Unifying characterization strategies for novel proteins and gene therapy products

David Colquhoun, PhD

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Biopharmaceutical technology with application flexibility

Multiple applications for multiple molecules on SCIEX LC/MS and CE systems

mAbs and next-generation protein therapeutics

Cell and gene therapy applications

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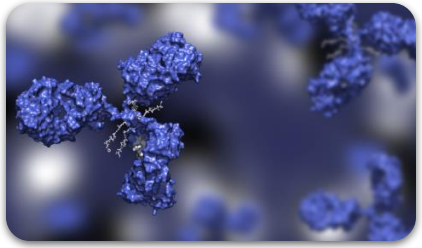
Key workflows for the analysis of protein therapeutics

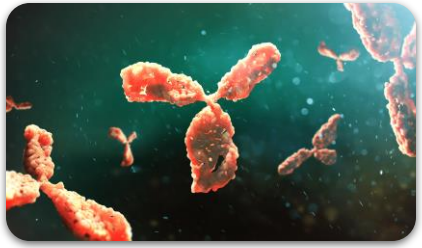
CHARACTERIZING PROTEIN-BASED PRODUCTS

Post-translational modifications

Deamidated amino acid isomerization and sulfated species

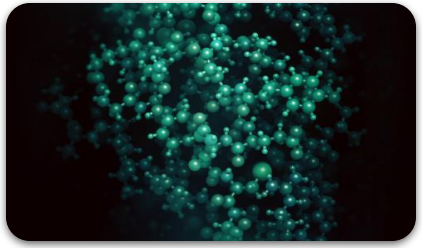


Glycosylation, glycation and AGEs screening



Fragments and impurities

Process- and product-related



Charge heterogeneity

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Key workflows for the analysis of protein therapeutics

CHARACTERIZING PROTEIN-BASED PRODUCTS

Post-translational modifications

Deamidated amino acid isomerization and sulfated species

- Deamidation of asparagine leads to an increase in negative charge to the antibody[1] can greatly affect the structure, function and stability of protein therapeutics[1]
  - Effects of deamidation on stability or function of mAbs[1], adeno-associated virus (AAV) capsid proteins[2] and SARS-CoV-2 spike protein[3] have been reported
- Sulfation has an impact on antigen binding and biological activity of mAbs
  - Tyrosine sulfation proteoforms affected the potency of a potential drug candidate for HIV-1 prevention, demonstrating the important modification is a potential CQA[4].

[1]Gupta S. *et al.* Oxidation and deamidation of monoclonal antibody products: potential impact on stability, biological Activity, and efficacy. *J Pharm Sci.* **2021**, 111: 903-918.
[2]Giles AR. *et al.* Deamidation of amino acids on the surface of adeno-associated virus capsids leads to charge heterogeneity and altered vector function. *Mol. Ther.* **2018**, 26:2848.
[3]Lorenzo R. *et al.* Deamidation drives molecular aging of the SARS-CoV-2 spike protein receptor-binding motif. *J. Biol. Chem* **2021**, 297:101175.
[4]Cindy X Cai, Nicole Doria-Rose, et al. (2022) Tyrosine O-sulfation proteoforms affect HIV-1 monoclonal antibody potency. Sci. Reports. 12: 8433.

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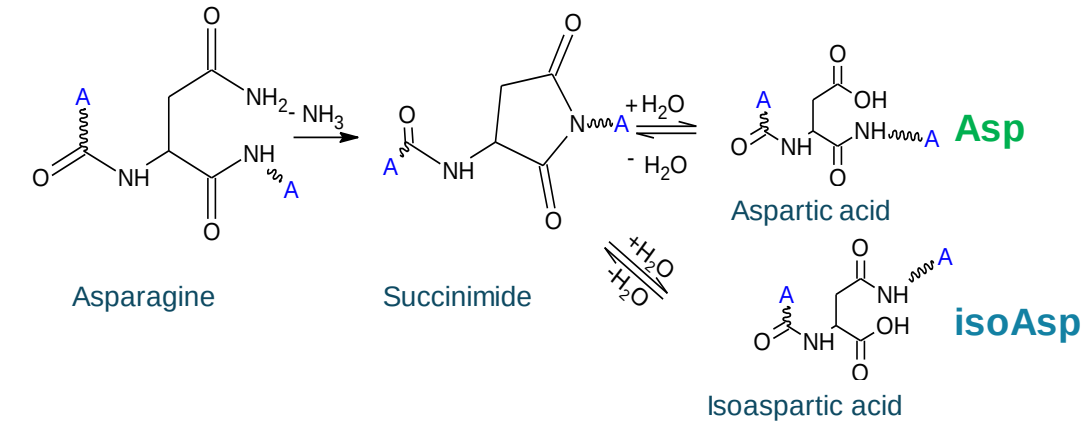
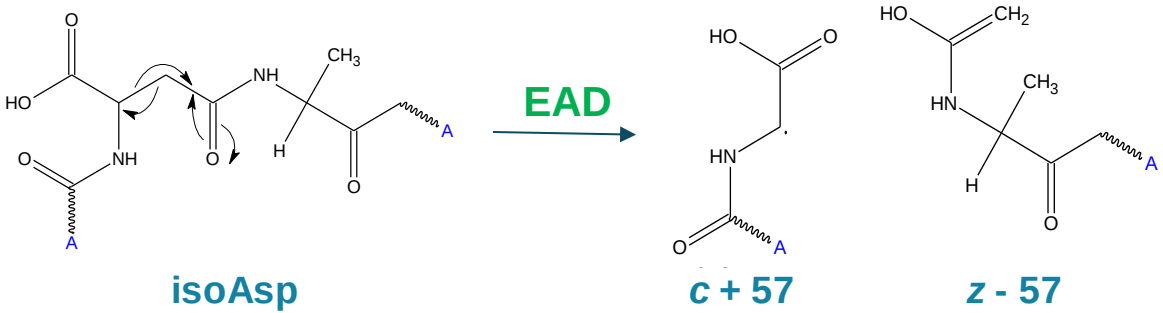
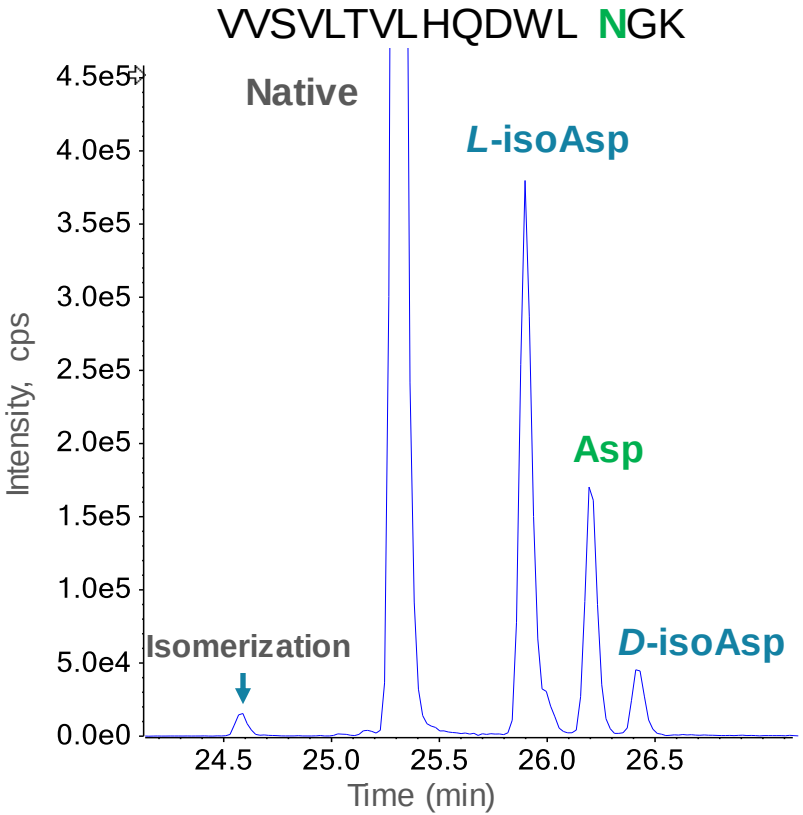
Differentiation of deamidation isomers

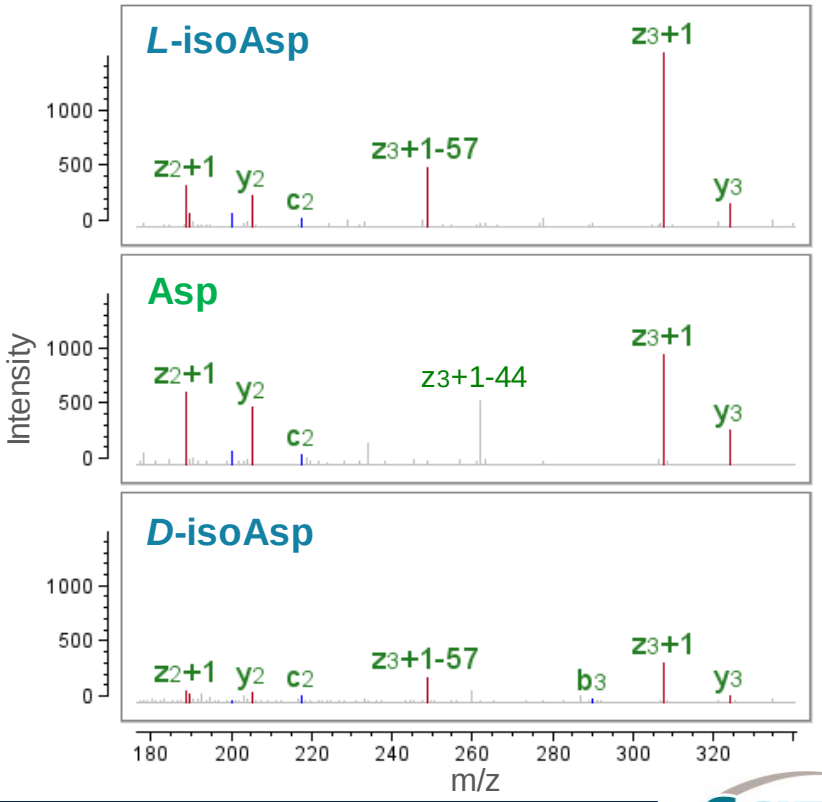




- Differentiation of Asp vs. isoAsp isomers is challenging when using their elution pattern alone or collision-based MS/MS approaches
- EAD generates signature fragments (c + 57 and z - 57 for isoAsp and z - 44 for Asp) for confident differentiation of these 2 amino acid isomers

Deamidation isomers in heat-stressed NISTmAb





Detection of signature fragments of Asp and isoAsp led to confident assignment of 3 deamidated peaks, thereby ensuring accurate quantification of these PQAs in multi-attribute monitoring MAM.

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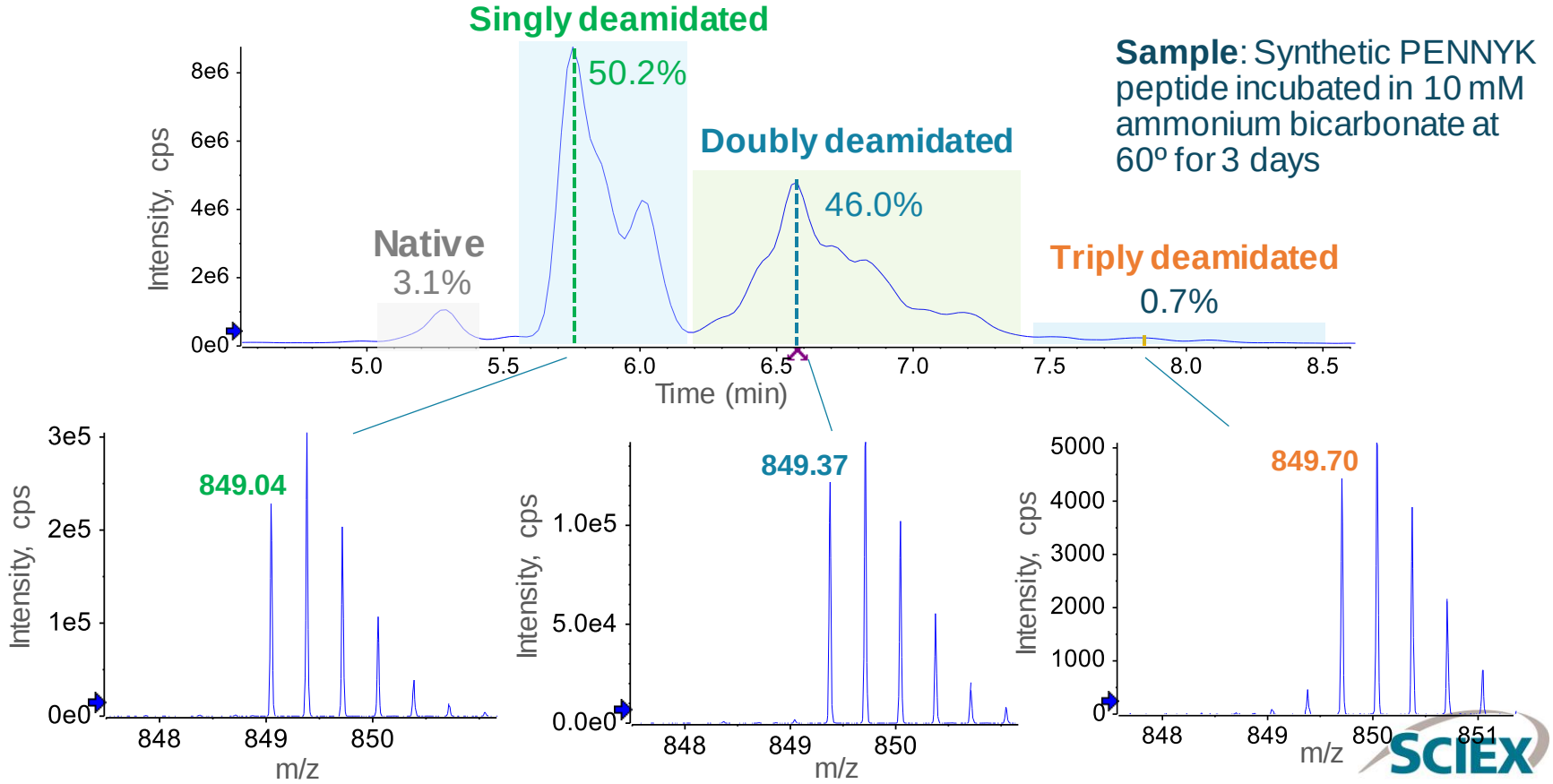
Elucidation of a complex deamidation profile using EAD



Sample: Synthetic PENNYK peptide incubated in 10 mM ammonium bicarbonate at 60º for 3 days

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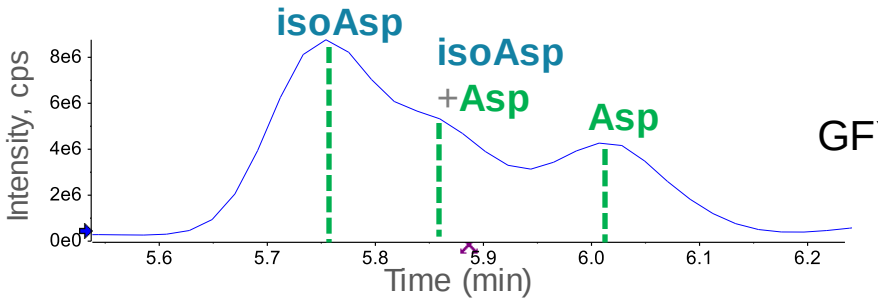
Singly deamidated species

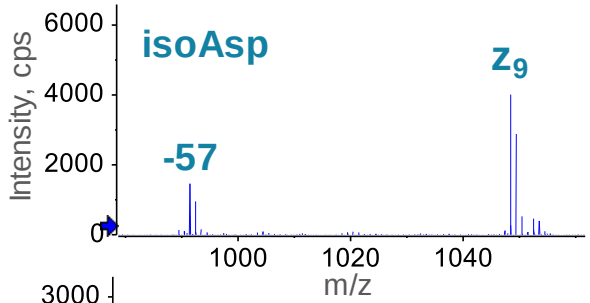


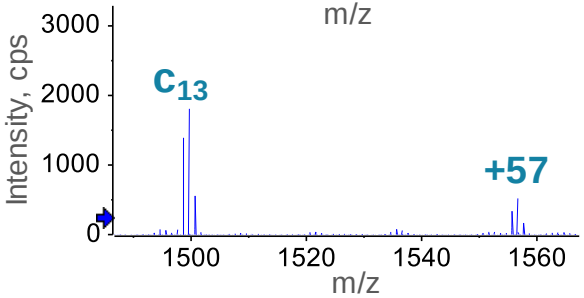
z_9

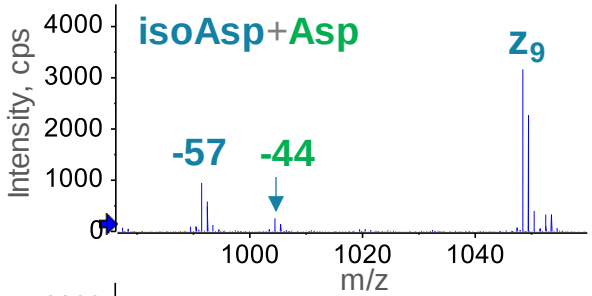
387

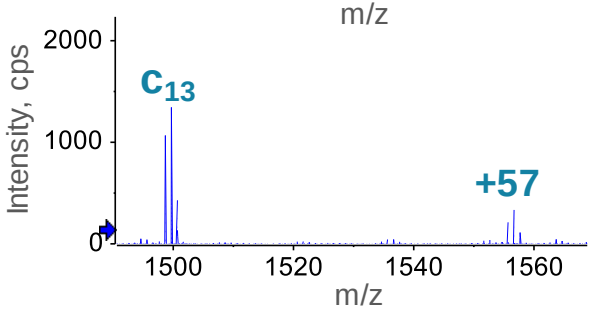
GFYPSDIAVEWES **N**GQPENNYK

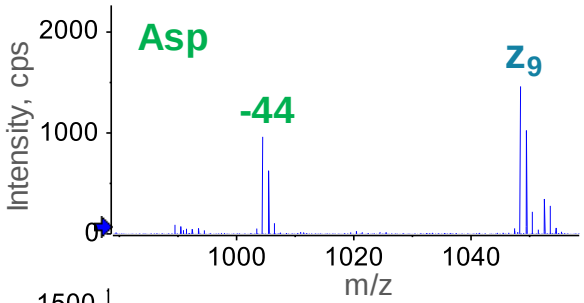
c_{13}

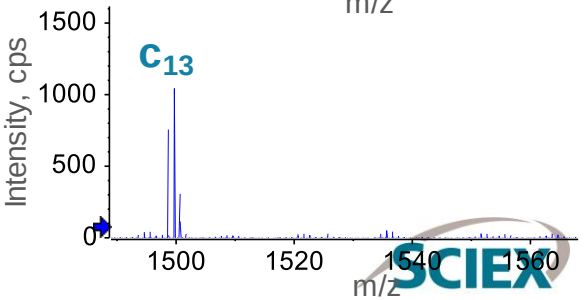












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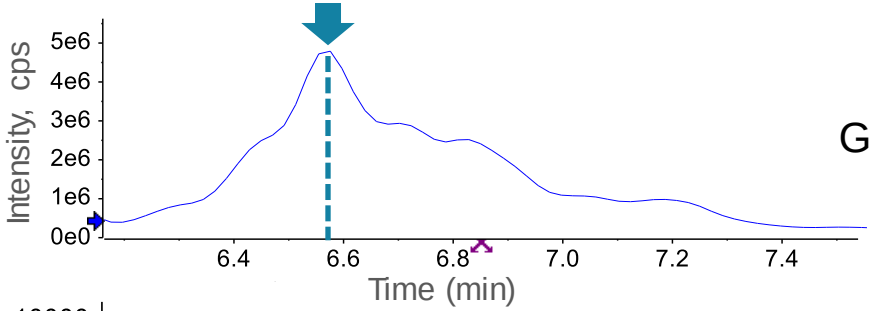
Doubly deamidated species

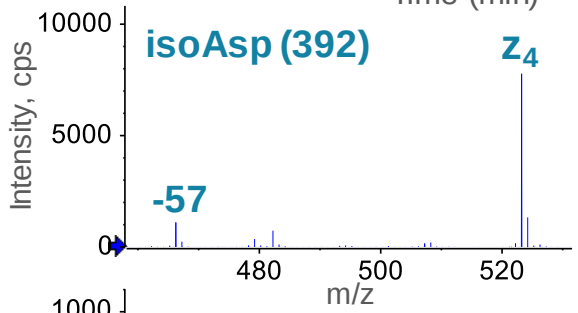


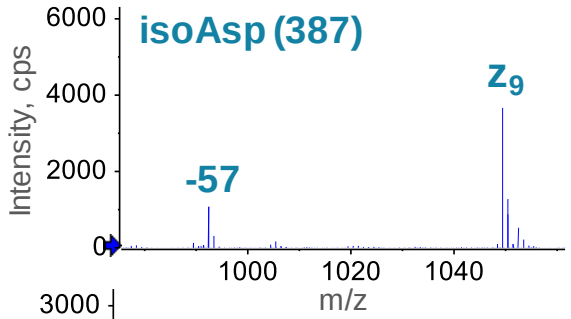
z_9 z_4

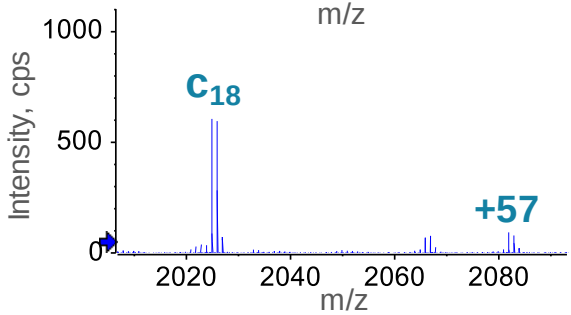
387 392

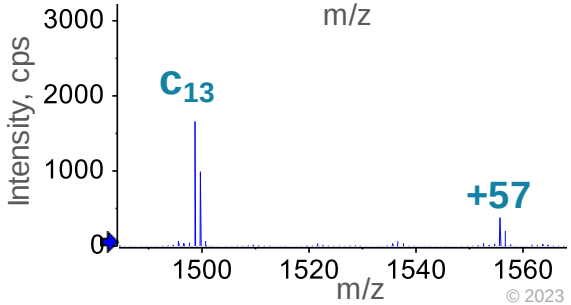
GFYPSDIAVEWES NGQPE NNYK

c_{13} c_{18}









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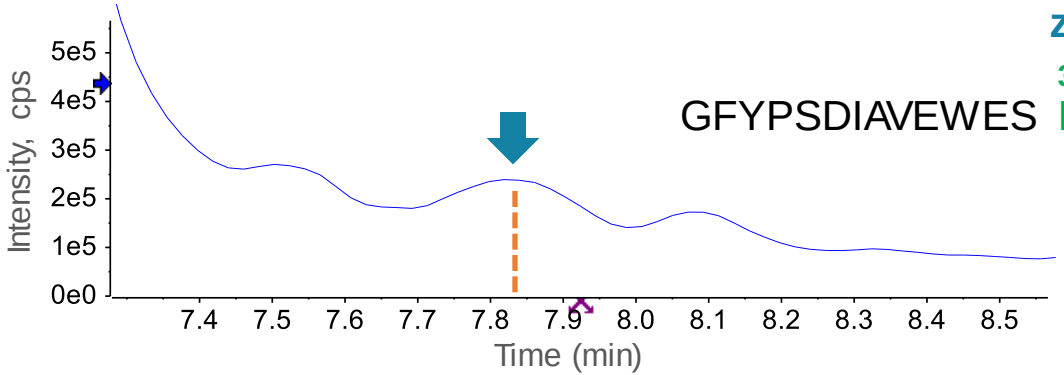
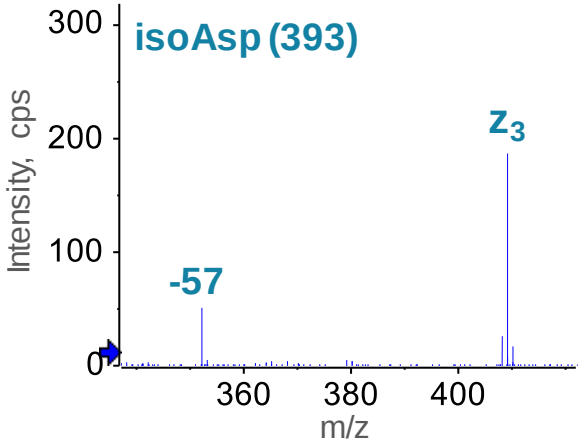
Triply deamidated species

