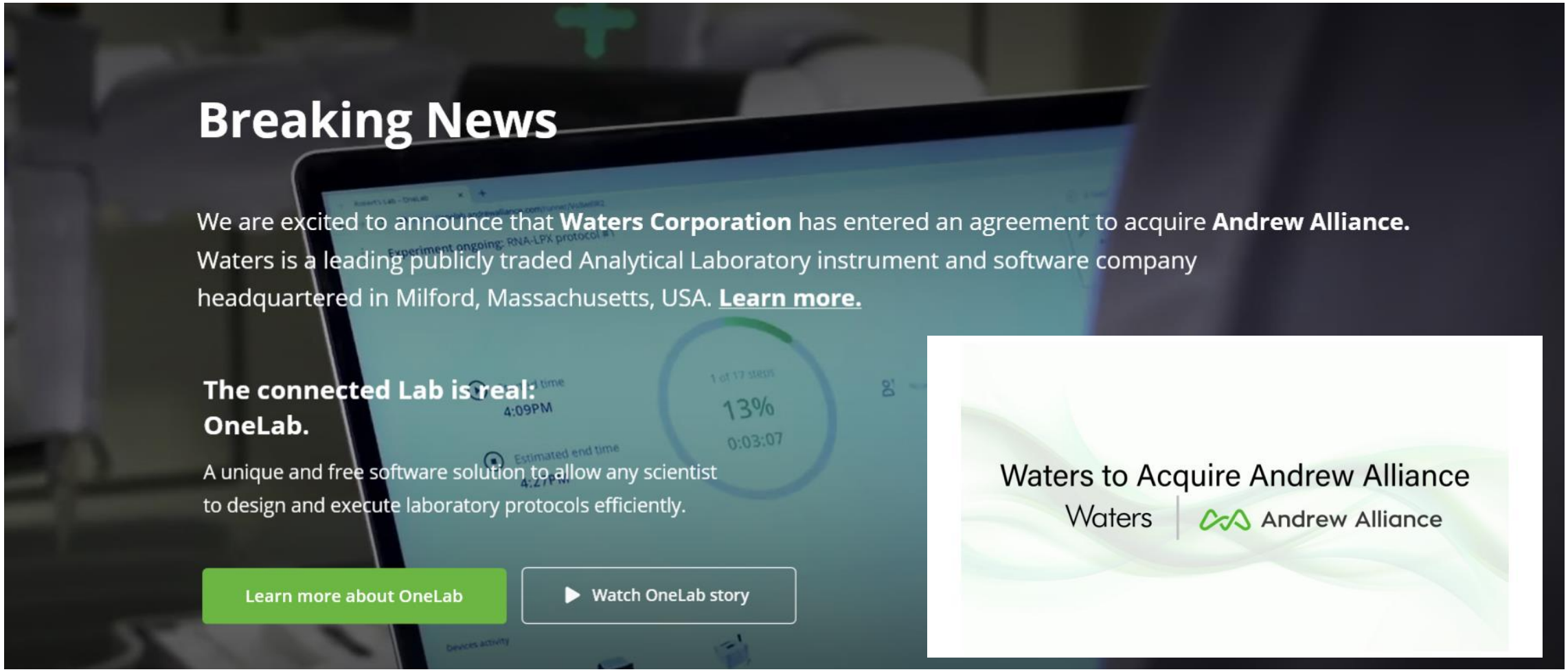
January 13, 2020: Announcing the acquisition of Andrew Alliance



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Andrew Alliance – Workflow Integration Technologies

Accessible Automation, Innovative Liquid Handling and Harmonized Lab Connectivity

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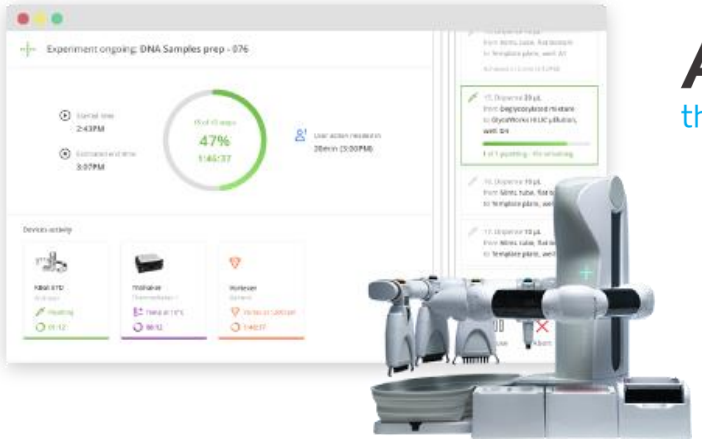
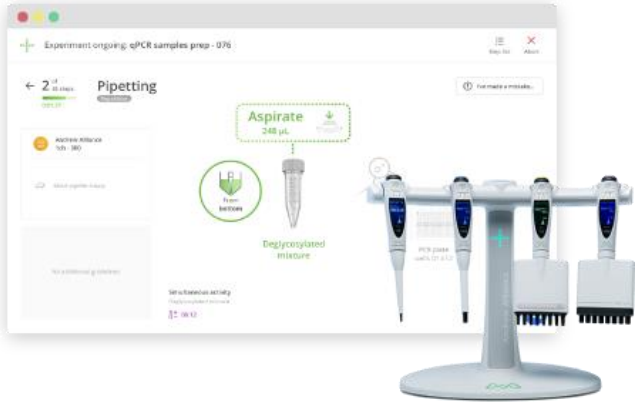
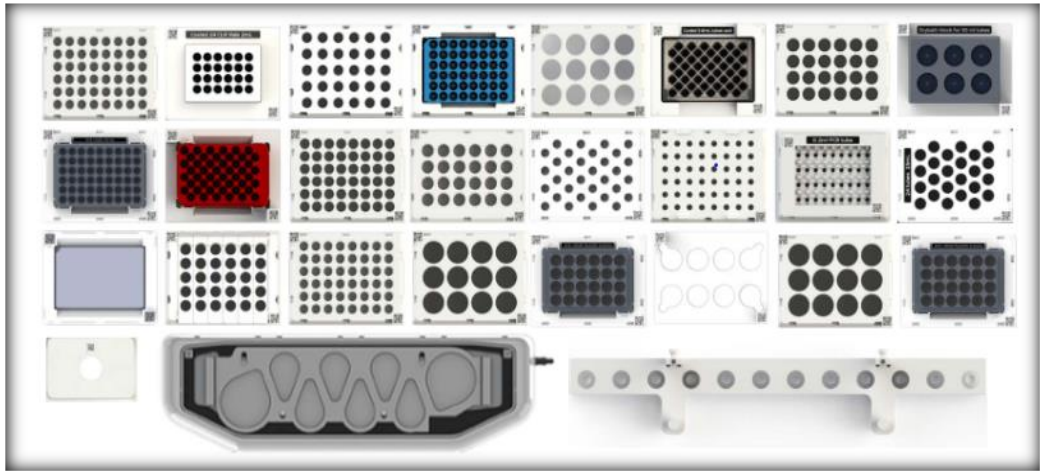


Andrew
the pipetting robot

& Domino Module Flexibility

Pipette
easy pipetting
Bluetooth®





OneLab
Connected Lab Ecosystem

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Expanding Waters Automation Solutions in 2020

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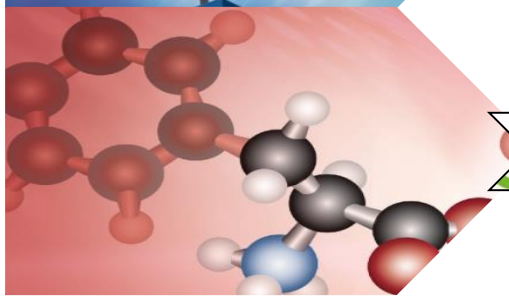
Andrew Alliance

Released N-Glycan Analysis
with GlycoWorks® *Rapi*Fluor-MS®



Andrew Alliance

Amino Acid Analysis with AccQ•Tag™ Ultra

- ***Kits*** *– Designed for automated derivatization*
- ***Standard*** *– Quantitative analysis of 26 AA*
- ***Scripts*** *– Optimized & verified on Andrew, Tecan & Hamilton*

Complements our partnerships with: TECAN. HAMILTON

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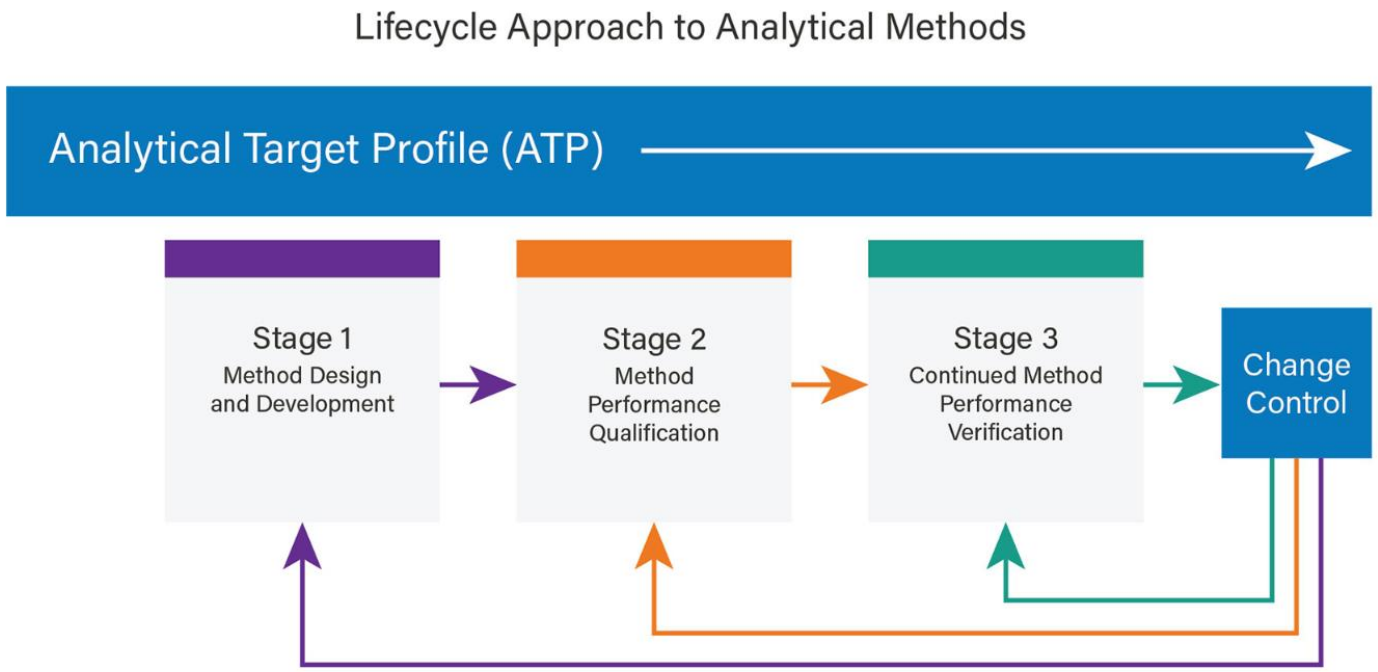
Increased focus on Method Life Cycle Management (MLCM)?

- USP draft chapter <1220> *The Analytical Procedure Lifecycle*, and ICH quality guidelines Q12, Q14 inform the MLCM framework.
- A structured approach for understanding and testing method performance, and continually assuring methods are fit for an intended purpose.
- Visit www.methods.waters.com for more about MLCM



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Separations span multiple LC classes over their lifetime?

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	ACQUITY UPLC H-Class H-Class Bio/ I-Class	ACQUITY Arc / Arc Bio (UHPLC)	Alliance HPLC
Chemistry Compatibility	≥ **2.1 mm** ID Columns ≥ **1.7 µm** Porous Particles	≥ **3.0 mm** ID Columns ≥ **2.5 µm** Porous Particles	≥ **4.6 mm** ID Columns ≥ **3.5 µm** Porous Particles
Detection	Optical (UV, PDA, FLR) and ACQUITY QDa Mass Detection		
MS Compatibility	SQD2, QQQ, TOF, QTof	SQD2	
Software Compatibility	Empower, MassLynx, UNIFI	Empower, MassLynx	
Common Role(s)	Characterization (Development) Monitoring (Late Development) Routine analysis (QA/QC)	Routine analysis (QA/QC) Monitoring (Late Development) Method Development & Transfer Characterization (Development)	Monitoring (Late Development) Routine analysis (QA/QC)

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ACQUITY Arc and Arc Bio Systems

Expanded Column Flexibility for Expanding Roles in the MLCM era.

Single instrument platforms for advanced method development and routine analysis deployment of both traditional HPLC and modern UHPLC separations

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Advancing Informatics Harmonization with Waters_connect

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Waters_connect platform

Evolution of the UNIFI architecture | Cloud-enabled | Data Integrity and Compliance built-in

For Discovery and Development Labs

Enables routine analysis in BioPharma with new workflows

UNIFI

The best new applications, maximizing the impact of analytical technology

waters_connect

For Development and QC Labs

Modern web-based capabilities that add value for Empower customers

Empower®

Enables 500,000+ Empower users with new applications and capabilities

Driving Digital Transformation

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A need for open but secure data exchange
Solution: The UNIFI Application Programmer's Interface

[TECHNOLOGY BRIEF]

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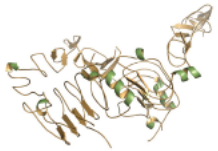
Using the UNIFI API to Enable Streamlined Data Export to Protein Metrics Byos Platform for Processing of Biotherapeutic Protein and Peptide LC-MS Data

Samantha Ippoliti and Ying Qing Yu
Waters Corporation, Milford, MA USA



Data collected with UNIFI can be securely accessed by the Protein Metrics Byos Bioinformatics platform, and other third-party applications, for extended protein characterization.

GOAL

Employ the Application Programming Interface (API) in UNIFI™ Scientific Information System v1.9.4 to enable third-party applications such as the Byos® platform (Protein Metrics) to access, read, and process data acquired by UNIFI.

BACKGROUND

Waters™ UNIFI-based LC-MS system solutions for biopharmaceutical analysis excel at automating the generation, processing, review, and reporting of biopharmaceutical characterization and monitoring data, including foundational tools for operating within compliant environments. Using the API functionality within UNIFI, analysts can further benefit from extended characterization capabilities offered by third-party software vendors. Byos (Protein Metrics, Cupertino, CA, USA), the first UNIFI API partner in the biopharmaceutical space, has created a vendor-neutral informatics platform of protein characterization tools designed to enable laboratories to streamline data comparisons across multiple vendor LC-MS platforms, and provide access to extended characterization workflows, supplementing those provided by Waters

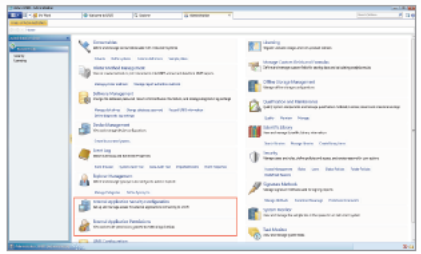
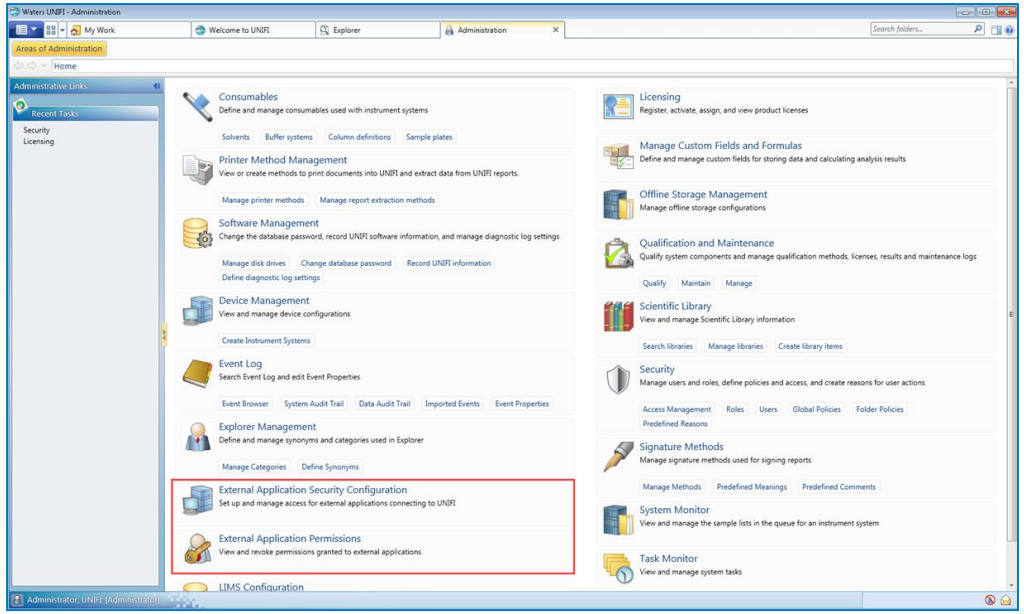
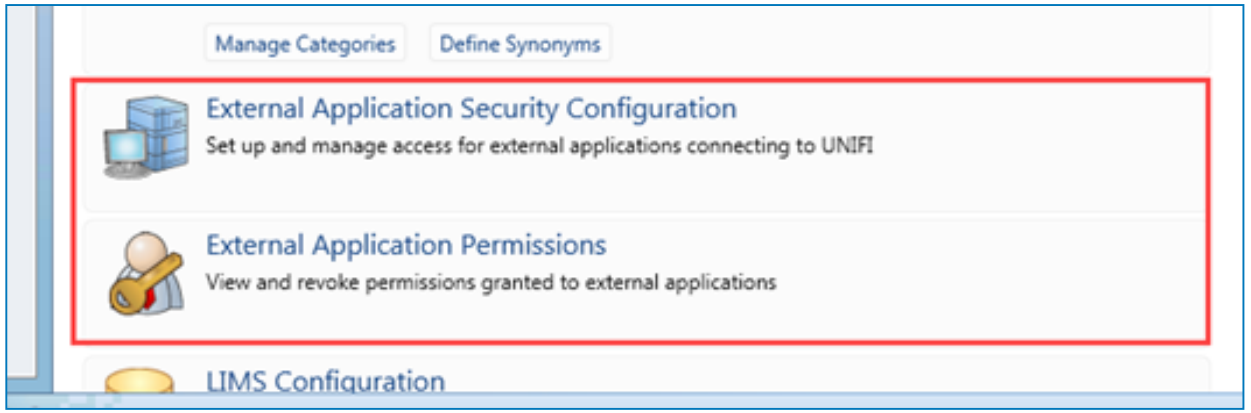


Figure 1. Installation of UNIFI API adds two additional modules to the Administration tab in UNIFI: External Application Security Configuration and External Application Permissions. It is here that the third-party application permissions are managed.

and other vendors. This API functionality allows the Byos application secure permissions-based access to the UNIFI database to extract raw data acquired by UNIFI, for use by the tools within the Byos software suite. This facilitates streamlined integration of UNIFI data with that from other data sources for commonplace workflows, such as intact/subunit protein mass and peptide mapping, as well as access to extended characterization tools for workflows, such as protein crosslinking analysis, not currently addressed in UNIFI or MassLynx™ today. Waters and Protein Metrics have developed a streamlined workflow for generating data in Byos-compatible formats, which circumvents the need for manually exporting data to generic file formats for subsequent manual import into the Byos platform, enabling a better user experience on both platforms.





