

Microchip CE-MS for rapid, deep and sensitive analysis of biopharmaceuticals

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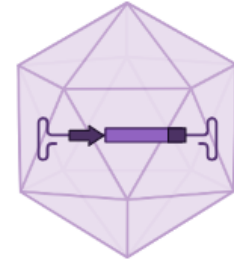
NIBRT Overview



- ❑ Unique facility dedicated to address the training and research needs of the global biopharmaceutical industry based in Dublin, Ireland.
- ❑ Competency based training experience in an environment that replicates modern industrial bioprocessing facilities.
- ❑ Research with impact – developing solutions to address real challenges faced within the biopharmaceutical industry.
- ❑ Facility expansion for advanced therapy research and training and new early-stage development facility opened in 2023.

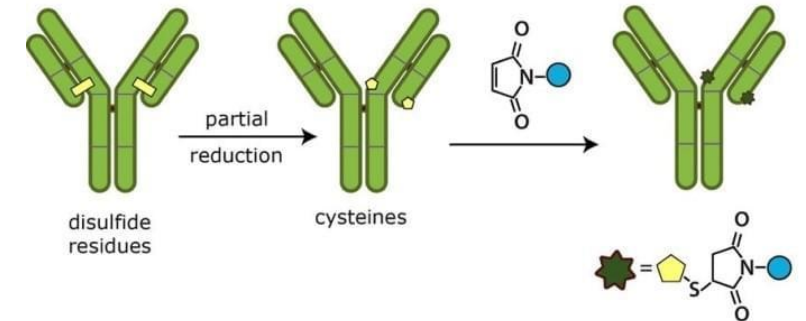
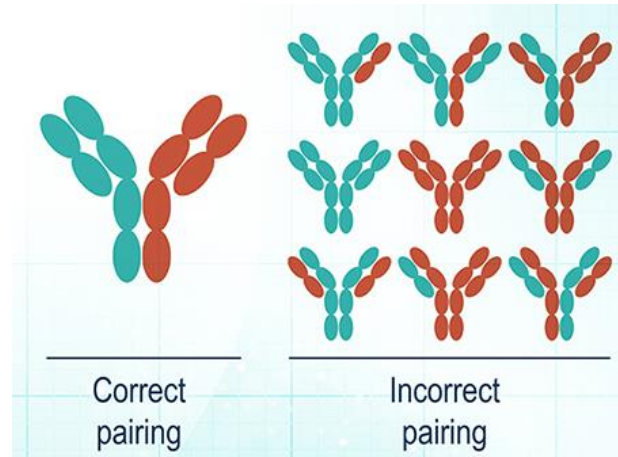
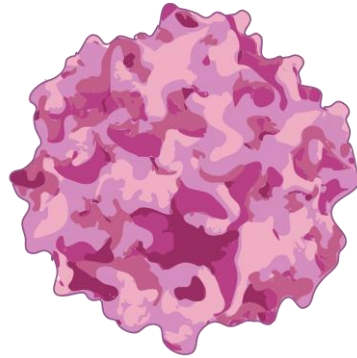


Complexity of Biopharmaceuticals landscape



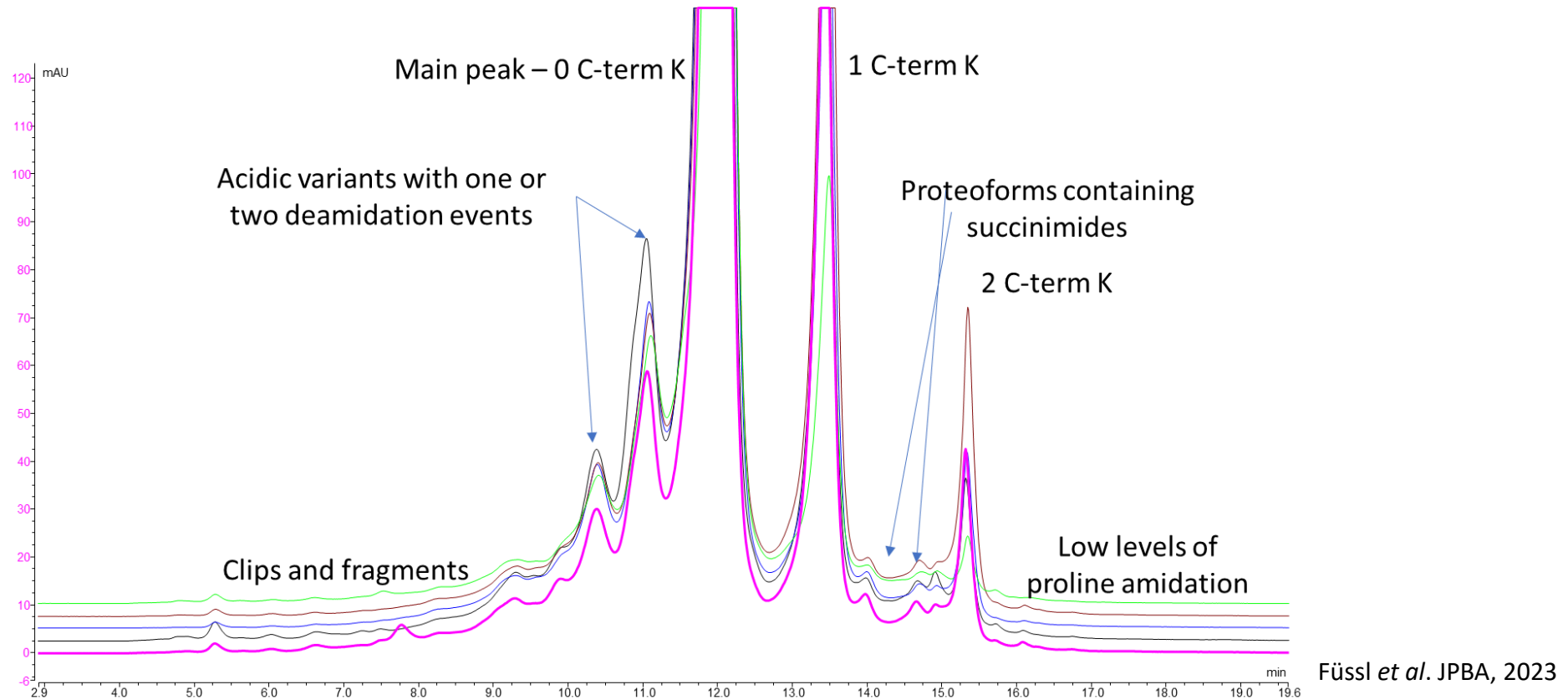
- ❑ Proteins exist in a variety of forms rather than one individual form due to macro- and microheterogeneity as a consequence of differential posttranslational modification (PTMs).
- ❑ New modalities come with different analytical challenges and bottom-up approaches cannot provide all the answers.

Need for Native Intact Analysis



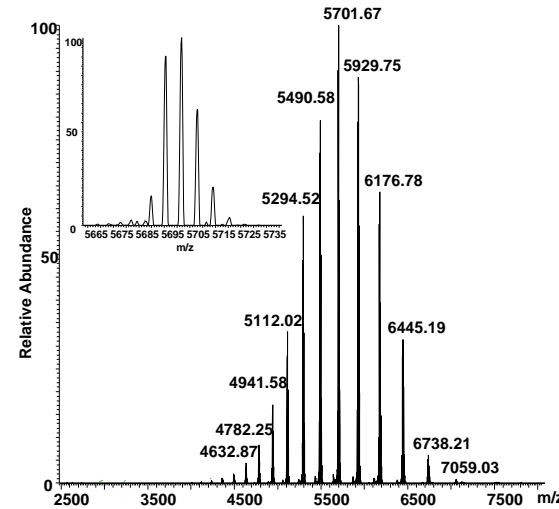
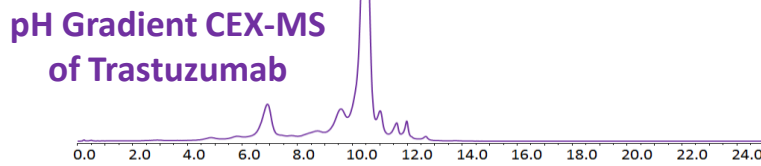
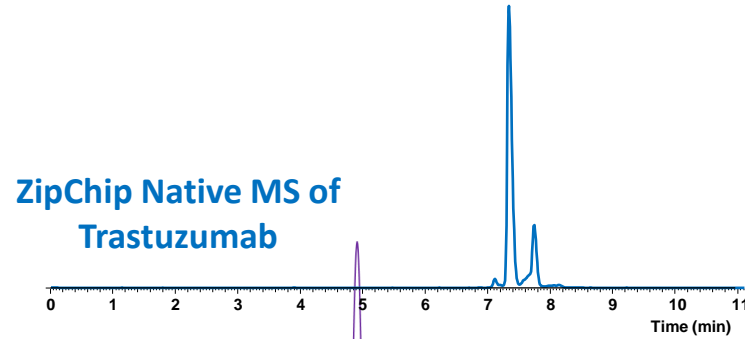
- ❑ Proteins exist in a variety of forms rather than one individual form due to macro- and microheterogeneity as a consequence of differential posttranslational modification (PTMs).
- ❑ New modalities come with different analytical challenges and bottom-up approaches cannot provide all the answers.

Need for Native Intact Analysis

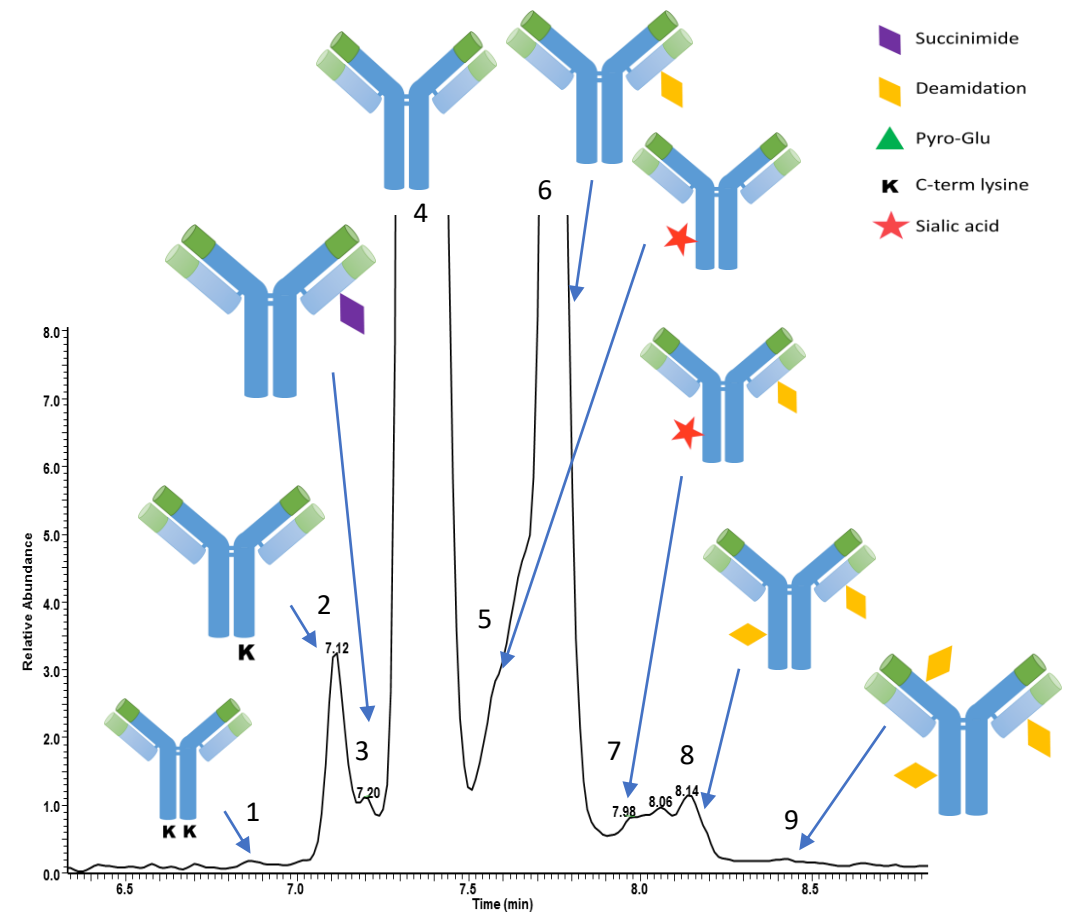


- ❑ Charge variant profile can only be obtained separating proteoforms in their native state. Direct MS identification can enhance speed of analysis and confidence in the identification.