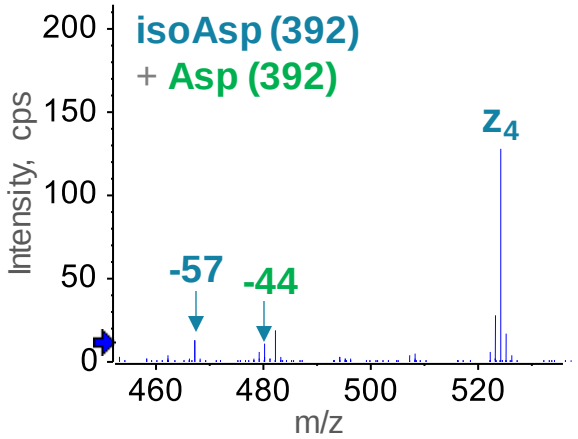


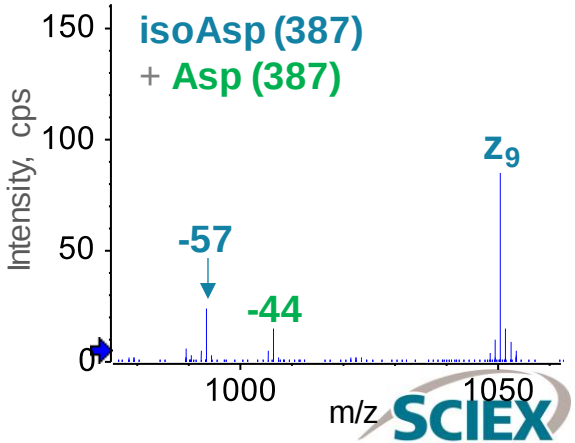
z_9 z_4 z_3

387 392 393

GFYPSDIAVEWES NGQPE N NYK







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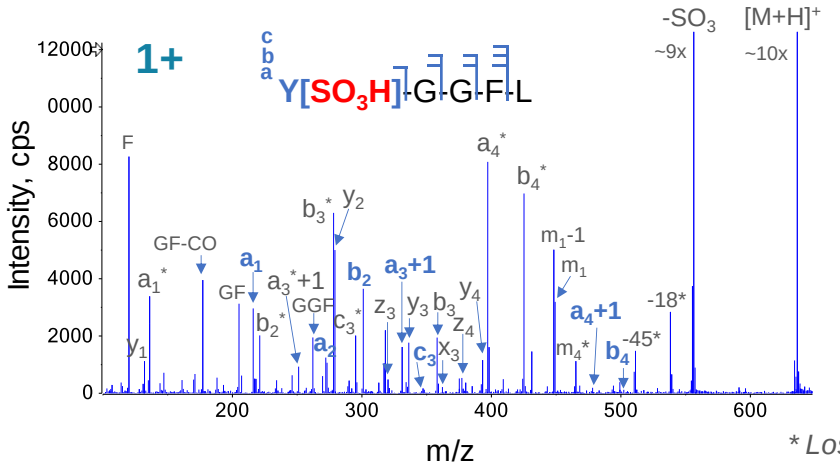
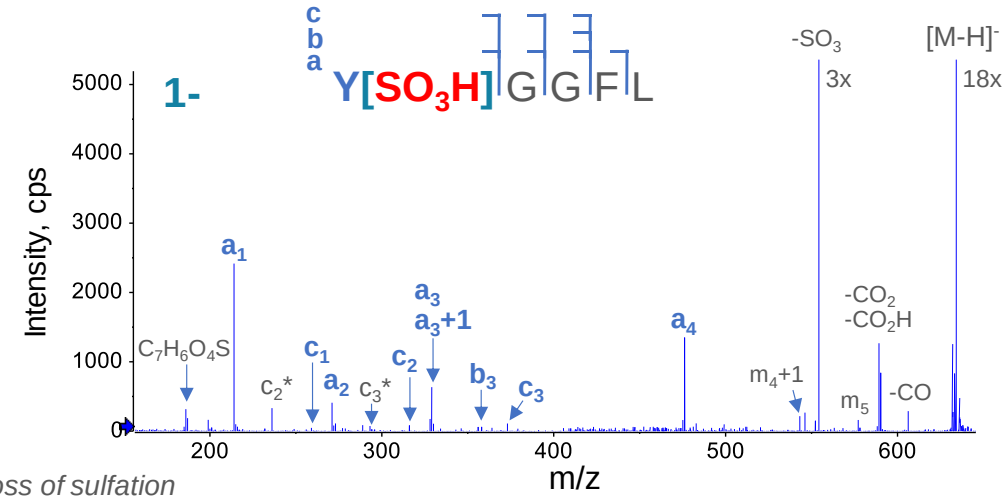
Positive and negative EAD of sulfated peptides

Positive EAD (15 eV)



Negative EAD (24 eV)



** Loss of sulfation*

The tunability of electron KE in EAD enabled the detection of sulfate-containing fragments (dominated by *a* type) in both positive and negative modes, leading to accurate localization of this challenging modification.

Accurate localization of labile tyrosine sulfation in peptides using electron activation dissociation (EAD). SCIEX technical note, RUO-MKT-02-14045-A.
Accurate localization of labile tyrosine sulfation using negative electron activated dissociation (EAD). SCIEX technical note, RUO-MKT-02-15175-A

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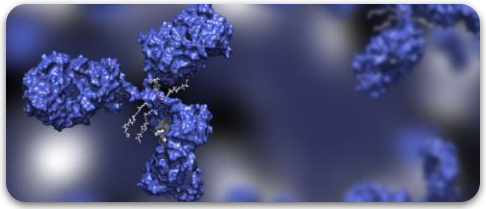
Glycosylation, glycation, and advanced glycation end-products

CHARACTERIZING PROTEIN-BASED PRODUCTS



Glycosylation, glycation and AGEs screening

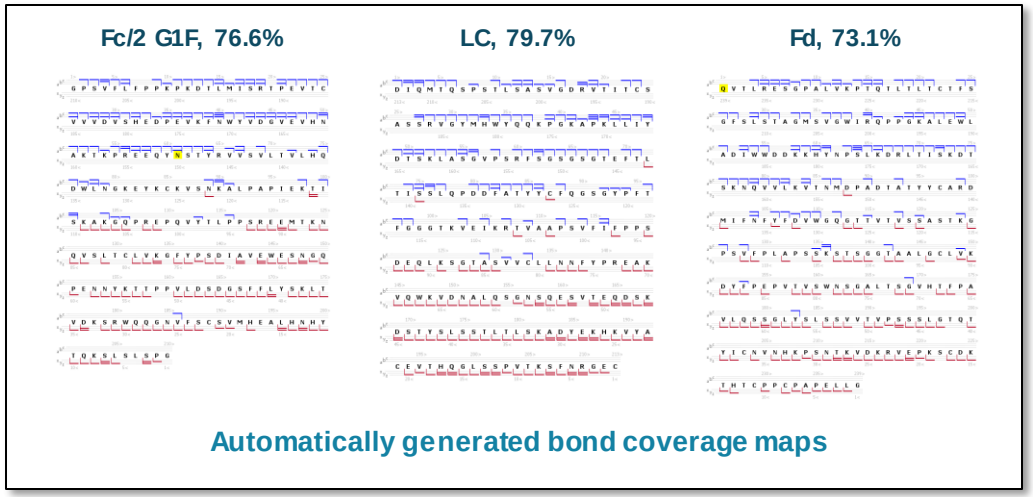
- Glycosylation is a common post-translational modification (PTM) which plays a critical role in antibody effector functions
  - Comprehensive characterization of N- and O-linked glycosylation in protein therapeutics is essential for ensuring drug safety and efficacy
- Glycation is a common non-enzymatic modification that can occur during fermentation and/or storage.[1] Protein therapeutics modified with glycation may undergo degradation to produce AGEs.
  - Glycation and AGEs increase product heterogeneity and can lead to protein aggregation and the expression of AGEs-specific receptors and cause adverse immune responses *in vivo*

[1]Anna Robotham and John Kelly (2020) LC-MS characterization of antibody-based therapeutics: recent highlights and future prospects. Approaches to the Purification, Analysis and Characterization of Antibody-Based Therapeutics. Chapter 1: 1-33.

Biologics Explorer software 3.0: middle-down analysis of mAbs

MIDDLE-DOWN ANALYSIS OF MABS WITH THE ZENOTOF 7600 SYSTEM

- Streamlined middle-down workflow combines the benefits of EAD from the ZenoTOF 7600 system and automatic data analysis using Biologics Explorer software. It significantly reduces time and effort spent on method development and provides reproducibly high sequence coverages for mAb subunits in **a single** injection.



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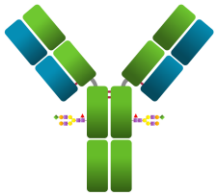
Biologics Explorer software 3.0: middle-down analysis of mAbs

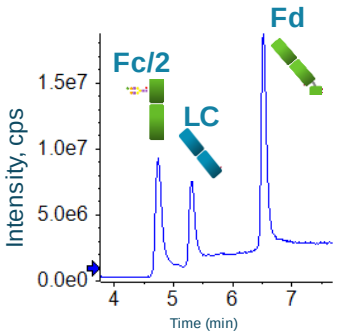
SIMPLE, PRODUCTIVE WORKFLOW FOR SEQUENCE & PTM CONFIRMATION



mAb

IdeS

DTT

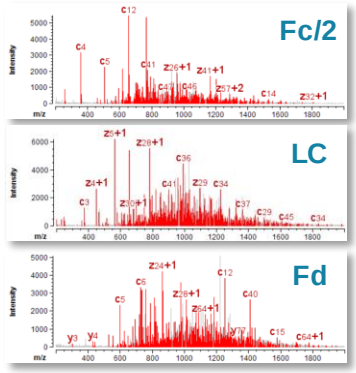


EAD

ZenoTOF 7600 system

+

Biologics Explorer software



Automatically annotated EAD spectra

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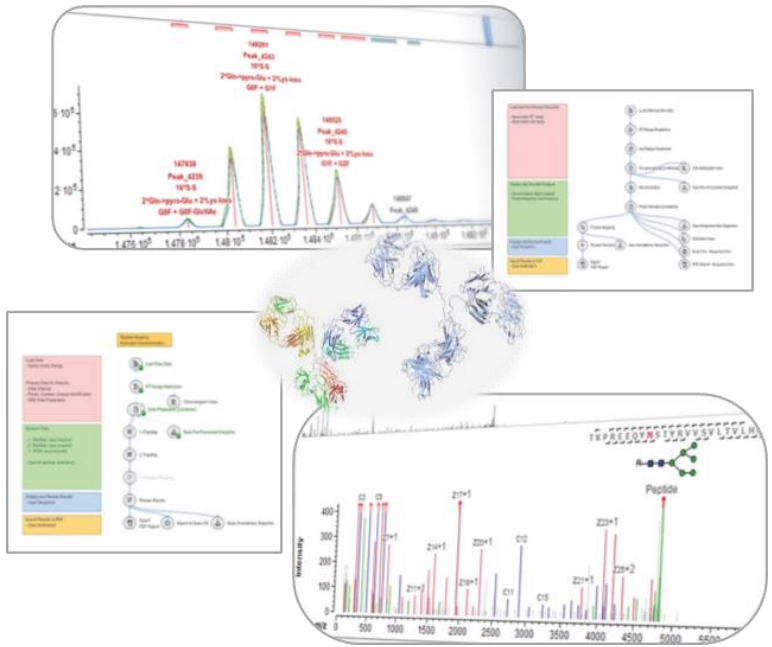
Biologics Explorer software

NEXT GENERATION BIOPHARMA SOFTWARE

Biologics Explorer software delivers highly accurate and informative workflows for full characterization of protein biotherapeutics.

Current workflows include:

- Middle-down analysis
- Intact and subunit analysis
- Peptide mapping by EAD or CID
- Disulfide bond analysis
- PTM determination, including MAM



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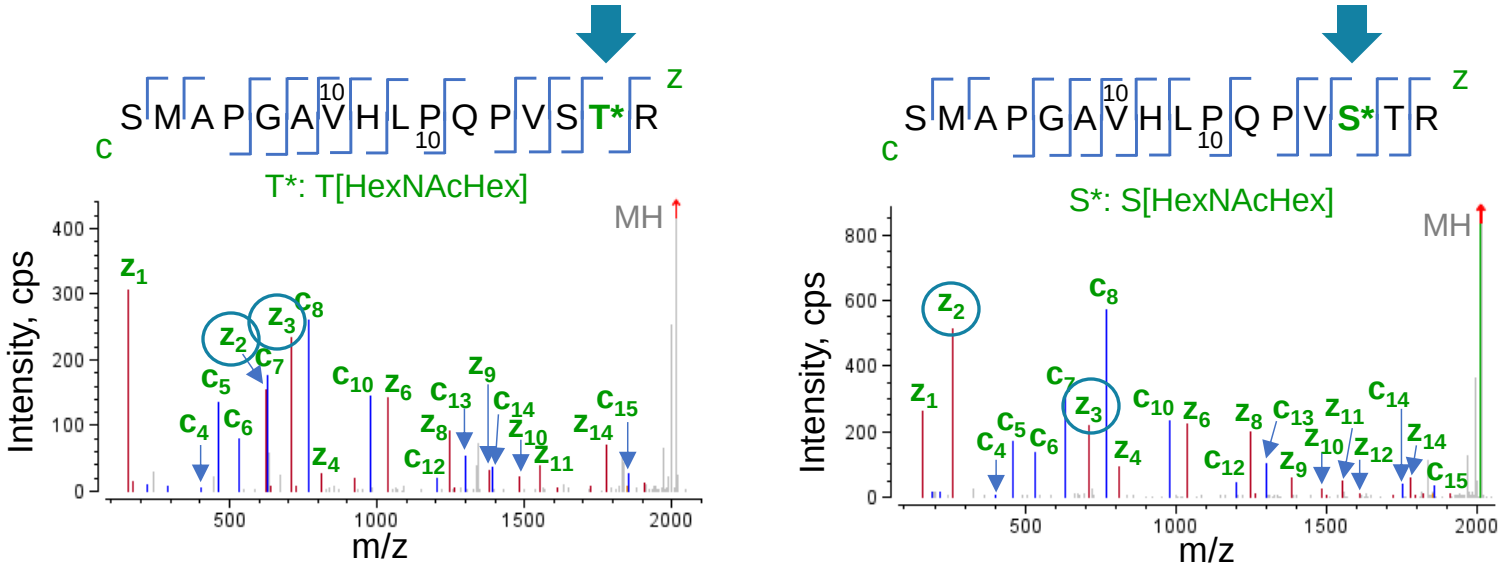
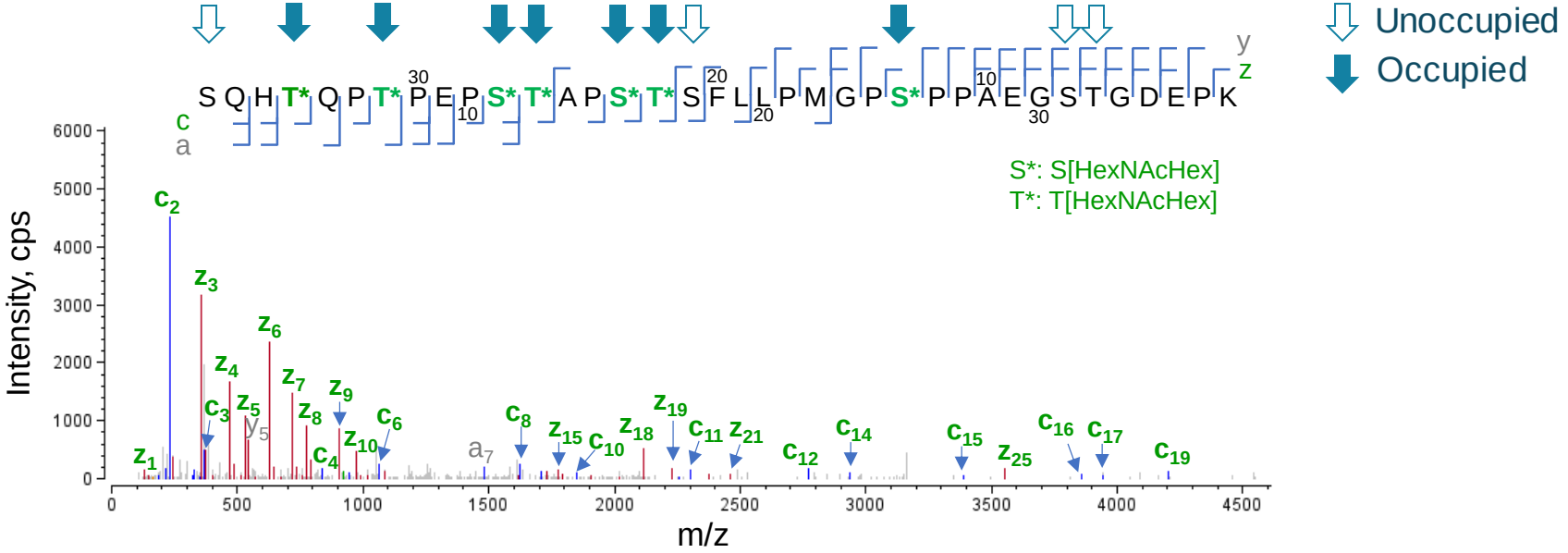
Glycan localization and isomer differentiation



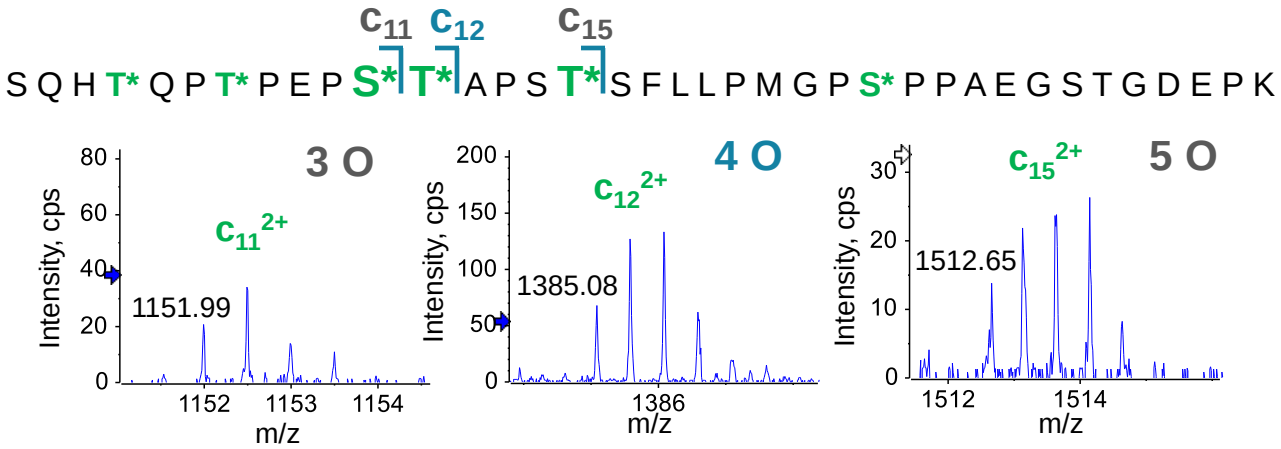
EAD resulted in excellent fragmentation of the O-linked glycopeptide backbone and the formation of glycan-containing fragments, allowing accurate localization of the O-linked glycan and confident differentiation of two positional isomers (S vs. T glycosylated)

Accurate localization of multiple O-linked glycans



Excellent EAD data led to accurate localization of glycosylation for etanercept glycopeptides carrying as many as 7 O-linked glycan moieties.