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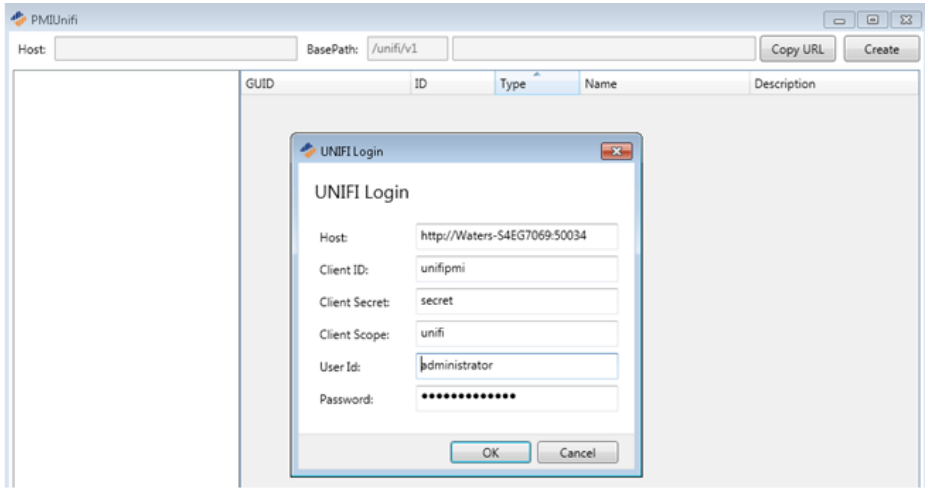
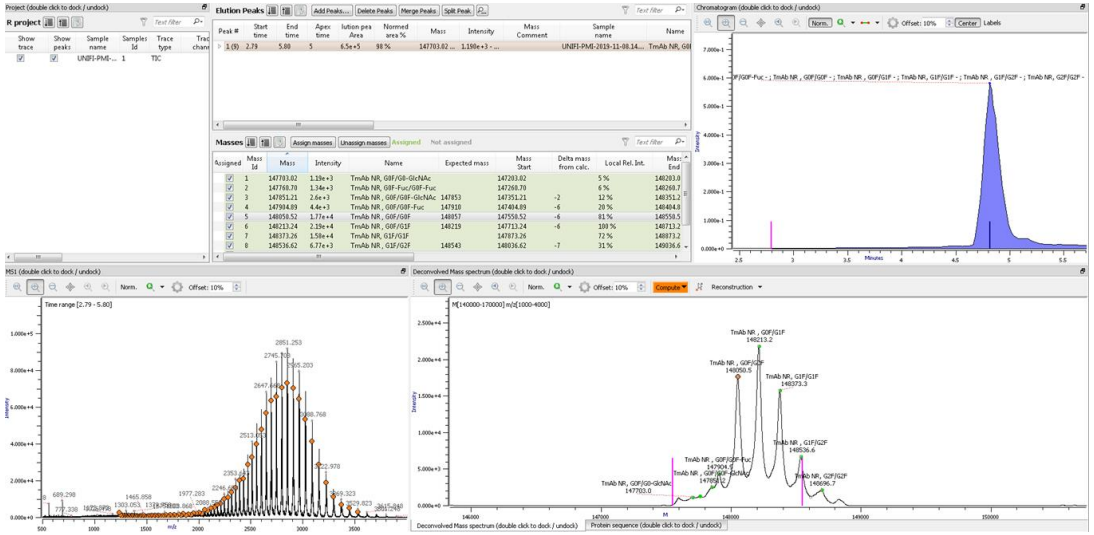
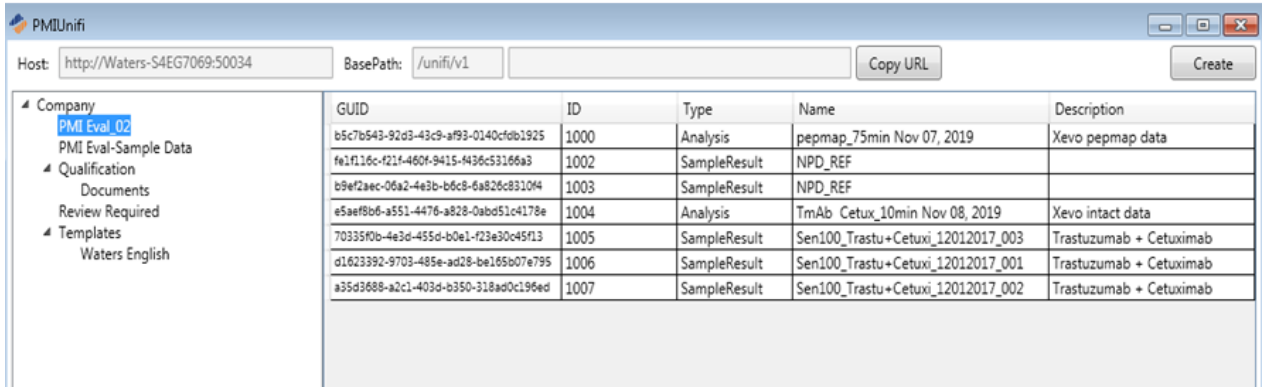
Extended Analysis of UNIFI acquired data on the Protein Metrics Byos Informatics Platform







Waters
UNIFI™

Byos®
PROTEIN METRICS

- Intact and Subunit Analysis
- PTM Analysis
- HCP Analysis
- Disulfide Bond Analysis
- Sequence Variant Analysis
- MAM Analysis
- Peptide Mapping
- Glycosylation profiling
- Etc...

Waters UNIFI data → Now available to be processed by Protein Metrics Byos®

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What would make MS more accessible?

96% say more user-friendly MS would benefit biopharma analysis.

Over half say user-friendliness would

- Reduce errors
- Enable less reliance on specialists
- Empower better, faster decision-making

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Scientists say an optimal workflow demands more MS capability.

Scientists ideally capable of performing MS-based biopharma analysis: **45%**

Scientists actually capable of performing MS-based biopharma analysis: **25%**

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As Easy to Deploy as an Optical Detector

+ QDa

QDa Mass Detector

- Empower® and MassLynx™ Control
- Minimal operator training required
- Qualification Service available
- 110/220V operation – no need for special outlet
- Workhorse with low maintenance requirement

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WCBP 2019… One Year Ago

"The inherent complexity of biopharma therapies, combined with rising regulatory standards, are driving more intensive and widespread testing requirements."

"Waters designed the BioAccord System as a fit-for-purpose LC-MS biopharmaceutical solution to deliver rich mass spectrometry data for improved productivity and effective decision-making."

Chris O'Connell, Chairman and CEO, Waters Corporation

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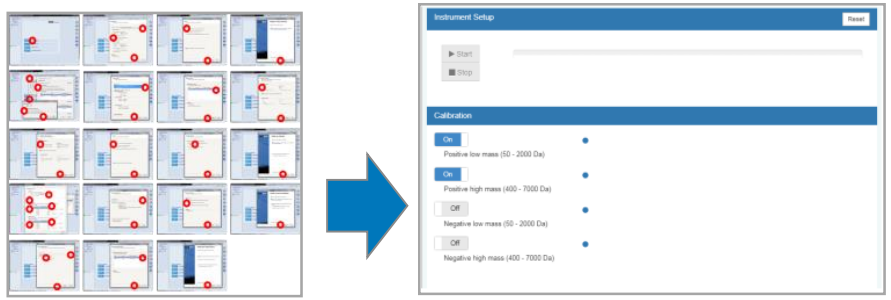
Delivering benefit — we listened to our customers

Robust by design

Single-button start up

Intelligent diagnostics



Simplified interface

End user evaluation

Focus on training

Systems integration testing

Consolidated shipment

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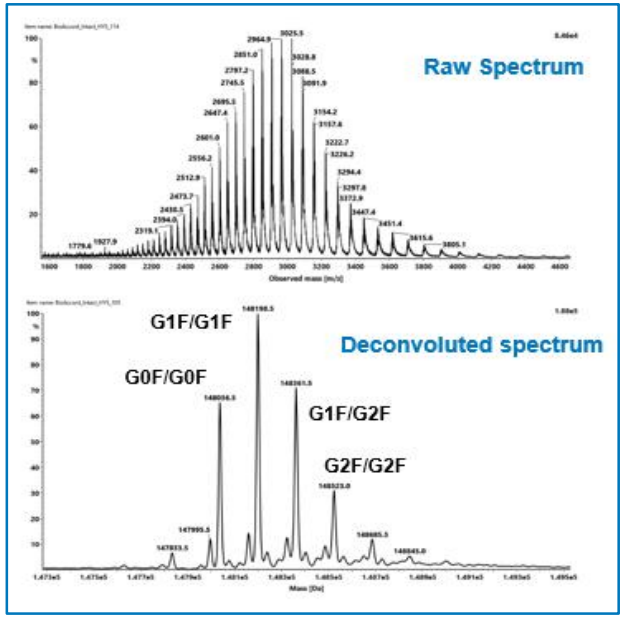
Streamlined Biopharmaceutical Workflows on BioAccord

Acquisition → Processing → Reporting

UNIFI WORKFLOW



Intact Mass

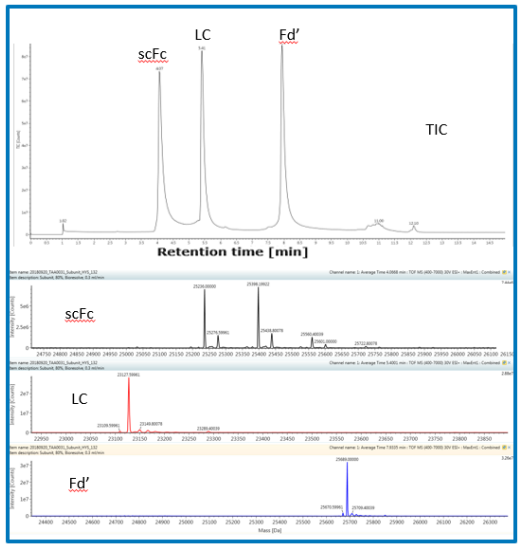
Native & Denatured
(to 7000 *m/z*)

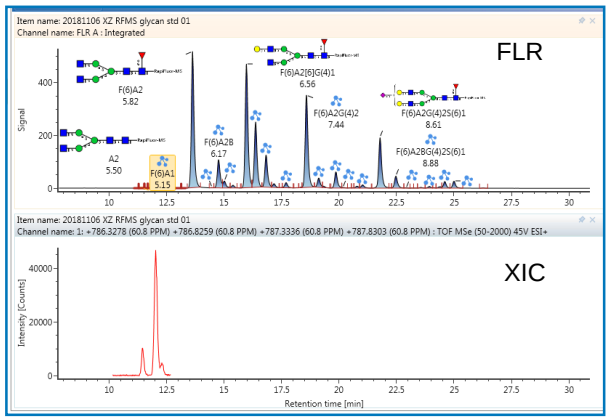
Released Glycan

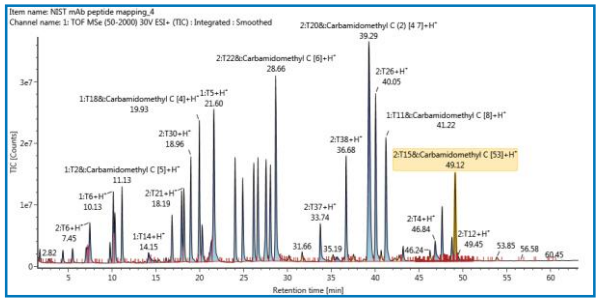
Subunit Mass

21 CFR Part 11
Compliance-ready

Peptide Mapping & Monitoring







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Routine Mass Detection:
The Key to Faster Biopharma Decision Making

The eBook is divided into two sections:

- **Section 1: The ACQUITY QDa mass detector and Empower**

In this section, we include key application notes and links to additional content that demonstrate how the ACQUITY QDa with Empower is being applied today, and the value it can bring to routine biopharmaceutical analysis and monitoring applications.

- **Section 2: The BioAccord System with the ACQUITY RDa mass detector & UNIFI**

In this section, we include key application notes and links to additional content that demonstrate the three primary biopharmaceutical analysis workflows that can be run by non-MS experts using the BioAccord System, including 1) Intact/subunit analysis, 2) released glycan analysis, and 3) peptide mapping/multi-attribute monitoring.

Acquity QDa

Empower

BioAccord™ SYSTEM

UNIFI SCIENTIFIC INFORMATION SYSTEM

Contents

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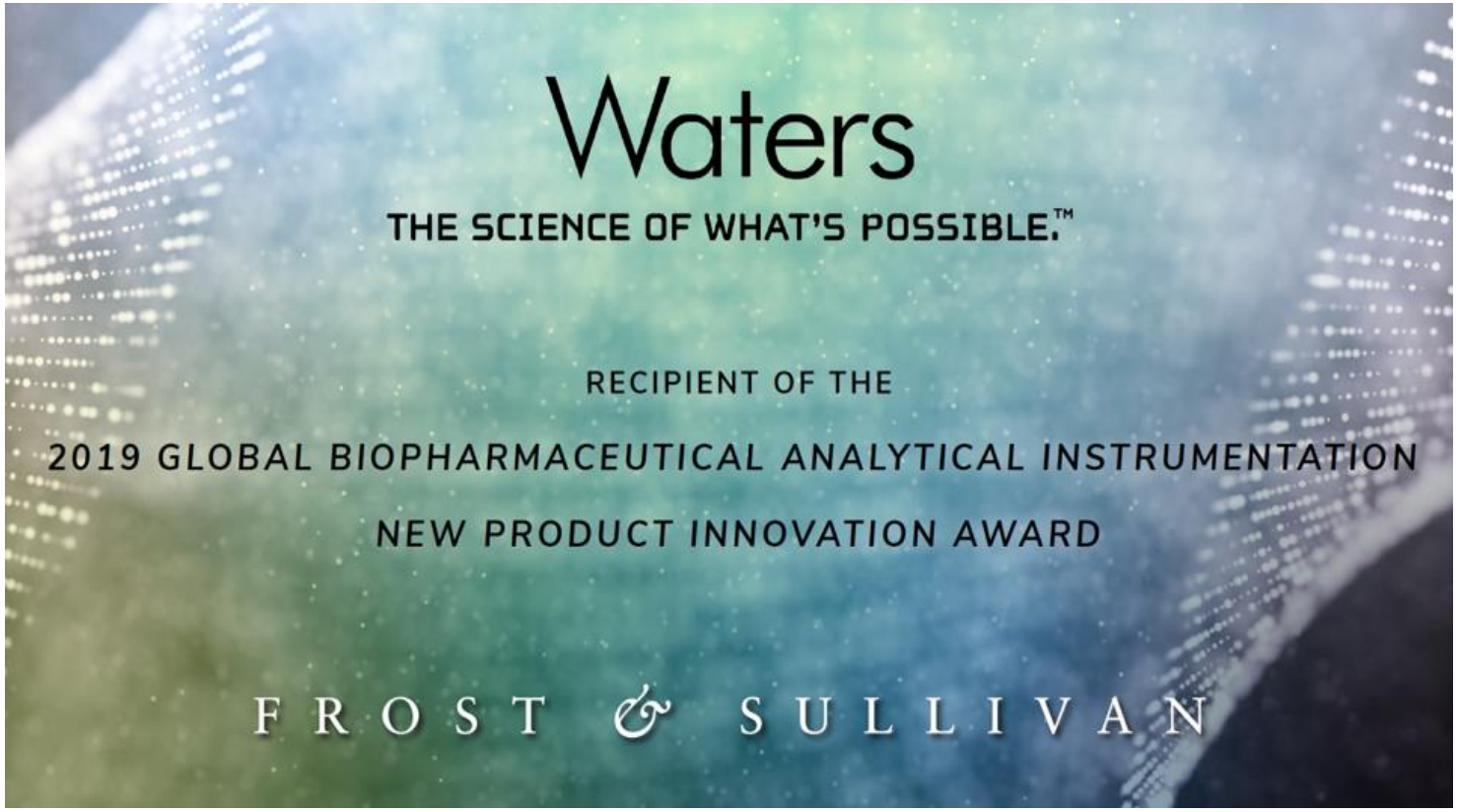
BioAccord LC/MS System:
Winner, 2019 Frost & Sullivan Product Innovation Award



Waters Corporation Lauded by Frost & Sullivan for Developing the First Truly Smart Mass Spectrometer, the BioAccord LC-MS System

BPAwards | Jan 8 2020 | Best Practice Awards | News

"Frost & Sullivan recognizes Waters Corporation with the 2019 Global New Product Innovation Award for its smart BioAccord Liquid Chromatography-Mass Spectrometry (LC-MS) System. It is the first smart MS-enabled biopharmaceutical solution with intelligent software and chemistries, which deliver high levels of reliability, sensitivity, and detection."

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BioAccord LC/MS System: Exceptional Customer Feedback

Scientists' Choice Award
Best New Drug Discovery and Development Product of 2019

/ Products / BioAccord LC-MS System

BioAccord LC-MS System by Waters

Manufacturer Waters | Available Worldwide

4.9 / 5.0 | 5 reviews | Write your own review

SCIENTISTS' CHOICE AWARDS 20 NOMINEE

The First SmartMS-Enabled Biopharma Solution The Waters BioAccord System is an integrated system that simplifies high performance LC-MS biopharmaceutical analysis for every user. An easy-to-use system solution that puts the power to make decisions directly in your hands; a self-calibrating, self-optimizing, self-sufficient tool that equips you with high quality data you can use to tackle the challenges you face every day during biopharmaceutical development.

ts have reviewed this product

Value for money

Write your own review

Peng Zhai
Status: Reviewer
Member since: 2019
Organization: Beijing QL Biopharm

"It is part of our daily work and a reliable friend to answer quality questions."

Rating: 5.0

Ease of use
After sales service
Value for money

Application Area: Analyzing samples for recombinant proteins and peptides

"BioAccord is a very user friendly LC/MS system that only takes a very short time to get working. The result is always consistent and reliable. The service and support from Waters is always fast and helpful."

Review date: 12 Dec 2019 | BioAccord LC-MS System

Francesco Lanucara
Status: Reviewer
Member since: 2019
Organization: Allergan

"Reliable instrument from characterization to QC."

Rating: 5.0

Ease of use
After sales service
Value for money

Application Area: Biopharmaceuticals analysis

"Extremely robust and easy to use, the BioAccord provides a reliable platform for routine analysis of biopharmaceuticals, whilst at the same time ensuring data integrity and providing seamless automated workflows to maximize throughput"

Review date: 12 Dec 2019 | BioAccord LC-MS System

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BioAccord Support for Protein and Oligonucleotide Mass Confirmation

MassPrep OST Oligo Standard 186004135

Manage Components

Create Protein | Modify Protein | Create Mass | Import | Delete

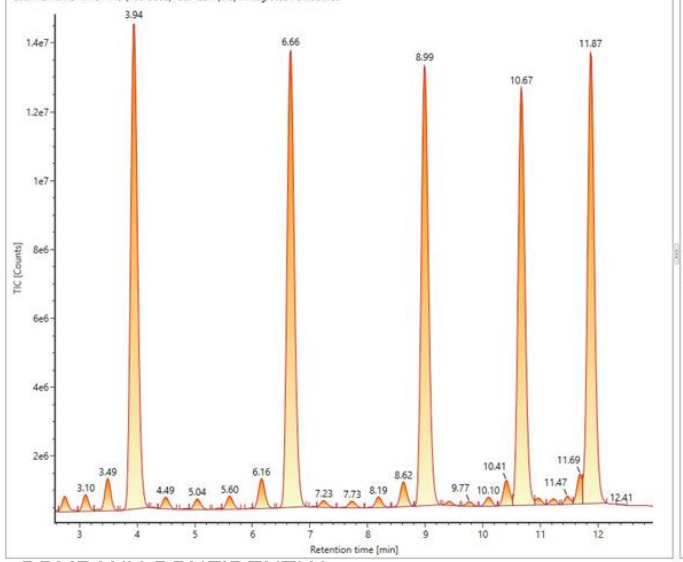
	Component name	Expected RT (min)	Time window (min)	Expected mass (Da)	Formula	Description
1	dT15	3.94	0.3	4500.9331		
2	dT20	6.66	0.3	6021.8990		
3	dT25	8.99	0.3	7542.8649		
4	dT30	10.67	0.3	9063.8308		
5	dT35	11.87	0.3	10584.7967		

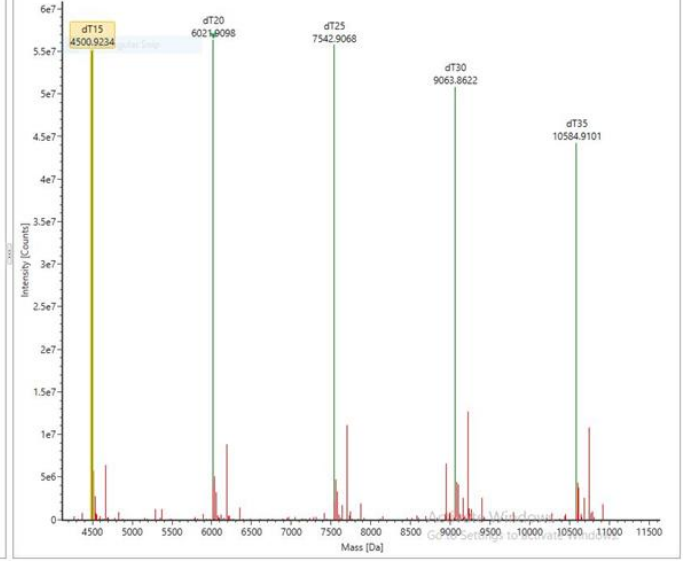
← expected average masses

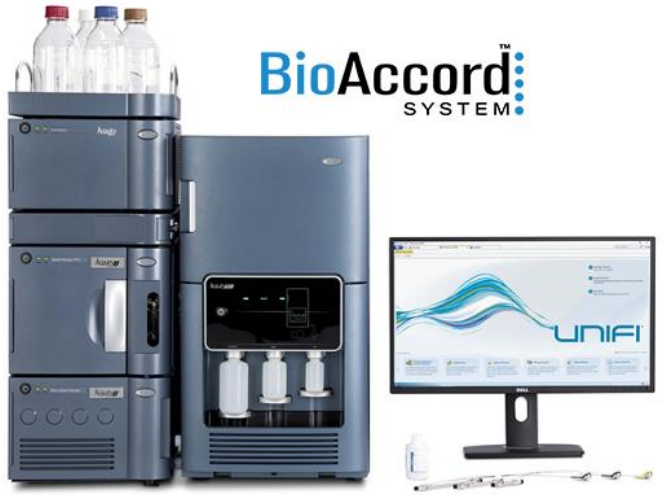
Component Summary

	Protein name	Modifiers	Response	Observed mass (Da)	Expected mass (Da)	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	Alternate assignments
1	dT15		55043004	4500.9234	4500.93310	-9.7	-2.1	3.93	No
2	dT20		56370438	6021.9098	6021.89900	10.8	1.8	6.67	No
3	dT25		55759395	7542.9068	7542.86490	41.9	5.6	8.98	No
4	dT30		50822917	9063.8622	9063.83080	31.4	3.5	10.67	No
5	dT35		44240738	10584.9101	10584.79670	113.4	10.7	11.87	No