ZipChip-MS, a Simple Method for Charge Variant Profiling

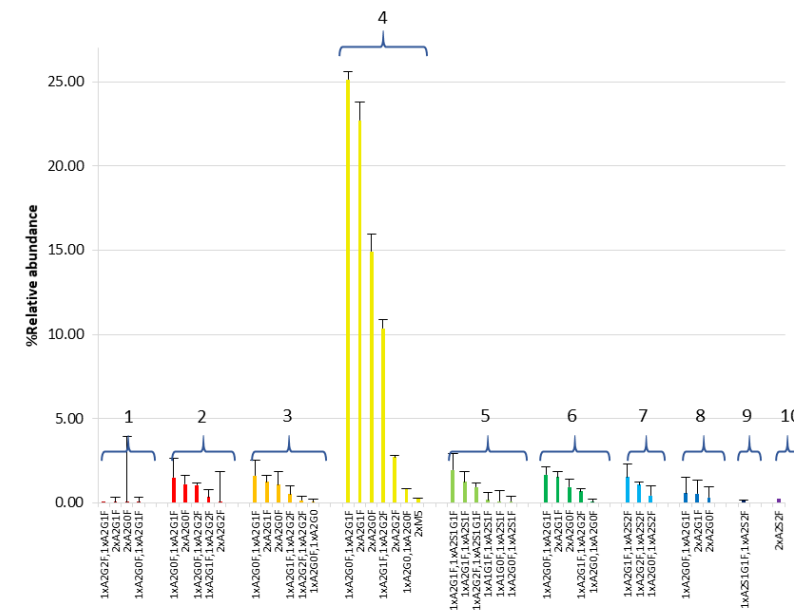
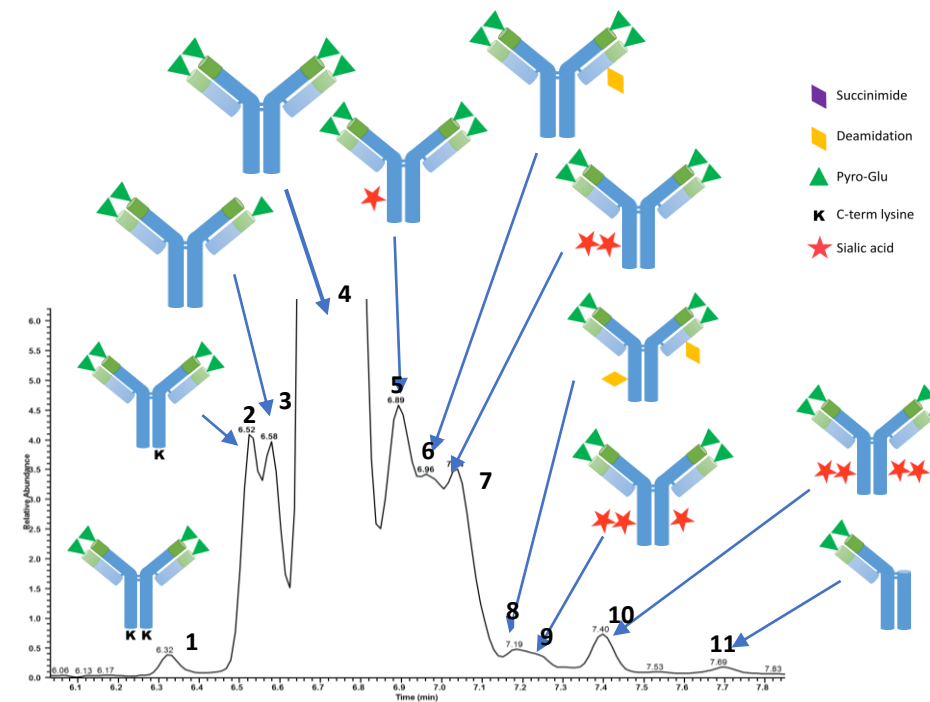
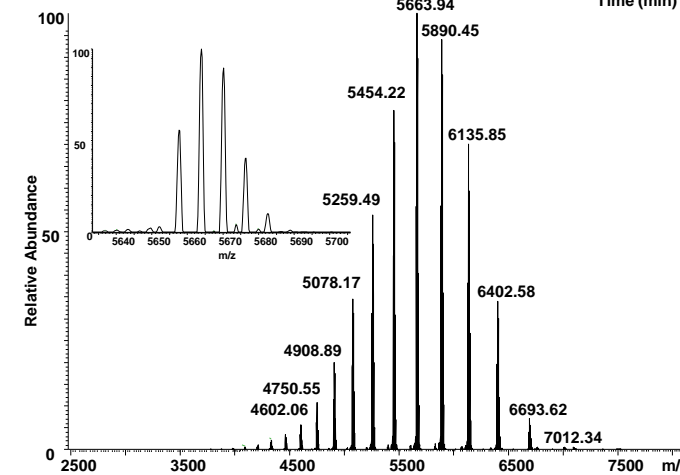
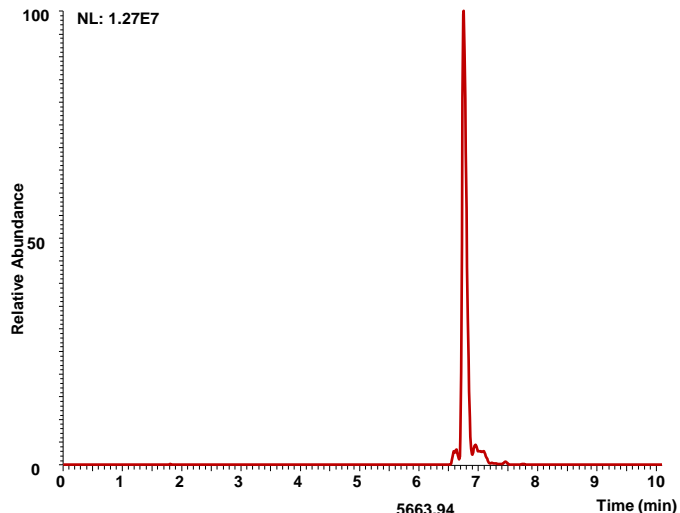


- The HRN ZipChip kit provides a simple out of the box solution for charge variant profiling with minimal optimisation required.
- Separation based on differences in net surface charge, pH gradient CEX-MS: ΔpI , ZipChip: differential electrophoretic mobility.

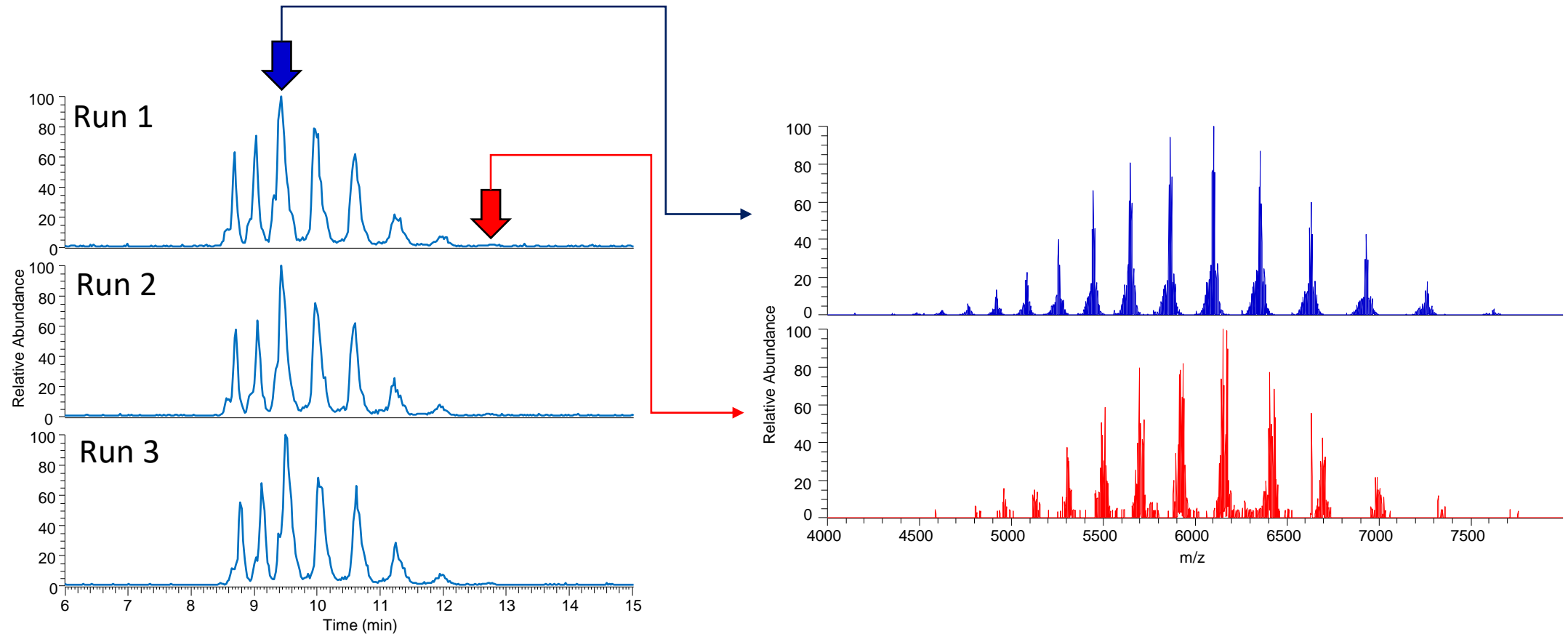


Platform-like Method for Any Molecule

45 different proteoforms for rituximab detected, including 10 different charge variants with their intrinsic N-glycan heterogeneity and a mAb fragment

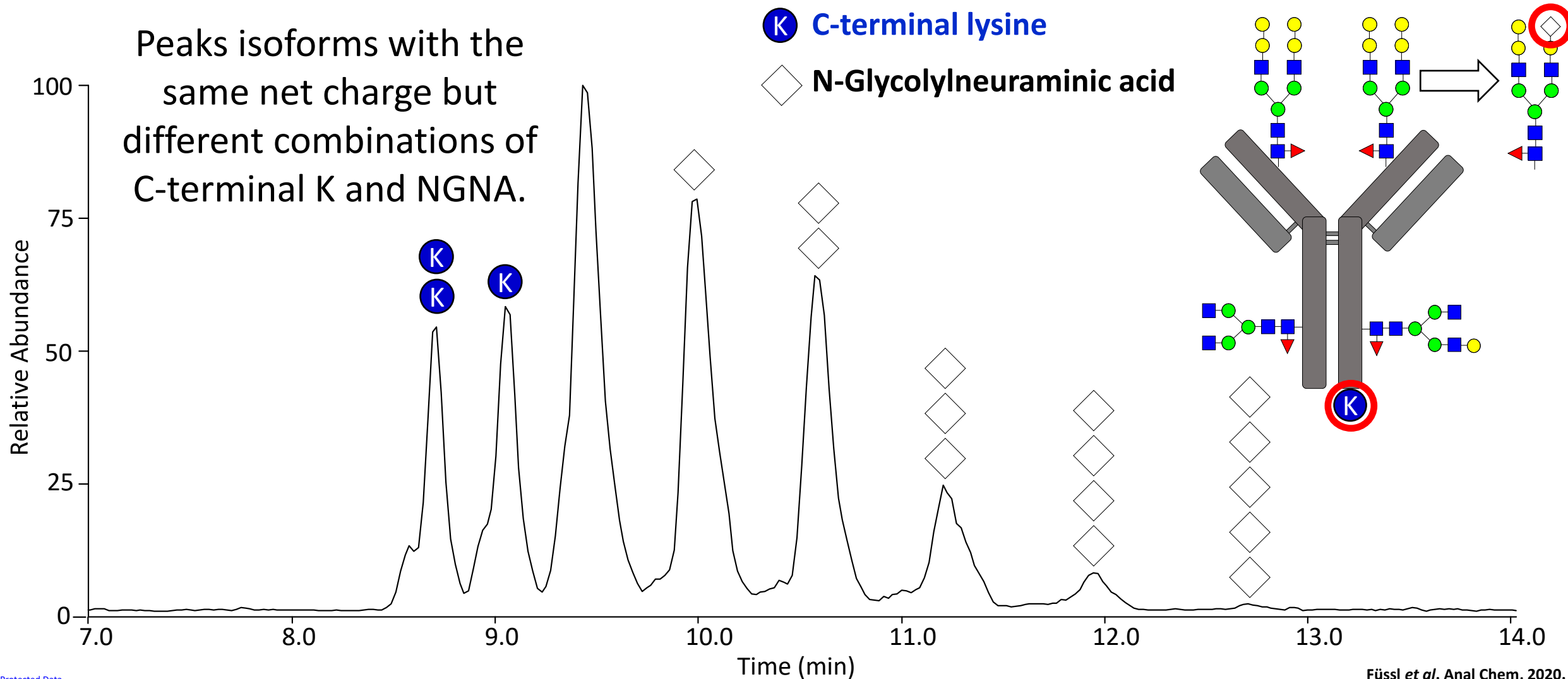


Sensitivity on ZipChip - Cetuximab

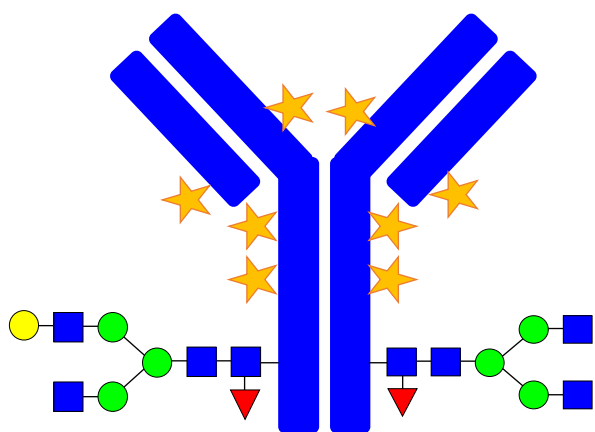


Operational flowrates of ZipChip are equivalent to those used in nanospray infusion, which generates excellent experimental sensitivity from sample on chip in the 1-2 ng range.