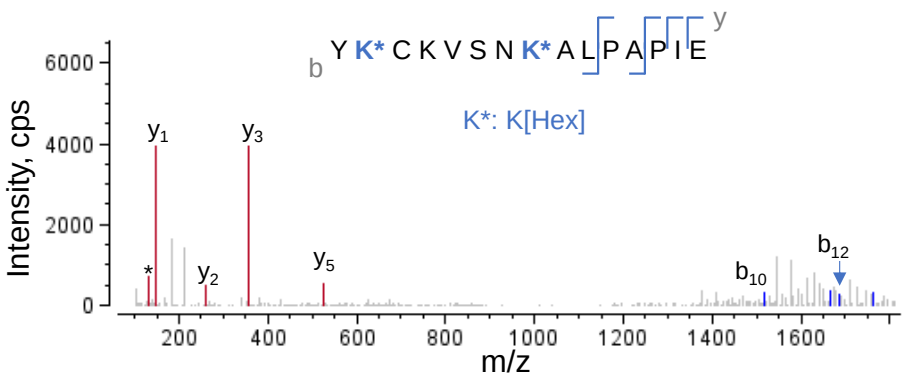
Differentiation of positional isomers



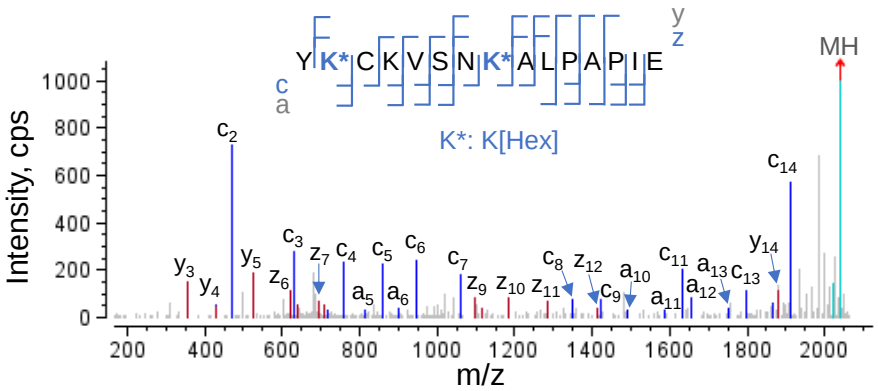
2 isomeric glycopeptides carrying 6 O-linked glycans can be confidently differentiated by EAD despite a minor difference in the position of 1 O-linked glycan

Challenges with glycation and AGE characterization

Poor fragmentation by CID



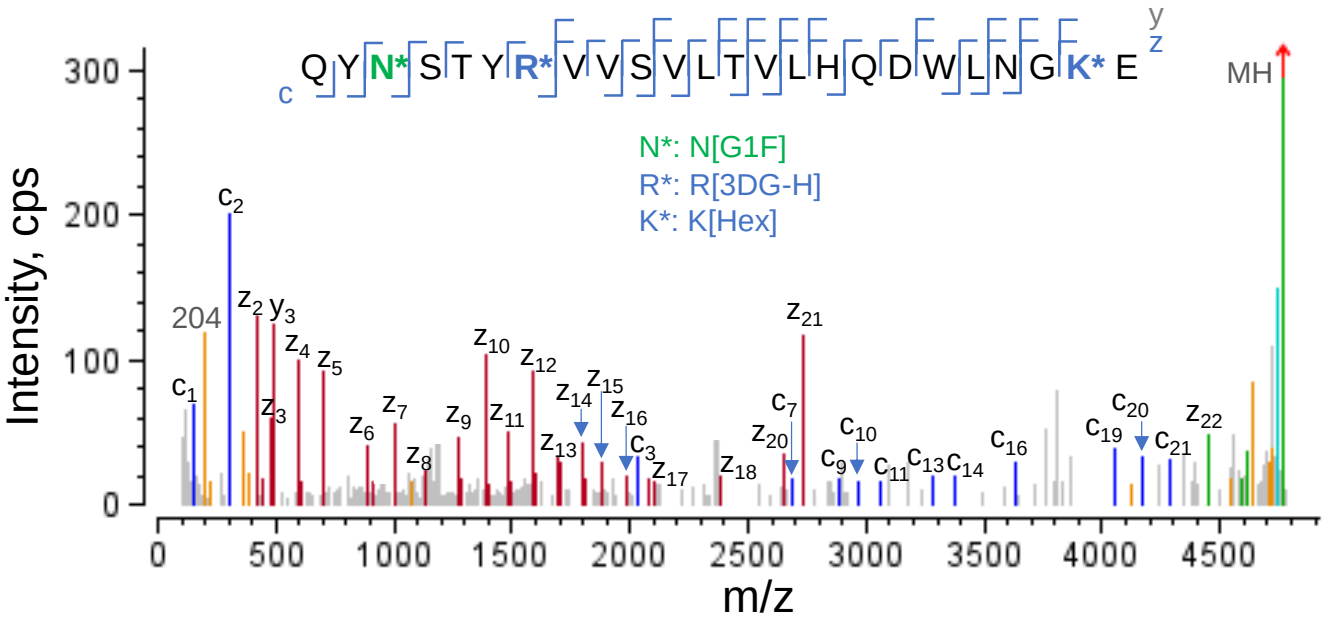
Excellent fragmentation by EAD



- Glycated peptides and AGEs are difficult to fragment by CID
- CID leads to preferential cleavage of H_2O from the hexose moiety and low yield of sequence ions
- Enzymatic digestion of glycated or AGE species, in which Lys and/or Arg residues are modified, leads to the formation of many long peptides containing the glycation and/or AGE moieties. The length of these species poses an additional challenge to CID.

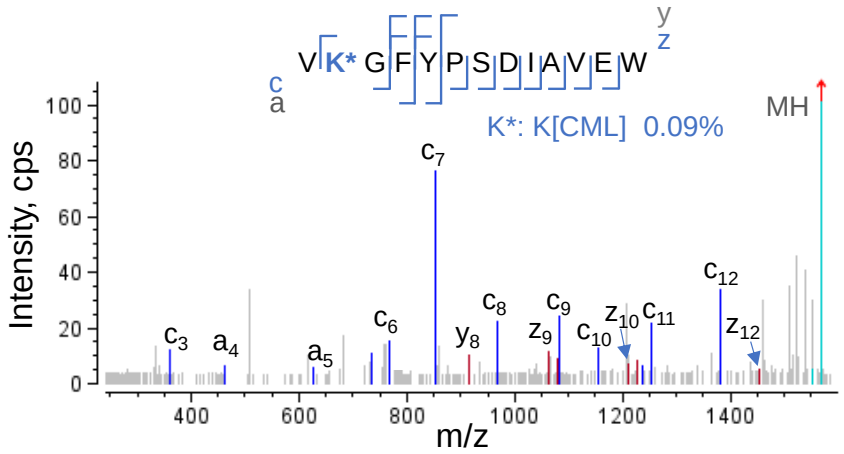
Comprehensive characterization of glycation in protein therapeutics using electron activated dissociation (EAD). SCIEX technical note, RUO-MKT-02-15020-A.
Comprehensive characterization of advanced glycation end products (AGEs) in protein therapeutics using electron activated dissociation (EAD). SCIEX technical note, RUO-MKT-02-15088-A.

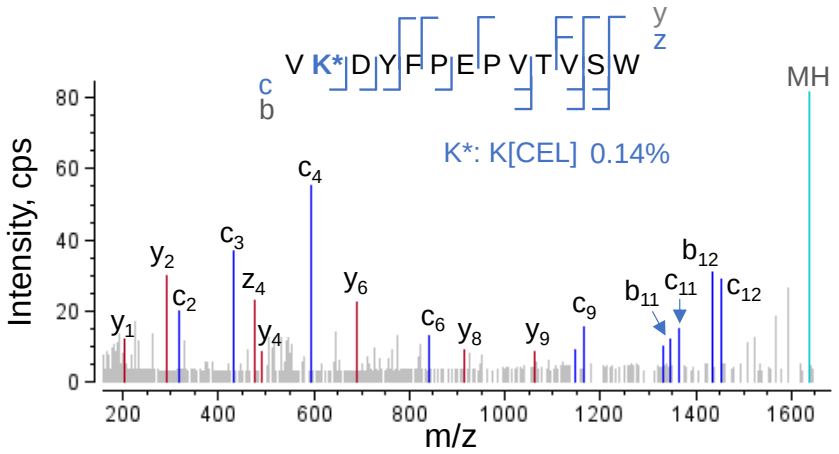
Simultaneous localization of multiple modifications



Excellent EAD data allowed for simultaneous localization of 3 modifications, including 1 N-linked glycan (G1F) on an Asn residue, 1 AGE moiety (3DG-H) on an Arg residue, and 1 glycation residue (Hex) on a Lys residue. Such depth of information cannot be achieved using CID.

EAD of AGEs in low abundance





- Traditional low-energy ExD approaches suffer from low sensitivity
- EAD platform method provided excellent fragmentation of AGEs with relative abundance as low as ~0.1%, demonstrating the high sensitivity of the approach

CML: carboxymethyl, CEL: carboxyethyl

Released N-linked glycan analysis on the BioPhase 8800 system

BioPhase Fast Glycan Labeling and Analysis kit

BioPhase 8800 software 1.2 (or greater) with glycan analysis module

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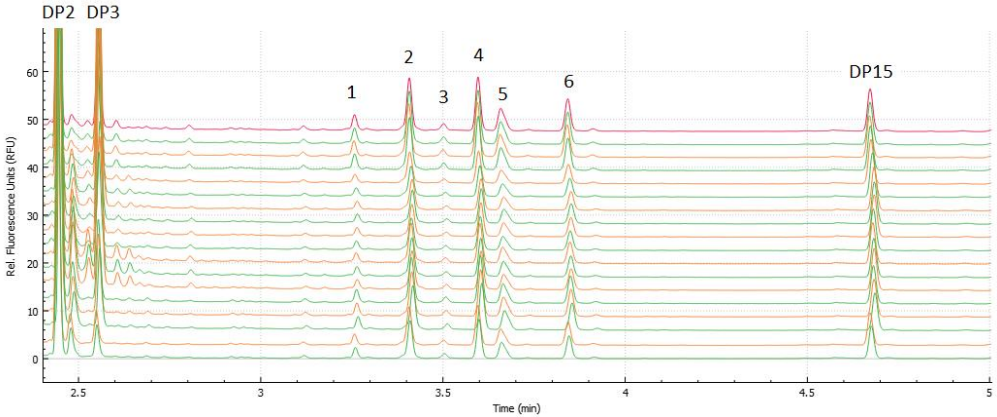
Screen your released glycans

FAST, PARALLEL SEPARATIONS WITH AUTOMATED SAMPLE PREP AND ANALYSIS CAPABILITIES

- Screen while maintaining resolution
  - 1 minute per sample, cycle time of 8 minutes per injection
  - <0.1 %RSD with glycan panel
- All-inclusive released N-glycan sample prep kit
- Automatable sample preparation
- Default or customizable glucose unit (GU) libraries
- Improved data processing



Glycan screening of human serum IgG on the BioPhase 8800 system. 1) M5, 2) FA2, 3) FA2B, 4) FA2(6)G1, 5) FA2(3)G1 and 6) FA2G2.

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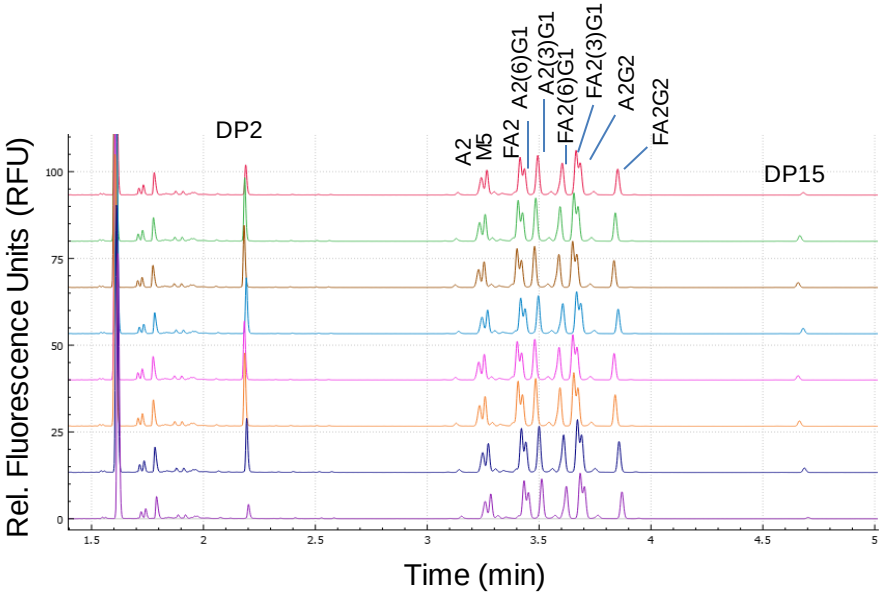
Highly reproducible glycan separations

<1 MIN PER SAMPLE WITH INTRA-CAPILLARY REPRODUCIBILITY < 0.1%



	Intra-capillary %RSD of GU for the 9-glycan panel (n=72)								
	A2	M5	FA2	A2(3)G1	A2(6)G1	FA2(3)G1	FA2(6)G1	A2G2	FA2G2
Capillary A	0.07	0.06	0.06	0.07	0.06	0.06	0.05	0.06	0.05
Capillary B	0.06	0.06	0.06	0.07	0.07	0.05	0.07	0.07	0.06
Capillary C	0.06	0.06	0.06	0.06	0.06	0.05	0.06	0.06	0.05
Capillary D	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05
Capillary E	0.07	0.07	0.07	0.07	0.07	0.06	0.05	0.07	0.05
Capillary F	0.06	0.07	0.06	0.07	0.06	0.06	0.05	0.06	0.05
Capillary G	0.07	0.08	0.08	0.08	0.08	0.06	0.07	0.07	0.08
Capillary H	0.08	0.08	0.07	0.09	0.09	0.07	0.07	0.08	0.06
Inter-capillary %RSD of GU (n=576)	**0.07**	**0.07**	**0.07**	**0.07**	**0.07**	**0.06**	**0.06**	**0.07**	**0.06**

8 representative electropherograms for a 9-glycan panel separation collected in parallel on the BioPhase 8800 system with high intra-capillary (n=72) and inter-capillary (n=576) reproducibility.

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Characterizing protein fragments and impurities

HIGH-RESOLUTION PROTEIN SIZING AND QUANTIFICATION

Fragments and impurities

Process- and product-related

- Determine product-related impurities including protein fragments and size variants
- Quantify process-related impurities including cell culture additives
- Key workflow:
  - Capillary gel electrophoresis using sodium dodecyl sulfate (SDS-CGE)

SCIEX
The Power of Precision

The BioPhase 8800 system

Robustness

Designed for robustness, hardware and software advances ensure repeatability and increased reliability

Software

Reimagined software makes getting results quick and easy. Simple drag-and-drop functionality for method and sequence creation complements innovative data analysis to accelerate characterization from start-to-finish

Flexibility

Flexible for your workflow requirements, switching between UV and LIF detection is simple and seamless. Integrated detection modules make it easy to go from one assay to the next, without sacrificing consistency or performance

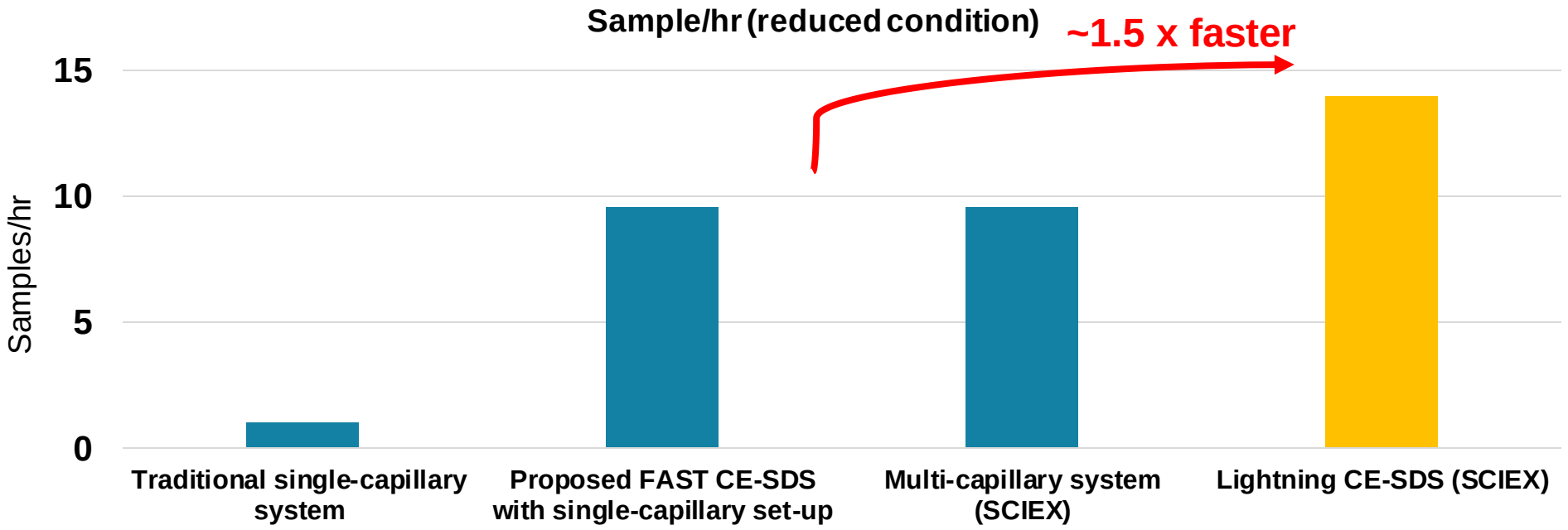
Compatibility

96-well plates are designed to ANSI/SLAS standards and are conveniently compatible with commercial liquid handling systems

Pre-assembled reagents/consumables

Simplify operation and minimize user error

Throughput improvement on CE-SDS assay



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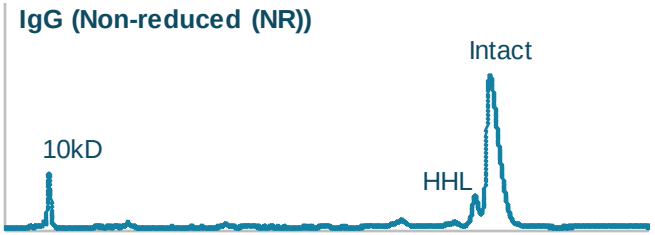
Original SCIEX CE-SDS method settings on the BioPhase 8800 system

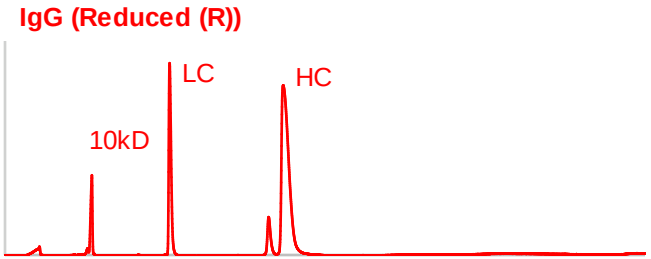
80 psi 2 min	Base
20 psi 5 min	Base
20 psi 5 min	Acid
20 psi 3 min	Water
80 psi 10 min	Gel

Rinsing (25 min)

35 min (NR)/25 min (R) at 15 kV

Separation





Cycle time 50–60 min (8 samples)

6.25 min (R) and 7.5 min (NR)/sample

High separation efficiency

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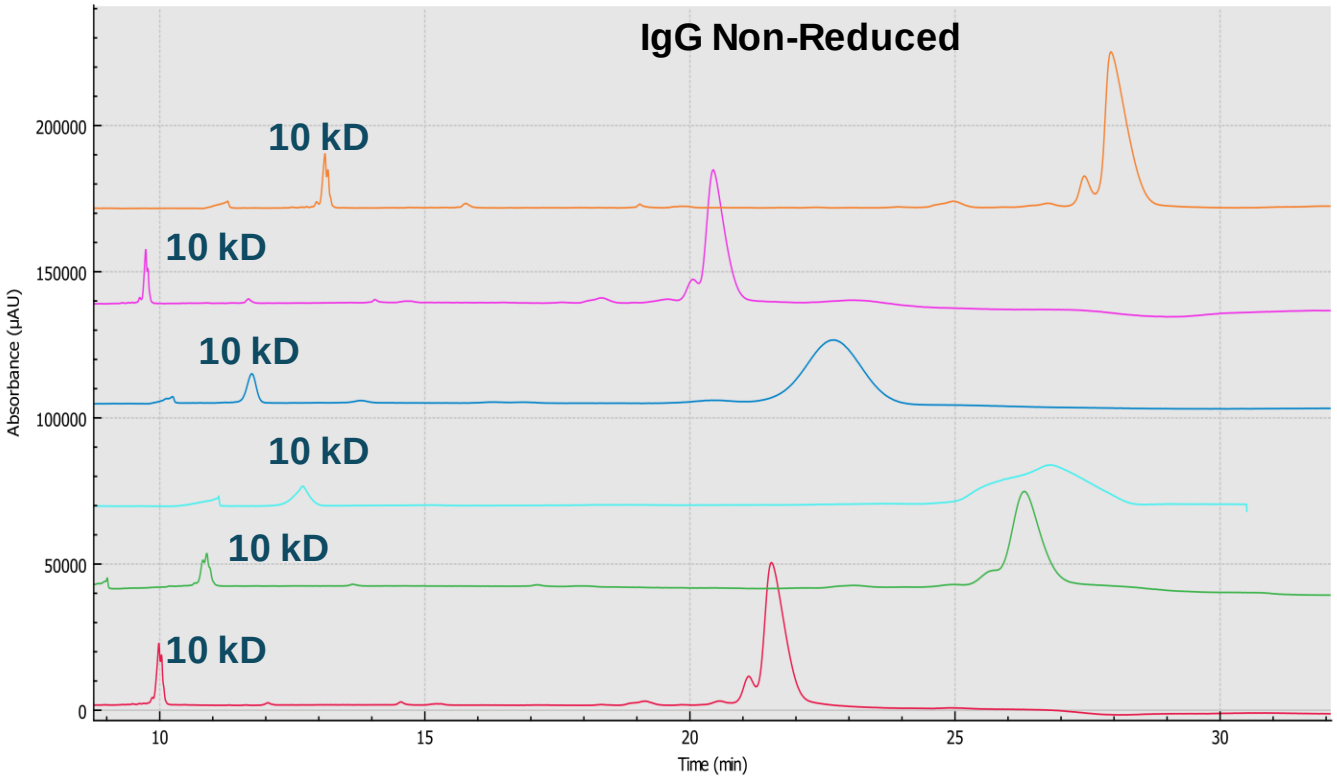
Decreasing cycle time by shortening separation time



Original (15 kV, 25°)

20 kV

15 kV + 1 psi

Pressure push, 15 kV only

Voltage push, 15 kV only

15 kV + 40°

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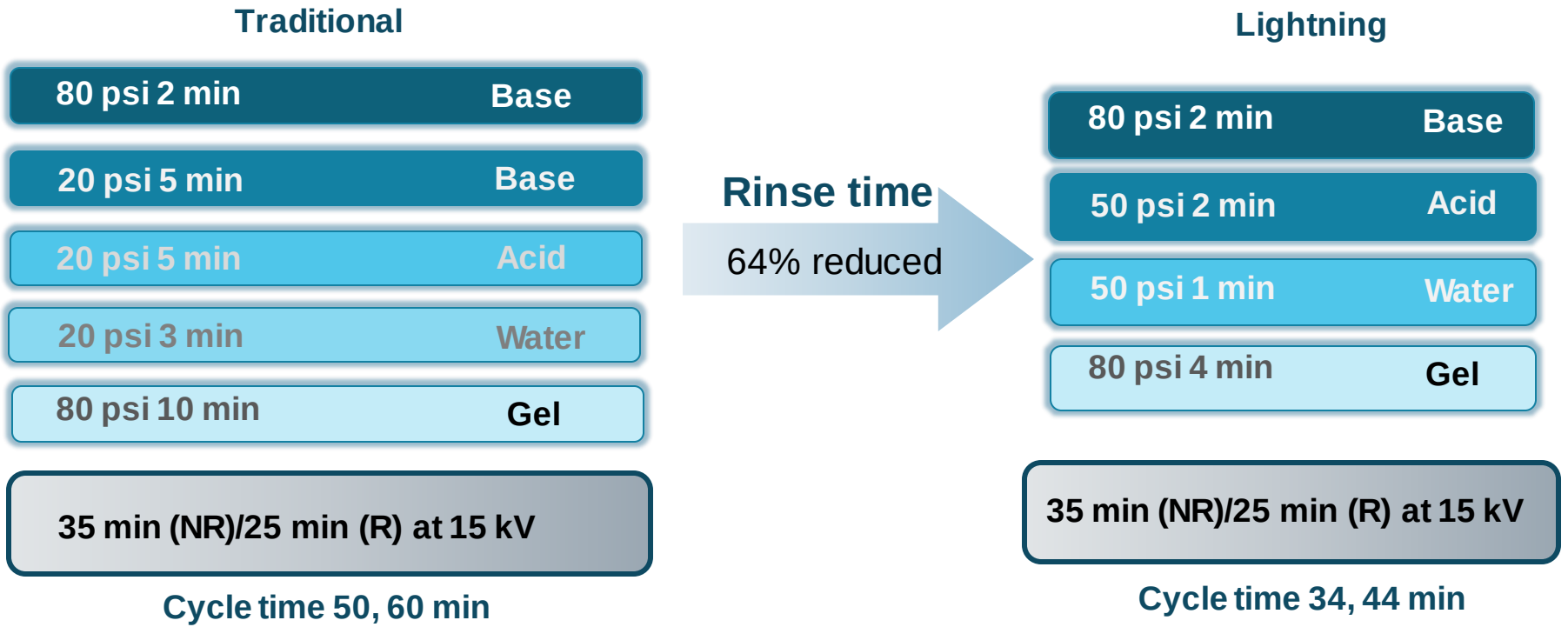
RUO-MKT-11-14733-A