Average mass accuracy error: 4.7 ppm







To Learn More Visit:
WWW.Waters.com/oligos

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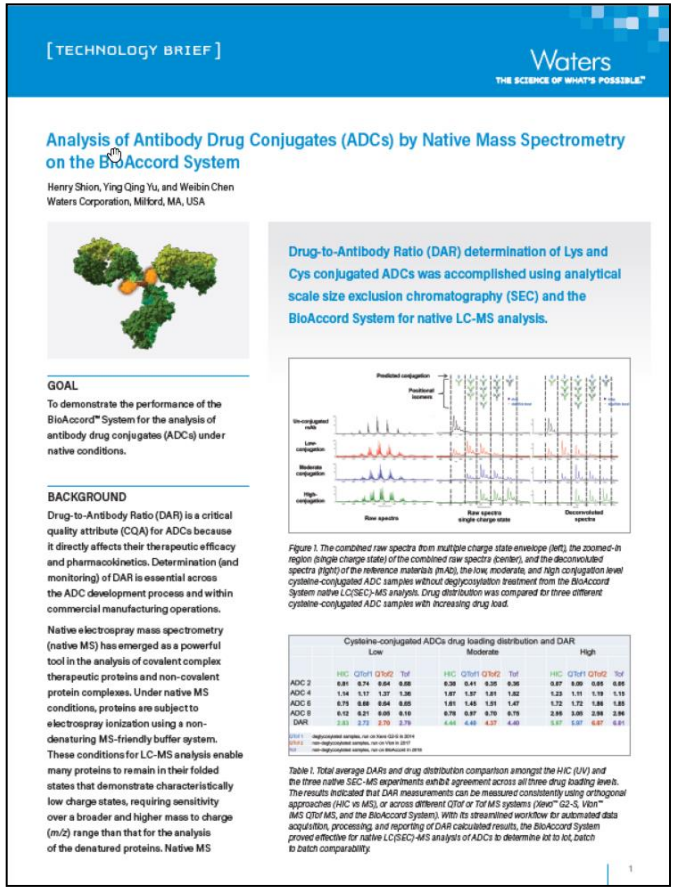
Native SEC-MS and IEX MS

SEC-Native MS of Cys and Lys ADCs



[TECHNOLOGY BRIEF]

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Analysis of Antibody Drug Conjugates (ADCs) by Native Mass Spectrometry on the BioAccord System

Henry Shion, Ying Qing Yu, and Weibin Chen
Waters Corporation, Milford, MA, USA

Drug-to-Antibody Ratio (DAR) determination of Lys and Cys conjugated ADCs was accomplished using analytical scale size exclusion chromatography (SEC) and the BioAccord System for native LC-MS analysis.

GOAL

To demonstrate the performance of the BioAccord™ System for the analysis of antibody drug conjugates (ADCs) under native conditions.

BACKGROUND

Drug-to-Antibody Ratio (DAR) is a critical quality attribute (CQA) for ADCs because it directly affects their therapeutic efficacy and pharmacokinetics. Determination (and monitoring) of DAR is essential across the ADC development process and within commercial manufacturing operations.

Native electrospray mass spectrometry (native MS) has emerged as a powerful tool in the analysis of covalent complex therapeutic proteins and non-covalent protein complexes. Under native MS conditions, proteins are subject to electrospray ionization using a non-denaturing MS-friendly buffer system. These conditions for LC-MS analysis enable many proteins to remain in their folded states that demonstrate characteristically low charge states, requiring sensitivity over a broader and higher mass to charge (m/z) range than that for the analysis of the denatured proteins. Native MS

Figure 1. The combined raw spectra from multiple charge state envelope (left), the zoomed-in region (single charge state) of the combined raw spectra (center), and the deconvoluted spectra (right) of the reference materials (mAb), the low, moderate, and high conjugation level cysteine-conjugated ADC samples without deglycosylation treatment from the BioAccord System native LC(SEC)-MS analysis. Drug distribution was compared for three different cysteine-conjugated ADC samples with increasing drug load.

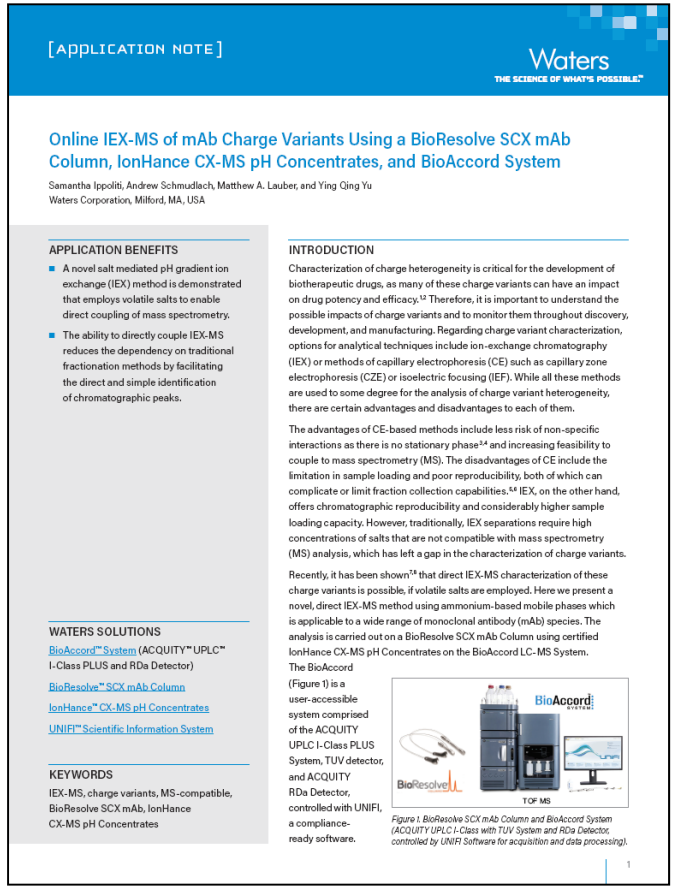
Cysteine-conjugated ADCs drug loading distribution and DAR

	Low				Moderate				High			
	HIC	QToF1	QToF2	Tof	HIC	QToF1	QToF2	Tof	HIC	QToF1	QToF2	Tof
ADC 2	0.81	0.74	0.64	0.88	0.39	0.41	0.35	0.35	0.07	0.09	0.05	0.05
ADC 4	1.94	1.17	1.07	1.26	1.67	1.57	1.51	1.62	1.23	1.11	1.19	1.15
ADC 6	0.75	0.68	0.84	0.85	1.61	1.45	1.51	1.47	1.72	1.72	1.86	1.85
ADC 8	0.12	0.21	0.05	0.10	0.78	0.97	0.70	0.75	2.95	3.05	2.98	2.96
DAR	3.61	2.72	3.70	3.79	4.44	4.40	4.37	4.40	5.97	5.97	5.87	5.81

Table 1. Total average DARs and drug distribution comparison amongst the HIC (UV) and the three native SEC-MS experiments exhibit agreement across all three drug loading levels. The results indicated that DAR measurements can be measured consistently using orthogonal approaches (HIC vs MS), or across different QToF or Tof MS systems (Xevo™ G2-S, Vion™ IMS QToF MS, and the BioAccord System). With its streamlined workflow for automated data acquisition, processing, and reporting of DAR calculated results, the BioAccord System proved effective for native LC(SEC)-MS analysis of ADCs to determine lot to lot, batch to batch comparability.

BioAccord™ SYSTEM

IEX-Native MS of Charge Variants



[APPLICATION NOTE]

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Online IEX-MS of mAb Charge Variants Using a BioResolve SCX mAb Column, IonHance CX-MS pH Concentrates, and BioAccord System

Samantha Ippoliti, Andrew Schmudlach, Matthew A. Lauber, and Ying Qing Yu
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- A novel salt mediated pH gradient ion exchange (IEX) method is demonstrated that employs volatile salts to enable direct coupling of mass spectrometry.
- The ability to directly couple IEX-MS reduces the dependency on traditional fractionation methods by facilitating the direct and simple identification of chromatographic peaks.

WATERS SOLUTIONS

BioAccord™ System (ACQUITY™ UPLC™ I-Class PLUS and RDa Detector)

BioResolve™ SCX mAb Column

IonHance™ CX-MS pH Concentrates

UNIFI™ Scientific Information System

KEYWORDS

IEX-MS, charge variants, MS-compatible, BioResolve SCX mAb, IonHance CX-MS pH Concentrates

INTRODUCTION

Characterization of charge heterogeneity is critical for the development of biotherapeutic drugs, as many of these charge variants can have an impact on drug potency and efficacy.[1,2] Therefore, it is important to understand the possible impacts of charge variants and to monitor them throughout discovery, development, and manufacturing. Regarding charge variant characterization, options for analytical techniques include ion-exchange chromatography (IEX) or methods of capillary electrophoresis (CE) such as capillary zone electrophoresis (CZE) or isoelectric focusing (IEF). While all these methods are used to some degree for the analysis of charge variant heterogeneity, there are certain advantages and disadvantages to each of them.

The advantages of CE-based methods include less risk of non-specific interactions as there is no stationary phase[3,4] and increasing feasibility to couple to mass spectrometry (MS). The disadvantages of CE include the limitation in sample loading and poor reproducibility, both of which can complicate or limit fraction collection capabilities.[5,6] IEX, on the other hand, offers chromatographic reproducibility and considerably higher sample loading capacity. However, traditionally, IEX separations require high concentrations of salts that are not compatible with mass spectrometry (MS) analysis, which has left a gap in the characterization of charge variants.

Recently, it has been shown[7,8] that direct IEX-MS characterization of these charge variants is possible, if volatile salts are employed. Here we present a novel, direct IEX-MS method using ammonium-based mobile phases which is applicable to a wide range of monoclonal antibody (mAb) species. The analysis is carried out on a BioResolve SCX mAb Column using certified IonHance CX-MS pH Concentrates on the BioAccord LC-MS System. The BioAccord (Figure 1) is a user-accessible system comprised of the ACQUITY UPLC I-Class PLUS System, TUV detector, and ACQUITY RDa Detector, controlled with UNIFI, a compliance-ready software.

Figure 1. BioResolve SCX mAb Column and BioAccord System (ACQUITY UPLC I-Class with TUV System and RDa Detector, controlled by UNIFI Software for acquisition and data processing).

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IonHance™

High Purity Mobile Phases for Maximum Data Quality

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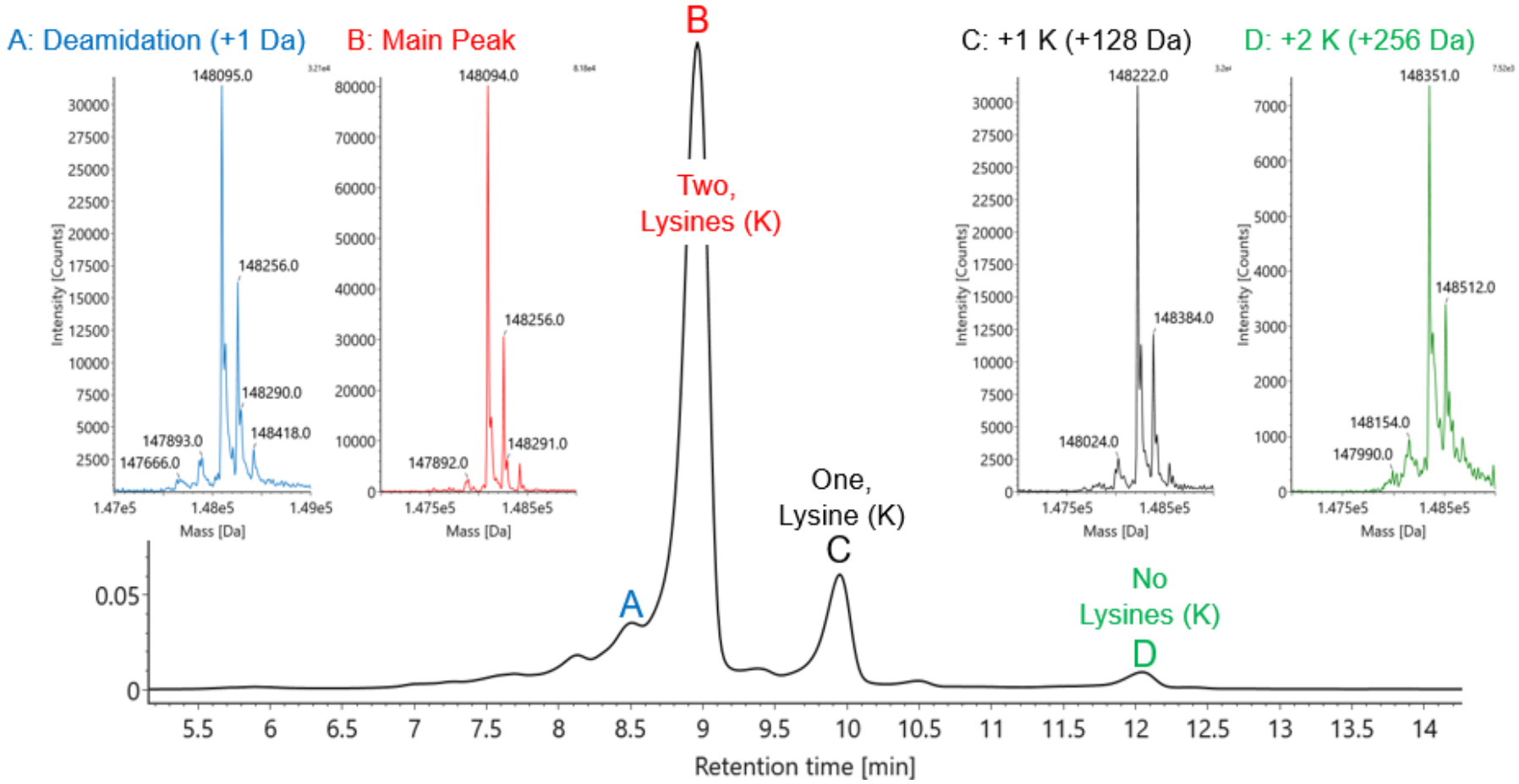

IonHance CX-MS pH Buffer Concentrates

- Reproducible LC-MS analyses of Intact mAbs and IdeS digests.
- Cleaner spectra, reduced noise, super charging, and ion suppression.

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Continued Innovations in mass spectrometry for improved biopharmaceutical characterization

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Research, Characterization, Advanced Characterization (HDX, ETD, IMS)

SELECT SERIES
Waters Advanced Mass Spectrometry

MassLynx

SYNAPT XS

MassLynx

Characterization and Attribute Monitoring

Xevo G2-XS QTof

UNIFI
MassLynx

Vion IMS QTof

UNIFI

Attribute Monitoring and Characterization *Light*

BioAccord™ SYSTEM

UNIFI

Chromatographic Mass Detection and Targeted Attribute Monitoring

Acquity QDa

Empower
MassLynx

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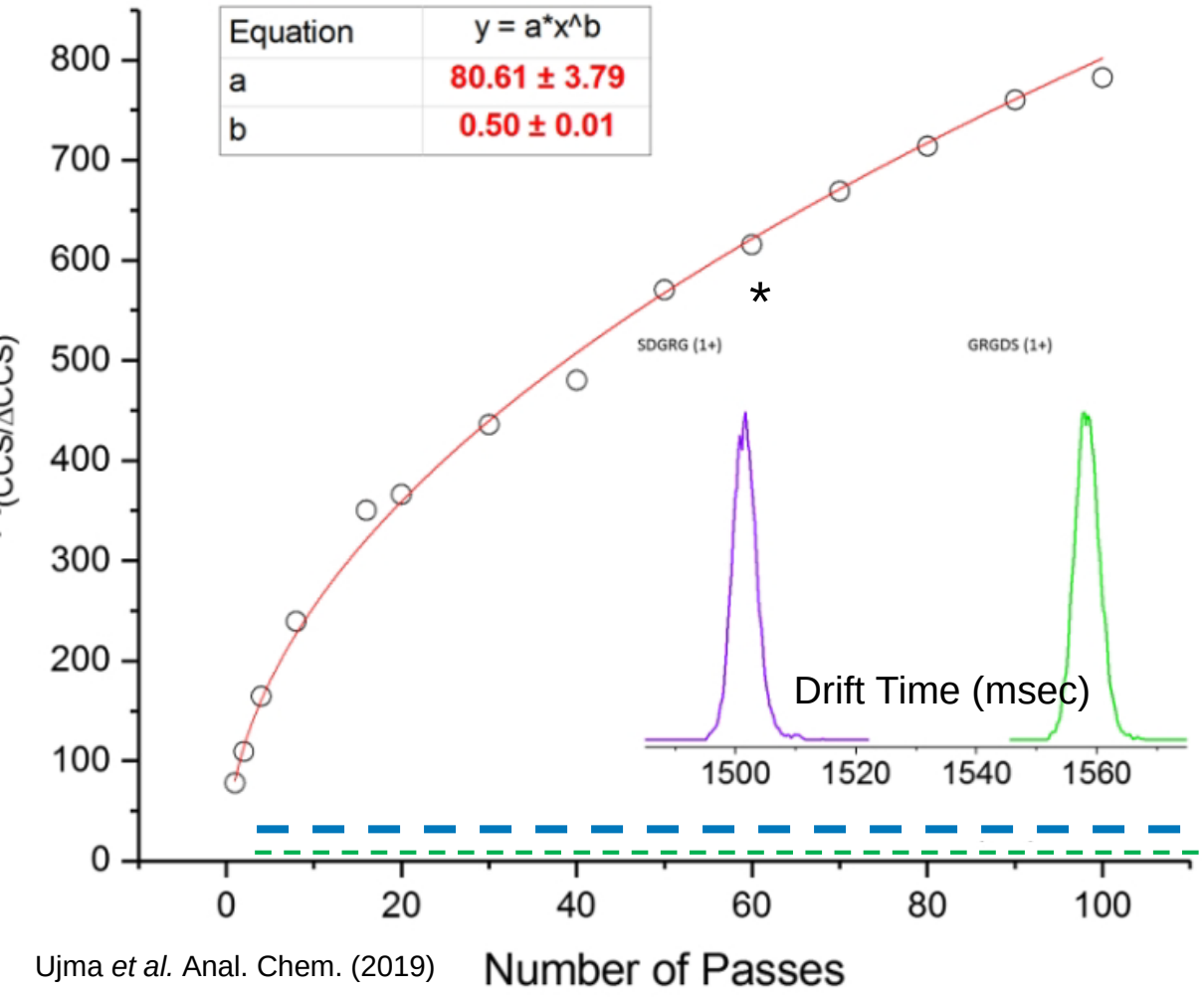
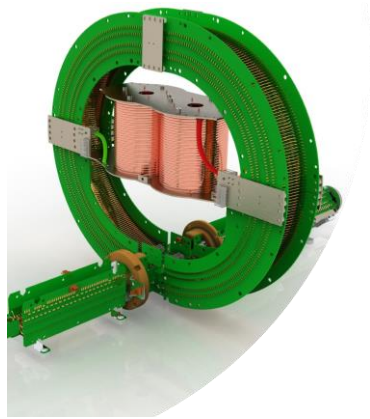
Scalable Ion mobility resolution



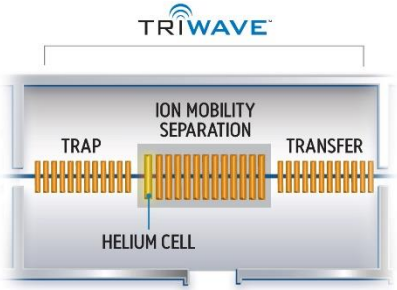
Ujma *et al.* Anal. Chem. (2019)

* IM selection used for n>18

2019 (Cyclic IMS)
Resolution ~750
100 m (100 passes)