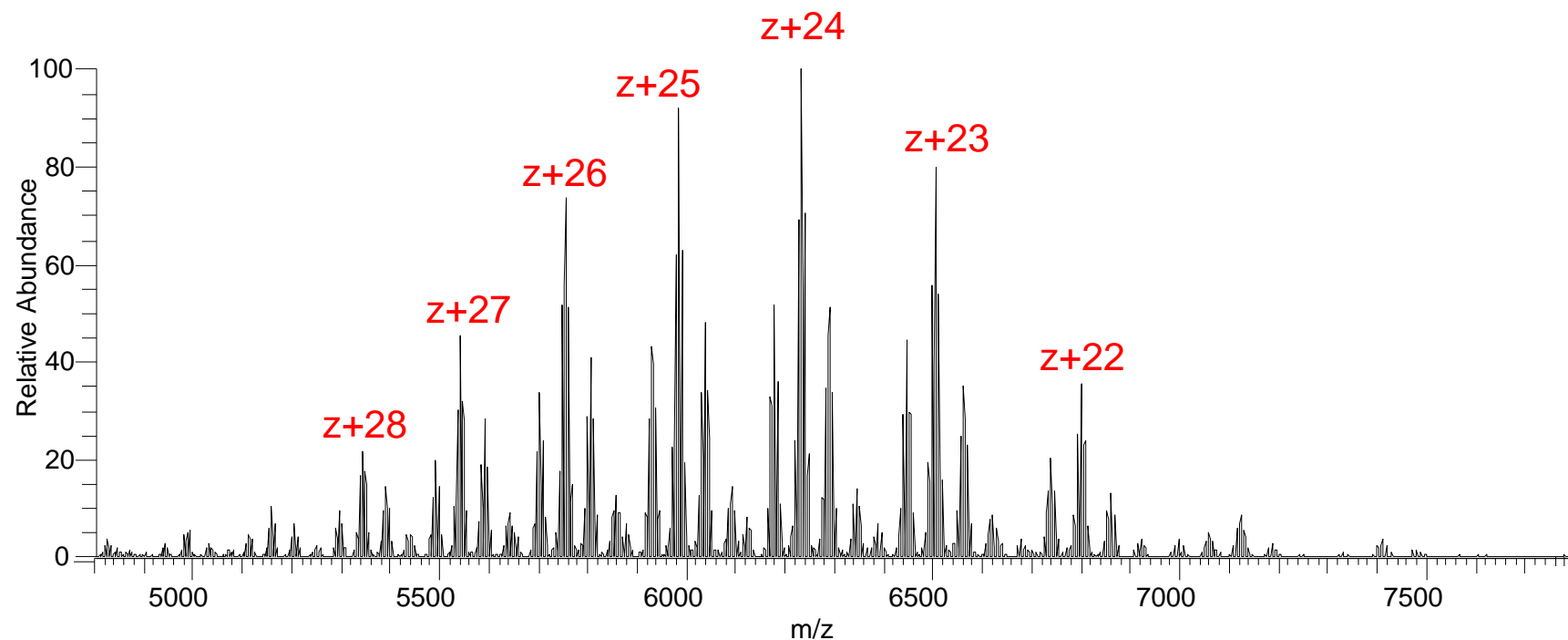
MS Detection Enables Proteoform Annotation



Platform Method for Antibody Drug Conjugates Too

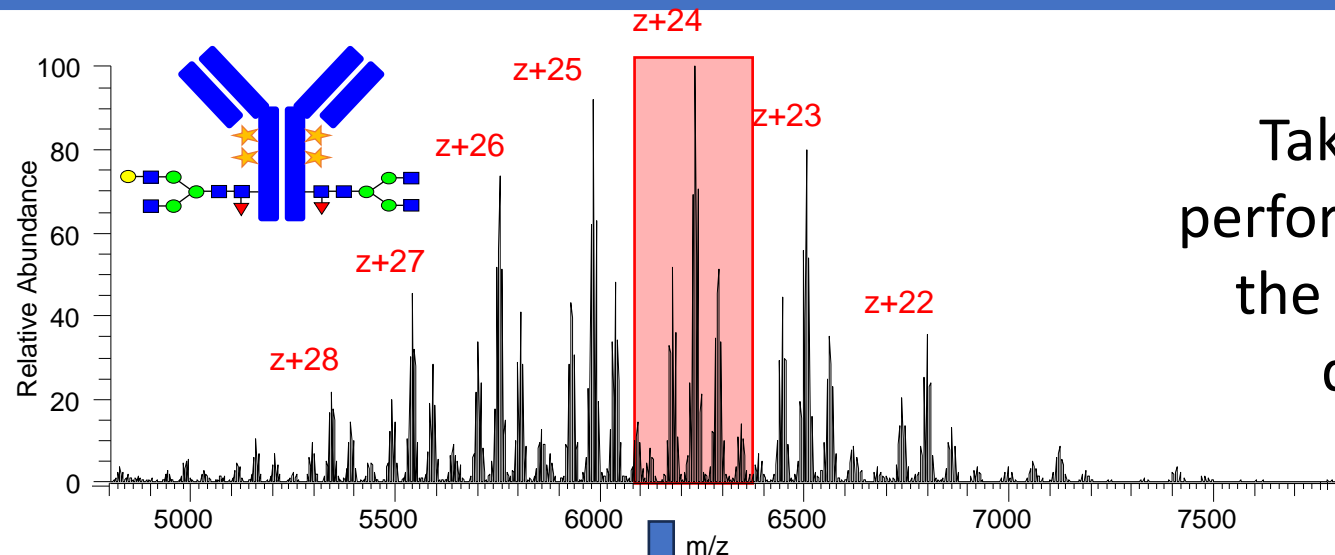


Millipore Sigma MSQC8

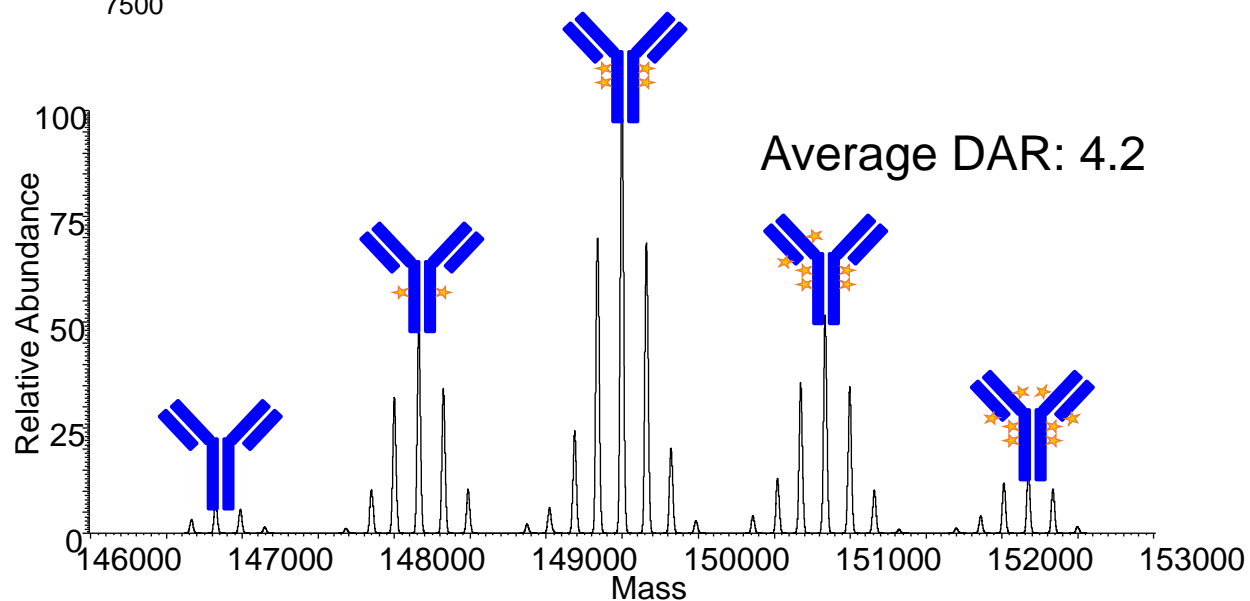
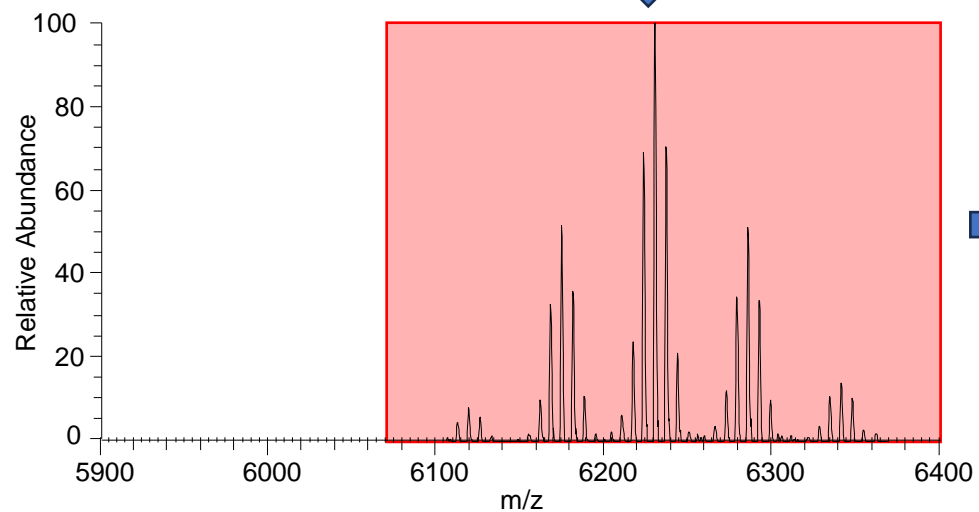


Molecular heterogeneity of MSQC8 cysteine linked ADC mimic evaluated using infusion first. High level of complexity visible due to attributes on the mAb itself, such as N-glycosylation in addition to linker payload conjugation