Decreasing cycle time by shortening rinse time



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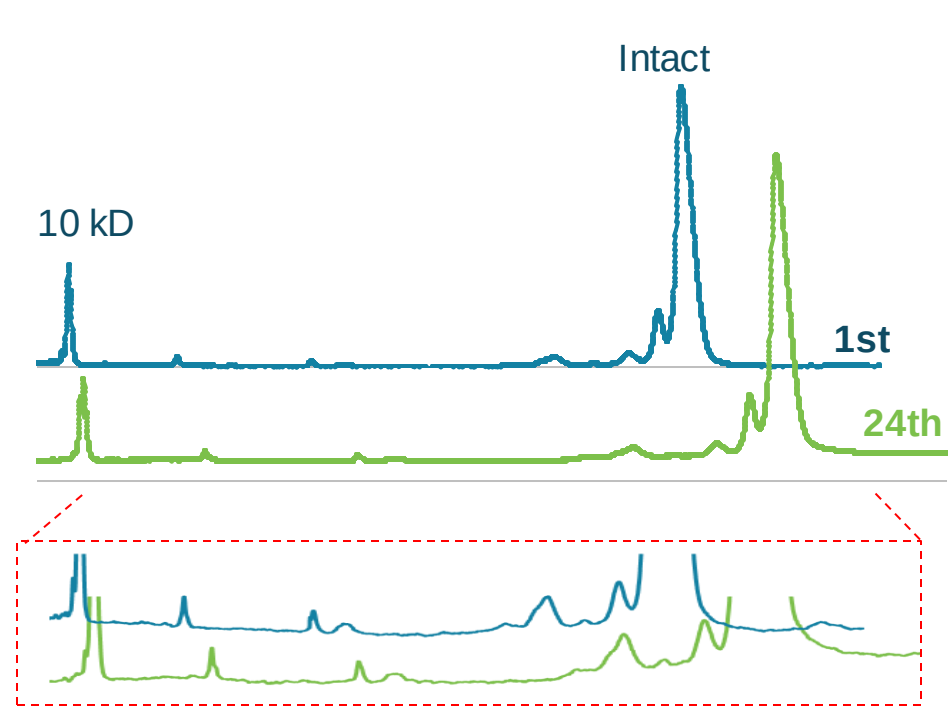
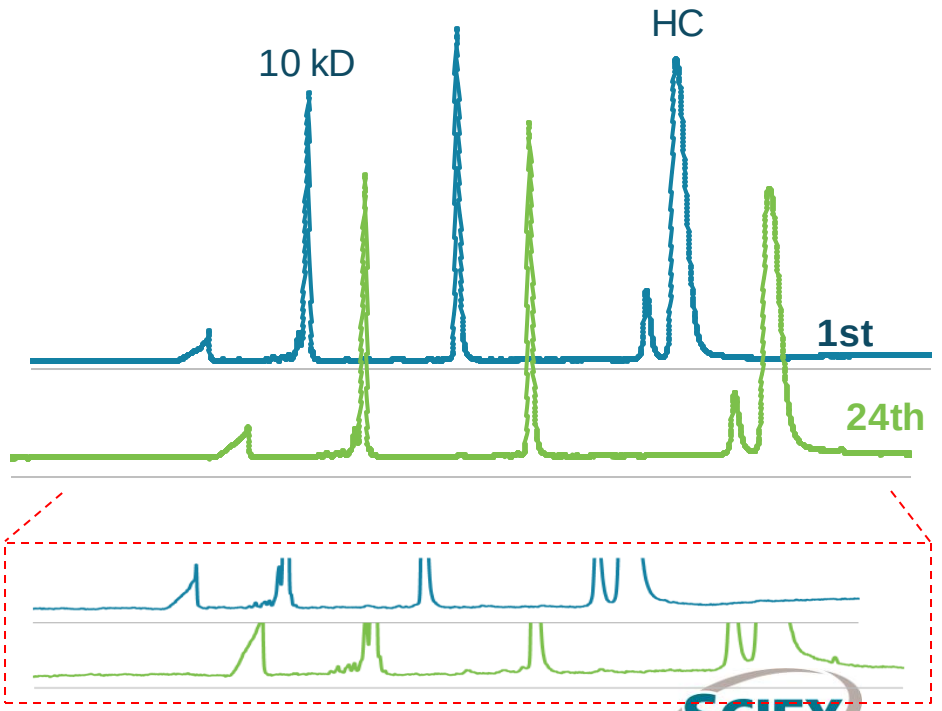
Throughput results

Condition	Method	Cycle time	Min/sample	Hours/plate	Samples/hour
Reduced IgG	***Lightning***	***34 min***	***4.3***	***6.9***	***14***
	Original	*50 min*	*6.25*	*10*	*9*
Non-reduced IgG	***Lightning***	***44 min***	***5.5***	***8.8***	***11***
	Original	*60 min*	*7.5*	*12*	*8*

Reproducibility of lightning CE-SDS

CONSISTENT SEPARATION PROFILE OVER 24 CONSECUTIVE INJECTIONS (192 REPS)





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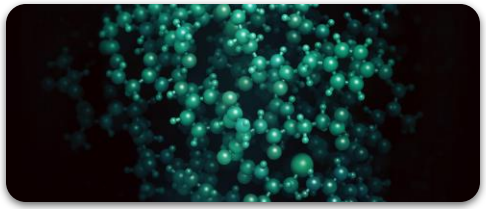
RUO-MKT-11-14733-A

Rapid characterization of protein therapeutic charge variants

HIGH PRECISION ANALYSIS OF CHARGE HETEROGENEITY



Charge heterogeneity

- Rapid characterization of protein therapeutic charge variants can be challenging for the growing number and wider variety of new modality drug candidates.
- Protein therapeutic charge variant assessment is essential at different manufacturing stages as they are subjected to instability, causing alterations in their primary amino acid sequence and variable post-translational modification (PTM)
- Key workflows:
  - Capillary isoelectric focusing (cIEF)
  - Imaged capillary isoelectric focusing coupled to mass spectrometry (icIEF-MS)

[1]Anna Robotham and John Kelly (2020) LC-MS characterization of antibody-based therapeutics: recent highlights and future prospects. Approaches to the Purification, Analysis and Characterization of Antibody-Based Therapeutics. Chapter 1: 1-33.

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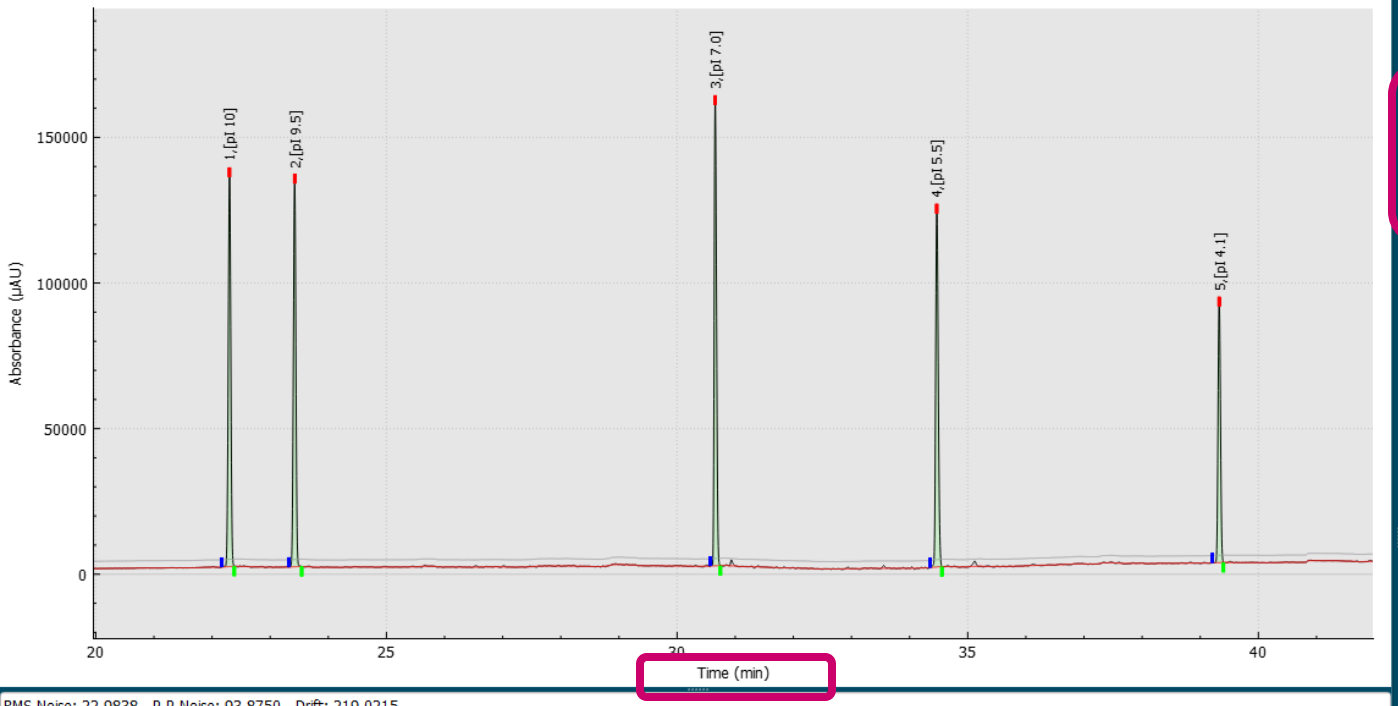
New cIEF features simplify analysis

BioPhase 8800 system

Single | Overlay



RMS Noise: 22.9838 - P-P Noise: 93.8750 - Drift: 219.0215

No.	Name	MT	MT*	Cal MT	Start	End	Height	Area	Area%	Corr. Area	Cor
1	[pI 10]	22.3000	22.3000	9.92	22.1667	22.3833	135421.4219	403553.4688	21.53	6032.19	
2	[pI 9.5]	23.4208	23.4208	9.53	23.3250	23.5417	133278.5625	389450.5938	20.78	5542.79	
3	[pI 7.0]	30.6542	30.6542	7.00	30.5750	30.7458	159902.7813	437809.4375	23.36	4760.74	
4	[pI 5.5]	34.4667	34.4667	5.67	34.3542	34.5542	123036.7969	380767.0938	20.32	3682.47	
5	[pI 4.1]	39.3250	39.3250	3.97	39.2083	39.4000	89614.5469	262596.9688	14.01	2225.87	

Analysis Parameters

Integration | Library | Post Analysis

Marker Table — External markers ...

Name	MT	Cal MT	Tol	Crit	Excl	Ref
pI 10	22.3000	10.0000	5%	Ctr		
pI 9.5	23.4000	9.5000	5%	Ctr		
pI 7.0	30.1250	7.0000	5%	Ctr		
pI 5.5	33.9958	5.5000	5%	Ctr		
pI 4.1	38.9125	4.1000	5%	Ctr		

Clear | Copy | Paste

X-axis Name: Isolectrict Point — Units: pI

Fit Type: Linear — Show Curve

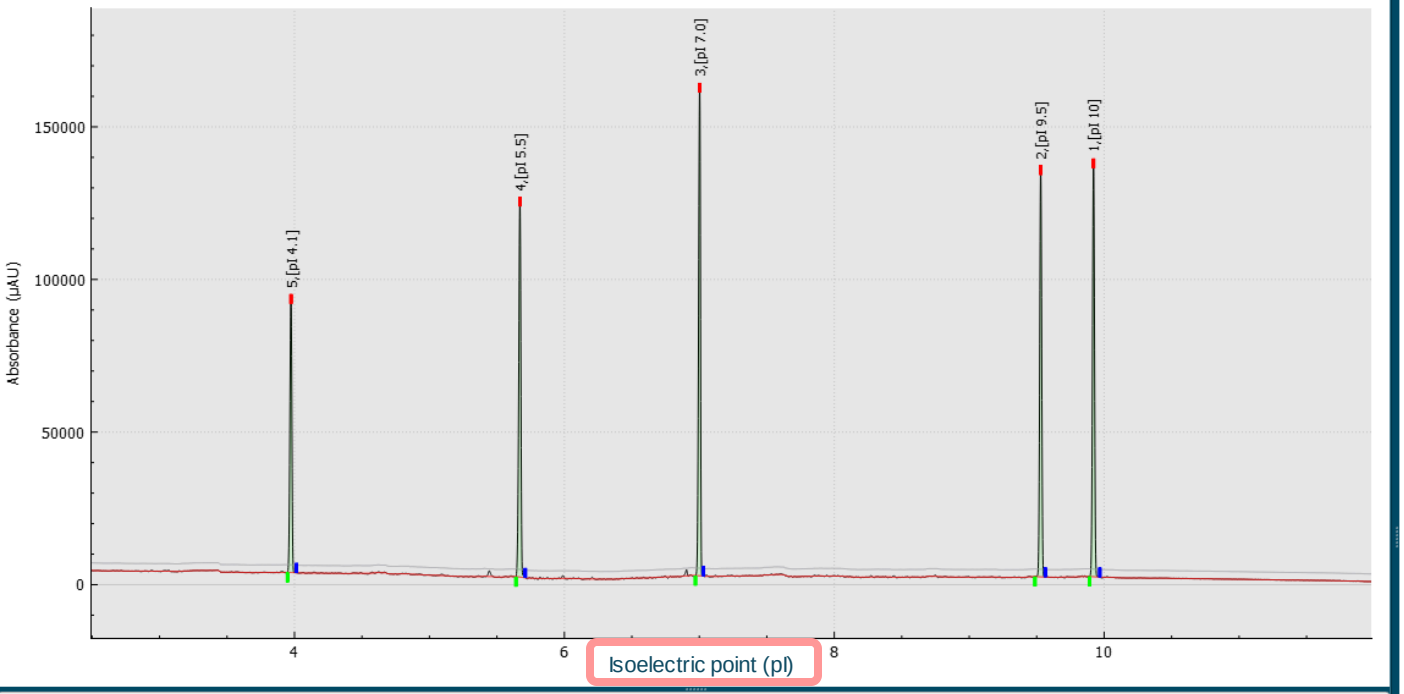
Peak Table - Identify by: Cal MT

Name	Cal MT	Tol	Crit	Excl	Ref

New cIEF features simplify analysis

BioPhase 8800 system

Single | Overlay



RMS Noise: 22.9838 - P-P Noise: 93.8750 - Drift: 219.0215

No.	Name	MT	MT*	Cal MT	Start	End	Height	Area	Area%	Corr. Area	Cor
1	[pI 10]	22.3000	22.3000	9.92	22.1667	22.3833	135421.4219	403553.4688	21.53	6032.19	
2	[pI 9.5]	23.4208	23.4208	9.53	23.3250	23.5417	133278.5625	389450.5938	20.78	5542.79	
3	[pI 7.0]	30.6542	30.6542	7.00	30.5750	30.7458	159902.7813	437809.4375	23.36	4760.74	
4	[pI 5.5]	34.4667	34.4667	5.67	34.3542	34.5542	123036.7969	380767.0938	20.32	3682.47	
5	[pI 4.1]	39.3250	39.3250	3.97	39.2083	39.4000	89614.5469	262596.9688	14.01	2225.87	

Analysis Parameters

Integration | Library | Post Analysis

Marker Table — External markers ...

Name	MT	Cal MT	Tol	Crit	Excl	Ref
pI 10	22.3000	10.0000	5%	Ctr		✓
pI 9.5	23.4000	9.5000	5%	Ctr		
pI 7.0	30.1250	7.0000	5%	Ctr		
pI 5.5	33.9958	5.5000	5%	Ctr		
pI 4.1	38.9125	4.1000	5%	Ctr		

Clear | Copy | Paste

X-axis Name: Isolectrict Point Units: pI

Fit Type: Linear Show Curve

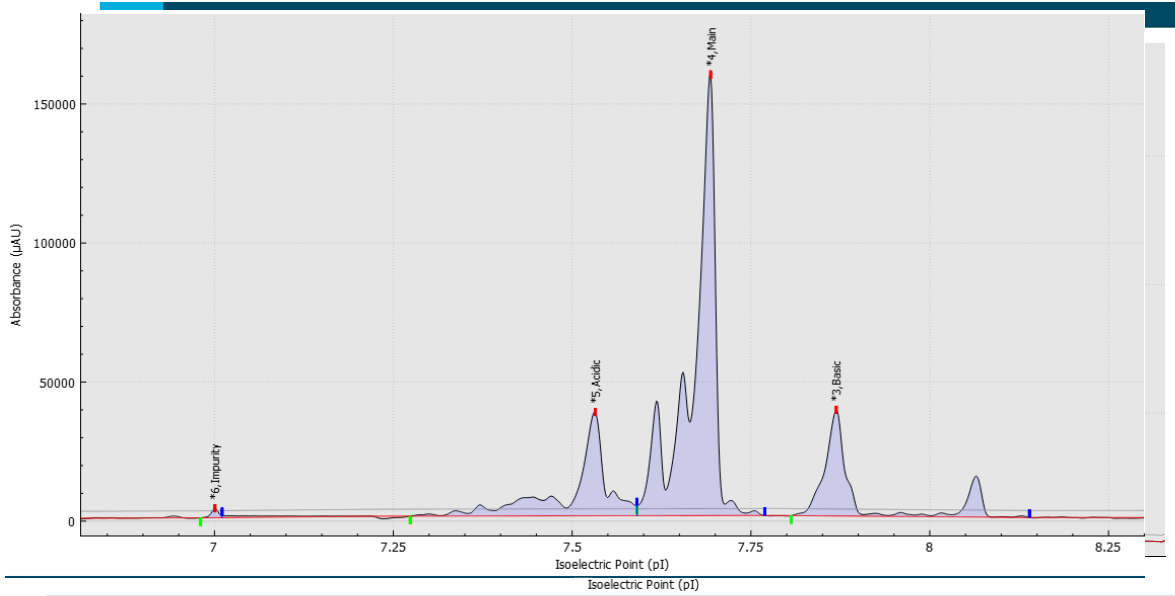
Peak Table - Identify by: Cal MT

Name	Cal MT	Tol	Crit	Excl	Ref

Peak grouping as you want it

BioPhase 8800 system

USP IgG



RMS Noise: 22.4327 - P-P Noise: 93.2120 - Drift: 147.2470

No.	Name	MT	MT*	Cal MT	Start	End	Height	Area	Area%	Corr. Area	Cor
1	[pI 10]	22.4167	22.4167	9.93	21.9458	22.6667	113792.3906	332226.0938	11.71	4940.17	
2	[pI 9.5]	23.5167	23.5167	9.55	23.4333	23.6958	108172.8047	294963.1563	10.40	4180.91	
3	Basic	28.4125	28.4125	7.87	27.6250	28.5958	38155.8203	284508.0000	10.03	3337.83	
4	Main	28.9250	28.9250	7.69	28.7042	29.2250	158585.8438	999726.1875	35.24	11520.90	
5	Acidic	29.3958	29.3958	7.53	29.2250	30.1500	37312.0195	341258.1875	12.03	3869.69	
6	Impurity	30.9458	30.9458	7.00	30.9167	31.0042	3393.1458	7055.9121	0.25	76.00	
7	[pI 5.5]	35.0000	35.0000	5.61	34.8708	35.1917	95073.0703	290573.4063	10.24	2767.37	
8	[pI 4.1]	39.6500	39.6500	4.02	39.5667	39.7250	95425.0469	286541.5938	10.10	2408.92	

Analysis Parameters

Integration | Library | Post Analysis

Marker Table — External markers ...