2009 (SYNAPT G2/*S*/*Si*)
2015 (Vion)
Resolution ~40
Triwave IMS Cell ~0.25 m



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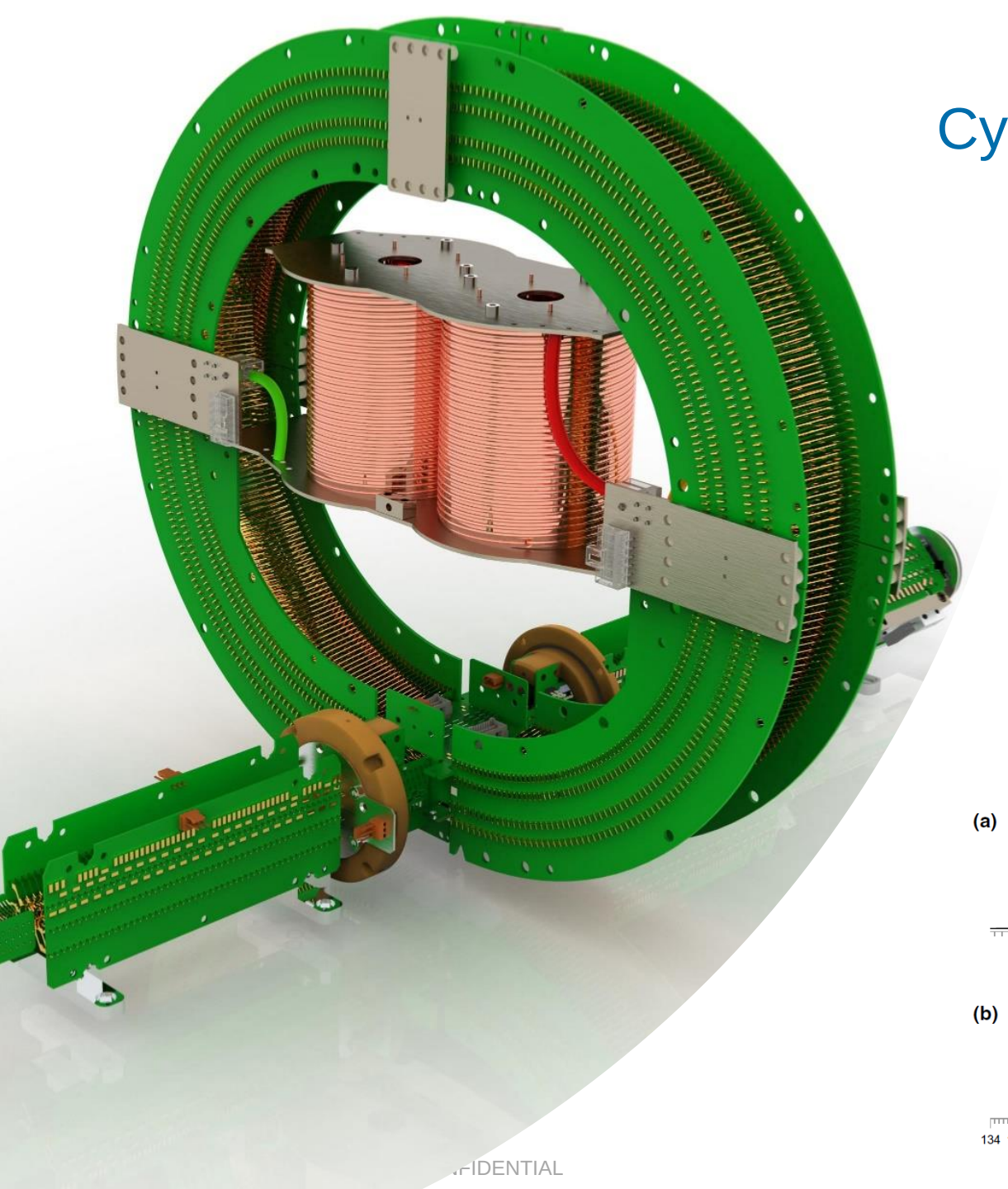

Cyclic IMS Experiments

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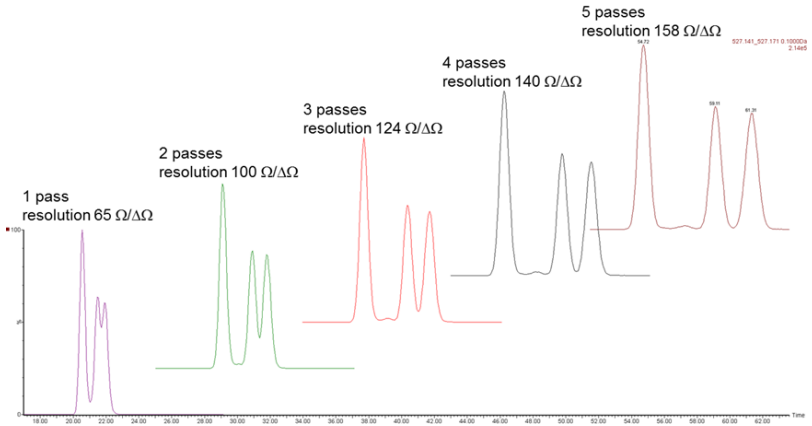
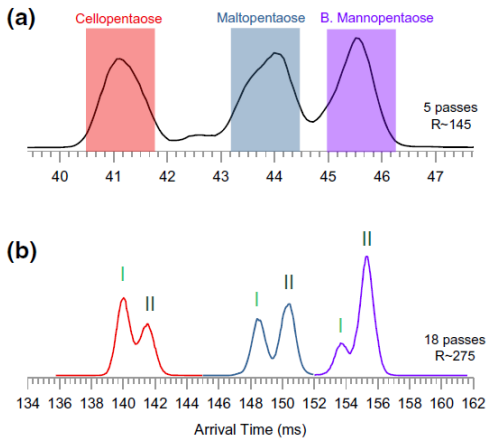
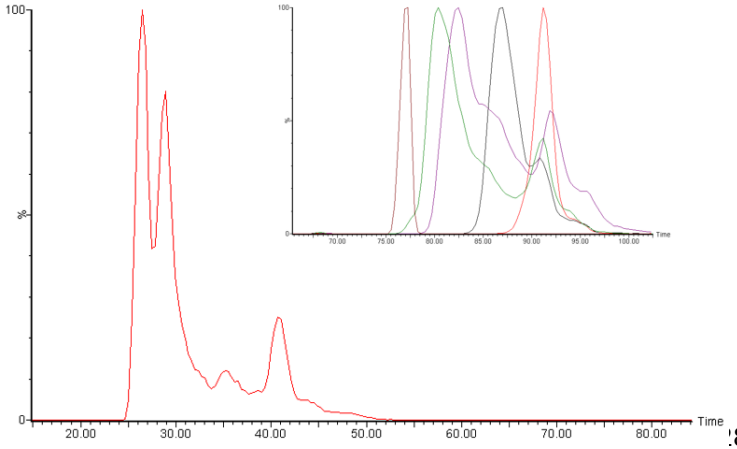
Single Pass and Multi-Pass



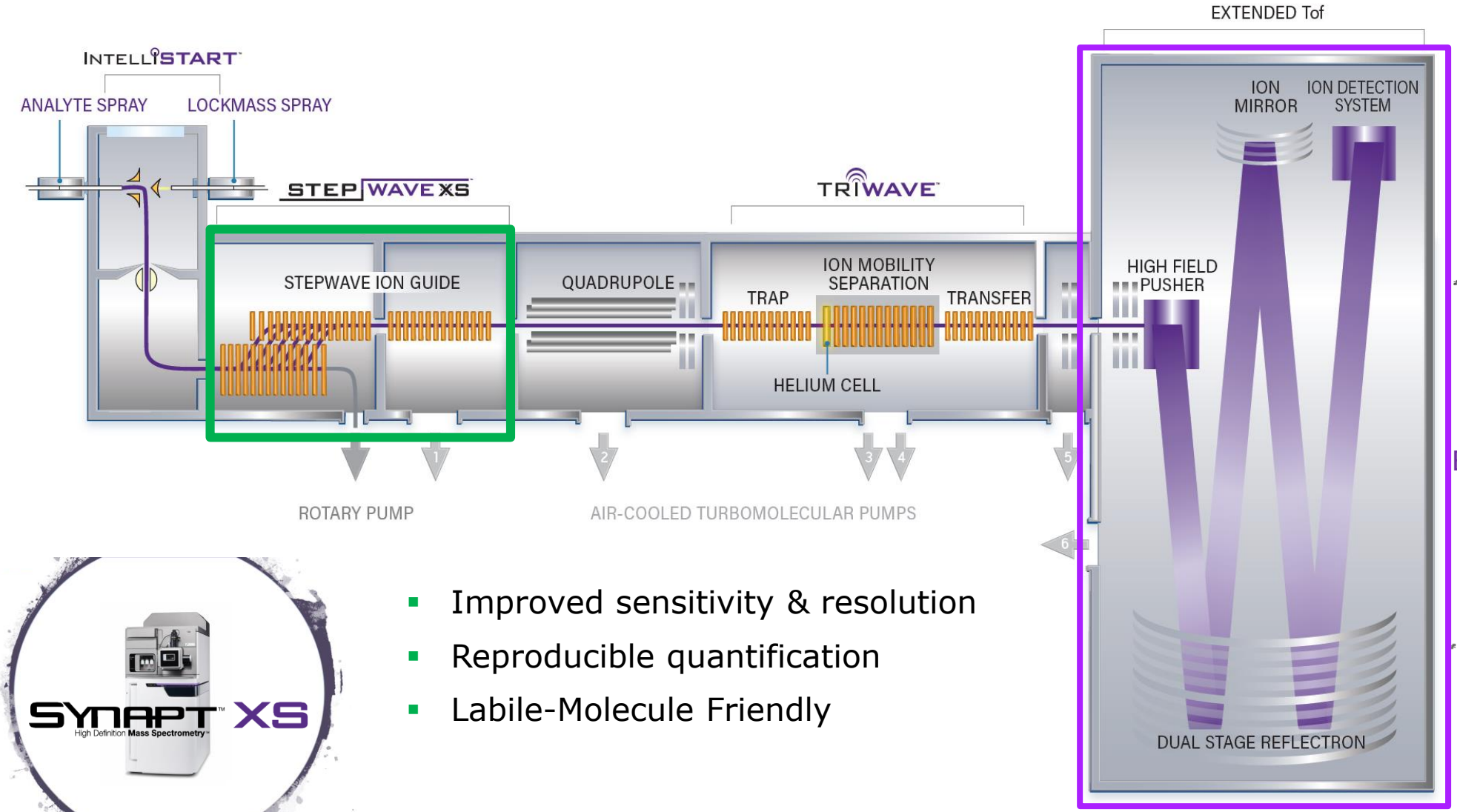
IMS^n Selection:



IMS^n with Activation:

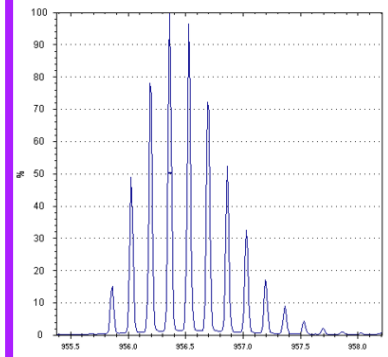


SYNAPT XS Enhancements

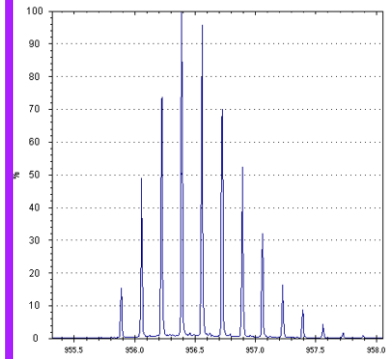


- Improved sensitivity & resolution
- Reproducible quantification
- Labile-Molecule Friendly

Resolution Mode (V) 25k



Enhanced Res Mode (W) >80k



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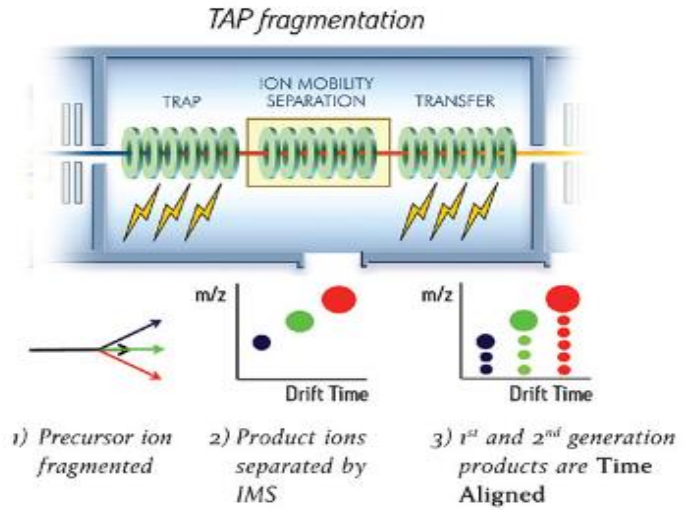
Triwave IMS

Flexible Fragmentation



Acquisition Modes

- Fast-DDA
- HD-DDA
- Tof-MRM
- HD-MRM
- MS^E
- $HDMS^E$
- $UDMS^E$
- SONAR
- TAP
- ETD*

*option

Options

- UPLC
- nano/micro UPLC
- UPC^2
- 2D-LC
- APGC
- HDX
- DESI
- MALDI
- REIMS
- ASAP
- UniSpray

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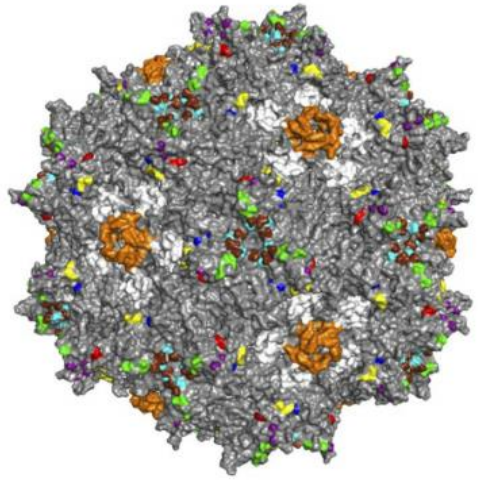
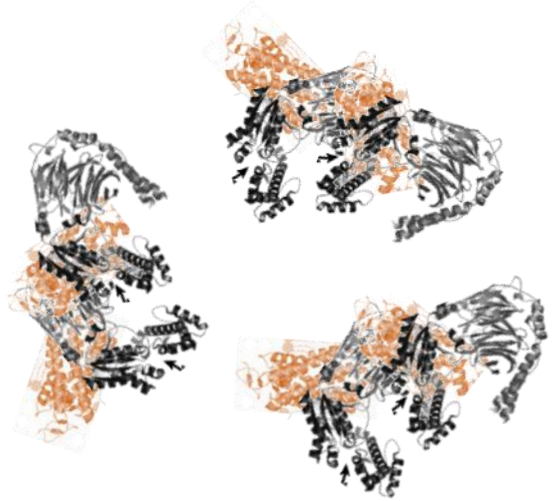
Developing analytical workflows supporting Adeno-Associated Virus (AAV) Capsid Analysis using LC and LC-MS

Ximo Zhang, Stephan Koza, Hua Yang, Weibin Chen

Waters Lunch Seminar

January 28, 2020





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Overview

- Introduction
  - Background and challenges in gene therapy analytical development
- Analysis of AAV capsid
  - Size variance of AAV capsids
    - SEC
  - Empty/full ratio
    - AEX
  - Characterization and monitoring of capsid proteins
    - RP-LC and LC/MS
- Conclusion and future work

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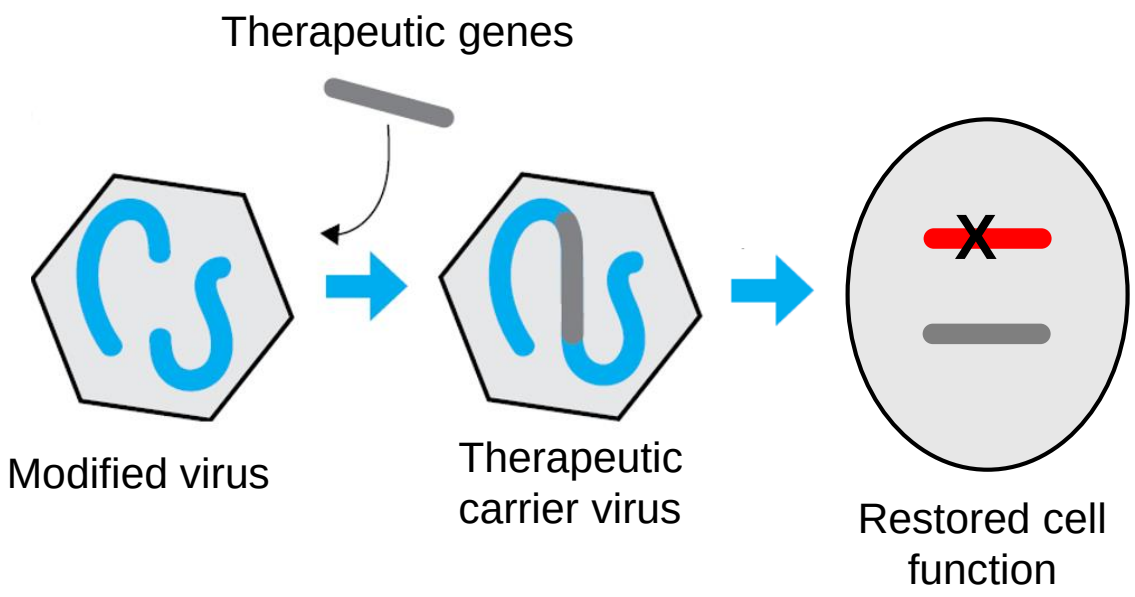
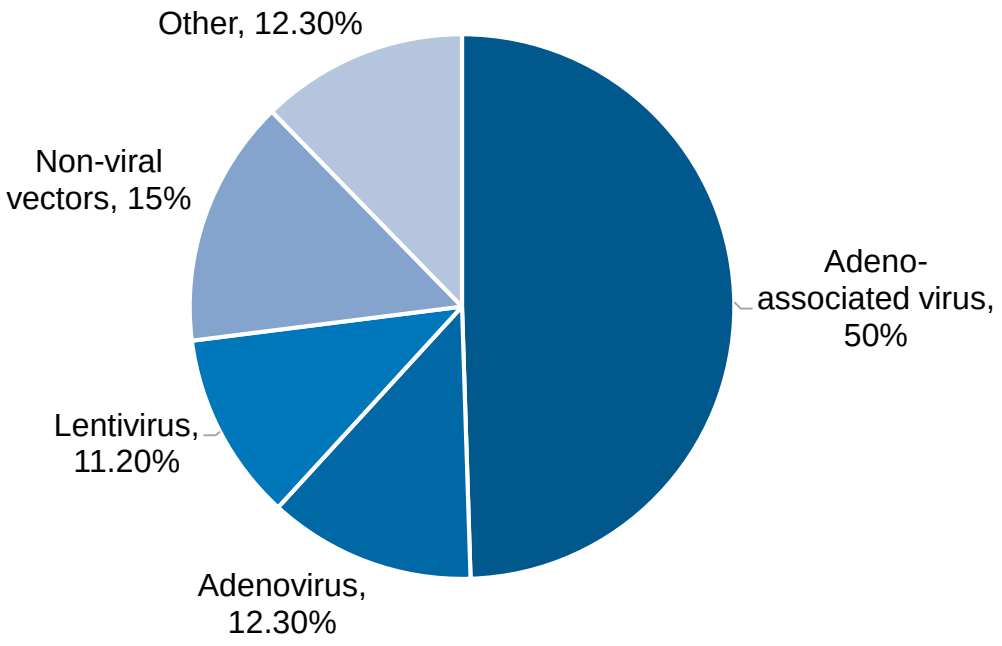
Gene therapy comes of age

- Gene therapy: use genes to treat genes
- Advances in gene editing
  - CRISPR
- 3 approved drugs in 2019



Type of vectors used in development pipeline



Gene therapy market report, Roots Analysis

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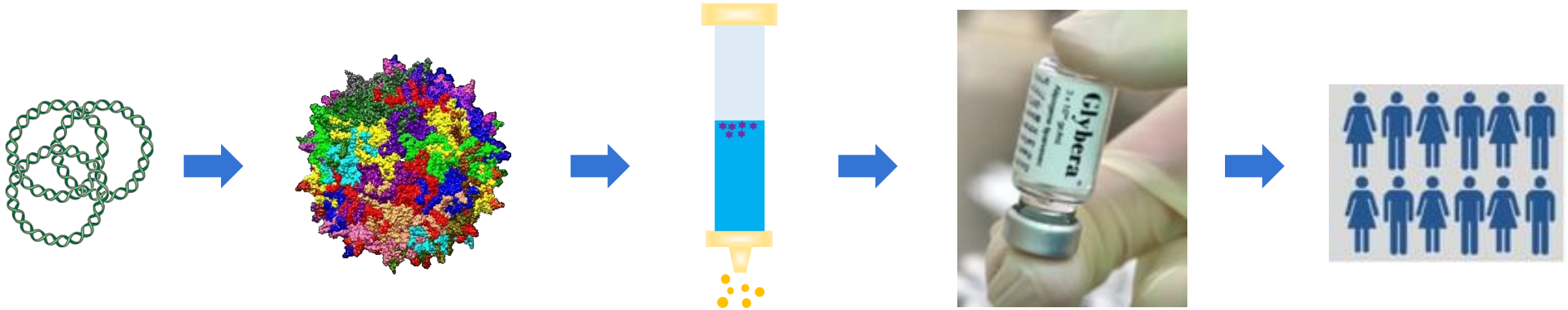
The development of gene therapy products calls for stage appropriate analytical tools