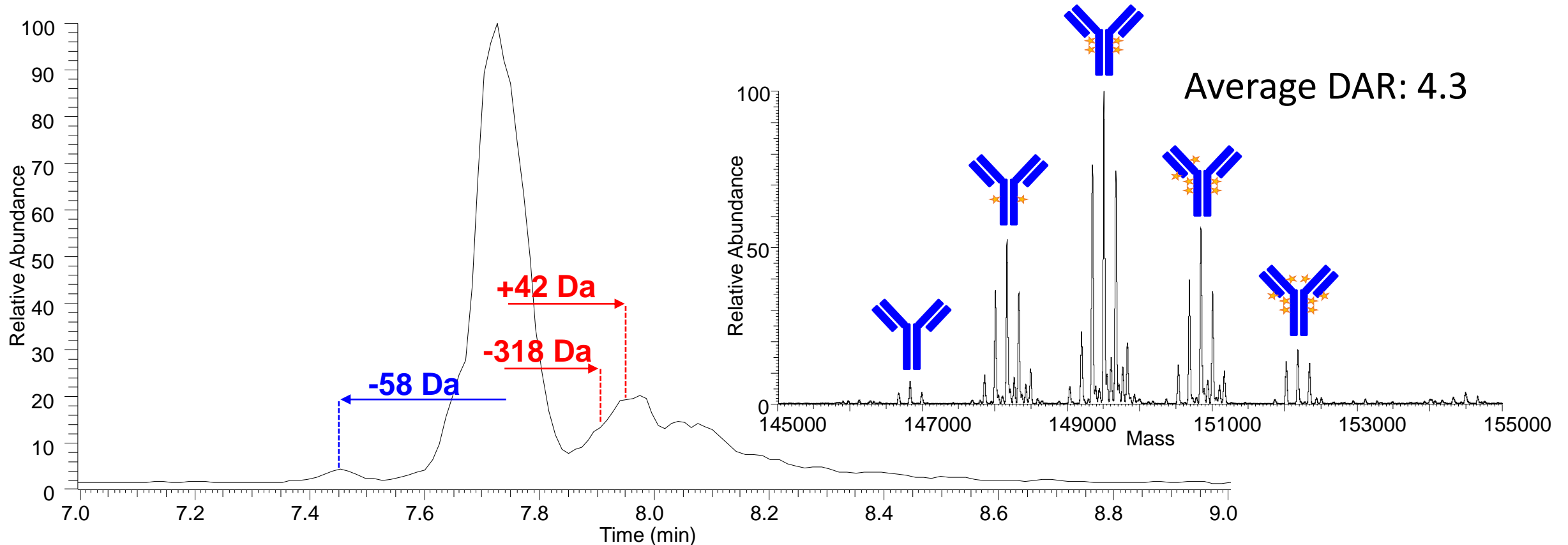
Exploring the Microheterogeneity



Taking just the $z = 24+$ charge state and performing deconvolution, we can determine the drug to antibody ratio (DAR) and also determine N-glycan heterogeneity

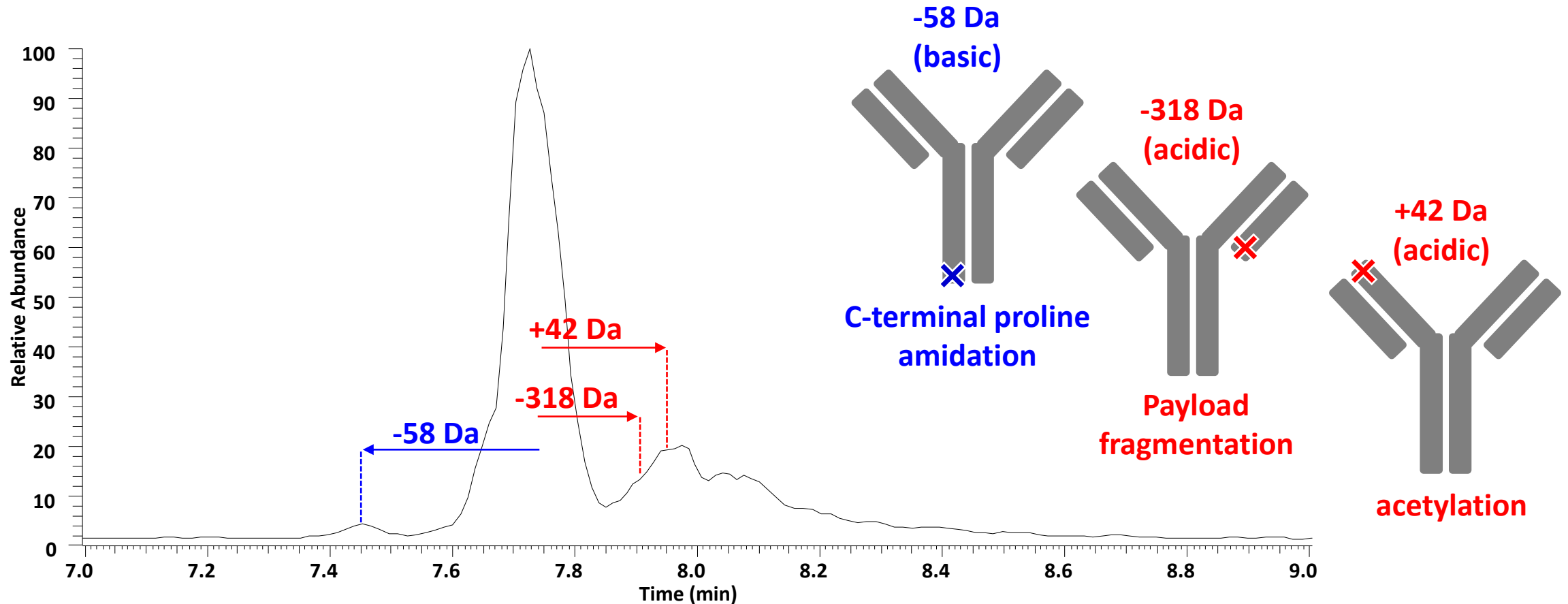


ZipChip-MS Analysis of MSQC8 ADC Mimic



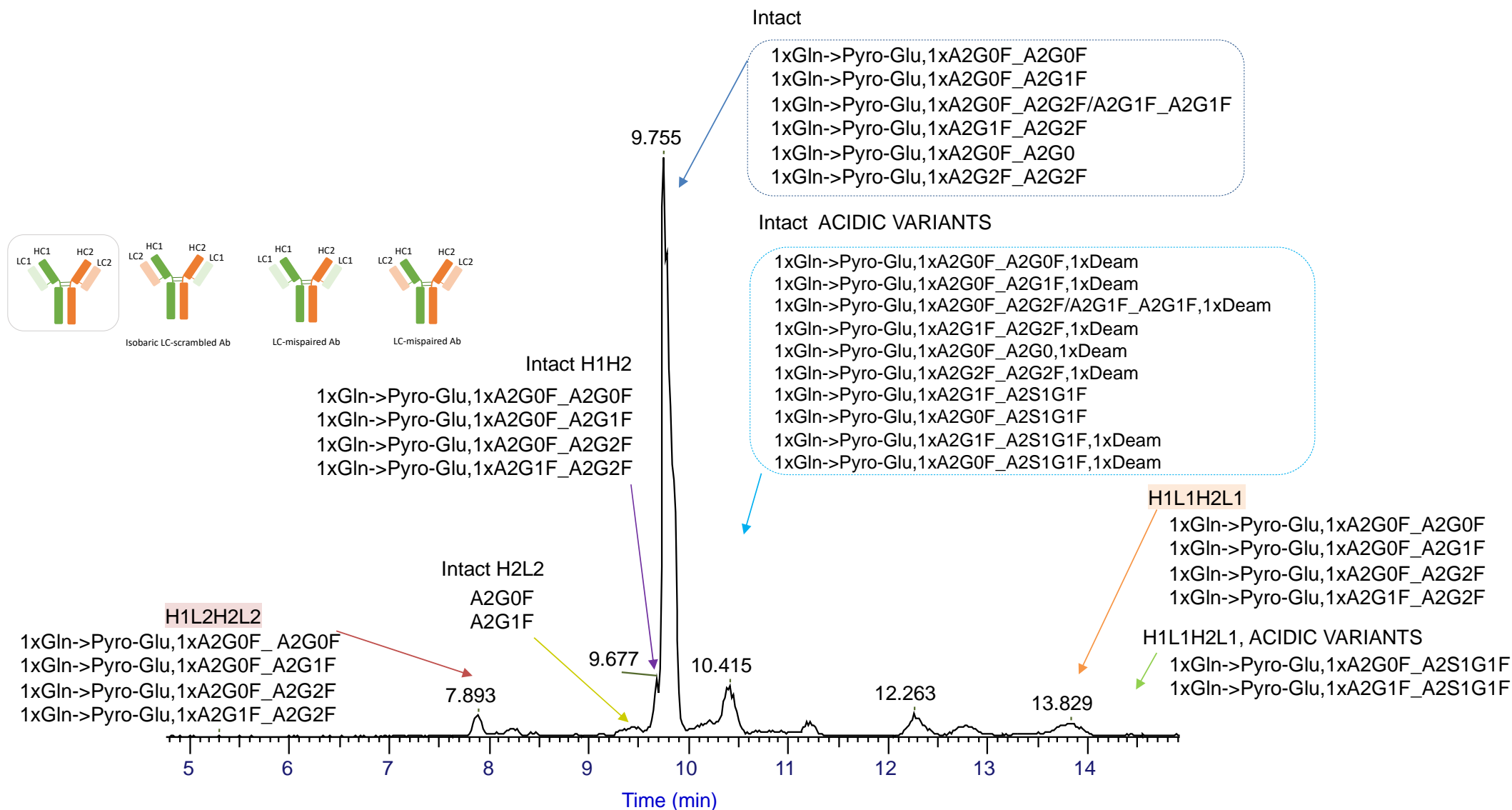
ZipChip-MS analysis on HRN chip reveals additional heterogeneity based on differential surface charge, mass shifts shown are relative to the dominant species in the main peak.

Annotation of ADC Charge Variants Following Pep Map

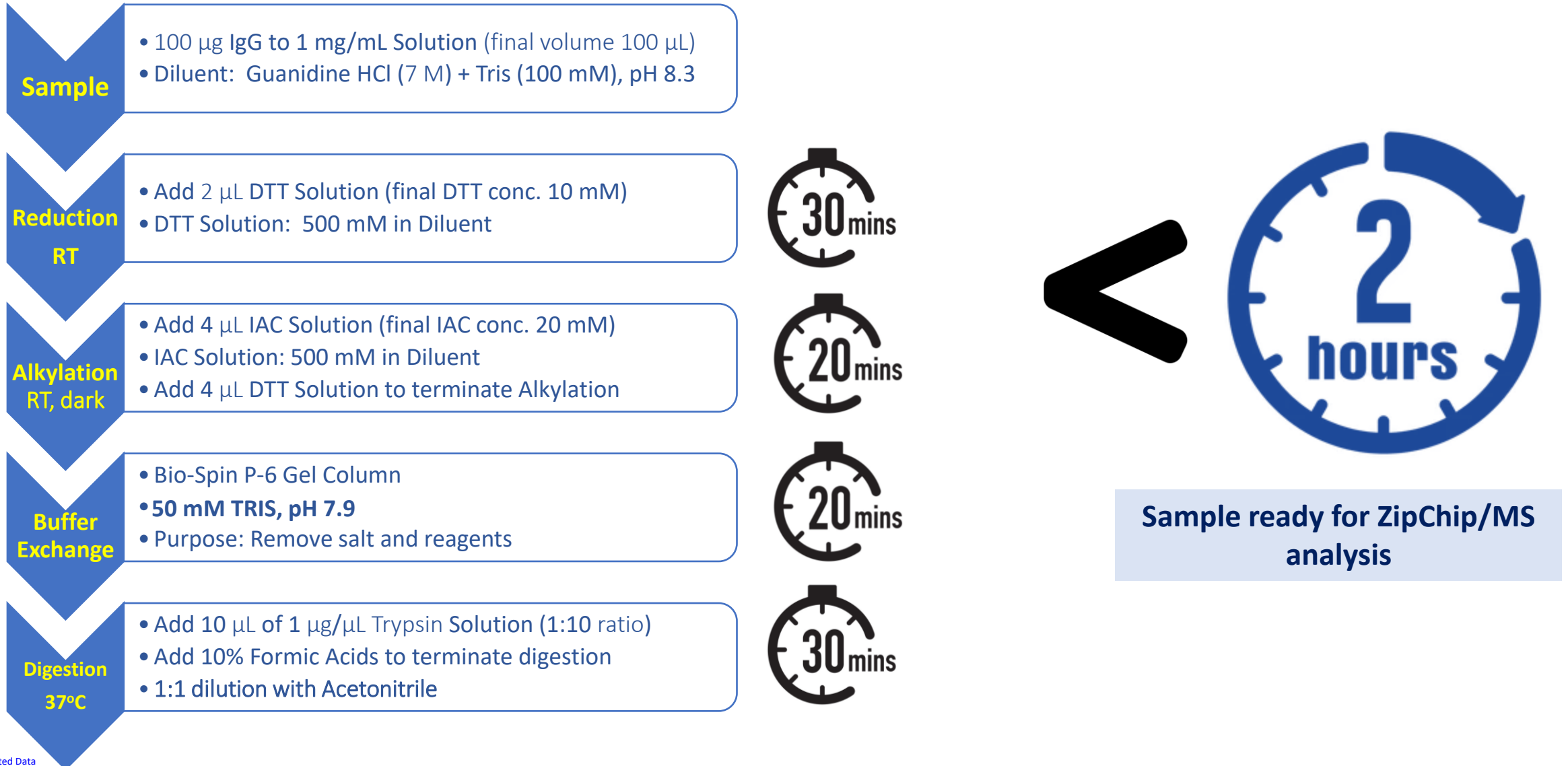


Peptide mapping was performed to assist with annotation of the observed charge variants, which were found to occur based on PTMs and payload fragmentation.