Name	MT	Cal MT	Tol	Crit	Excl	Ref
pI 10	22.2875	10.0000	5%	Ctr		
pI 9.5	23.4375	9.5000	5%	Ctr		
pI 5.5	35.2208	5.5000	5%	Ctr		
pI 4.1	40.0208	4.1000	5%	Ctr		

Clear | Copy | Paste

X-axis Name: Isoelectric Point Units: pI

Fit Type: Linear Show Curve

Peak Table - Identify by: Cal MT

Name	Cal MT	Tol	Crit	Excl	Ref
Impurity	7.0007	5%	Ctr		
Acidic	7.5251	5%	Ctr		
Main	7.6863	5%	Ctr		
Basic	7.8628	5%	Ctr		

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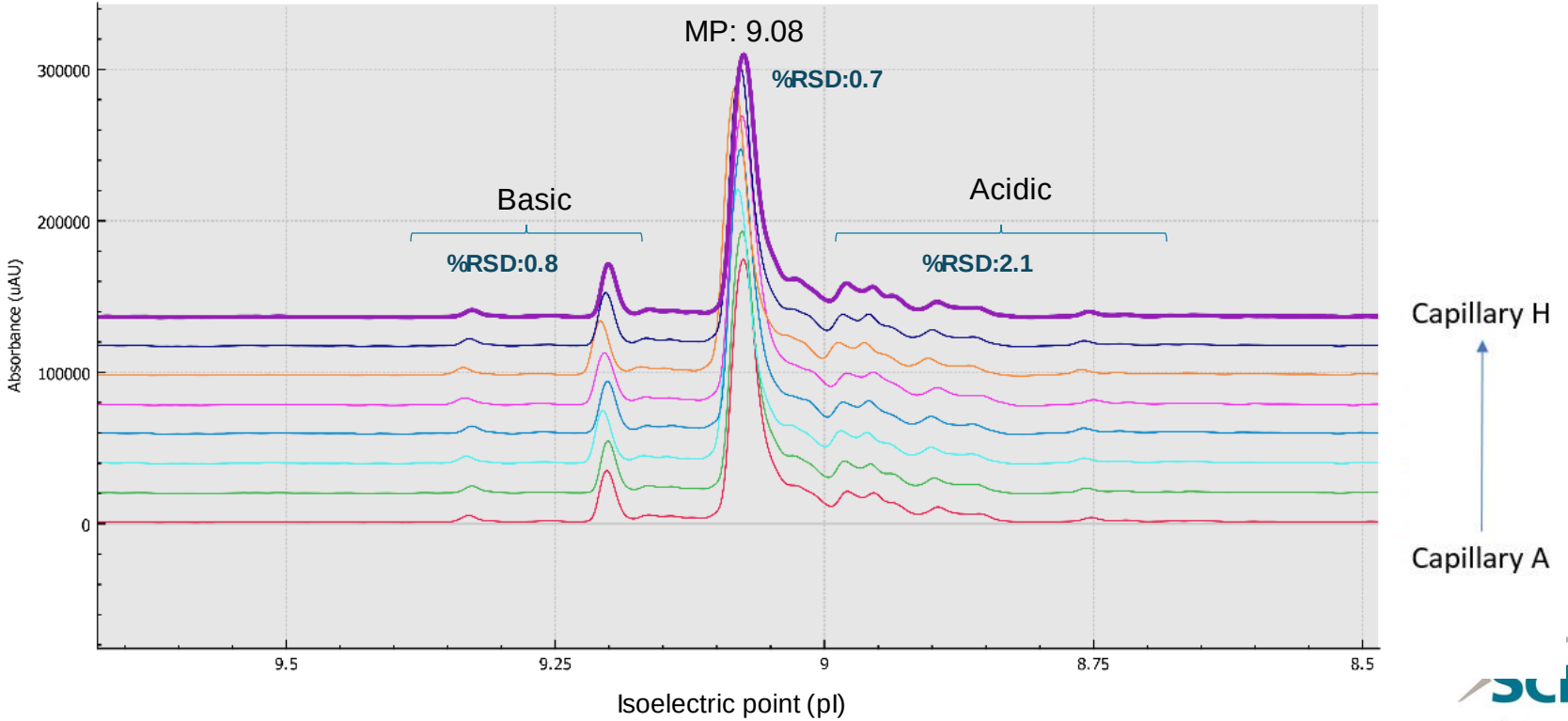
cIEF assay repeatability

BioPhase 8800 system

6 REPLICATE ANALYSIS FOR NIST IgG



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Reproducible cIEF data

BioPhase 8800 system

Capillary	Total corrected area		% Basic corrected area		% Main corrected area		% Acidic corrected area		Calibrated main pI	
	Avg	%RSD	Avg	%RSD	Avg	%RSD	Avg	%RSD	Avg	%RSD
A	19595.9	2.7	11.6	0.8	69.5	0.2	18.9	0.8	9.08	0.1
B	20016.0	4.0	11.7	1.7	68.6	0.4	19.6	1.0	9.08	0.0
C	20769.4	2.9	11.7	1.0	68.8	0.8	19.5	3.1	9.08	0.1
D	20908.0	0.2	11.8	2.0	67.9	0.3	20.3	0.2	9.08	0.0
E	22005.1	2.4	11.7	1.0	68.6	0.5	19.7	1.8	9.08	0.0
F	21339.3	1.7	11.7	0.7	68.6	0.6	19.6	1.8	9.08	0.0
G	20622.4	3.9	11.6	1.7	68.8	0.3	19.6	1.3	9.08	0.1
H	20010.0	3.7	11.5	1.1	68.4	0.9	20.1	3.7	9.08	0.1
Avg	20658.3		11.7		68.7		19.7		9.08	
%RSD	**3.8**		**0.8**		**0.7**		**2.1**		**0.02**	

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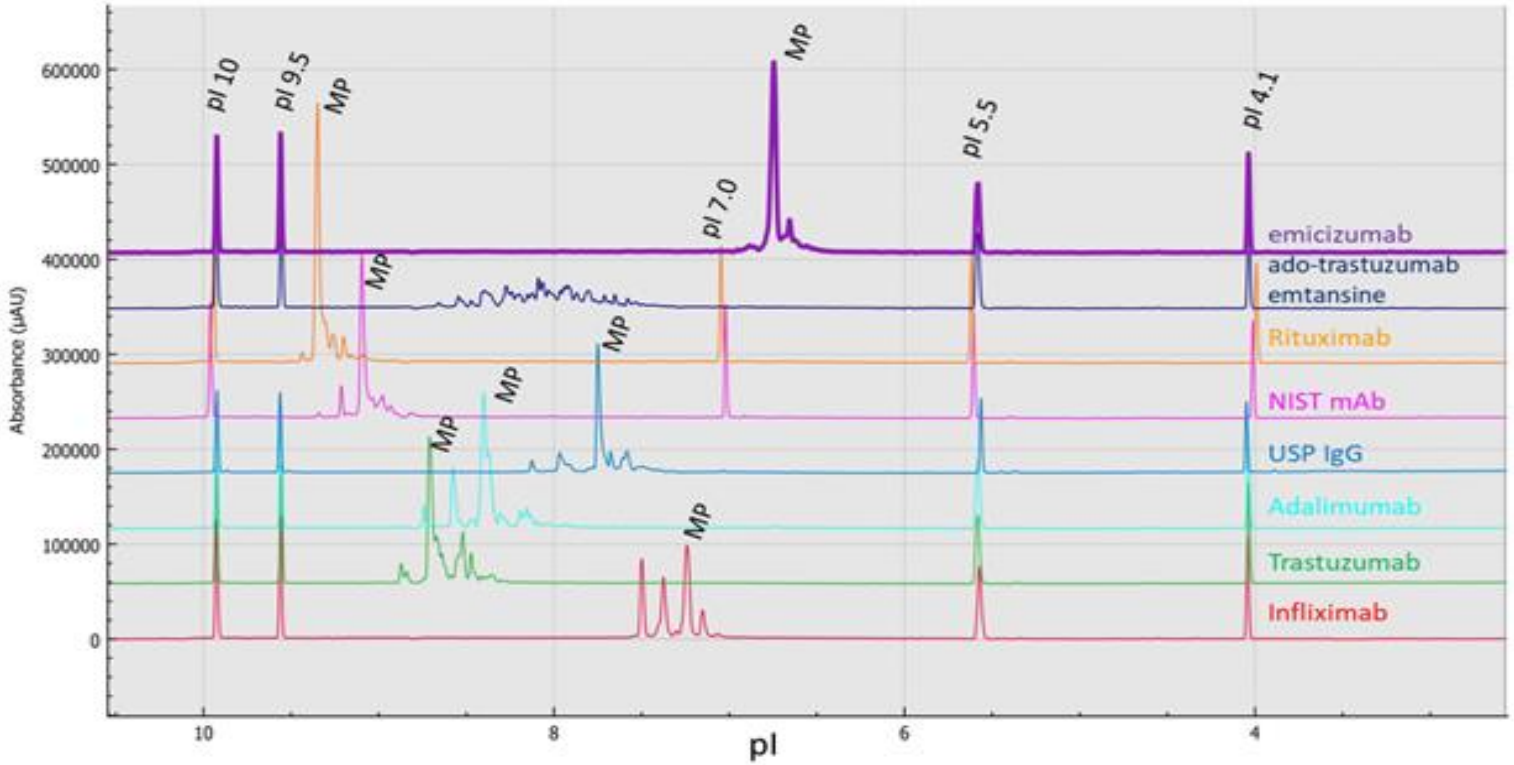
Reference molecules: NIST and USP

BioPhase 8800 system



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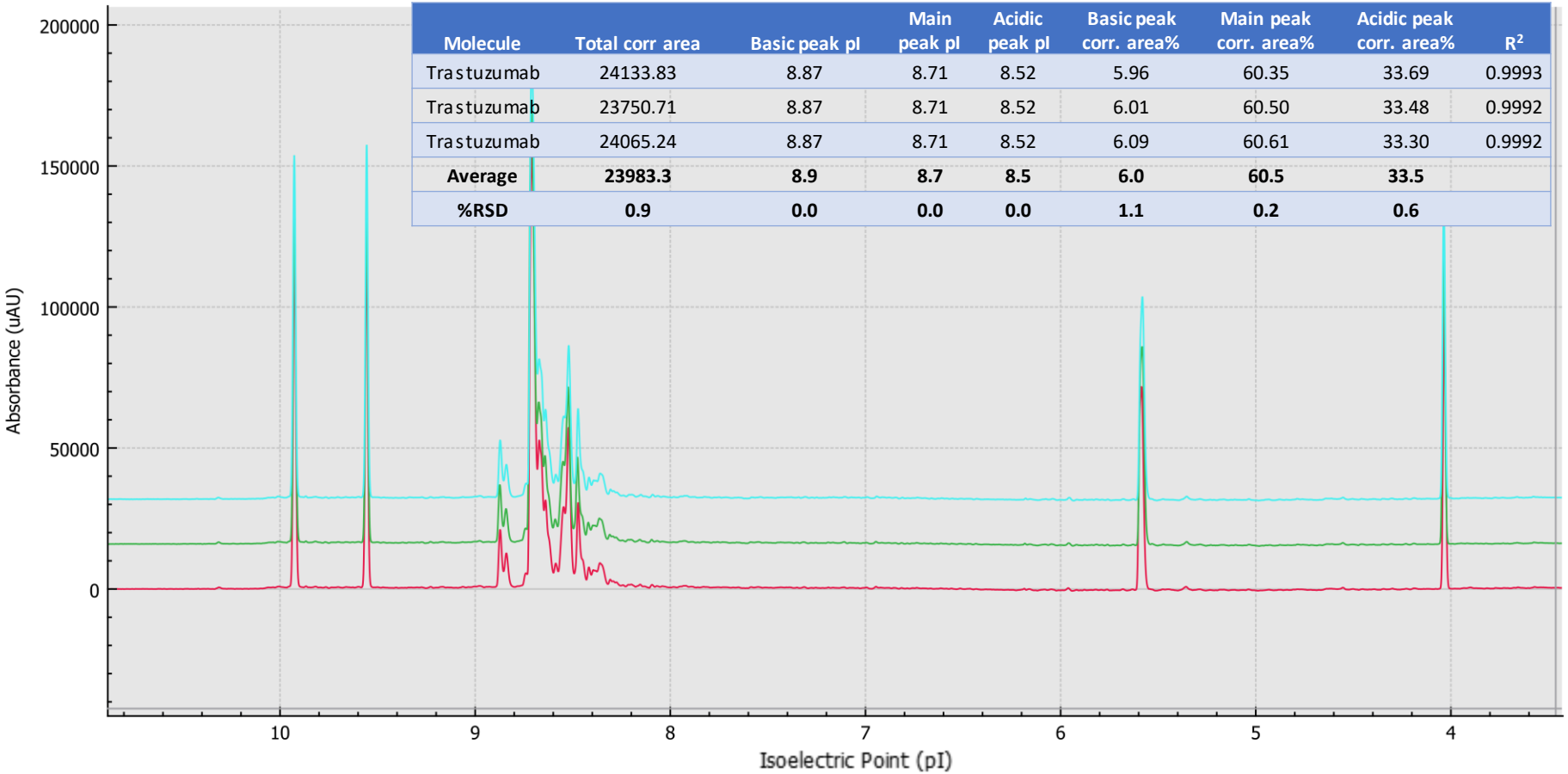
Trastuzumab

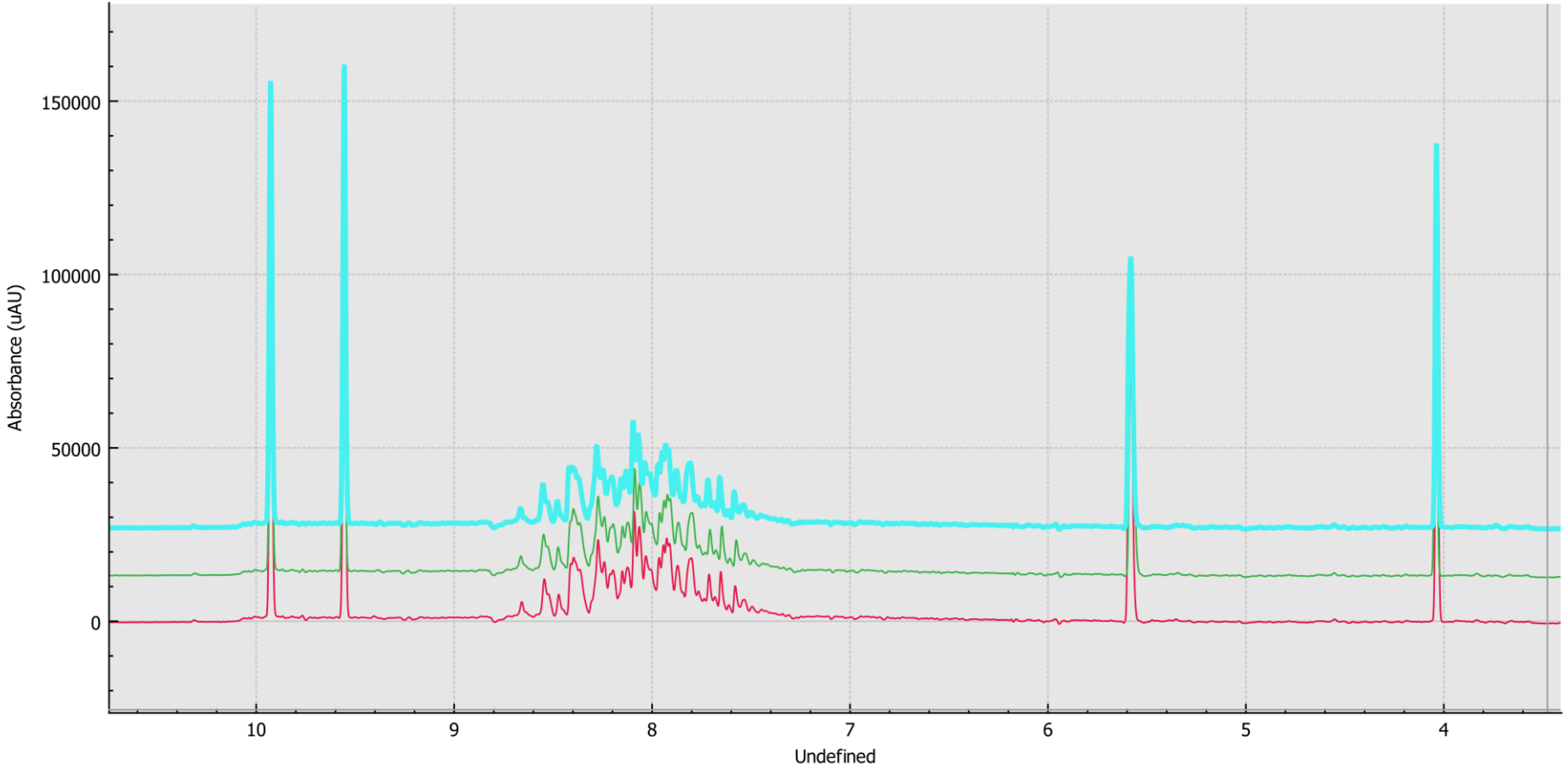
BioPhase 8800 system

Molecule	Total corr area	Basic peak pI	Main peak pI	Acidic peak pI	Basic peak corr. area%	Main peak corr. area%	Acidic peak corr. area%	R^2
Trastuzumab	24133.83	8.87	8.71	8.52	5.96	60.35	33.69	0.9993
Trastuzumab	23750.71	8.87	8.71	8.52	6.01	60.50	33.48	0.9992
Trastuzumab	24065.24	8.87	8.71	8.52	6.09	60.61	33.30	0.9992
Average	**23983.3**	**8.9**	**8.7**	**8.5**	**6.0**	**60.5**	**33.5**	
%RSD	**0.9**	**0.0**	**0.0**	**0.0**	**1.1**	**0.2**	**0.6**	



ADC: Ado-trastuzumab emtansine

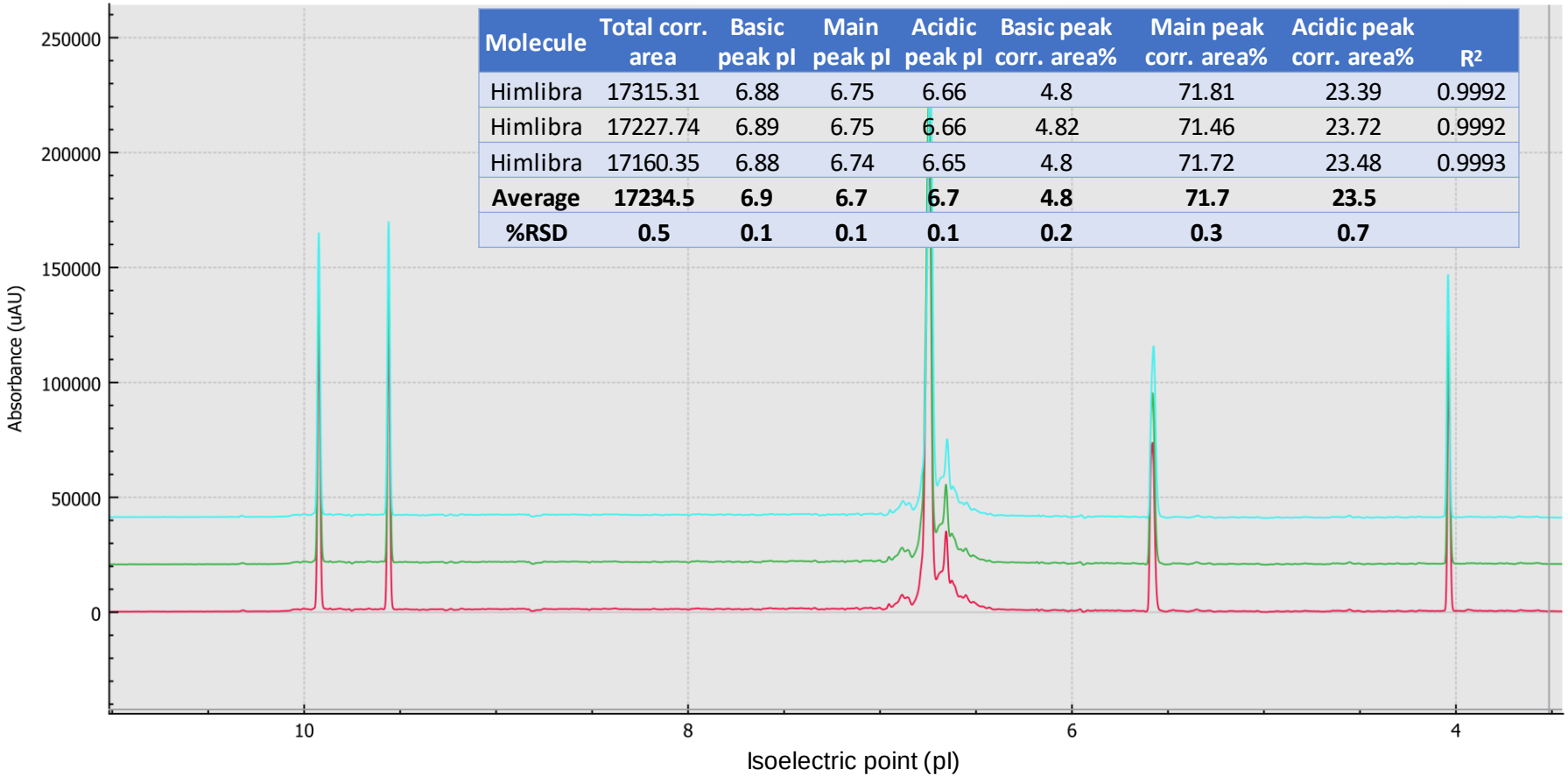
BioPhase 8800 system



Bi-specific: emicizumab-kxwh

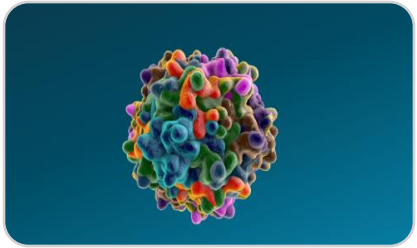
BioPhase 8800 system

Molecule	Total corr. area	Basic peak pI	Main peak pI	Acidic peak pI	Basic peak corr. area%	Main peak corr. area%	Acidic peak corr. area%	R^2
Himlibra	17315.31	6.88	6.75	6.66	4.8	71.81	23.39	0.9992
Himlibra	17227.74	6.89	6.75	6.66	4.82	71.46	23.72	0.9992
Himlibra	17160.35	6.88	6.74	6.65	4.8	71.72	23.48	0.9993
Average	**17234.5**	**6.9**	**6.7**	**6.7**	**4.8**	**71.7**	**23.5**	
%RSD	**0.5**	**0.1**	**0.1**	**0.1**	**0.2**	**0.3**	**0.7**	



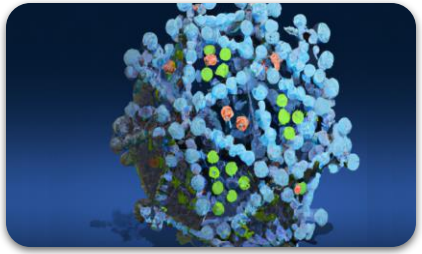
Key workflows for the analysis of AAV therapies

CHARACTERIZING AAV-BASED PRODUCTS



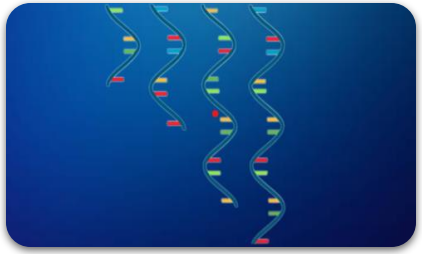
Capsid protein characterization

Intact, sub-unit and peptide mapping



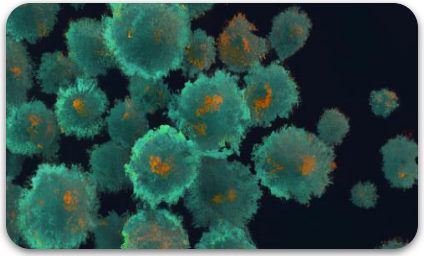
Viral protein purity

Drug substance and drug product analysis



Genome integrity

Transgene sizing and process related impurities



Full and empty

Determination of full capsid percent

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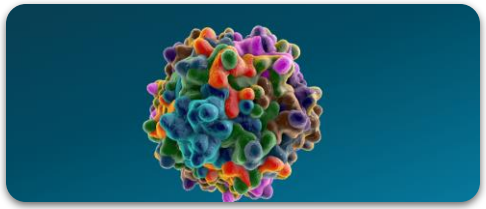
Intact mass analysis of capsid proteins

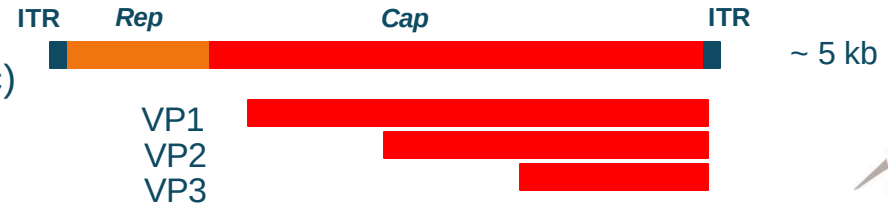
UTILIZING RECOMBINANT AAV8



Capsid protein characterization

Intact, sub-unit, and peptide mapping

- AAV capsid proteins are made up of viral protein 1 (VP1), viral protein 2 (VP2) and viral protein 3 (VP3)
  - Each is ~60-82 kDa protein derived from the same genome through alternative splicing
  - Characterization is necessary to confirm proper expression and identify modifications or impurities