Before giving the drug to real people:



Candidate selection → Gene editing encapsulation → Purification → Formulation → Clinical trials

Characterization

Process monitoring

Stability assessment

Release testing

http://www.21stcentech.com/biomedicine-update-progress-aidshiv-front/

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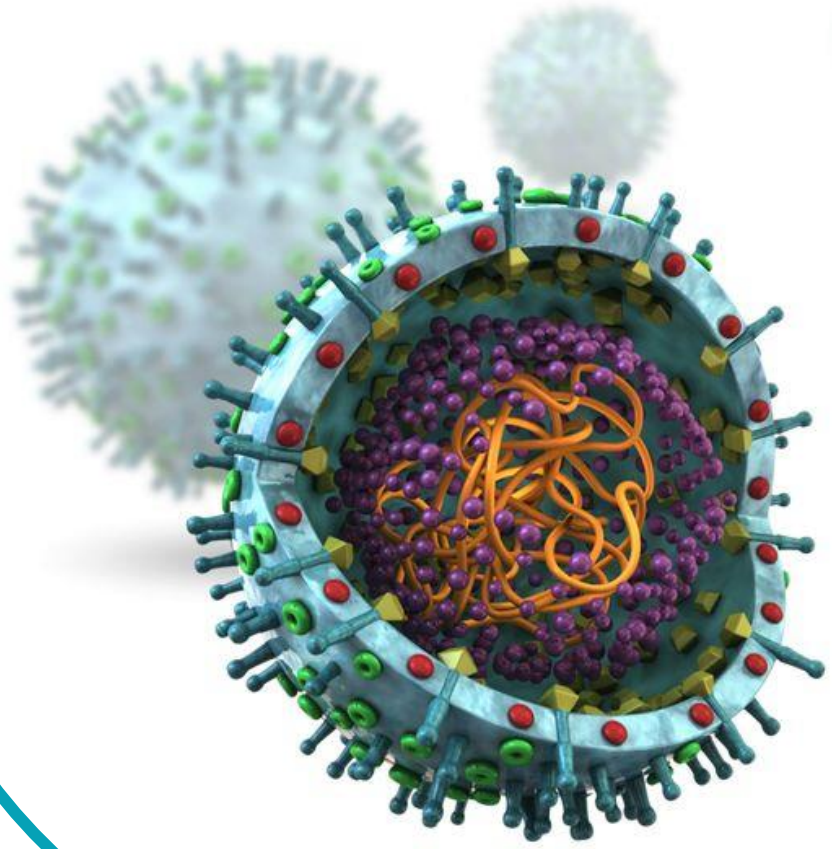
Key analytical characteristics of gene therapy products

Vector

- Capsid ID
- Empty/full ratio
- Purity profile
- Stability
- Batch to batch consistency



Therapeutic gene

- Identity
- Dosage strength
- Purity
- Potency
- Safety

For *ex vivo* GTP, cells also need to be controlled

http://www.21stcentech.com/biomedicine-update-progress-aidshiv-front/

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Key analytical characteristics of gene therapy products

Vector

- Capsid ID
- Empty/full ratio
- Purity profile
- Stability
- Batch to batch consistency

The goal(s) of analytics:

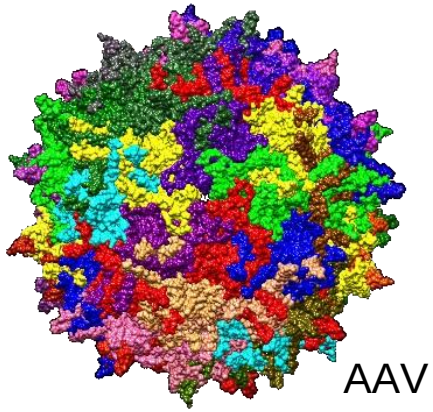
- Stage appropriate and streamlined methods
- Well characterized from early stage

…in reality:

- Time consuming tests
- Lack of established analytical platform
- Limited sample amount

Use AAV as a case study:

- Most popular vector
- 13 serotypes
- ~5 MDa/3 Kbp
- BSL-1



AAV vector

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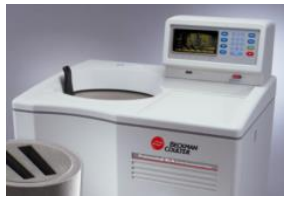
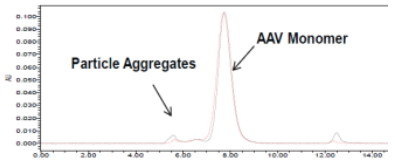
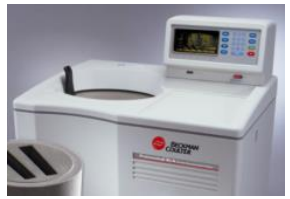
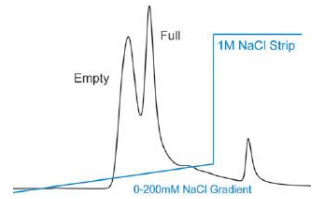
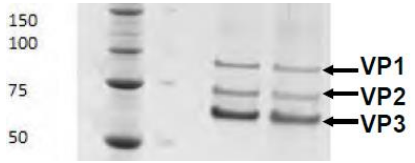
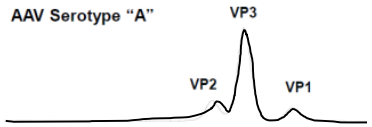
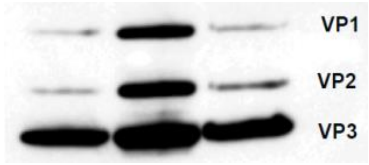
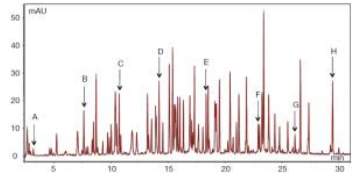
Advanced technologies are accelerating AAV development

	Traditional	Advanced
Aggregation	**AUC** Measure difference in Sedimentation Coefficient 	**SEC** Detect E/F ratio, and aggregation 
Empty/full ratio	**AUC** Measure difference in Sedimentation Coefficient 	**IEX** Measure difference in charge profile 
Purity	**SDS-PAGE** Measure difference in separation profile 	**RPLC** Measure difference in hydrophobicity profile 
Capsid identity	**Western Blot** Resource intensive 	**Peptide Mapping** Measure AA sequence and PTMs 

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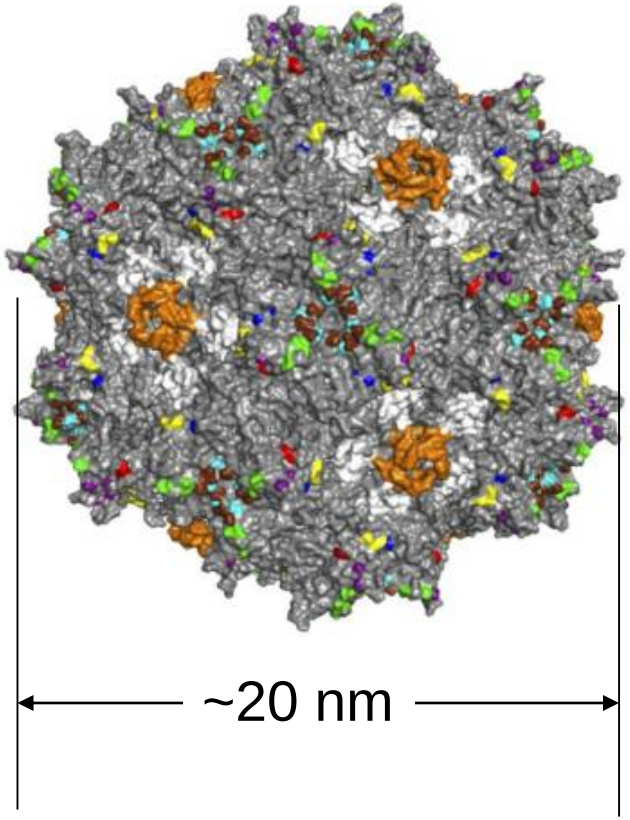
SEC Analysis: Molecular Weight is not the only factor

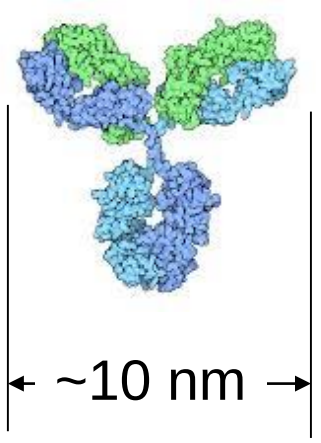
Dr. Steve Koza

3.7 MDa



MW = 25X mAb

0.15 MDa



Hydrodynamic radius is closer

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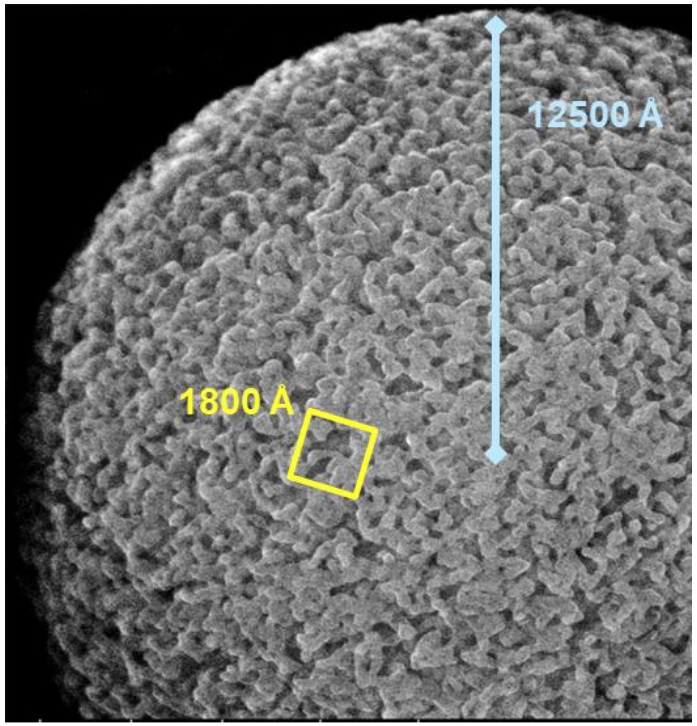
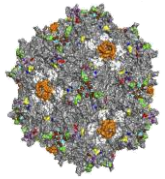
Larger viral capsids need larger pore size for SEC analysis



BEH SEC 450Å (2.5 µm)



AAV

~20 nm

Lentivirus

~90 nm

- Current SEC technology should be sufficient for AAV separation
- Larger pore size or other technology is needed for larger viral vectors

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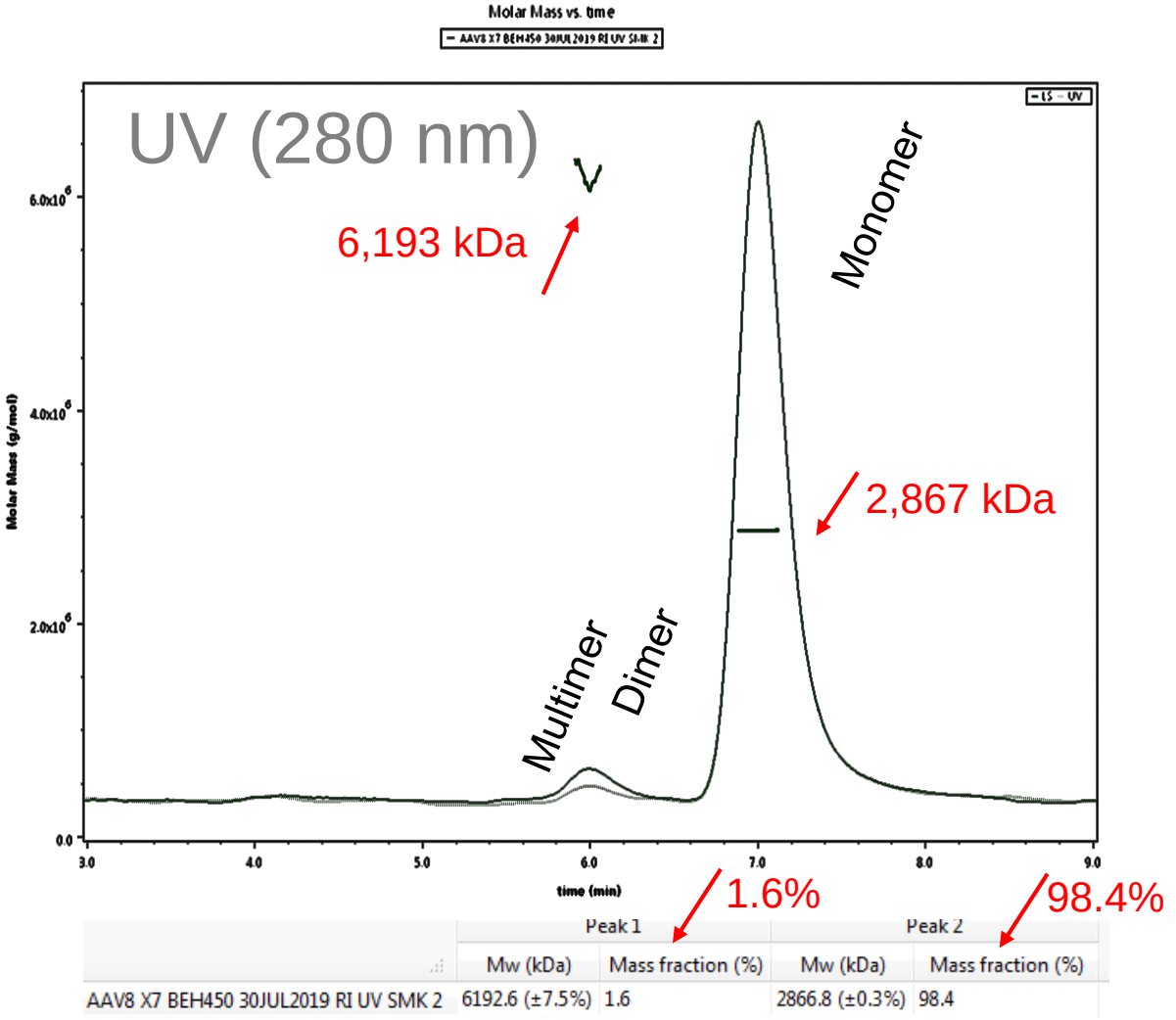
Current SEC technologies can separate the capsid monomer and aggregates

- Conditions:
  - ACQUITY BEH450 SEC column, 2.5 µm, 4.6X300 mm
  - PBS @ 0.35 mL/min
  - µDAWN MALS

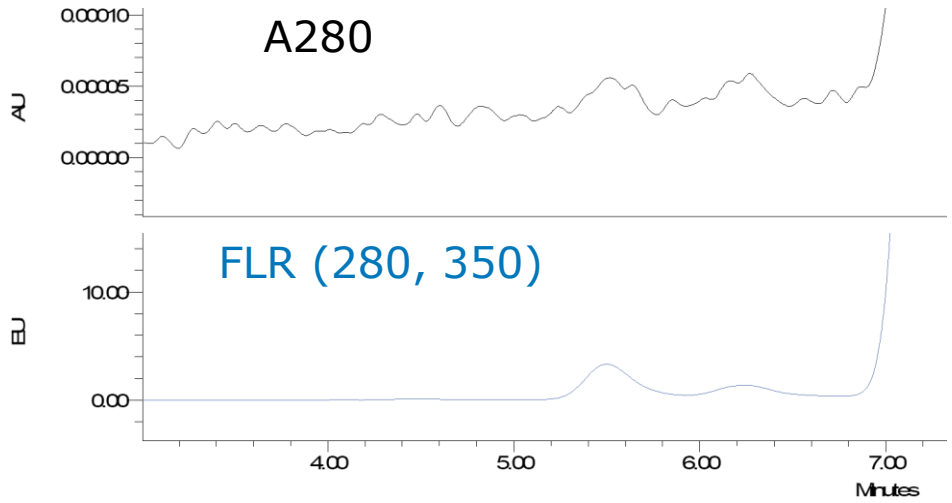


	Peak 1		Peak 2	
	Mw (kDa)	Mass fraction (%)	Mw (kDa)	Mass fraction (%)
AAV8 X7 BEH450 30JUL2019 RI UV SMK 2	6192.6 (±7.5%)	1.6	2866.8 (±0.3%)	98.4

AAV Null sample concentrated to ~ 8 E12 capsids/mL

Tips and tricks in developing SEC methods

- General method optimization:
  - KCl is more effective than NaCl for minimizing secondary interactions
  - Perchlorate, arginine, IPA, citrate (chelator),and MES (in place of PO4) had no significant benefits
- Low sample concentrations benefit from the use of FLR detector over UV
  - Measured HMW levels will be different for UV vs FLR



AAV Null Sample (~10 ug/mL)

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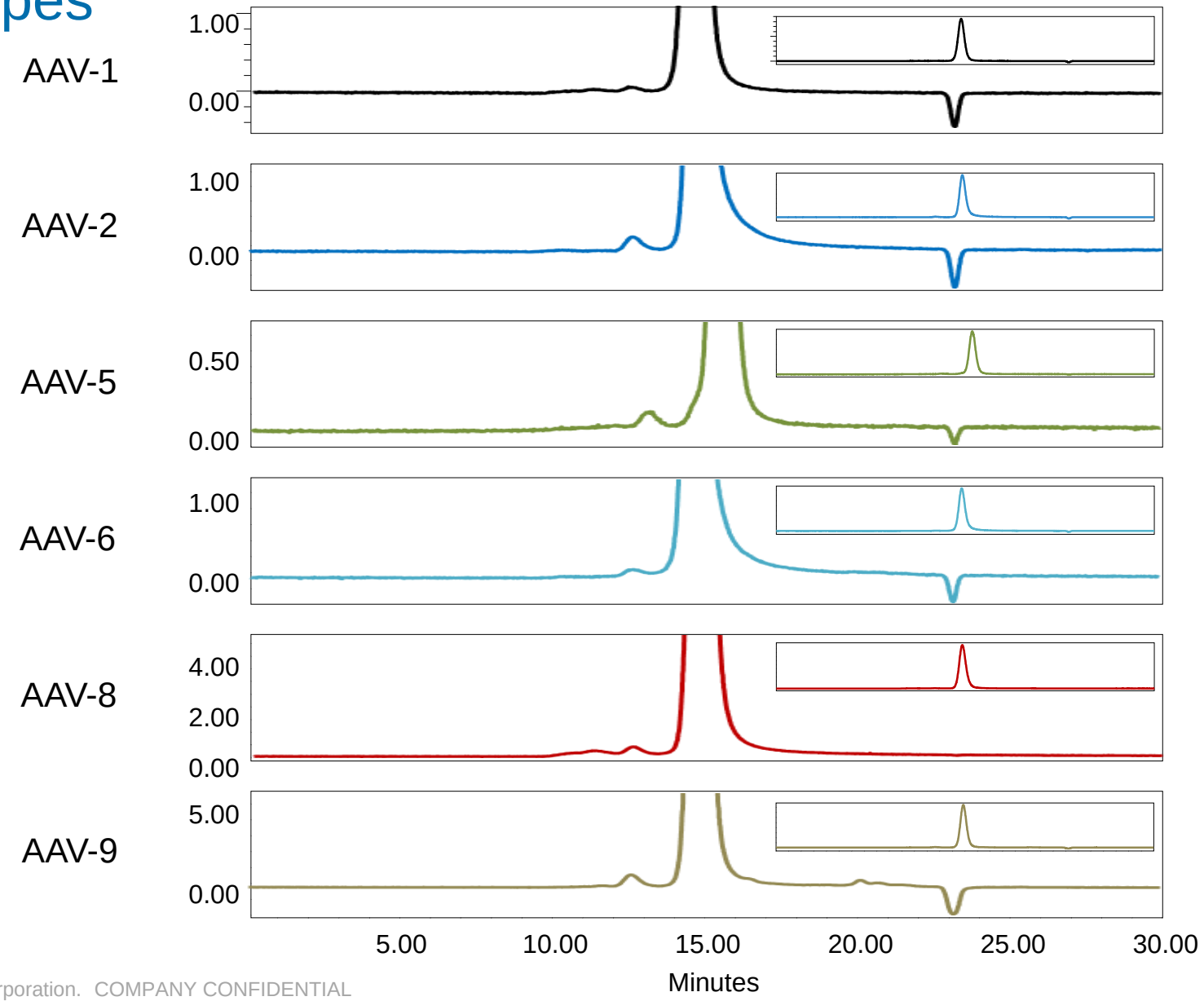
Optimized condition showed good separation for all tested serotypes



Optimized condition:

3.5 um BEH450 (7.8 X300 mm)