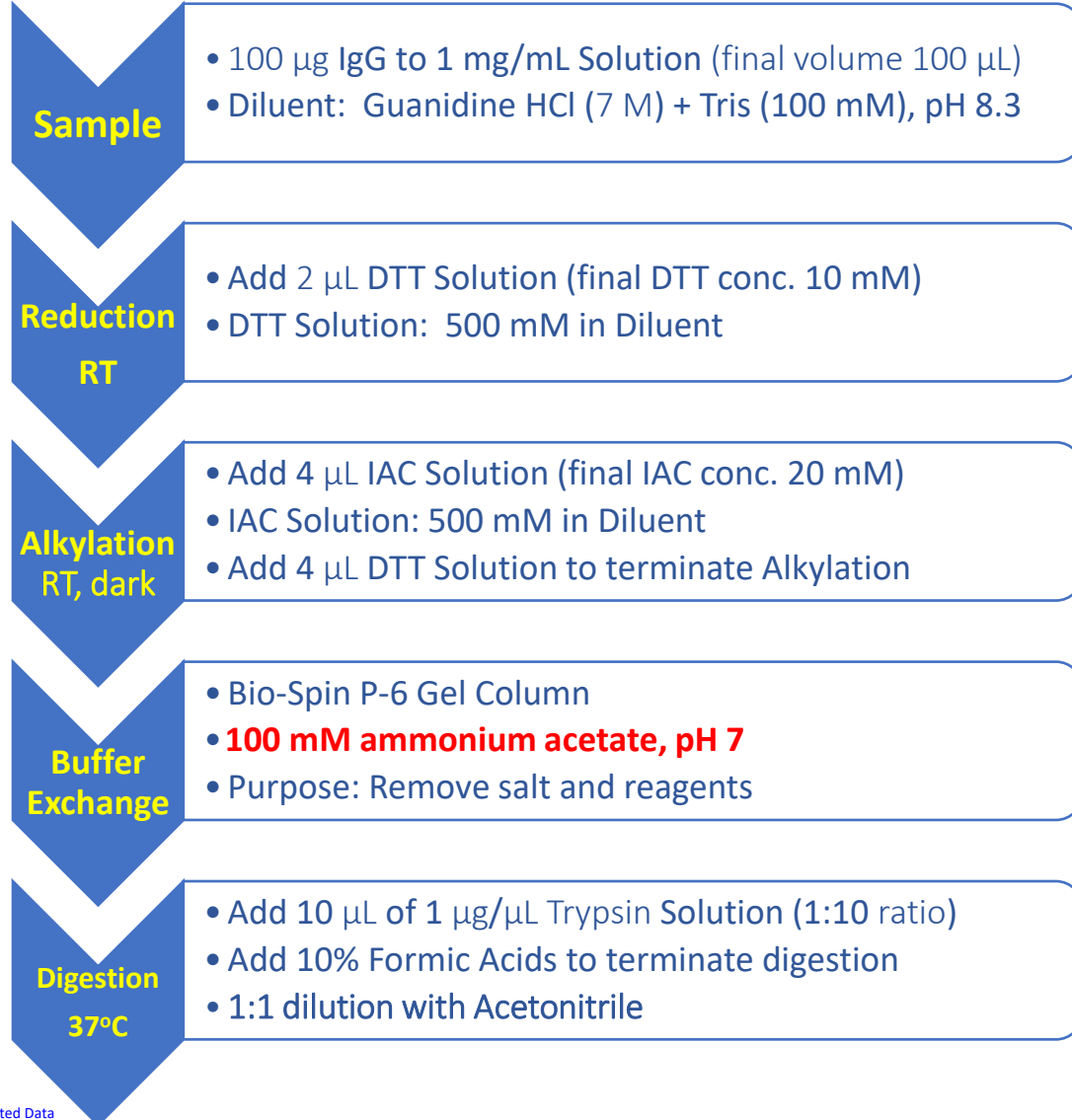
ZipChip-MS Analysis of Bispecific Antibodies



Peptide Mapping on ZipChip-MS – Sample Prep Workflow

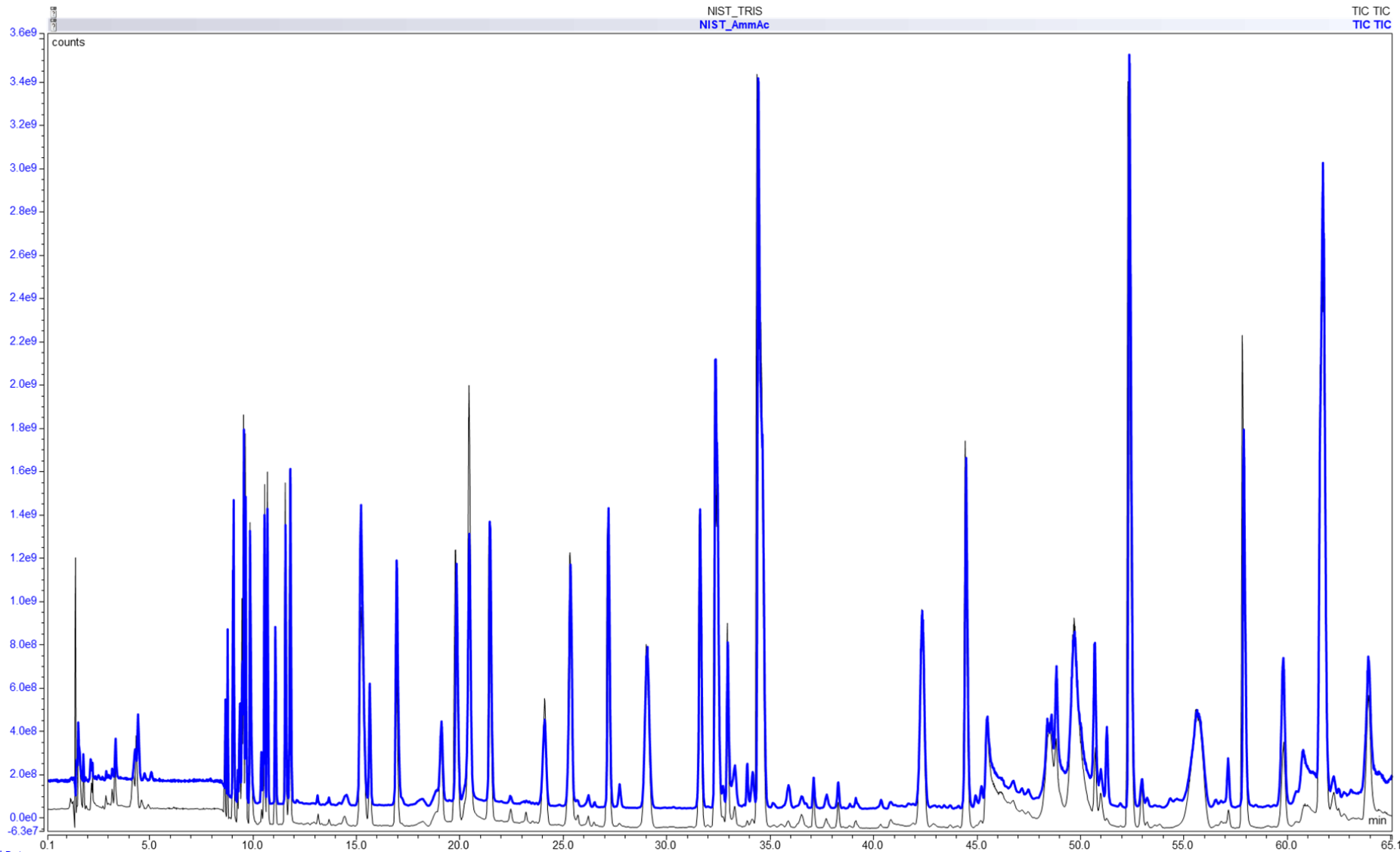


Peptide Mapping on ZipChip-MS – Sample Prep Workflow



Sample ready for ZipChip/MS analysis

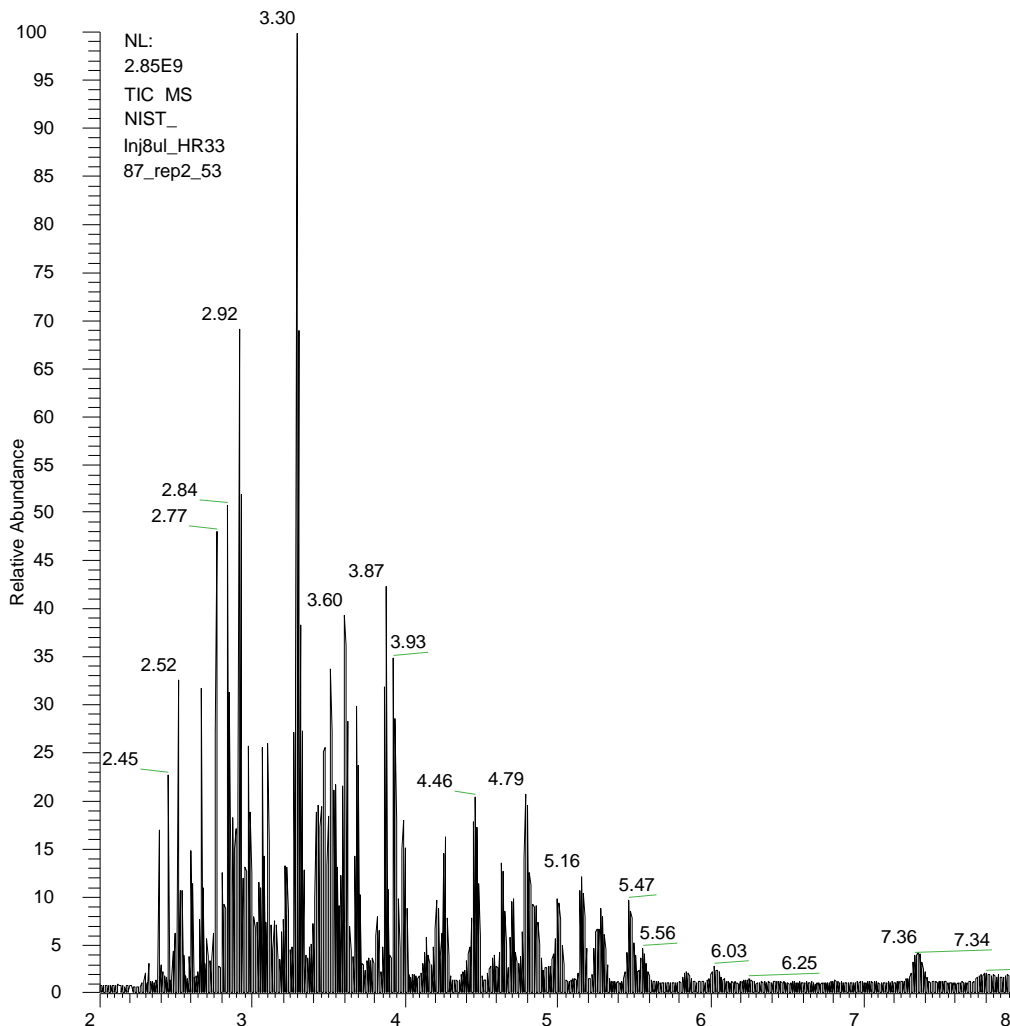
Verification of Digest Performance using LC-MS



- LC-MS comparison.
- Non-targeted MS search.
- 0 new components.
- Comparable PTMs.