AAV2 genome map (schematic)

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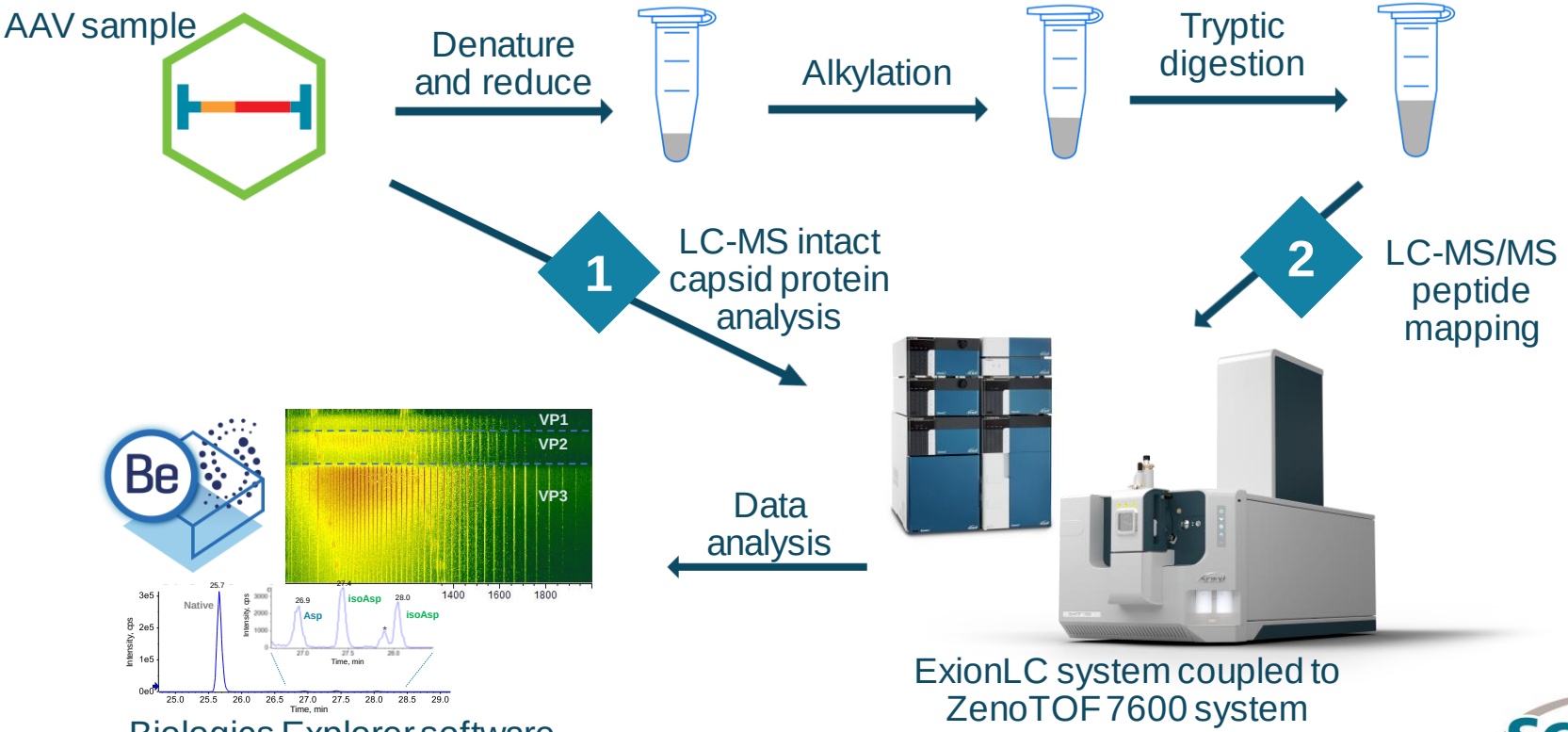
LC-MS workflows for capsid protein characterization



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LC-MS workflows for capsid protein characterization

AAV sample

Denature and reduce

Alkylation

Tryptic digestion

1

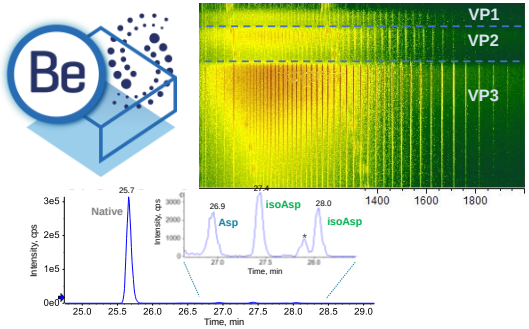
LC-MS intact capsid protein analysis

2

LC-MS/MS peptide mapping

ExionLC system coupled to ZenoTOF 7600 system

Data analysis



Biologics Explorer software

SCIEX
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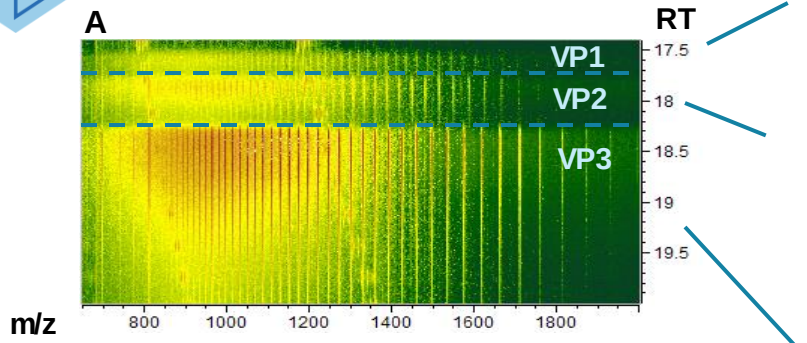
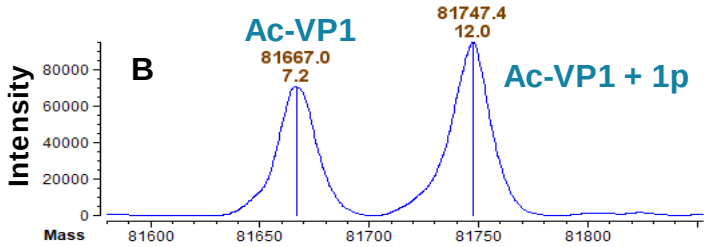
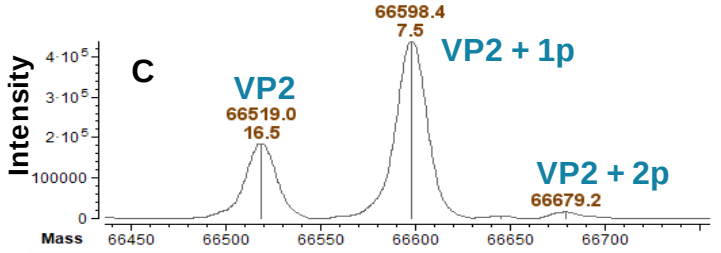
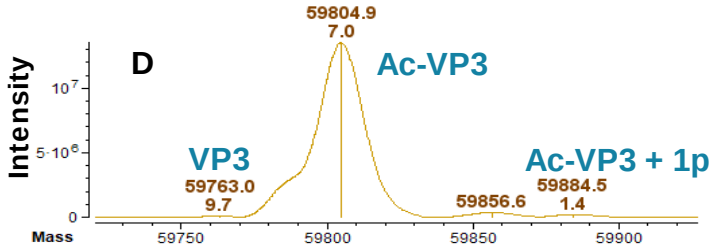
Intact mass analysis of AAVs

RECOMBINANT AAV8 CAPSID PROTEINS









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LC-MS workflows for capsid protein characterization

AAV sample

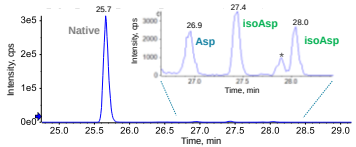
Denature and reduce

Alkylation

Tryptic digestion

1 LC-MS intact capsid protein analysis

2 LC-MS/MS peptide mapping



Biologics Explorer software

Data analysis

ExionLC system coupled to ZenoTOF 7600 system

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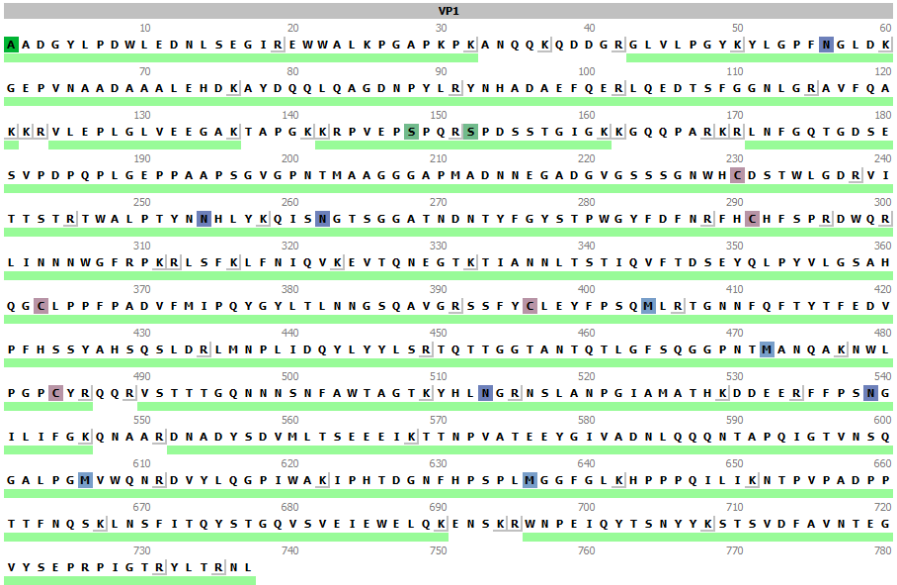
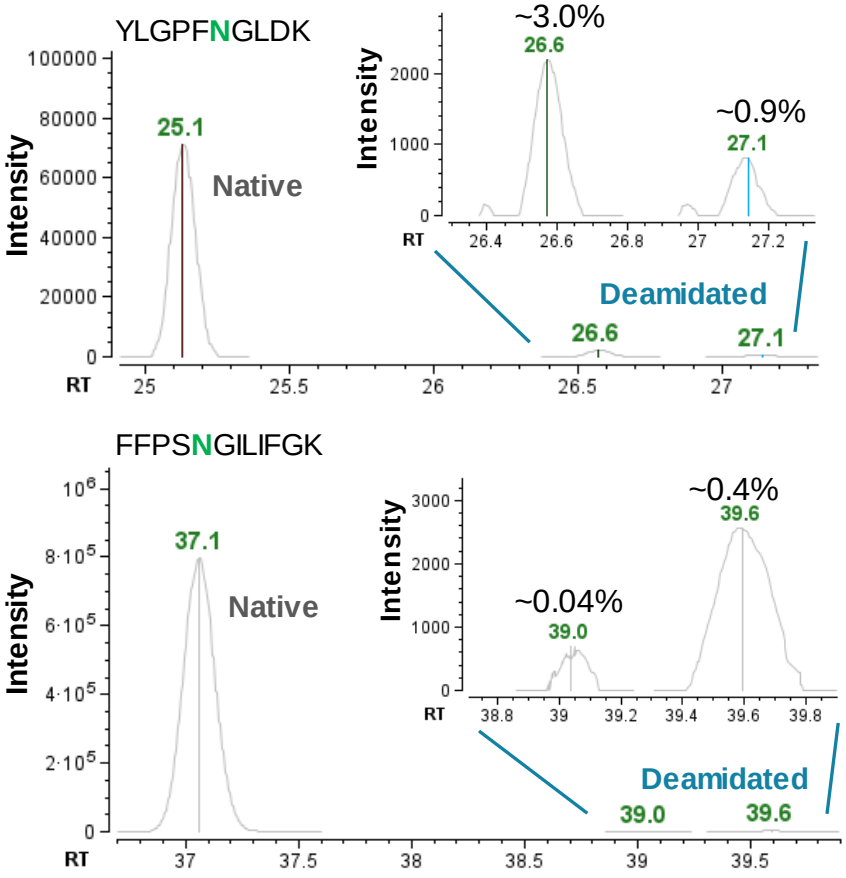
Peptide mapping analysis

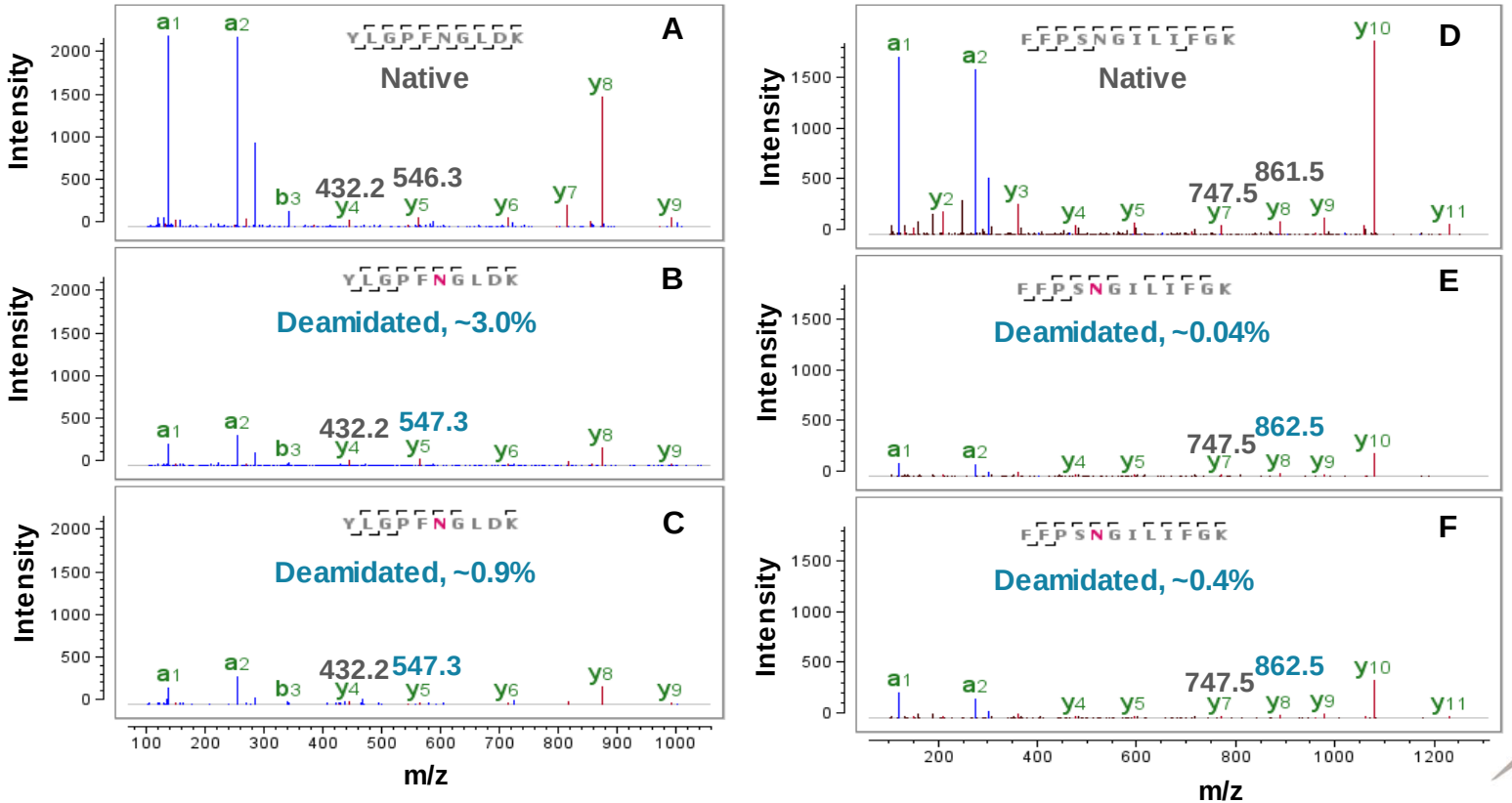
rAAV8, VP1, coverage 94.7%





PTM characterization

NATIVE AND DEAMIDATED SPECIES



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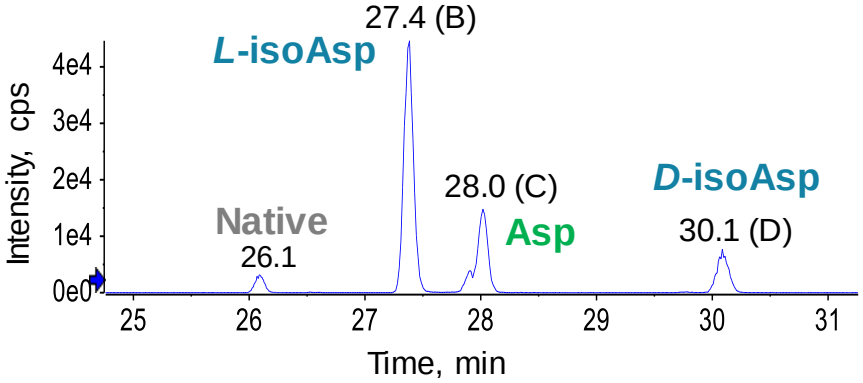
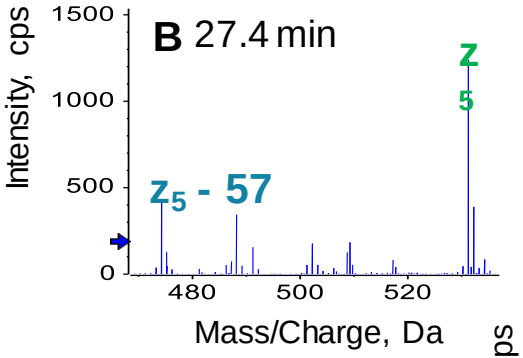
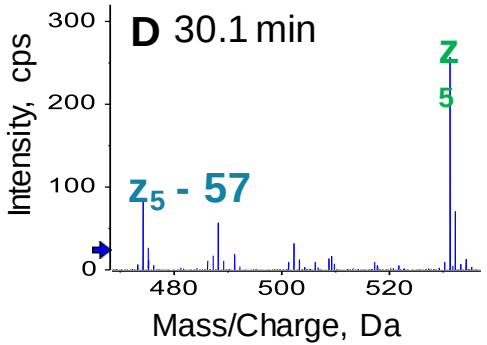
Superior PTM characterization

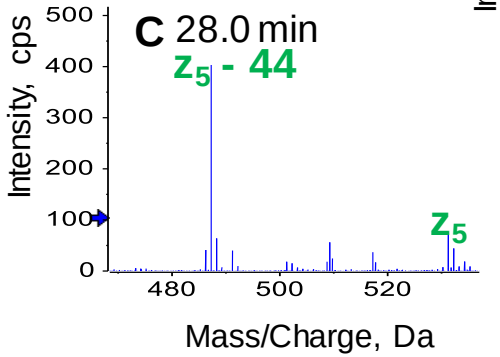
EAD SPECTRA OF YGPFNGLDK (2+)

- Retention time is not an accurate measure to differentiate between Asp and isoAsp
- However, the different deamidated species could be identified as Asp and isoAsp through Zeno EAD
  - Asp produces descriptive fragment *z*-44
  - IsoAsp produces descriptive fragment *z*-57









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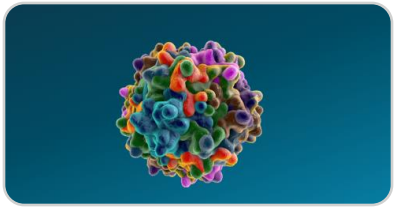
Understanding capsid protein quality

VIRAL PROTEIN RATIO AND IMPURITY ASSESSMENT



Viral protein monitoring

Quantitative ratio and impurity assessment

- Purity analysis of the AAV viral proteins is important for quality assurance and safety of AAV products
- High-sensitivity detection is required to analyze low-titer samples from process development
- Key workflow:
  - Capillary electrophoresis sodium dodecyl sulfate with laser induced fluorescence detection (CE-SDS-LIF)

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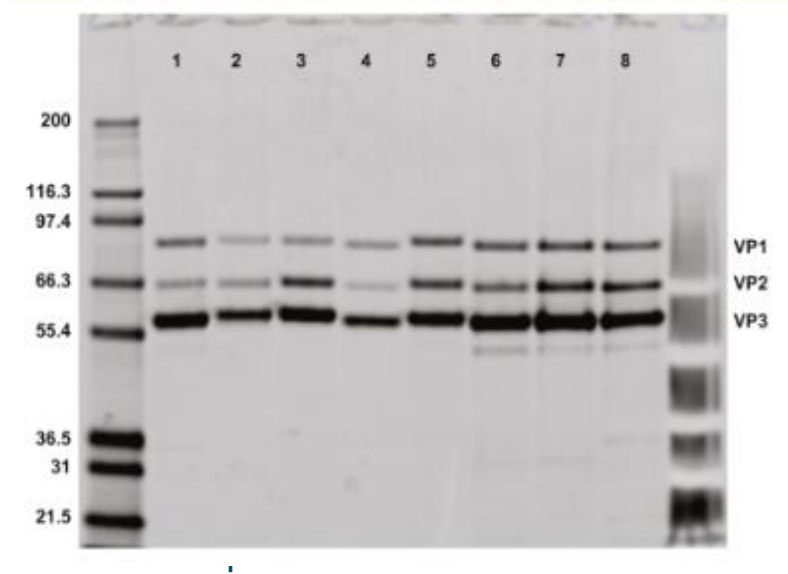
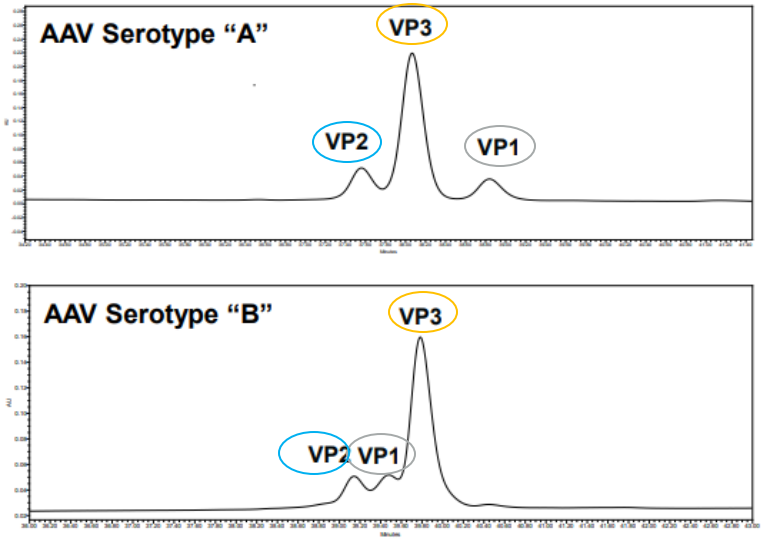
Technologies for AAV capsid purity analysis