Flow rate: 0.5 mL/min

Inj. Vol.: 3uL (1E12 to 10E12 cp/mL)

mobile phase: 20 mM Na_xH_yPO4, 150 mM KCl, pH 6.6

FLR: exc: 280 nm, emm: 350 nm

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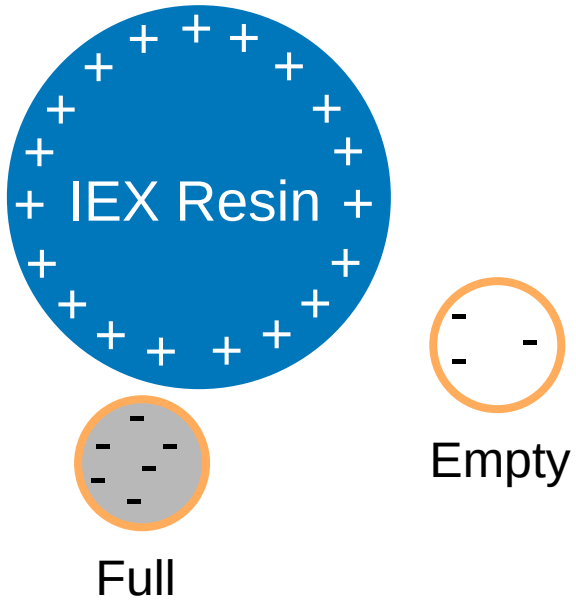
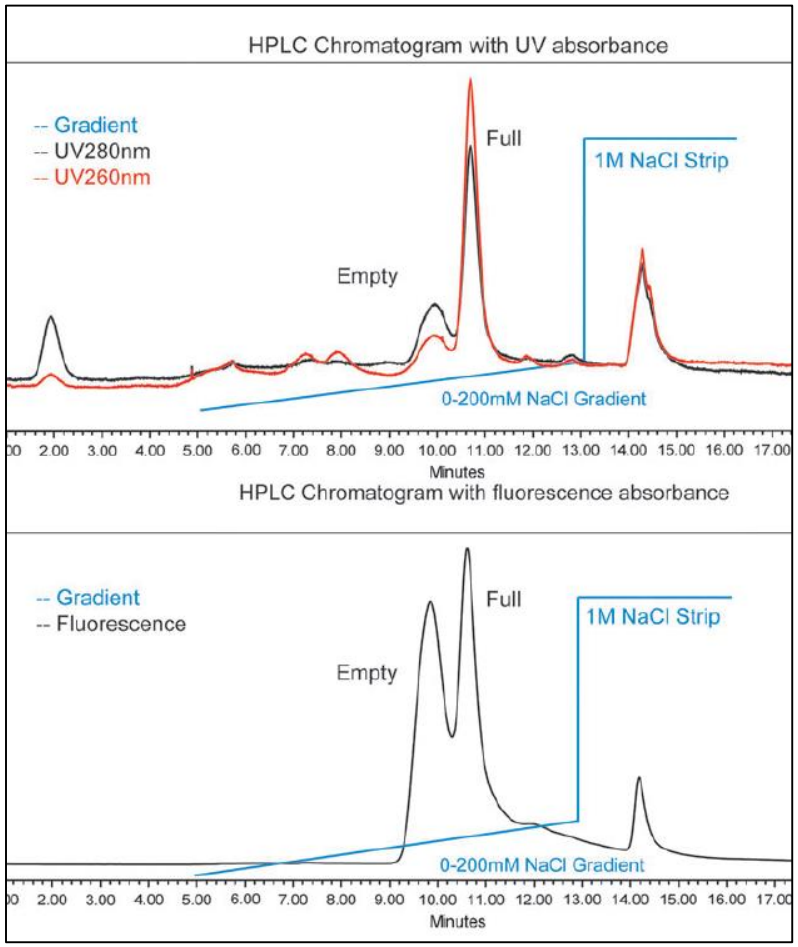
Full/empty ratio of AAV capsids can be determined by anion-exchange chromatography

- Multiple techniques are available:
  - AUC
    - Measure sedimental rate
    - Large sample consumption
    - Time consuming
  - Spectrometer
    - Less time consuming
    - Interference
  - IEX
    - Less sample consumption
    - Needs method development
  - CDMS
    - Less sample consumption
    - More definite measurement



Stronger binding due to the filled genome



X. Fu, et al. Human gene therapy, 2019, V30, 4

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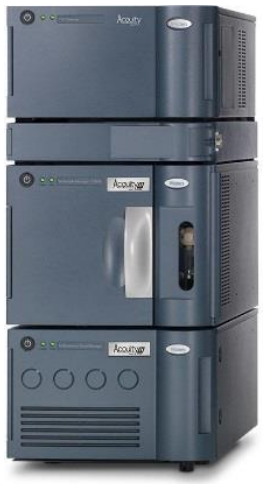
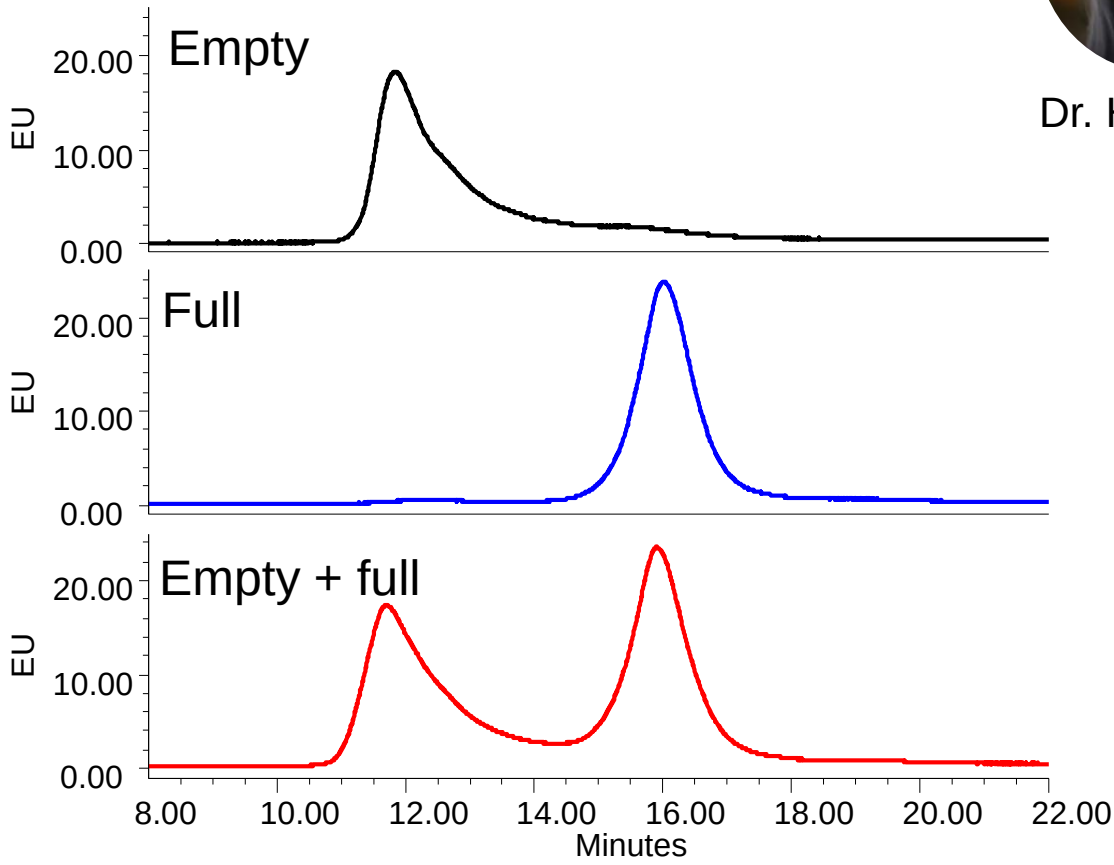
Full/empty ratio of AAV capsids can be determined by anion-exchange chromatography

- Conditions:
  - ACQUITY H-Class Plus Bio
  - ProteinPak Hi Res Q, 4.6 x 100 mm
  - Fluorescence detection
  - Gradient:
    - 100-300 mM Me_4NCl in 20 min
    - 70 mM Bis-tris buffer
    - pH 9.0
    - 0.4 mL/min



- Anion exchange chromatography:



Dr. Hua Yang

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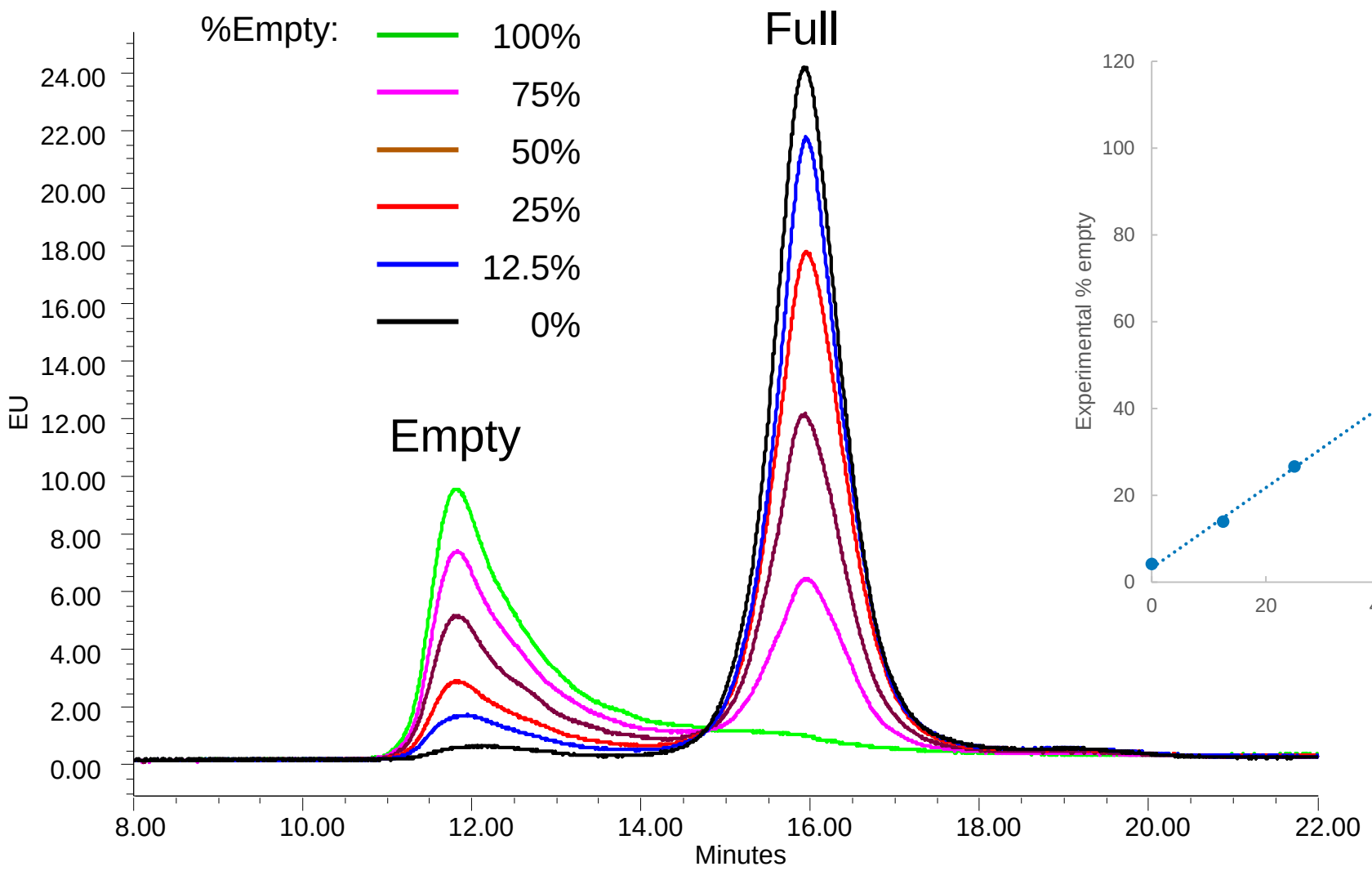
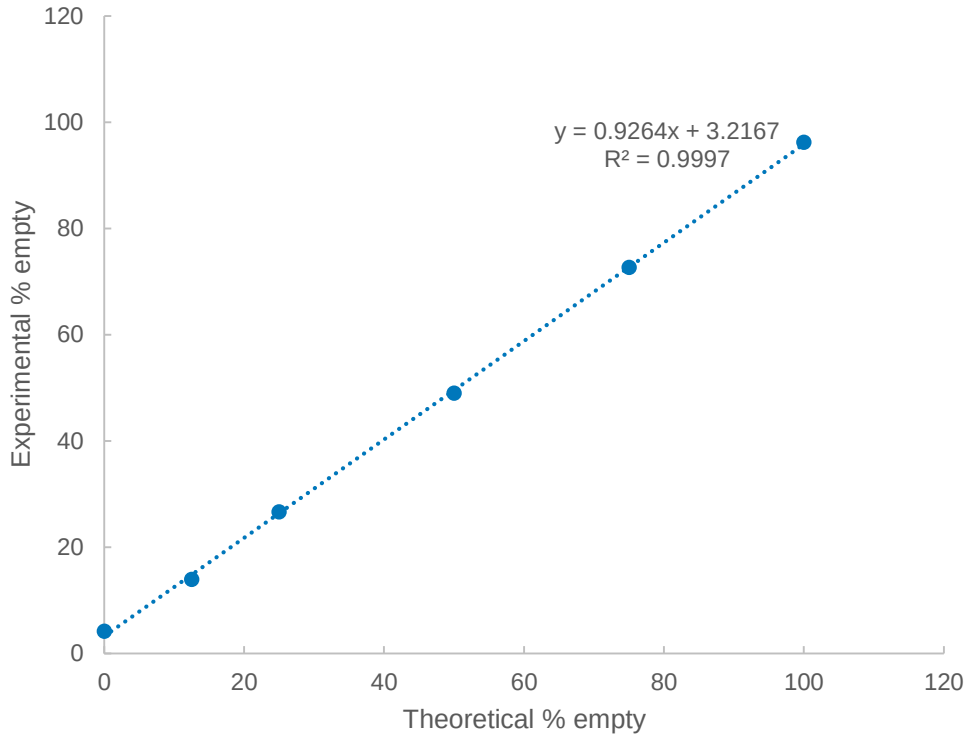
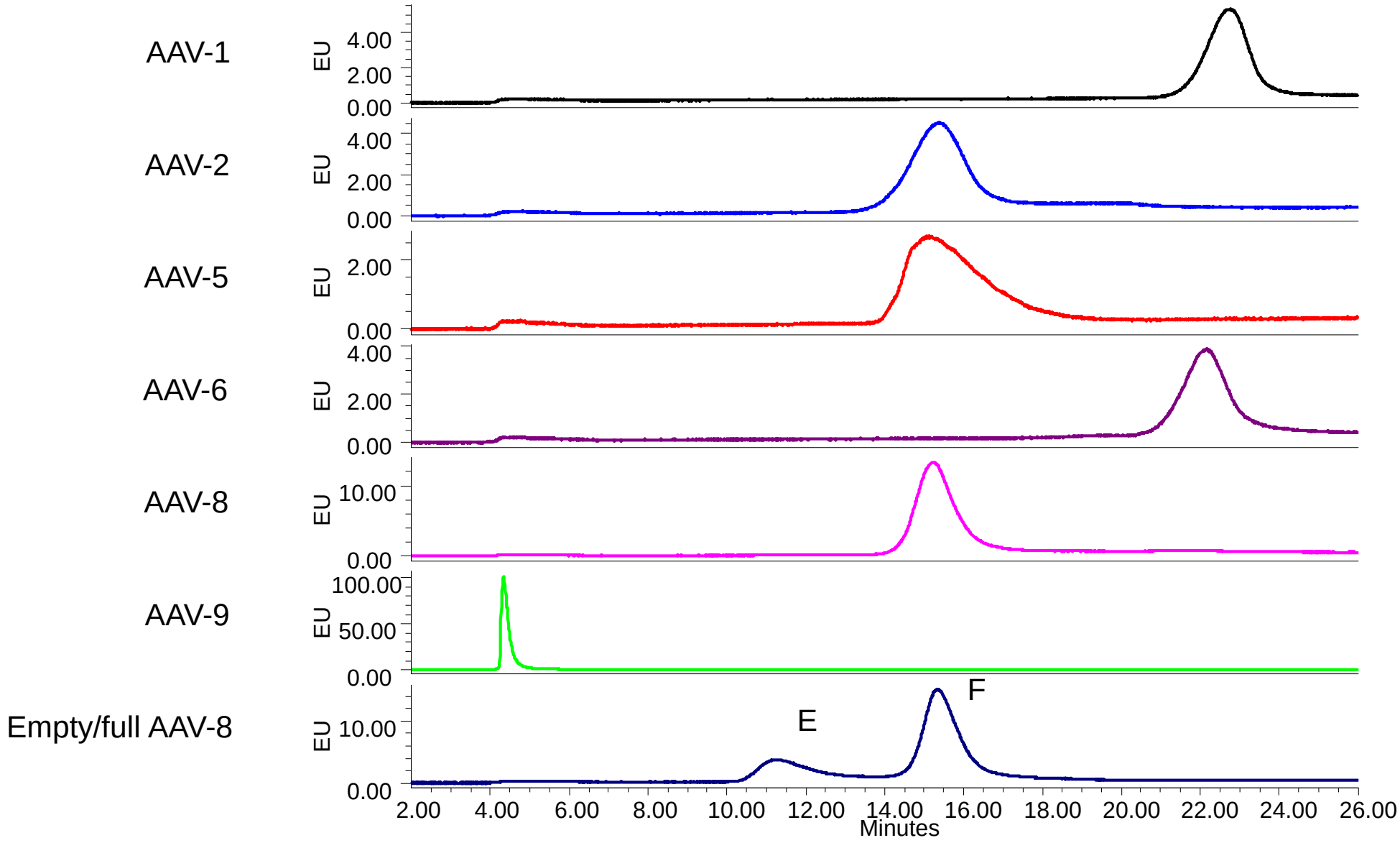
Good linearity enables quantification of 0-100% empty capsids by AEX

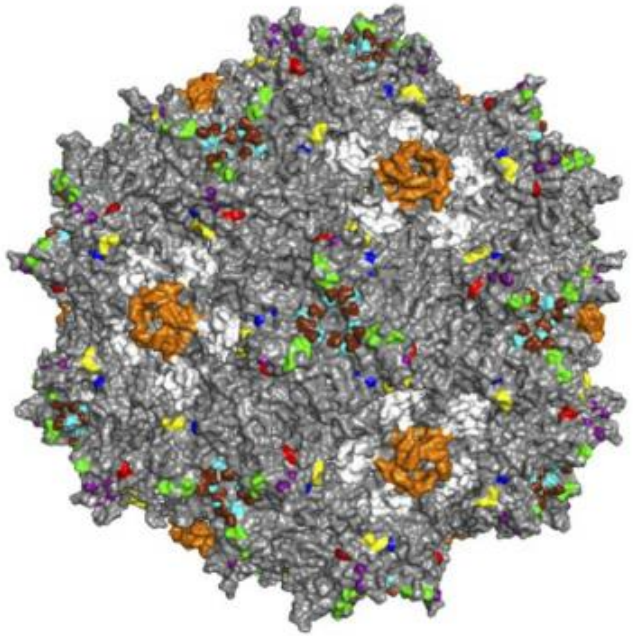




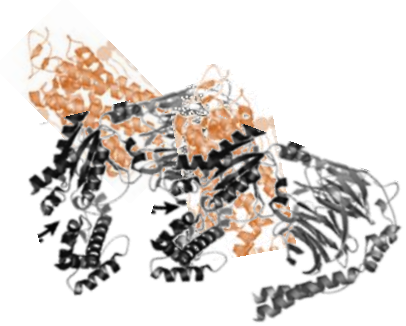
Testing a panel of AAV serotypes showed that optimization might be needed for each serotype



Capsid protein analysis by RPLC-MS reveals additional information but faces more challenges