Rapid, High Sensitivity, High Coverage Peptide Mapping

RT: 2.00 - 15.00



Sequence Coverage Map - User Defined

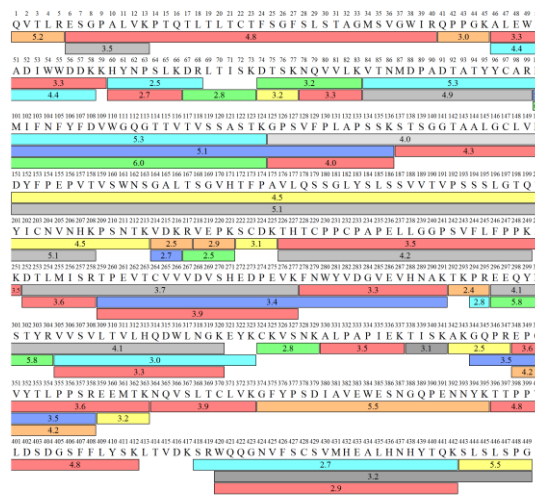
Created on 12/01/22
 Minimum MS Signal = 0
 Data File = NIST_newDigest30min_Inj8ul_HR3387_rep1_52.raw
 Protease = Trypsin

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:NISTmAb HC	154	66.8%	98.9%	59.70%
2:NISTmAb LC	67	33.2%	98.1%	40.30%
Unidentified	0	0.0%		

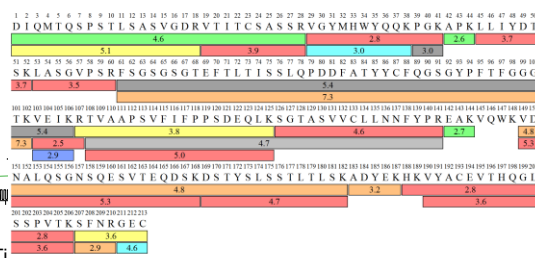
Minimum Recovery = 0%
 Minimum Recovery of Overlapping Peptides = 0%
 Minimum Confidence = 0
 Maximum Mass = 7000

Color code for peptide recovery
 50.0% - 20.0% - 10.0% - 5.0% - 2.0% - 1.0% - 0.5% - 0.2% - 0.1% - 0.0%
 good fair poor

NISTmAb HC



NISTmAb LC

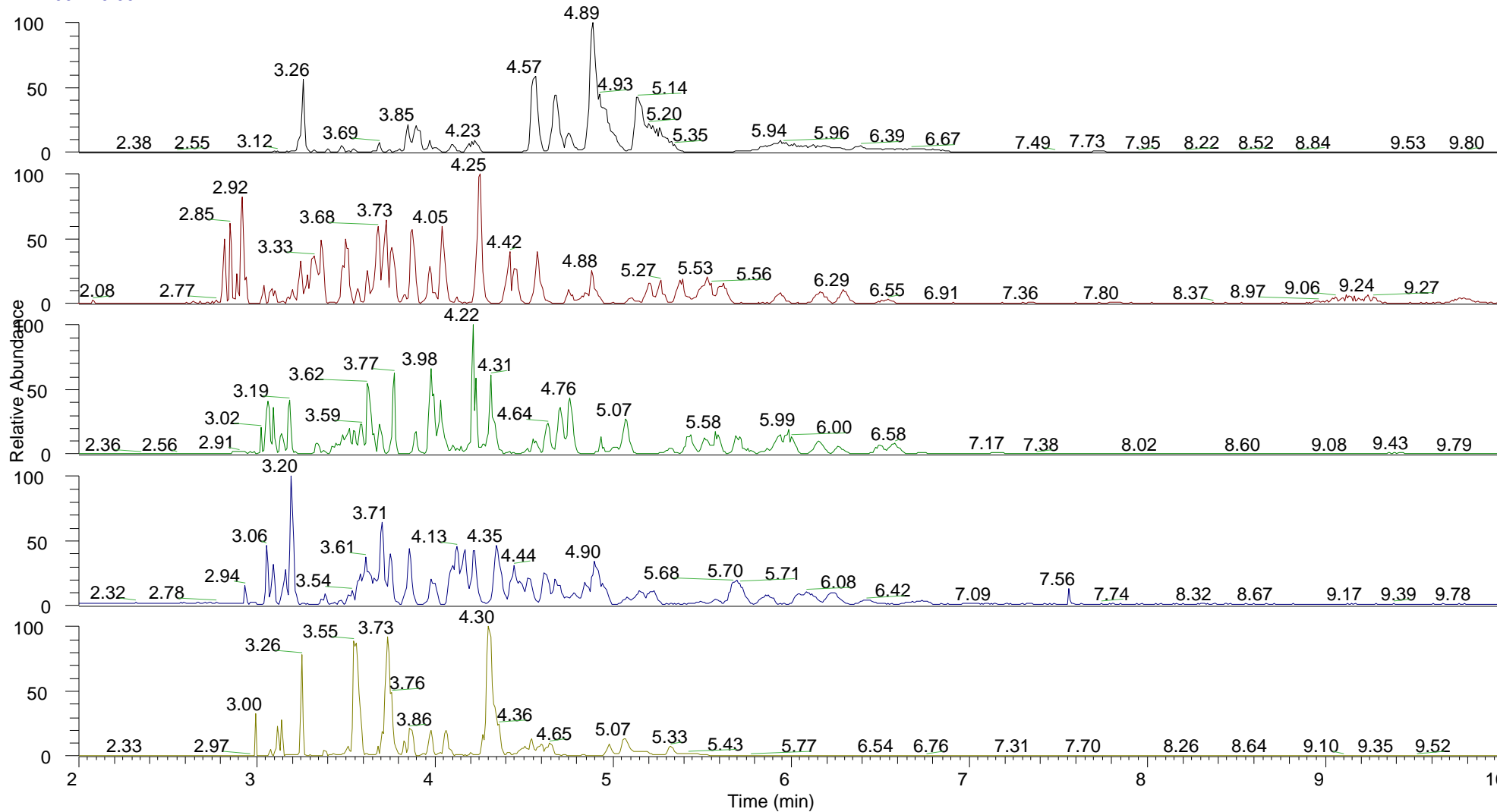


CHAIN	RESIDUE MODIFICATION	RELATIVE ABUNDANCE (%)	RSD (%)
HC	M255+Oxidation	1.99	7.1
HC	N300+A1G0	0.52	14.3
HC	N300+A1G0F	3.09	4.6
HC	N300+A2G0F	44.45	0.2
HC	N300+A2G1F	41.39	0.5
HC	N300+A2G2F	9.68	2.0
HC	N300+A1G1F	2.70	1.9
HC	N300+Unglycosylated	1.69	7.1
HC	M361+Oxidation	0.79	5.8
HC	N387+Deamidation	0.94	4.3
HC	N387+Succinimide1	0.71	1.0
HC	M431+Oxidation	1.27	7.4
LC	M4+Oxidation	2.05	18.7



Platform Peptide Mapping using ZipChip-MS

RT: 2.00 - 10.00



NL: 5.57E8
Base Peak F: FTMS + p ESI
Full ms
[200.0000-2000.0000] MS
Dulaglutide_HR6085_Rep2_51

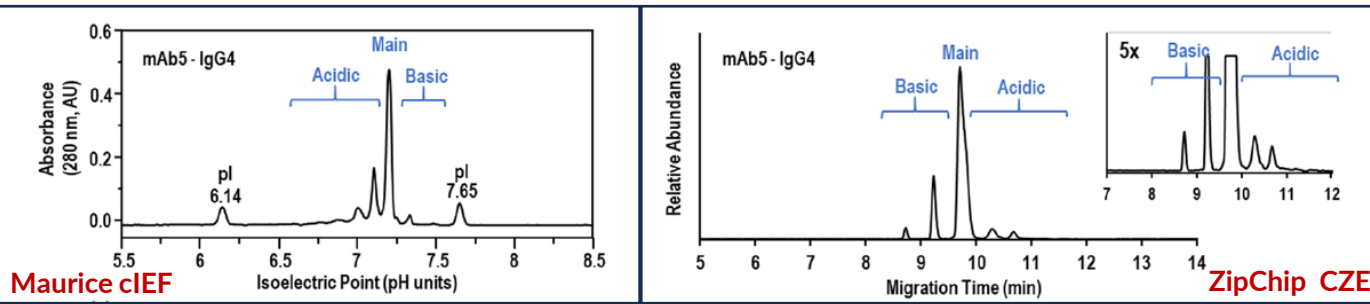
NL: 1.66E8
Base Peak F: FTMS + p ESI
Full ms
[200.0000-2000.0000] MS
nivolumab_hr6254_rep2_66

NL: 2.71E8
Base Peak F: FTMS + p ESI
Full ms
[200.0000-2000.0000] MS
rituximab_hr6254_rep2_63

NL: 1.06E8
Base Peak F: FTMS + p ESI
Full ms
[200.0000-2000.0000] MS
trastuzumab_hr6254_rep2_60

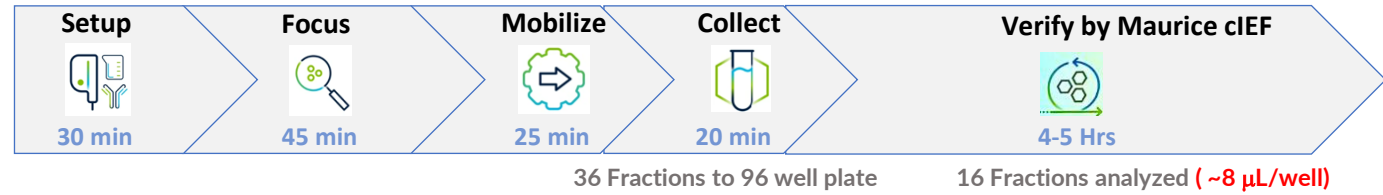
NL: 5.55E8
Base Peak F: FTMS + p ESI
Full ms
[200.0000-2000.0000] MS
aflibercept_hr6085_rep2_48

ZipChip and Maurice Flex



J. Pharm. Biomed. Anal. 2023, Vol 223, P20

Fraction Collection by MauriceFlex
(30 μ L/well)



Fraction Analysis by ZipChip
(22 μ L/well)

Intact Mass Analysis

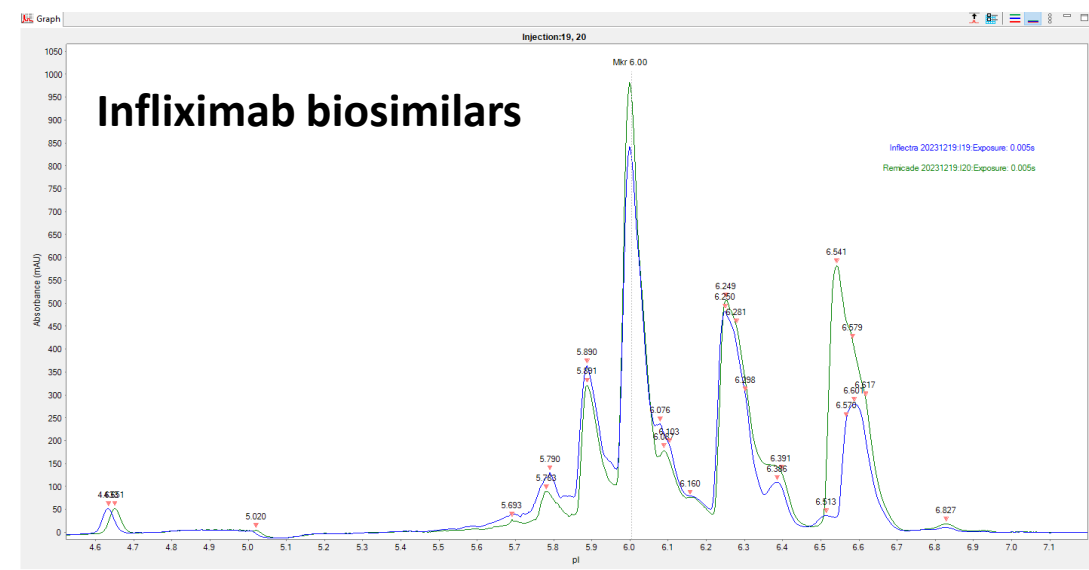
- Rapid characterization of fractions
- Size confirmation by MW
- Profile Glycoforms

1 min analysis, 4 min cycle time

Peptide Mapping Analysis (SPE)

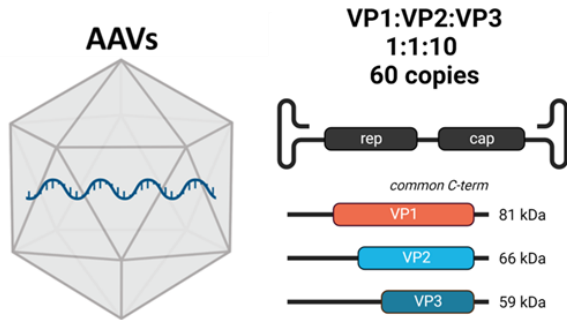
- The ultimate analysis of fractions
- Site specific localization of PTM
- AA Sequence confirmation of protein