CHALLENGES WITH EXISTING METHODOLOGIES

- SDS-PAGE



- RP-HPLC



  - Serotype-specific resolution

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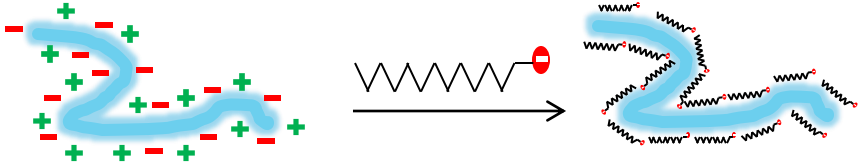
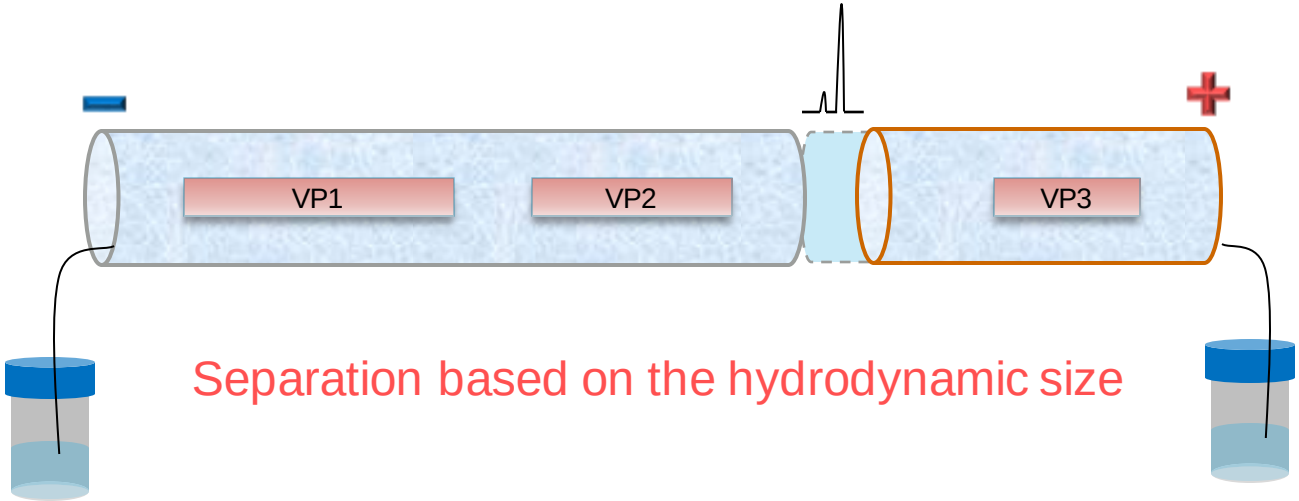
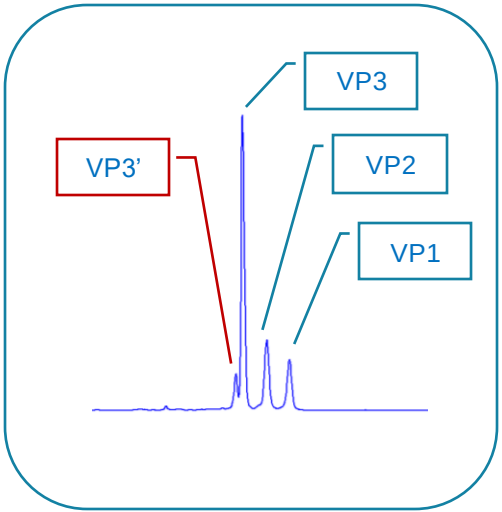
Capillary electrophoresis with sodium dodecyl sulfate

CE-SDS ANALYSIS OF VIRAL CAPSID PROTEINS







Separation based on the hydrodynamic size

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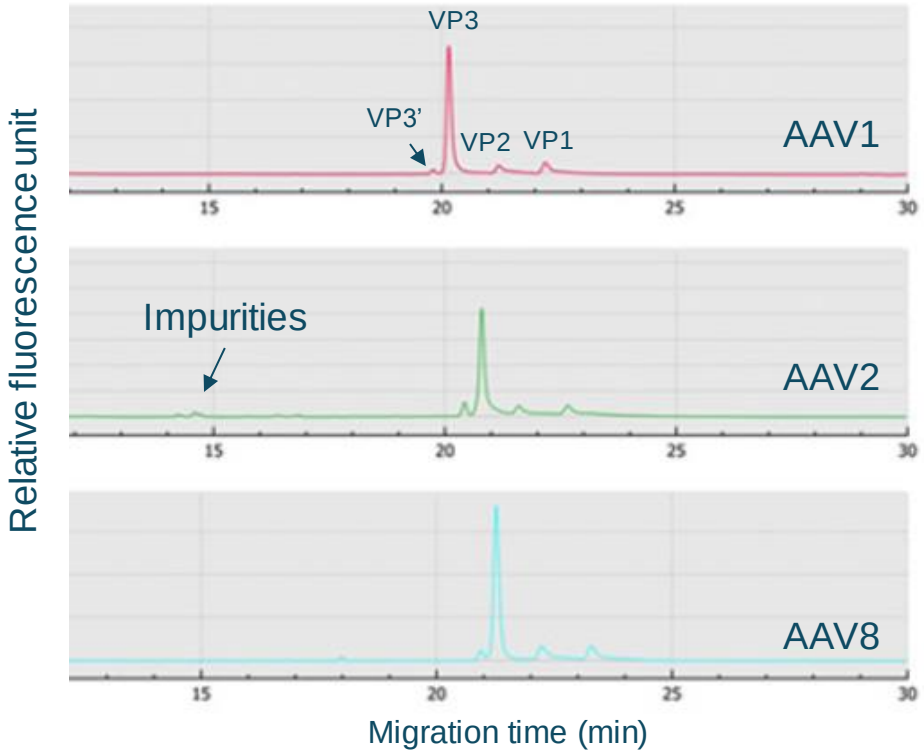
AAV viral protein characterization

1 WORKFLOW FOR MULTIPLE AAV SEROTYPES



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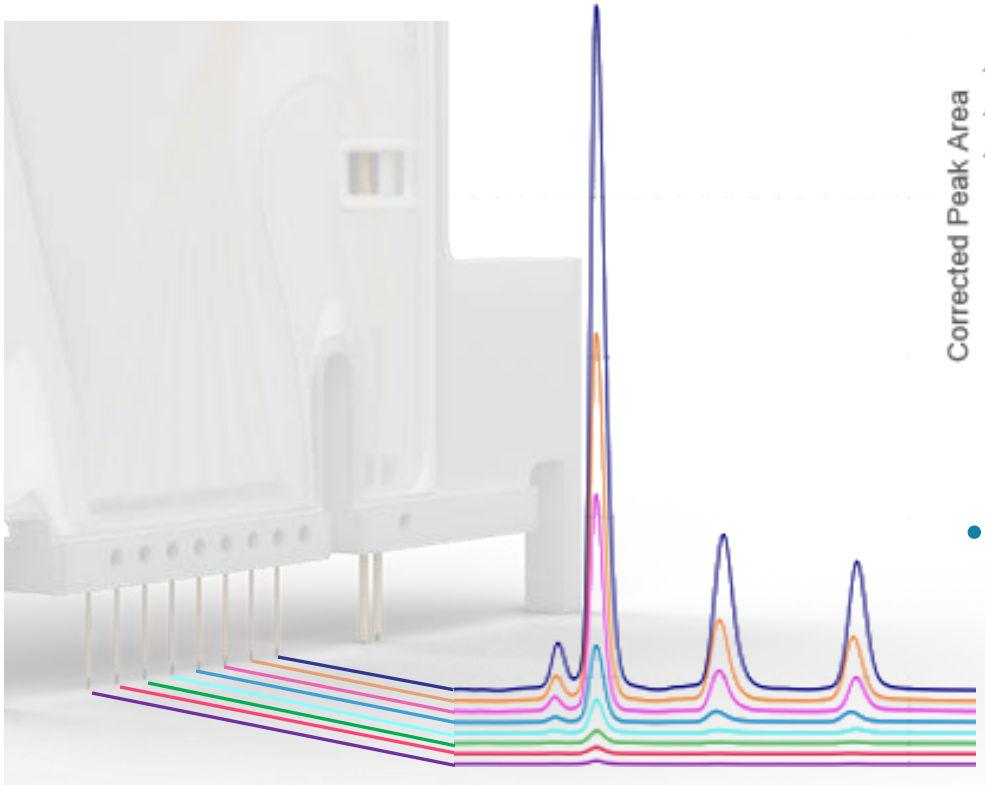
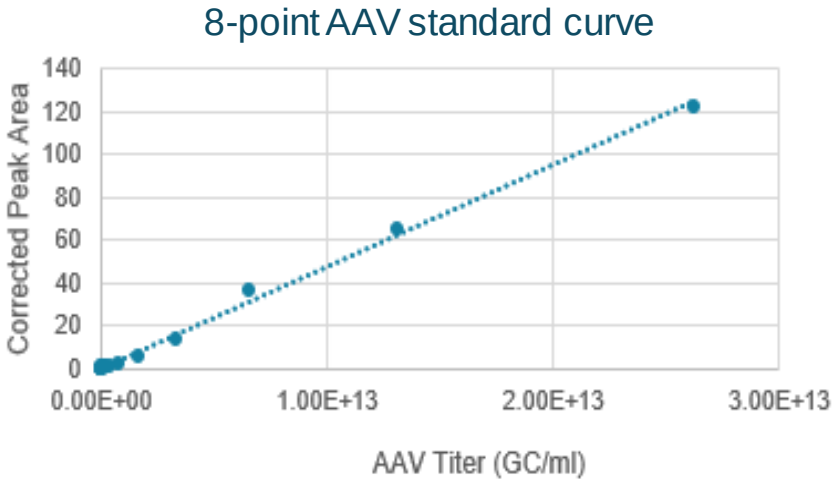
AAV titer determination

FAST STANDARD CURVE GENERATION





- A single separation run provides data for an 8-point standard curve

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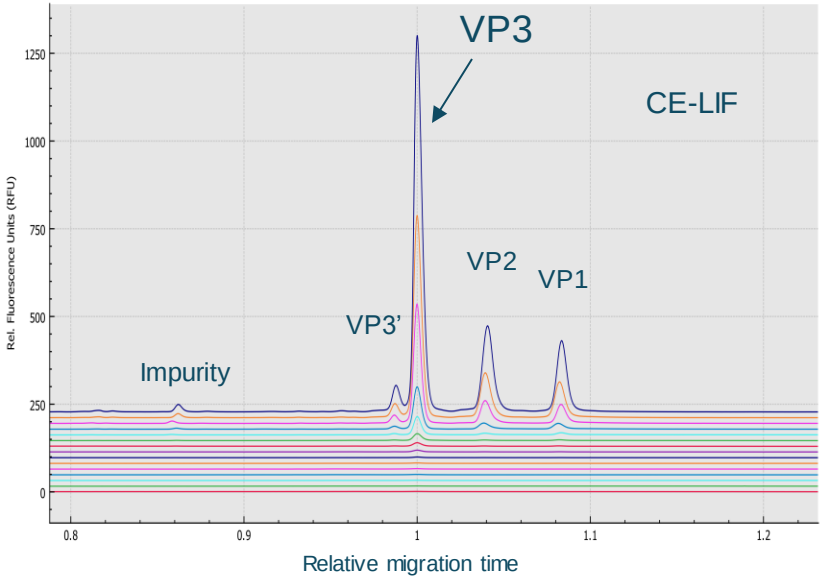
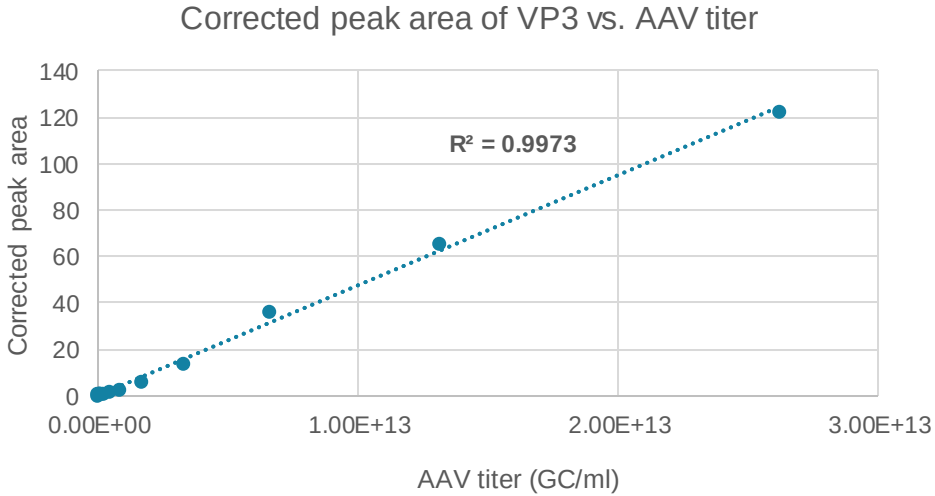
AAV capsid protein titer determination

Separation of p503-labeled AAV capsid proteins from serially diluted standard with known titer



Standard curve



LOD: 1.60E+9 GC/mL LOQ: 6.40E+9 GC/mL

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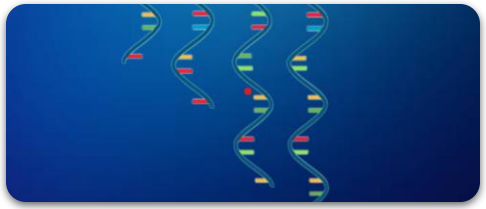
Genome integrity and sizing

PRODUCT- AND PROCESS-RELATED IMPURITIES ANALYSIS



Genome integrity

Transgene sizing and process related impurities

- Quality of the transgene inside a viral vector impacts the infectivity, efficacy and safety of the gene therapy
- The transgene in the genome cassette could be
  - Not present (empty capsid)
  - Not present (partial capsid)
  - Truncated (partial capsid)
  - Not present, but capsid has contaminant fragments from host cell or plasmid (nuclease-resistant)
- Key workflow:
  - Capillary gel electrophoresis with laser-induced fluorescence detection (CGE-LIF)

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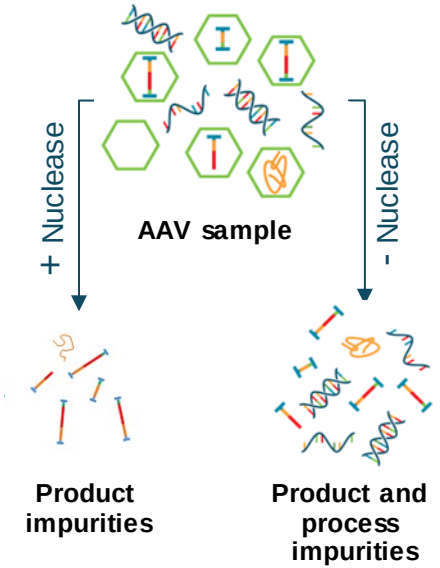
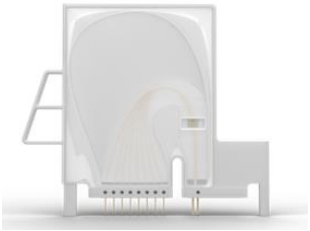
AAV genome integrity analysis workflows

Easy sample preparation



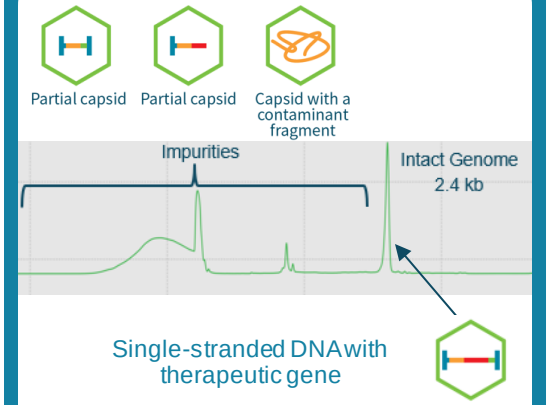
CGE workflow

BioPhase 8800 system



BioPhase BFS Capillary Cartridge

Automated result generation



LIF detection

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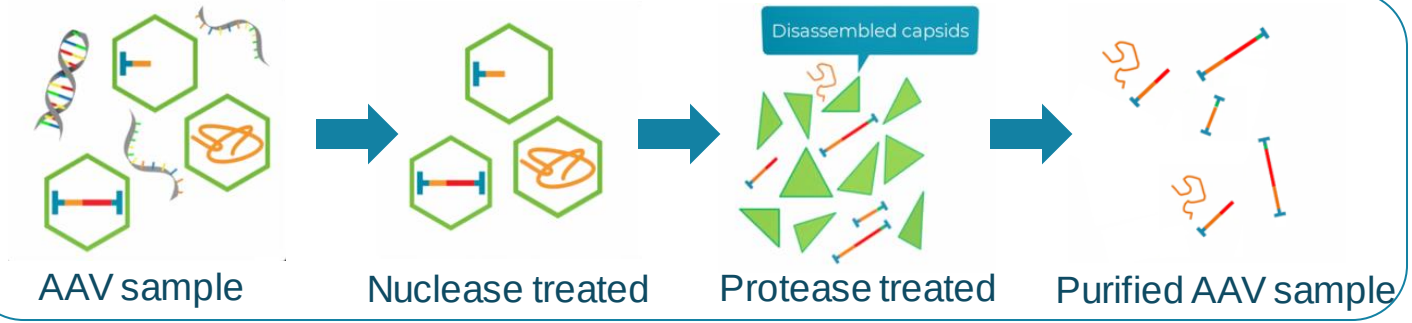
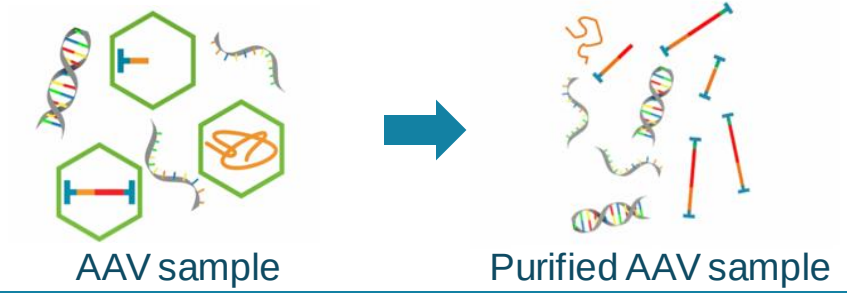
AAV genome integrity analysis workflows

TWO SAMPLE PREPARATION OPTIONS FOR PRODUCT AND PROCESS IMPURITIES

Product-related impurities



Product- and process-related impurities



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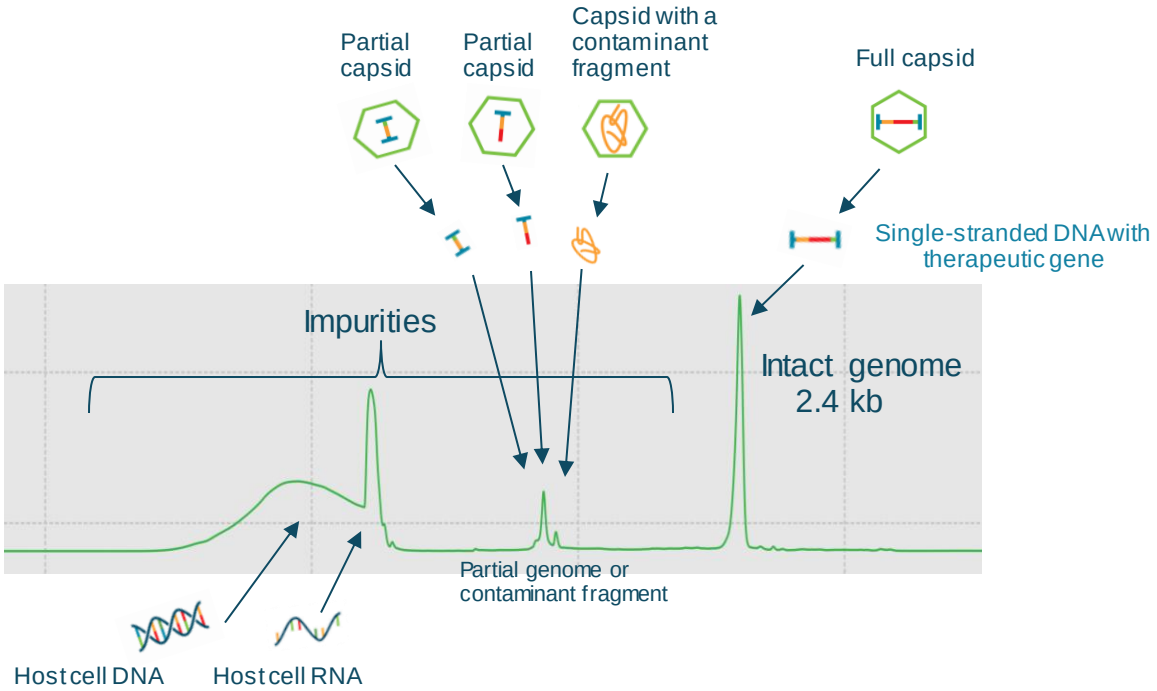
High-resolution genome integrity analysis



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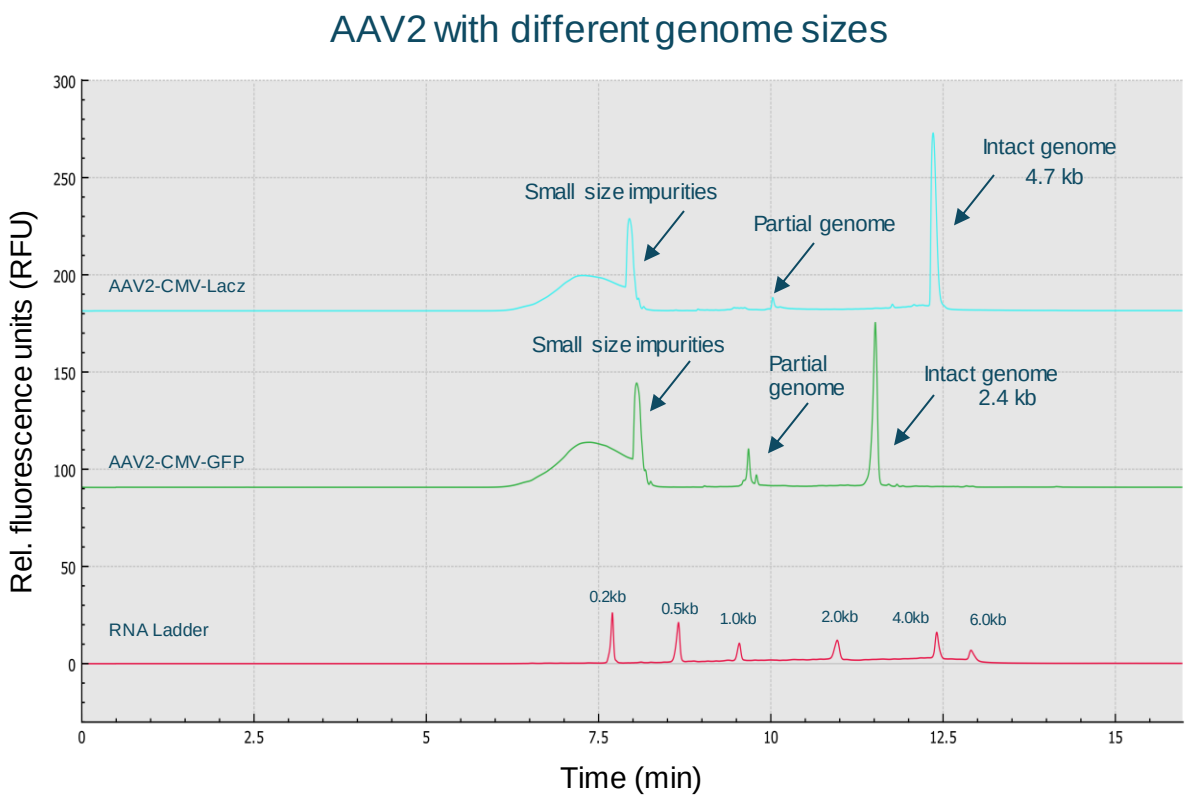
High-resolution genome integrity analysis