Dissociate



VP1:VP2:VP3 = 1:1:10

Ratio, Identity & Purity

10-50X lower concentration

=> Method development

Surfactant

=> Sample prep

Lack of prior knowledge

=> Technology evaluation

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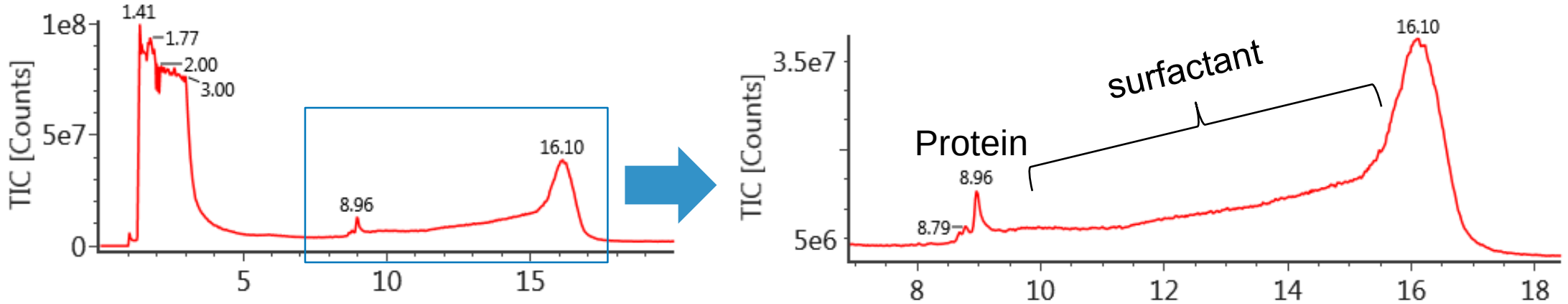
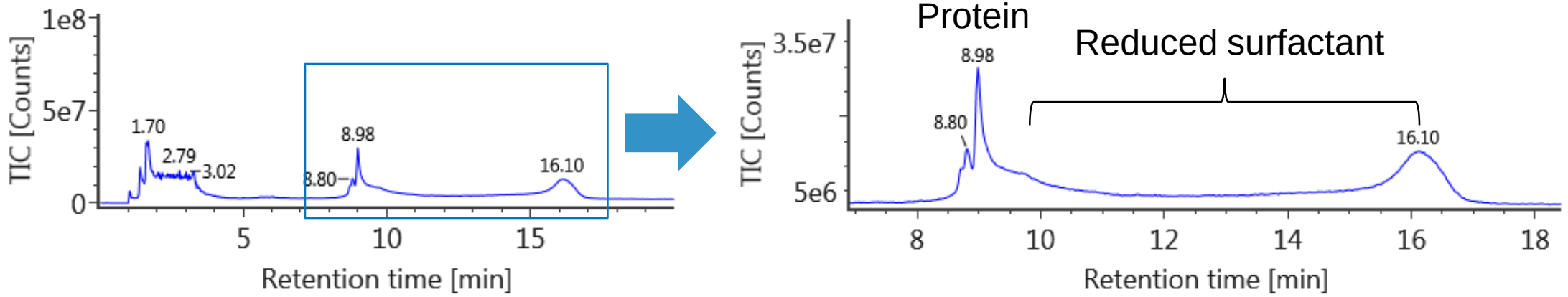
Surfactant removal is beneficial for formulated and in-process AAV samples

Capsid protein separation of formulated AAV

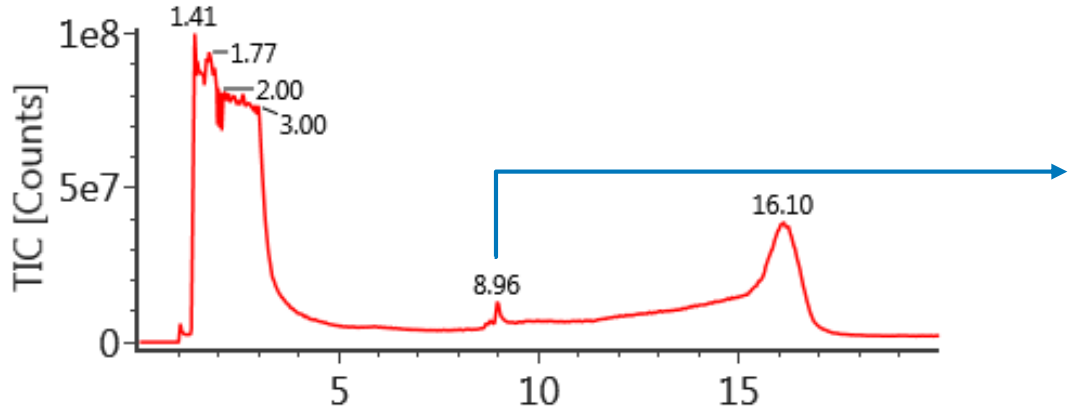


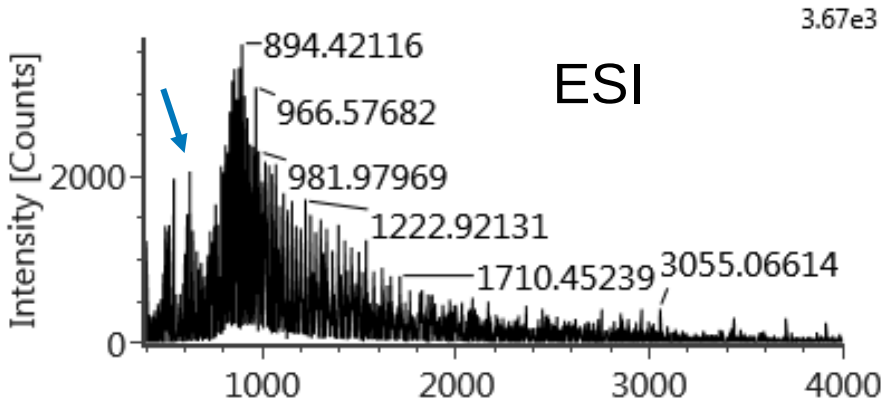
After removing surfactant:



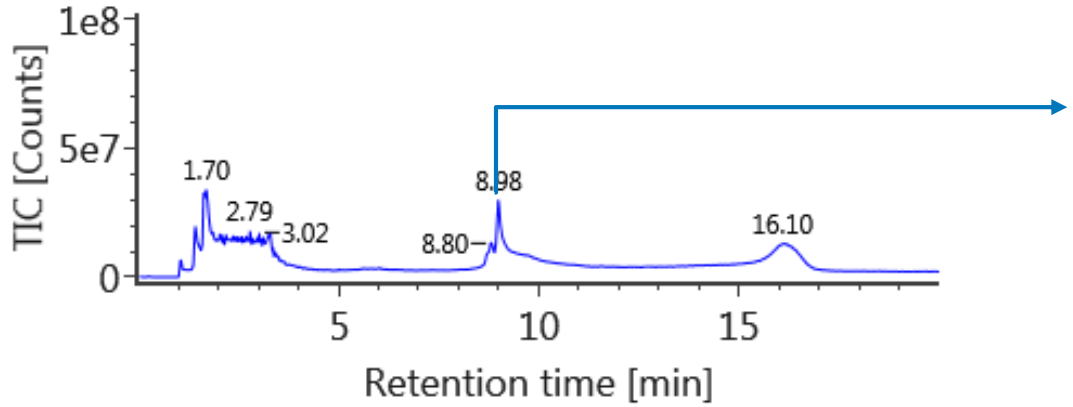
Sample prep removes the surfactant, improving MS signal

Capsid protein separation of formulated AAV samples

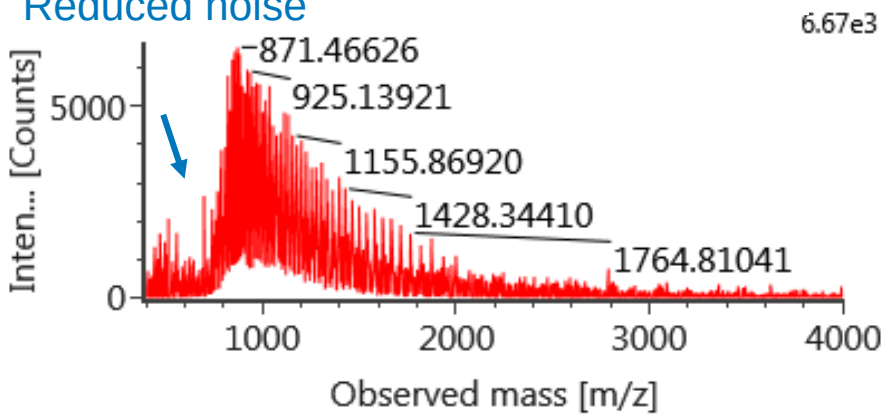




After removing surfactant:

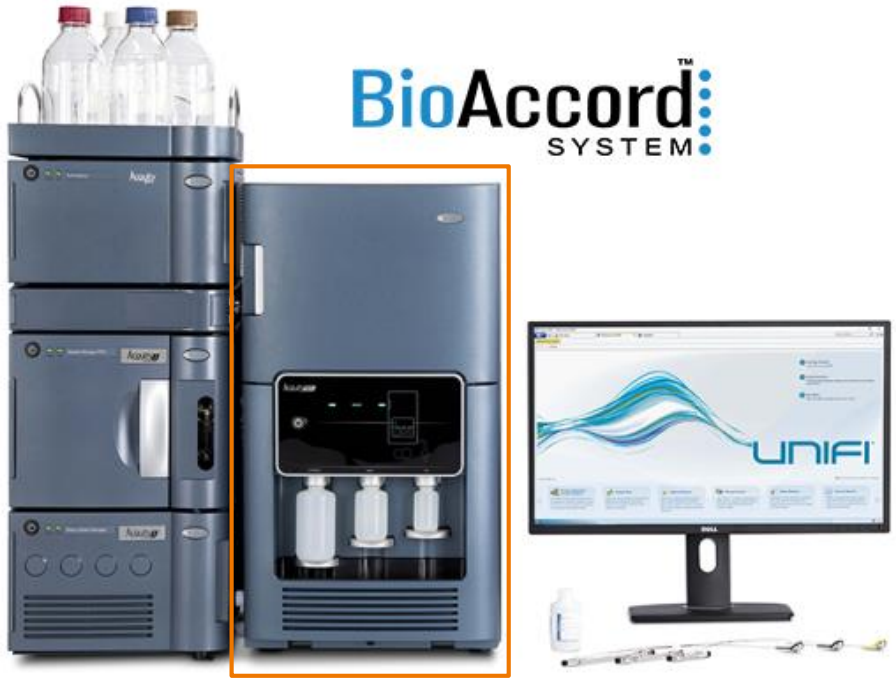


Reduced noise

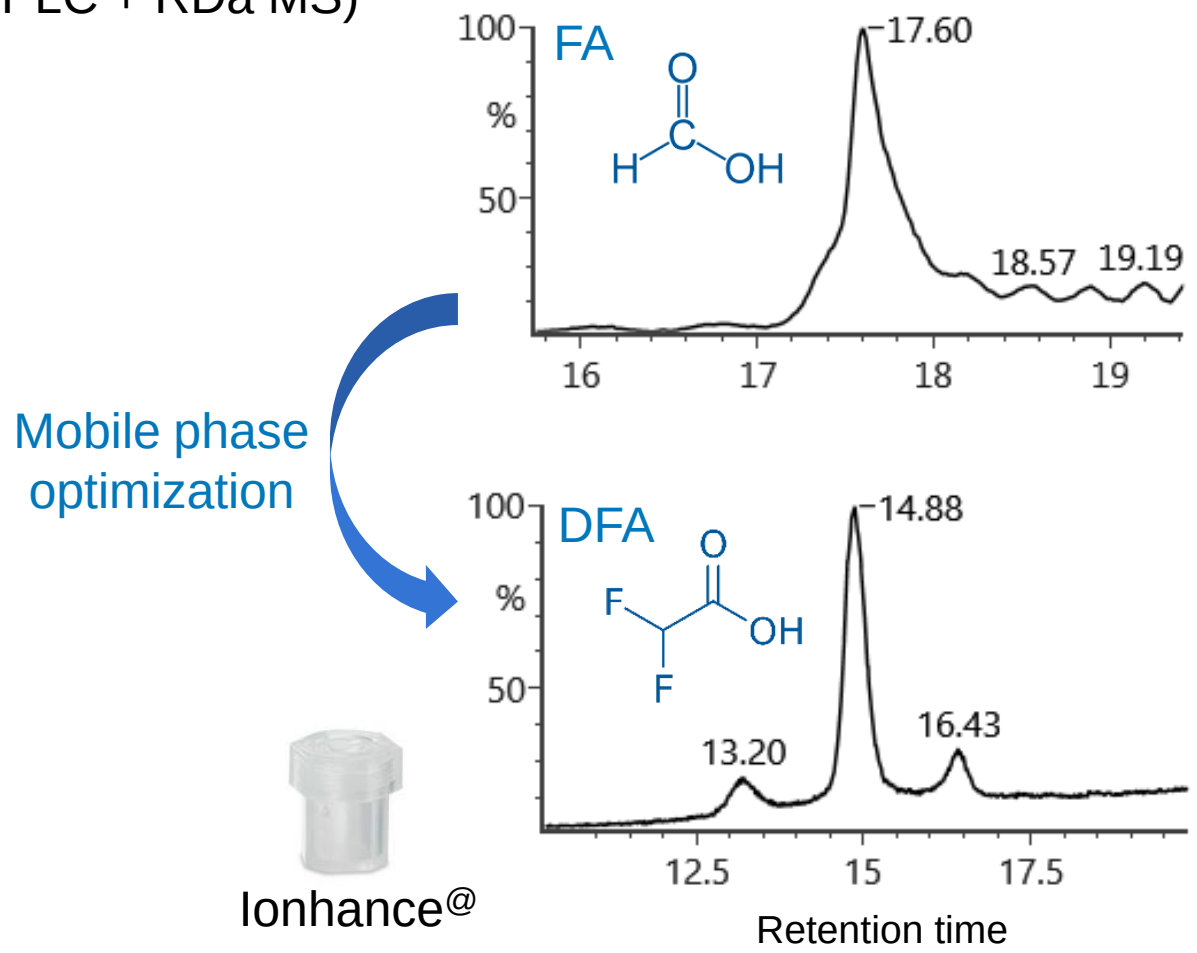


Method development is required to achieve high resolution separation of capsid proteins

- Conditions:
  - BioAccord system (Acquity I-Class Plus UPLC + RDa MS)
  - BEH C8 column, 2.1x100 mm
  - 0.1% formic acid



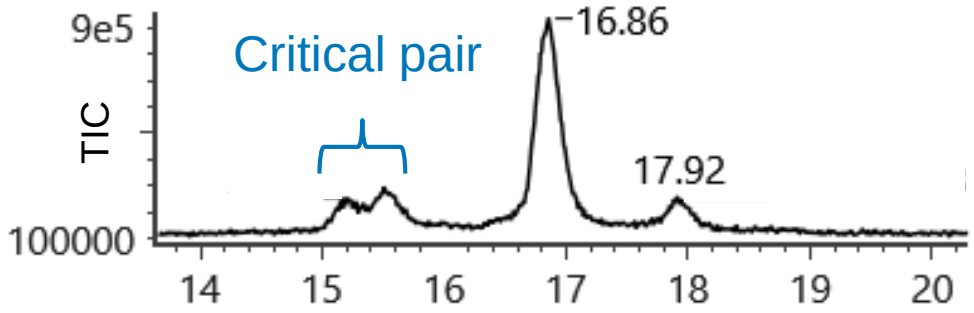
- Separation of AAV-B capsid proteins:



Ionhance@

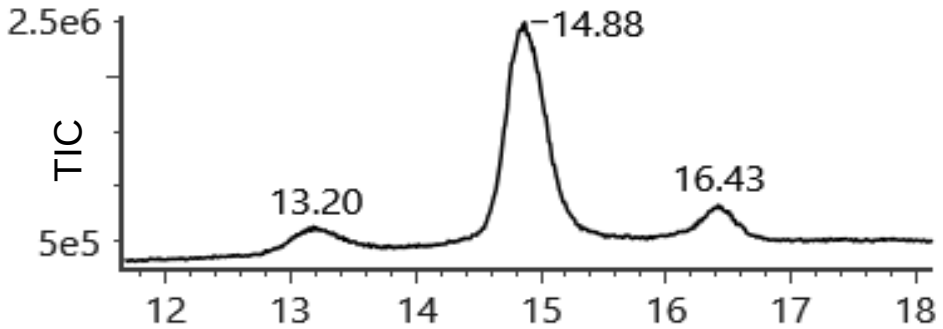
Column chemistry also has impacts the separation

C4, 300 Å



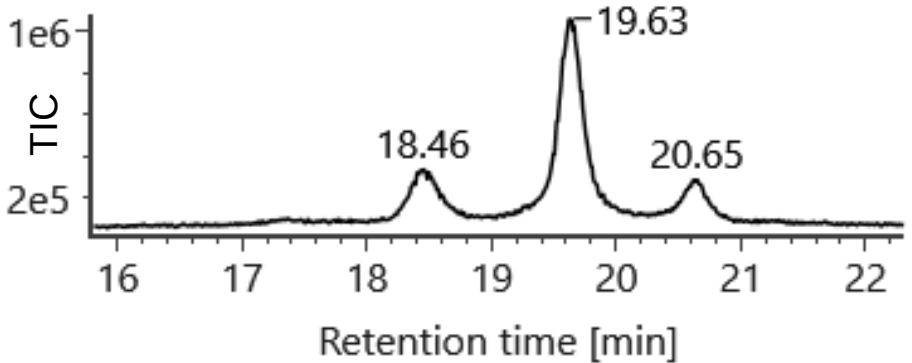
Better selectivity

C8, 130 Å



Broadened peaks

C18, 300 Å



High resolution separations enable mass measurement of individual capsid proteins

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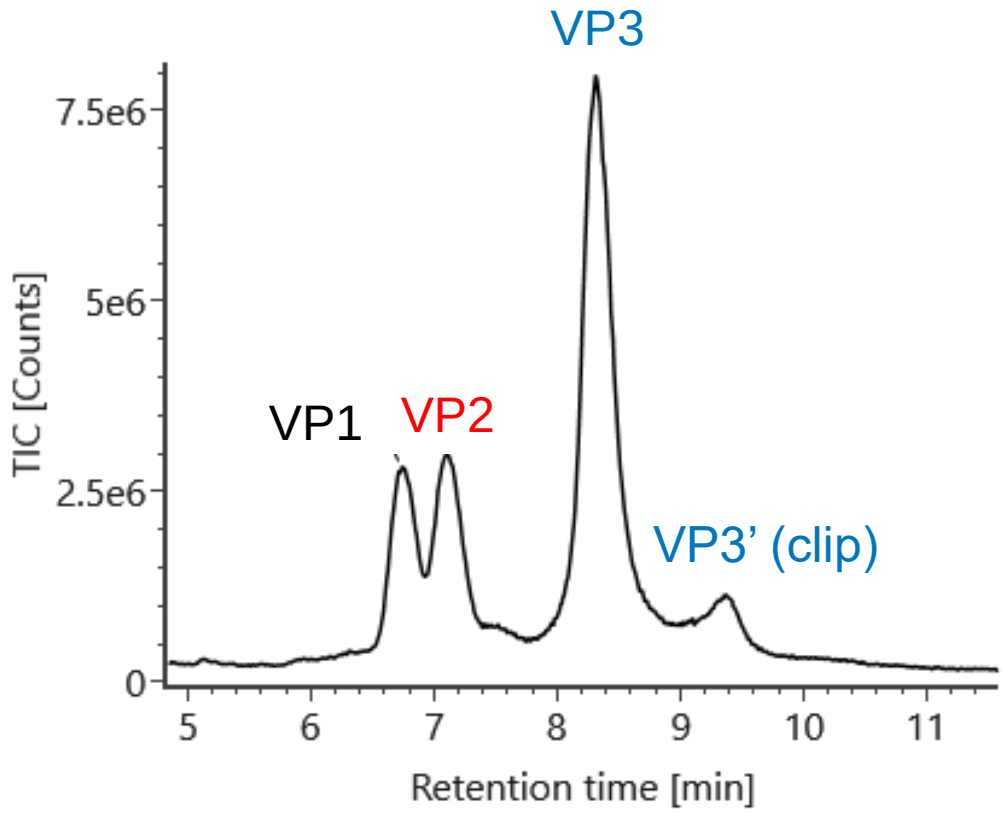
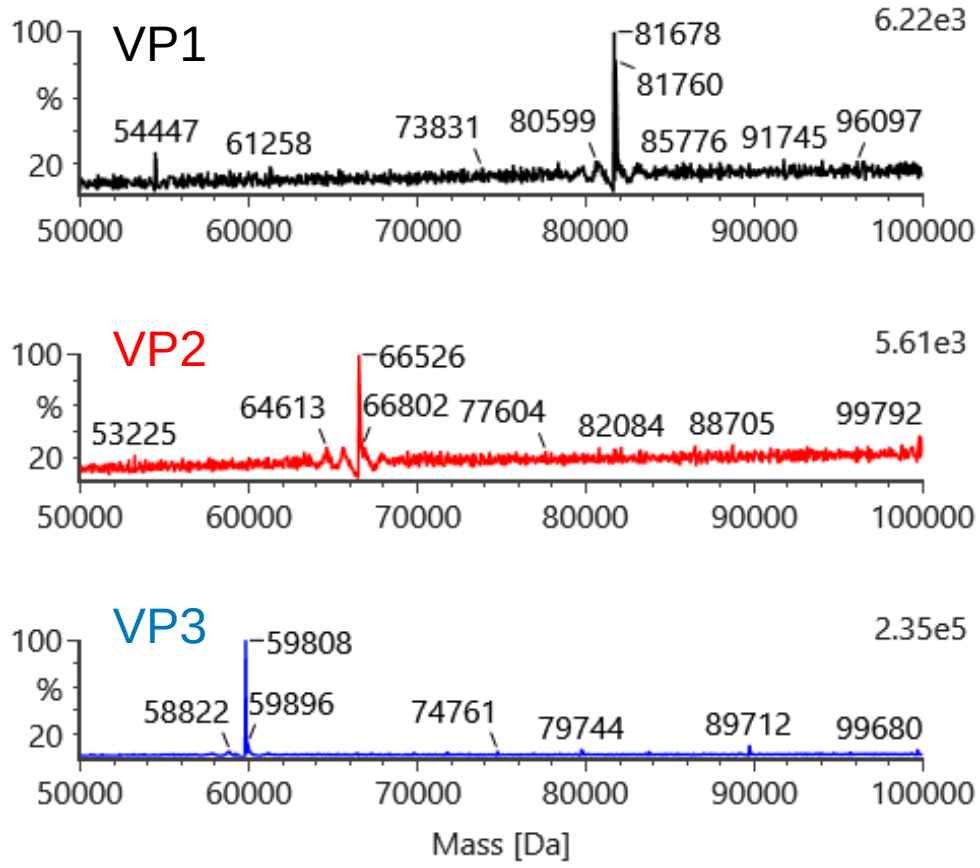
RPLC-MS of AAV Capsid Proteins