SPE ZC analysis, ~25 min cycle time

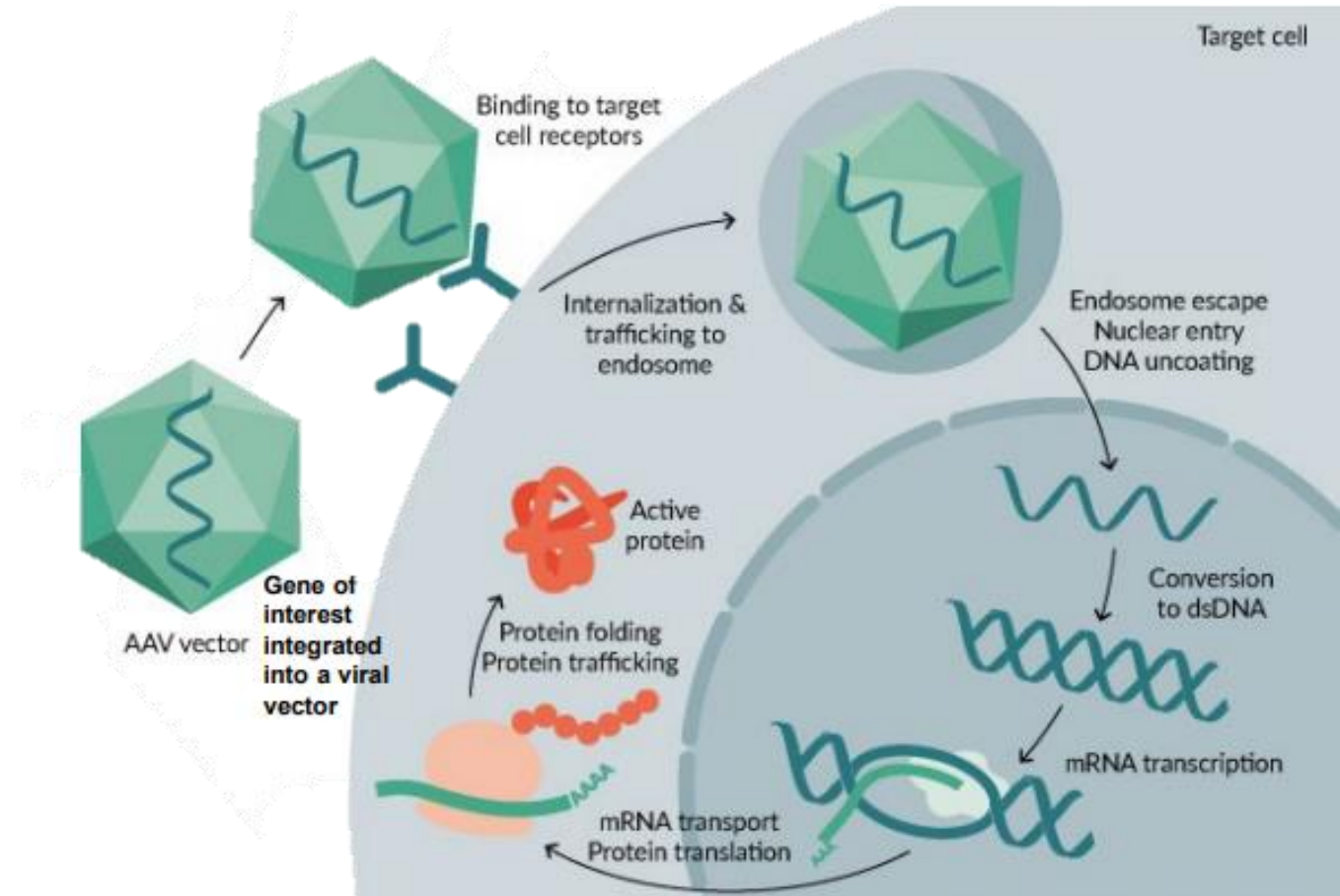


Adeno-Associated Virus (AAV)-based Gene Therapy

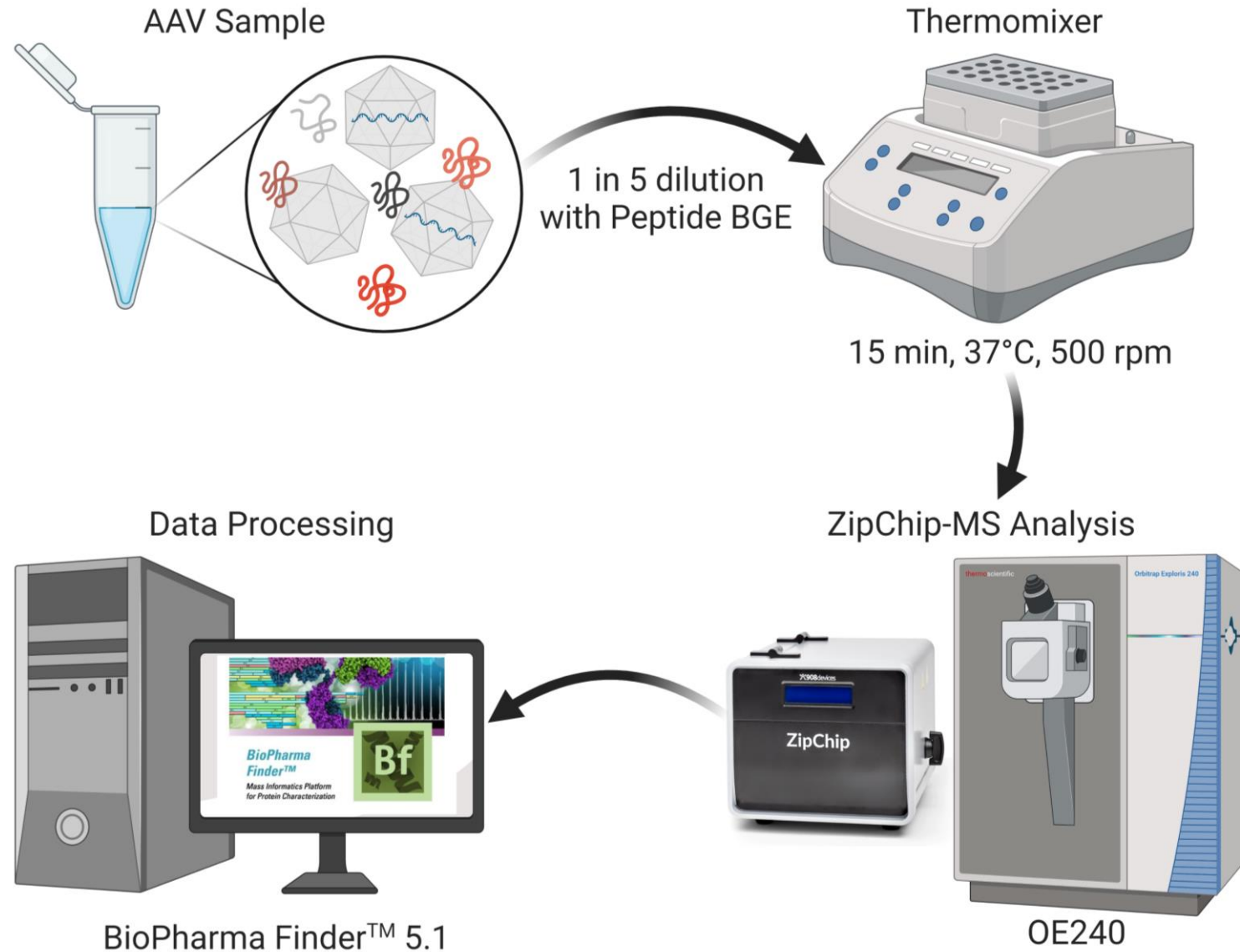
Treats genetic diseases, caused by absent or defective genes



Properties	AAV
Examples	Serotypes AAV 1-10
Size	25 nm
Genome	ssDNA
Packaging Capacity	4.7 kb
Transduction	Medium
Biosafety Level	BSL-1
Immunogenicity	Low
Advantages	Low immunogenicity, nonpathogenic, no known cause of disease in humans
Disadvantages	Small packaging capacity, low production yields, requires helper gene for replication

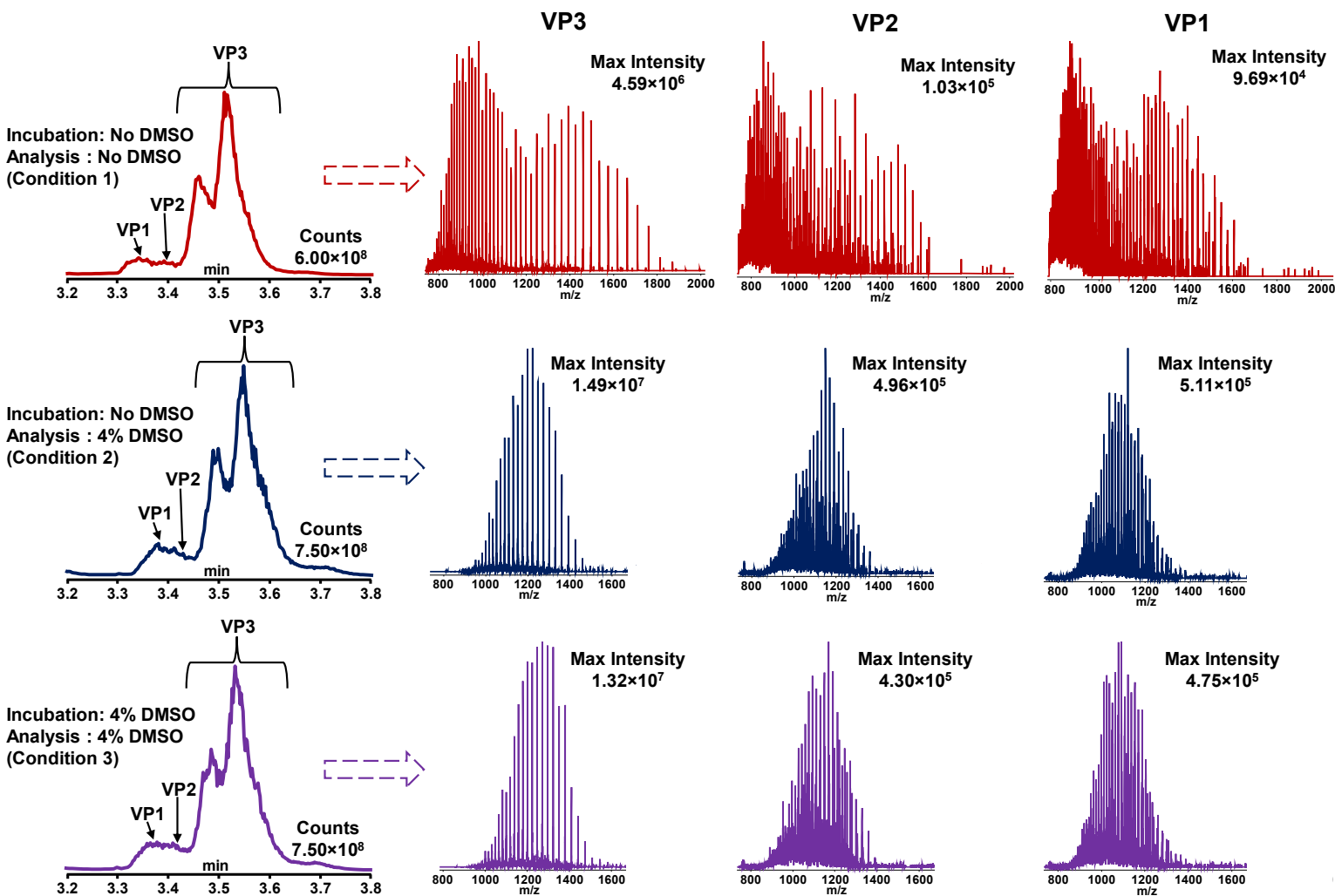


AAV Viral Capsid Protein (VP) Analysis



Does DMSO impact MS signal and Identifications?