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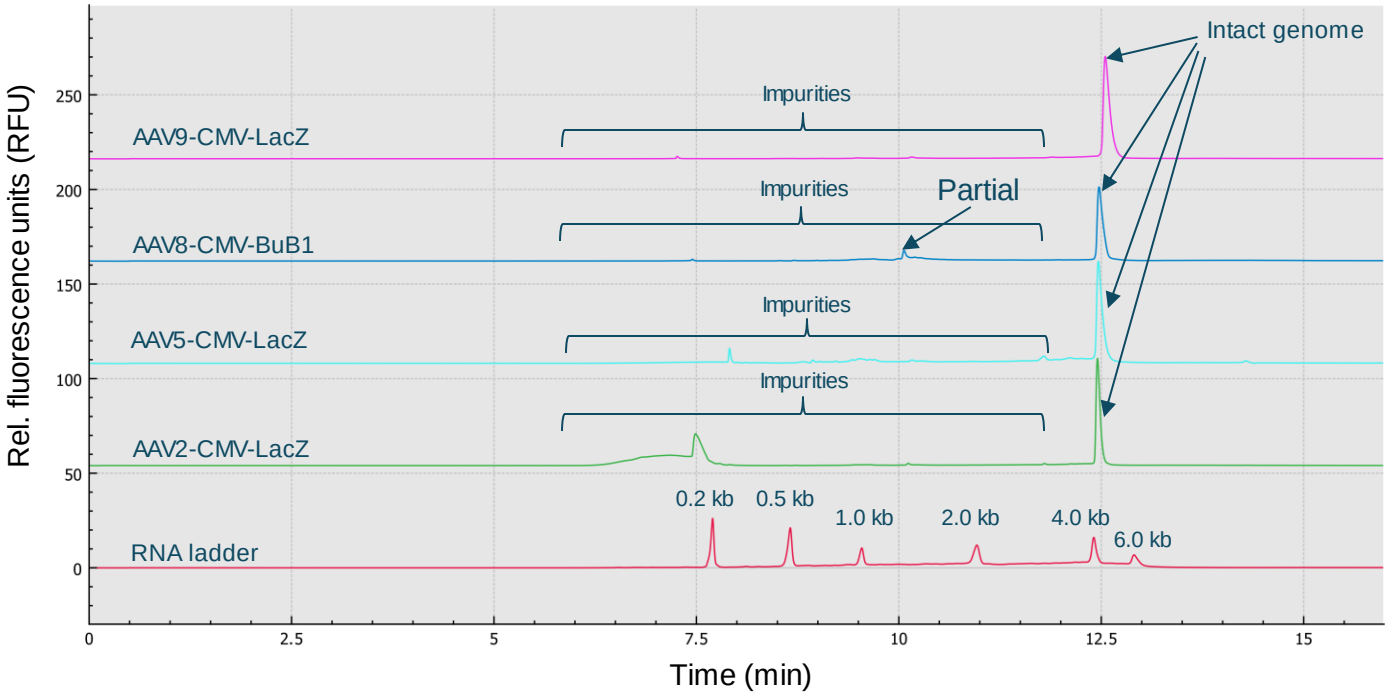
AAV genome integrity analysis for multiple AAV serotypes

HIGH-THROUGHPUT, SEROTYPE-INDEPENDENT WORKFLOW



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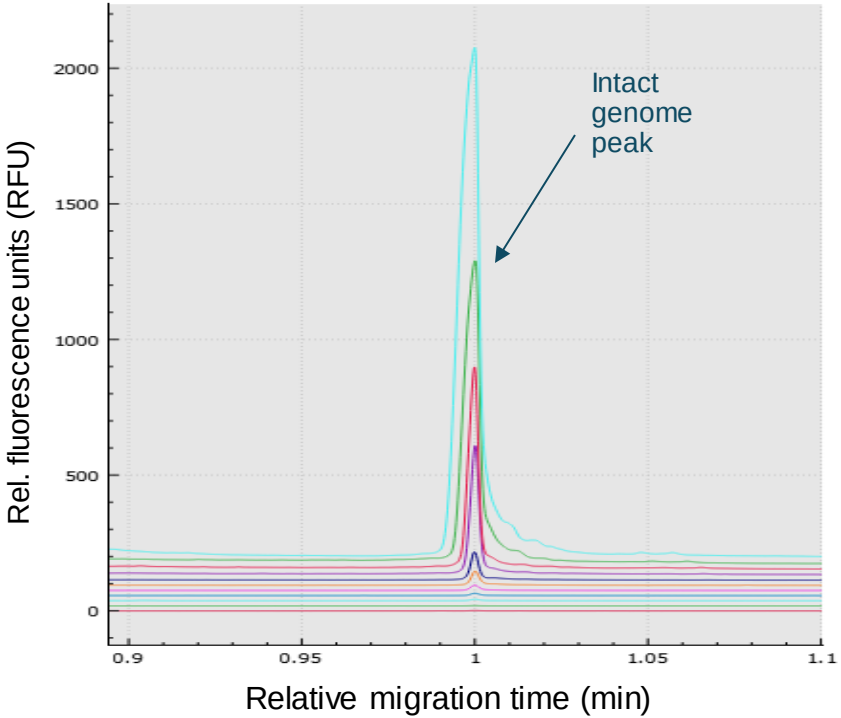
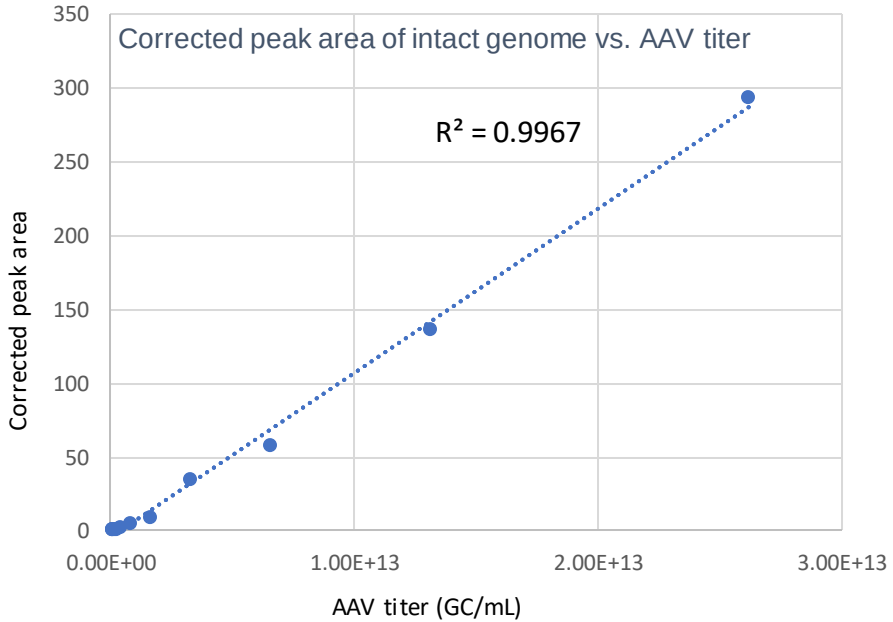
AAV genome titer determination on the BioPhase 8800 system

Separation of AAV genome extracted from serially diluted standard with known titer



Standard curve



LOD: 1.28E+10 GC/mL

LOQ: 2.56E+10 GC/mL

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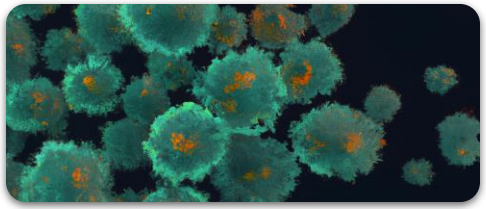
Full and empty capsid ratio monitoring

ANALYSIS OF THE ASSEMBLED DRUG SUBSTANCE



Full and empty

Determination of full capsid percent

- Full capsids deliver intact transgene into target cells
- Full capsids directly impact the potency of AAV therapeutics
- Monitoring full and empty capsid ratio is important during process optimization to remove empty and partial capsids for better efficacy and safety of AAV-based gene therapy
- Key workflow:
  - Calculating empty-to-full product ratio from protein and genome titer assays

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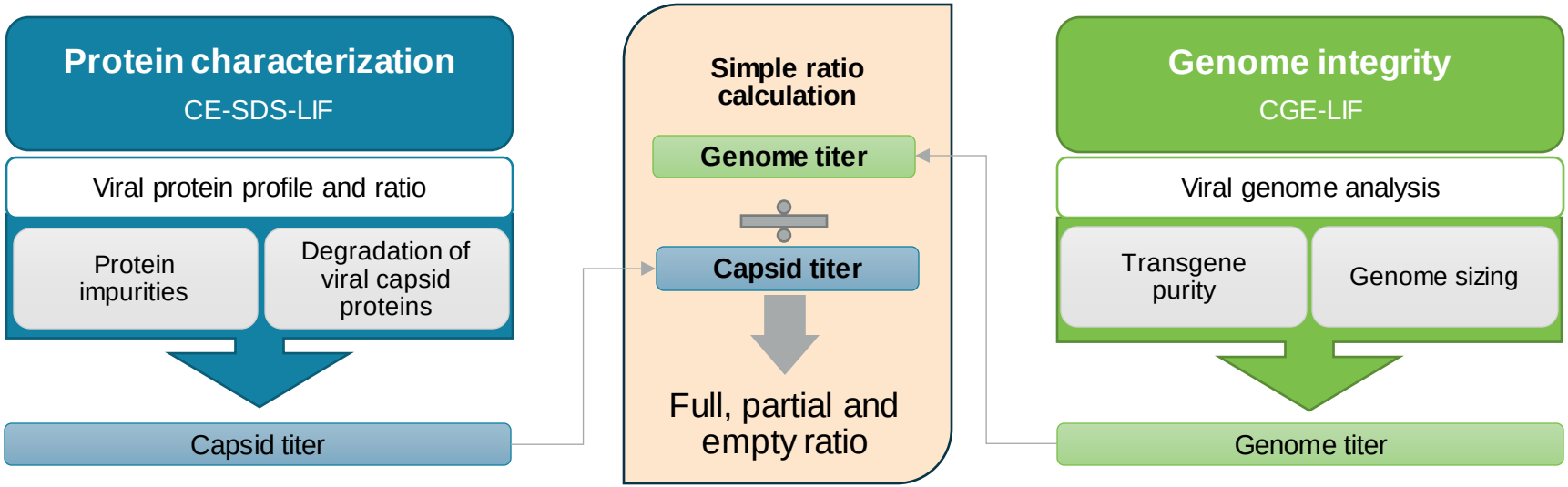
Full and empty capsid workflow

- Comprehensive analysis of **AAV critical quality attributes** (CQAs) using a high-throughput method on a single **multi-capillary** electrophoresis (CE) platform



A novel approach for high-resolution **full and empty AAV capsid analysis**

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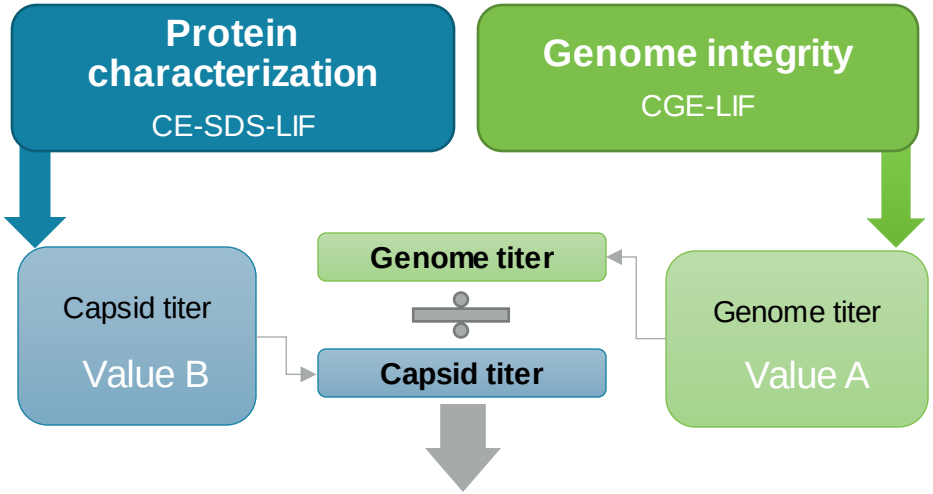
Full and empty ratio analysis with the BioPhase 8800 system

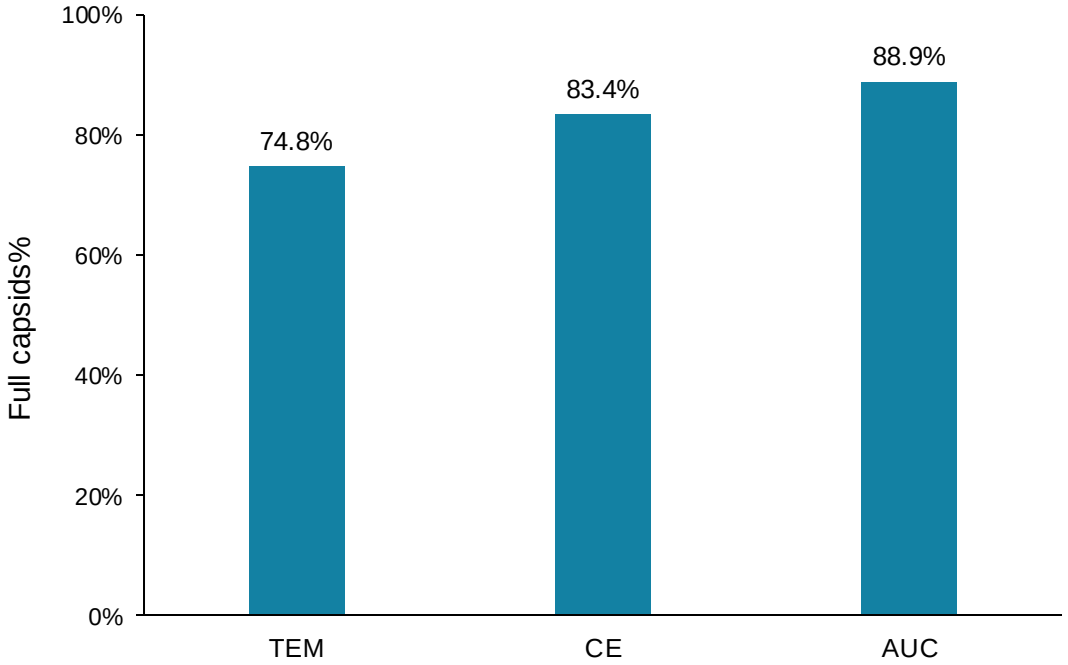
- AAV8 reference standard from Vigene was used as the test sample
- Value A from genome integrity analysis by CE: 2.21E+12 GC/mL
- Value B from AAV capsid analysis by CE: 2.65E+12 GC/mL
- Full capsids% in the AAV8 test sample:

  (2.21E+12 GC/mL / 2.65E+12 GC/mL)*100 = 83.40%



$$\frac{\text{Value A}}{\text{Value B}} \text{ X } 100 = \text{Full capsids \%}$$

Comparison of methods for determining full and empty ratio



Reference: Vigene webinars:

https://www.vigenebio.com/news-events/join-vigenes-webinars-about-gmp-production-of-aav-lentivirus-and-plasmids-at-asgct-virtual-conference/#AAVRMCase

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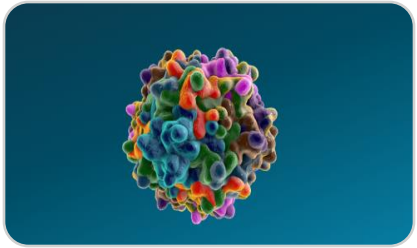
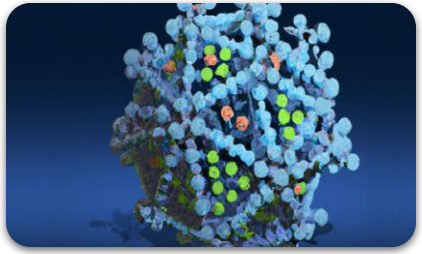
Key workflows for the analysis of AAV therapies

CHARACTERIZING AAV-BASED PRODUCTS



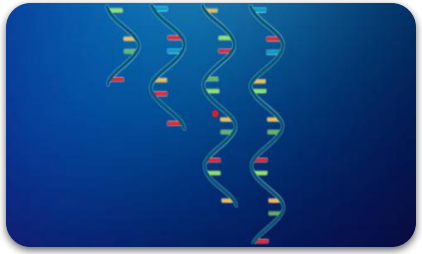
Capsid protein characterization

Intact, sub-unit and peptide mapping



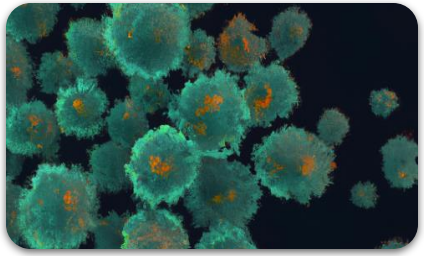
Viral protein purity

Drug substance and drug product analysis



Genome integrity

Transgene sizing and process related impurities



Full and empty

Determination of full capsid percent

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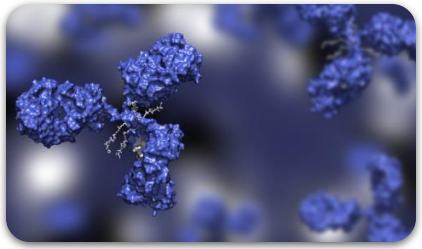
Key workflows for the analysis of protein therapeutics

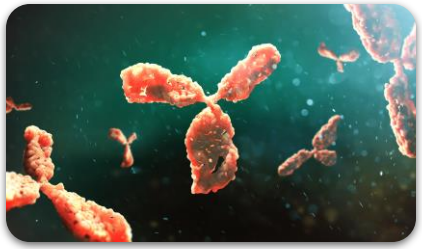
CHARACTERIZING PROTEIN-BASED PRODUCTS

Post-translational modifications

Deamidated amino acid isomerization and sulfated species

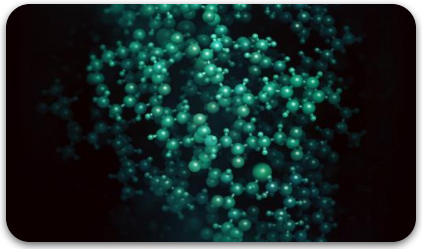


Glycosylation, glycation and AGEs screening



Fragments and impurities

Process- and product-related



Charge heterogeneity

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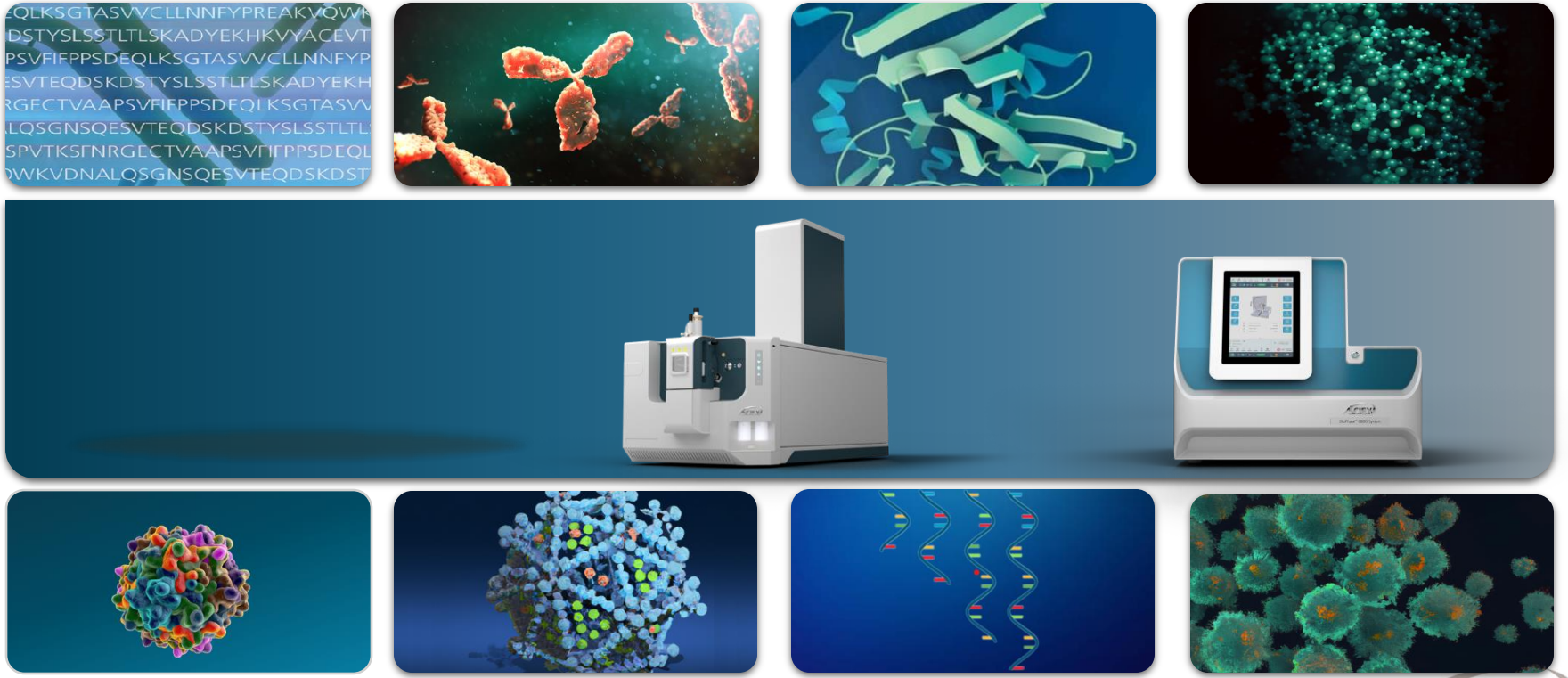
Unified workflows for novel proteins & gene therapy products



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