Deconvoluted Spectra



Column: Acquity BEH C4 column, 2.1x100 mm

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High resolution separations enable mass measurement of capsid protein modifications

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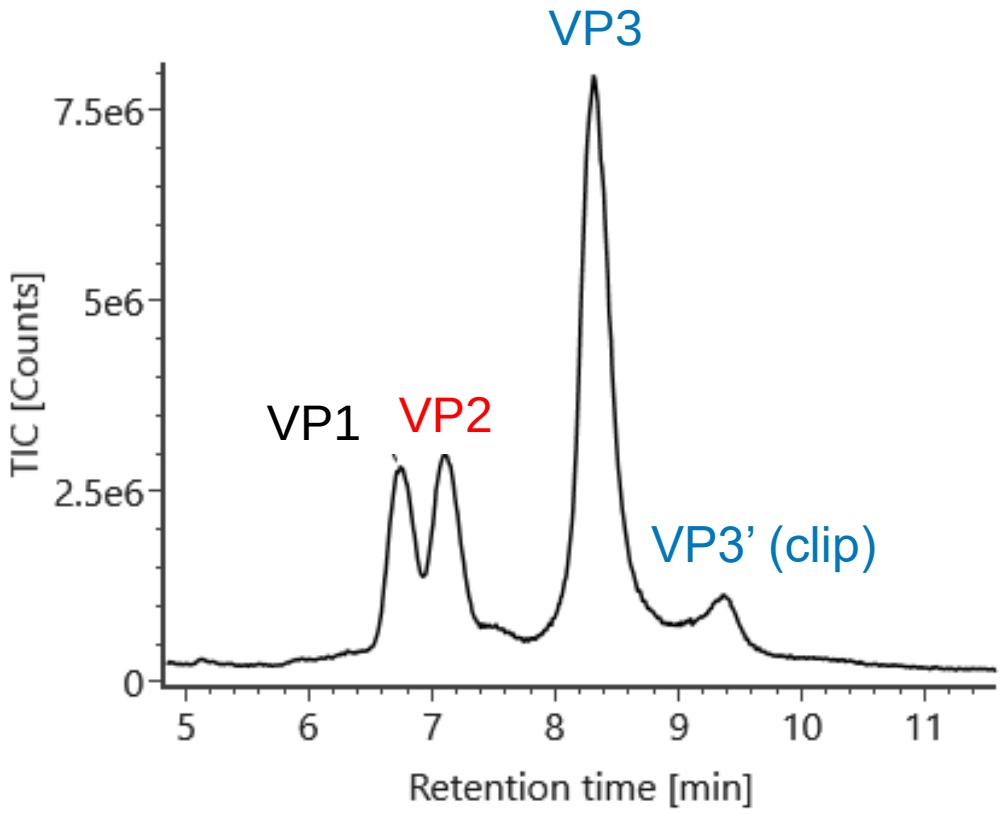
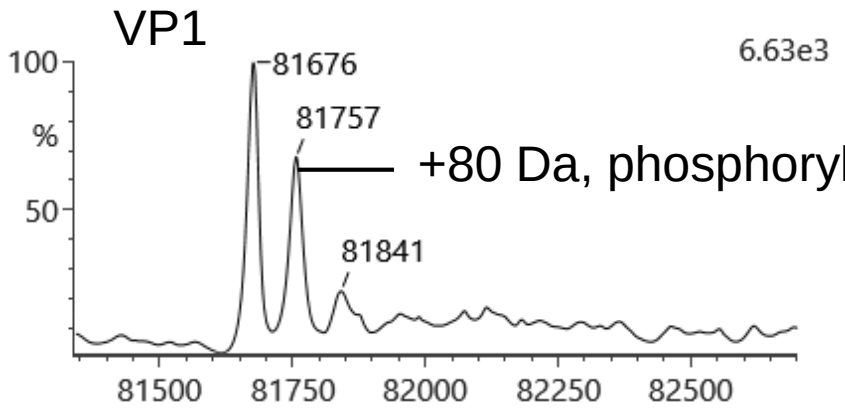
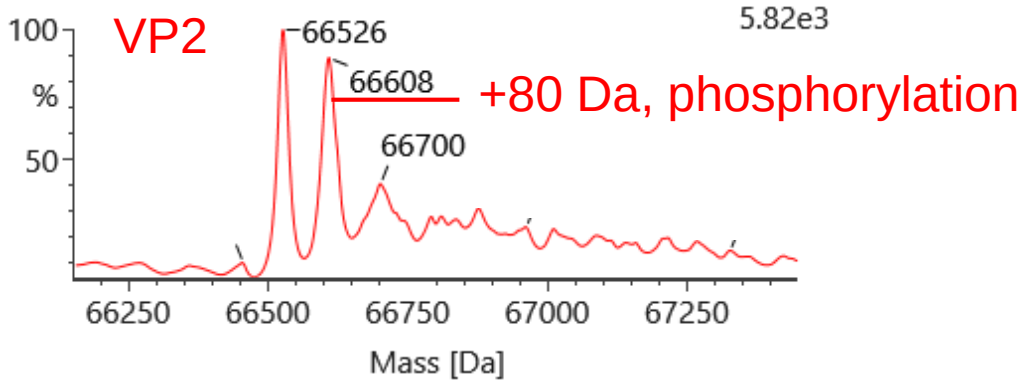
RPLC-MS of AAV Capsid Proteins



Column: Acquity BEH C4 column, 2.1x100 mm

Deconvoluted Spectra





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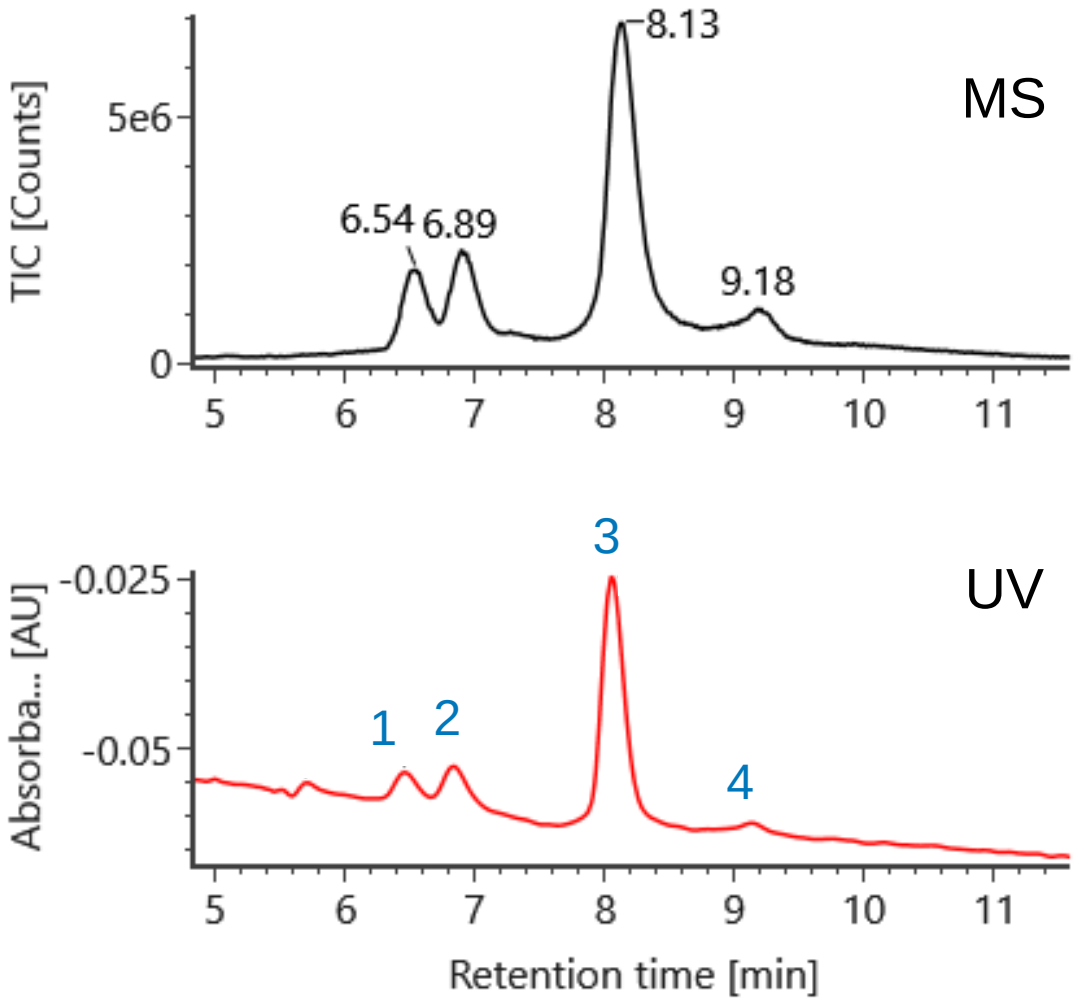
LC-UV-MS can be used to measure the protein ratios and confirm the capsid protein identity



Identified based on deconvoluted MS Speatra

Peak	Abundance (%)	Tentative identity	Observed mass (Da)
1	8.8	VP1	81,676
2	13.0	VP2	66,526
3	67.5	VP3	59,808
4	5.9	VP3 clip	50,598

Calculated based on integrated peak areas

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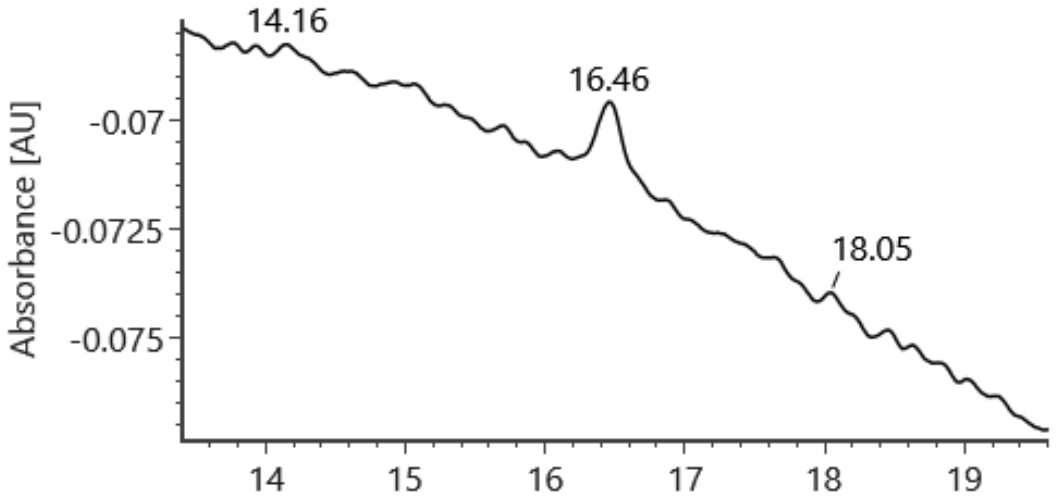
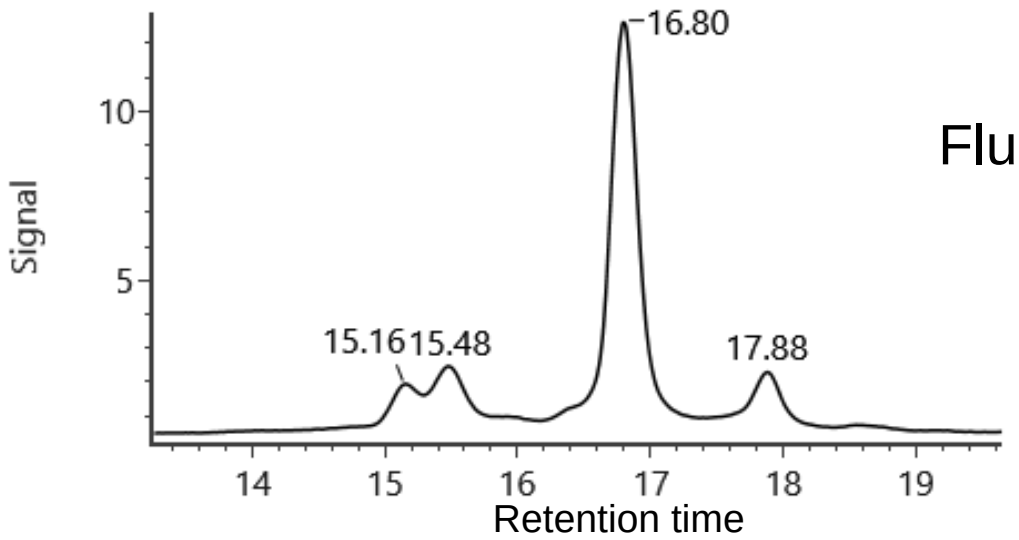
Minimizing sample: Fluorescence can be used for higher sensitivity capsid protein monitoring



UV



Fluorescence

> 20X higher S/N

0.08 µg injected on column

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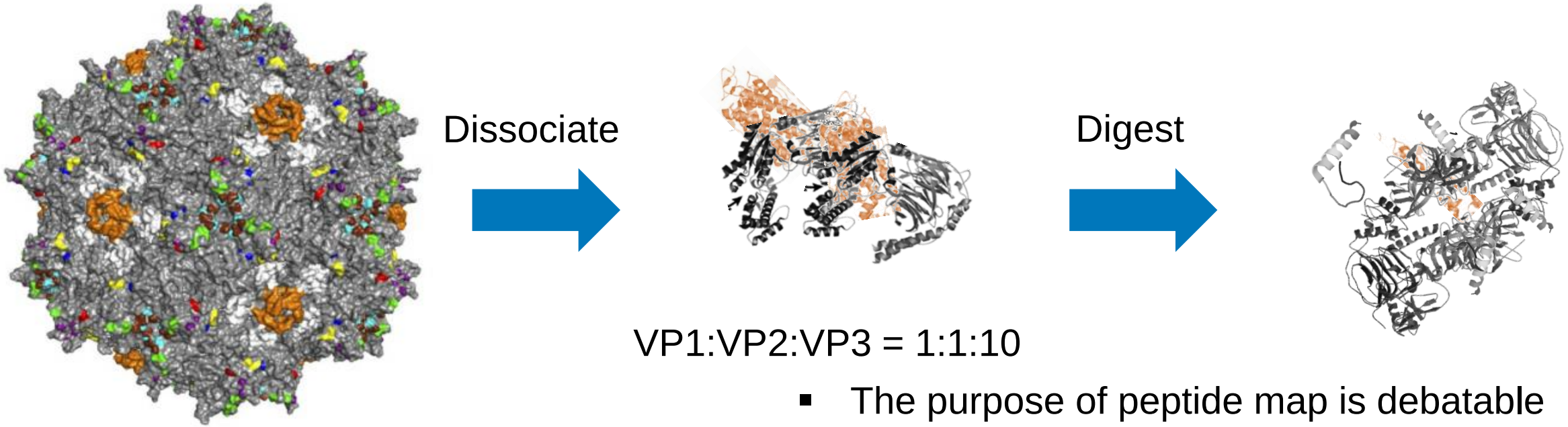
Peptide mapping can facilitate characterization of additional modifications



VP1:VP2:VP3 = 1:1:10

- The purpose of peptide map is debatable
- Characterization of potential PTMs
  - *Phosphorylation, N-acetylation, glycosylation, disulfide bonding, deamidation and oxidation*

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Multiple AAV peptide mapping challenges to be resolved

- Challenges with sample
  - Limited sample amount (µg/mL)
  - Surfactants in formulation
  - Overlapping amino acid sequences

- Challenges with digestion approach
  - *Digest pooled protein?*
    - Uncertainty as to which proteins are modified
    - Requires wider dynamic range to detect VP1 and VP2 specific variants
  - *Or digest isolated VP proteins?*
    - Complexity and time
    - Greater sample consumption due to lower recoveries
- The goal is to generate a reproducible and reliable digestion protocol from µg's of proteins

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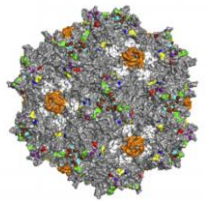
Summary and Future Work

- Conventional large pore SEC (450A) particles are ideally suited to aggregation and fragment analysis of AAV particles, but are unlikely to address Lentivirus and other larger viral vectors.
- AEX methods optimized on one AAV serotype, work well to screen other serotypes for Empty/Full analysis, but may need optimization to build quantitative assays for those other serotypes
- DFA as a RPLC-MS modifier demonstrated superior chromatographic and MS performance for AAV capsid protein analysis.
- Fluorescence detection appears more effective at obtaining max sensitivity and dynamic range for AAV analysis, but collection of UV A260/280 data would provide particle load information.
- Peptide level analysis of AAV is complicated by low sample amounts and overlapping sequences, and typical biotherapeutic peptide map workflows will need redevelopment for map optimization.



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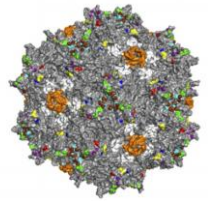
Thank you for your attention!

Scientific Operations at Waters

- Corey Reed
- Henry Shion
- Hua Yang
- Lindsey Organtini
- Samantha Ippoliti
- Steve Koza
- Ximo Zhang
- Ying Qing Yu
- Weibin Chen

We thank our collaborator Danny Gailbraith from BioReliance for access to samples and serotype information.



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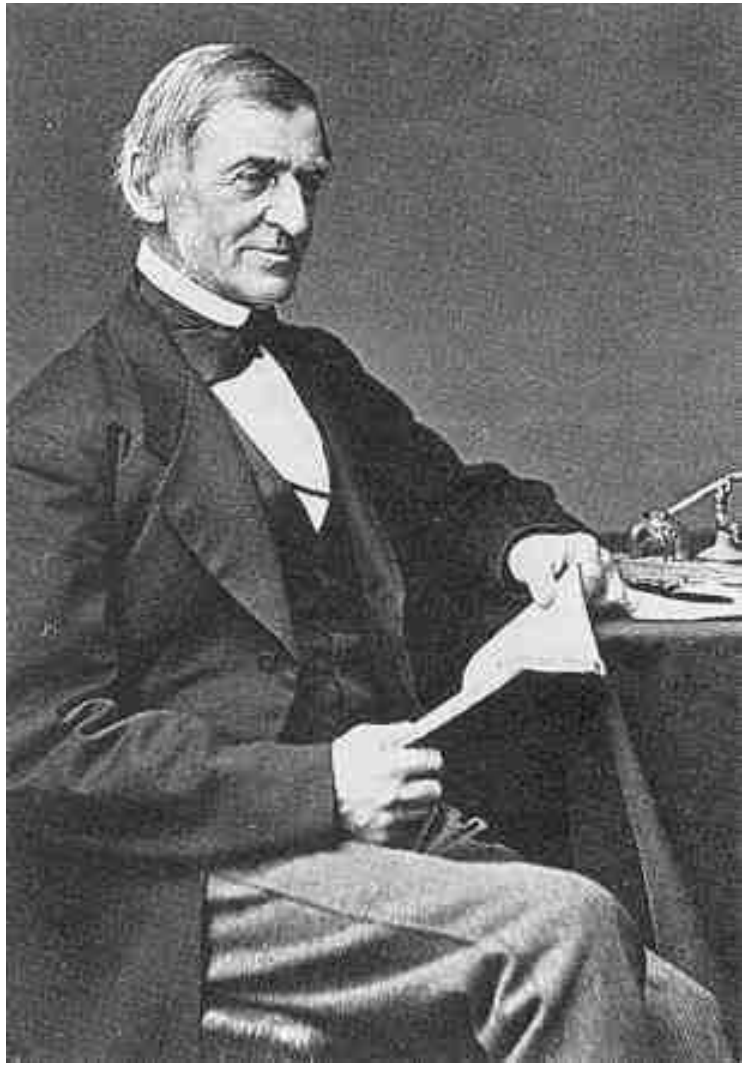
What has become clear to you since we last met?

Do not go where the path may lead, go instead where there is no path and leave a trail.

Ralph Waldo Emerson
b. 1803 - d.1882



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