Improved MS Spectra Intensity & Reduction of Bimodal Signal



Effect of DMSO exemplified using acetylated VP3 ((Ac)VP3) and VP2

Condition	(Ac)VP3			VP2		
	1	2	3	1	2	3
Ave. Sum Intensity	2.34×10^{10}	3.54×10^{10}	3.36×10^{10}	8.90×10^7	3.67×10^8	3.30×10^8
Ave. # of Charge States	53.20	30.40	30.00	44.80	24.40	24.25
Ave. Min. Charge State	31.20	37.40	37.40	44.00	48.60	49.25
Ave. Max. Charge State	83.40	66.80	66.40	88.60	72.00	74.75

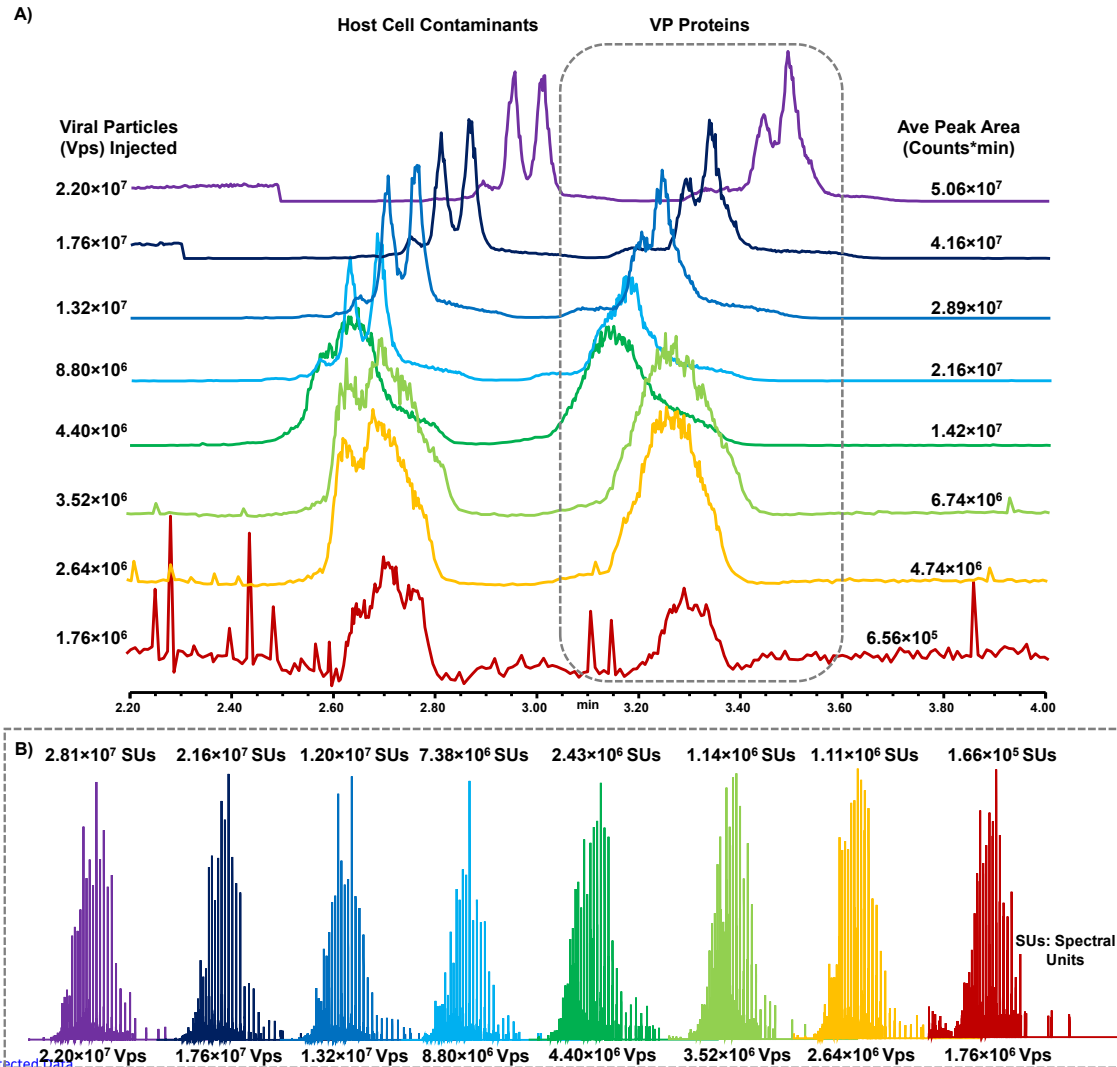
Identified AAV8 viral capsid proteins (VPs) under investigated sample analysis conditions

Capsid Viral Proteins (Theoretical mass)	Matched Mass Error (ppm)			Relative Abundance (%)			Fractional Abundance (%)			Quality Score		
	1	2	3	1	2	3	1	2	3	1	2	3
(Ac)VP1 + 2x P (81,826.67 Da)	6.7 ^b	0.7 ^a	-	0.06	0.56	-	0.01	0.07	-	34.33	74.78	-
(Ac)VP1 + 1x P (81,746.69 Da)	1.7	0.0	2.1	0.57	2.05	1.83	0.09	0.24	0.21	149.73	127.34	115.08
(Ac)VP1 (81,666.71 Da)	6.4	1.6	1.0	0.43	1.12	1.26	0.07	0.13	0.14	168.28	94.43	117.70
VP2 + 1x P (66,598.08 Da)	2.7	1.2	8.3	0.51	1.85	1.74	0.08	0.22	0.20	107.14	91.33	92.65
VP2 (66,518.10 Da)	6.0	3.4	3.1 ^a	0.25	0.94	0.81	0.04	0.11	0.09	81.58	98.99	83.38
(Ac)VP3 + 1x P (59,884.66 Da)	1.5	15.3	14.3	1.24	5.81	5.25	0.19	0.68	0.60	60.41	75.34	77.02
(Ac)VP3 (59,804.68 Da)	3.9	5.8	5.9	65.15 ^c	90.87 ^c	81.87 ^c	10.11	10.64	9.39	262.41	122.59	119.71
VP3 (59,762.65 Da)	9.5	14.9	13.5	28.58	47.77	43.82	4.43	5.60	5.03	238.27	114.04	114.50
VP3 Fragment (59,506.81 Da)	1.6	2.5	5.0	0.87	3.13	2.61	0.13	0.37	0.30	79.64	85.42	81.09
A213(Ac)-VP3 Variant (59,191.98 Da)	3.3 ^a	1.7	5.1	0.87	3.79	3.48	0.14	0.44	0.40	55.24	75.60	83.71

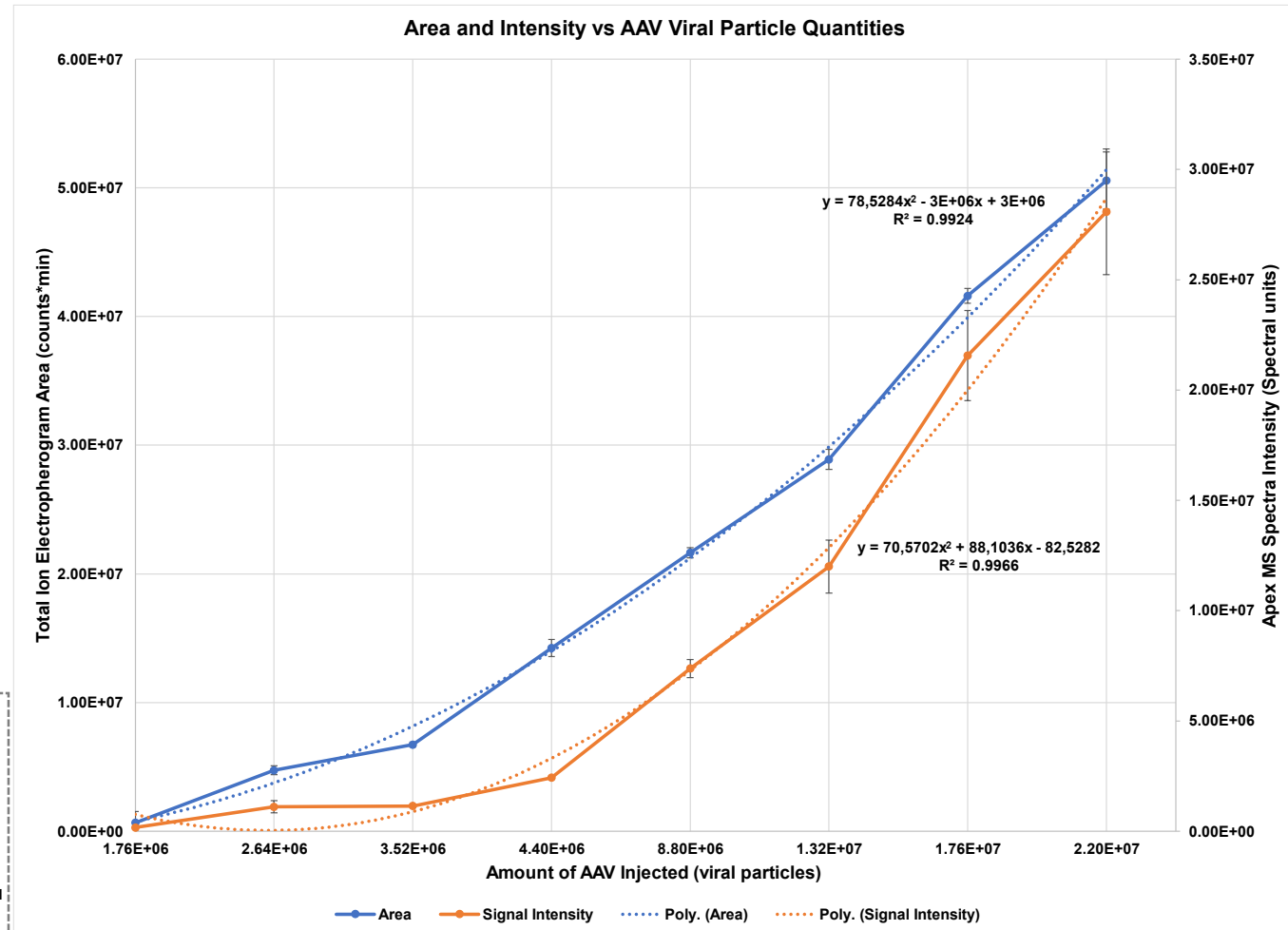
^a Only found in 4 of the 5 replicate injections; ^b Only found in 3 of the 5 replicate injections; ^c (Ac)VP3 is the most abundant VP. The most abundant feature is an unknown component detected in the host cell contaminants peaks

Limit of Detection (LoD) Testing

Total ion electropherograms (TIE) and MS signal intensity



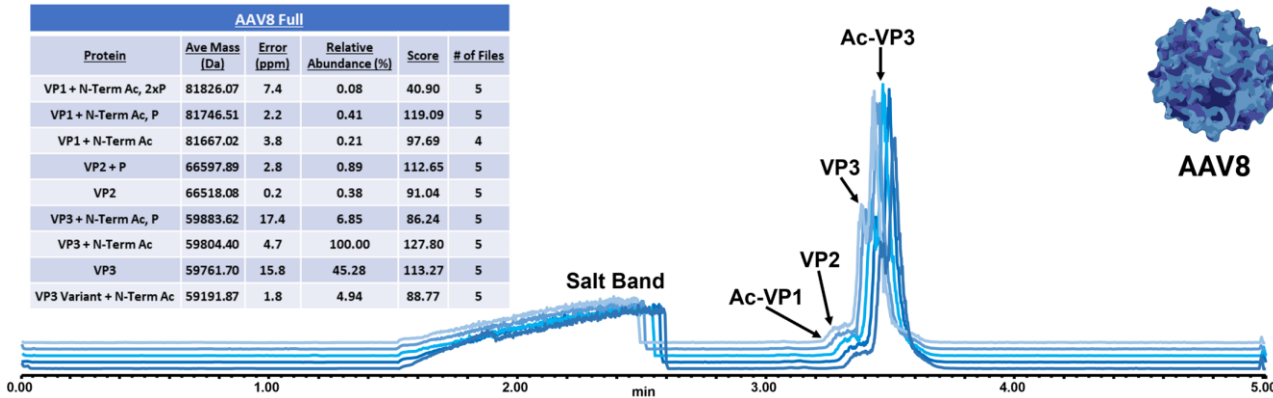
Plot of TIE area and intensity versus # of viral particles



Adapted from Figure 2 and Figure S1 of Smith et. al ABC, 2023

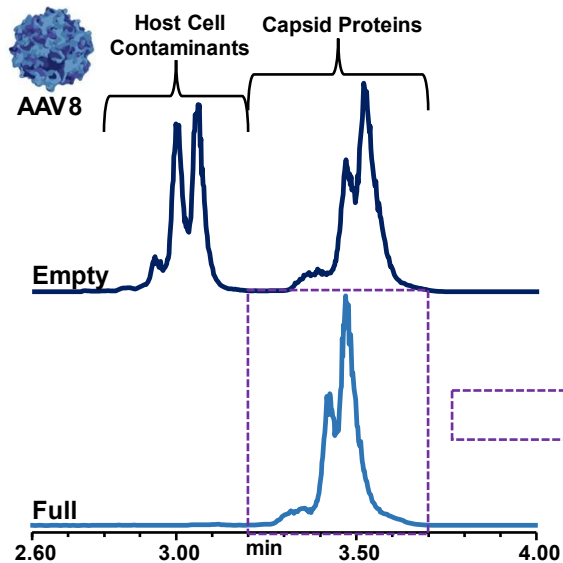
VP Separation on ZipChip Platform with AAV8

5 minute VP Separation

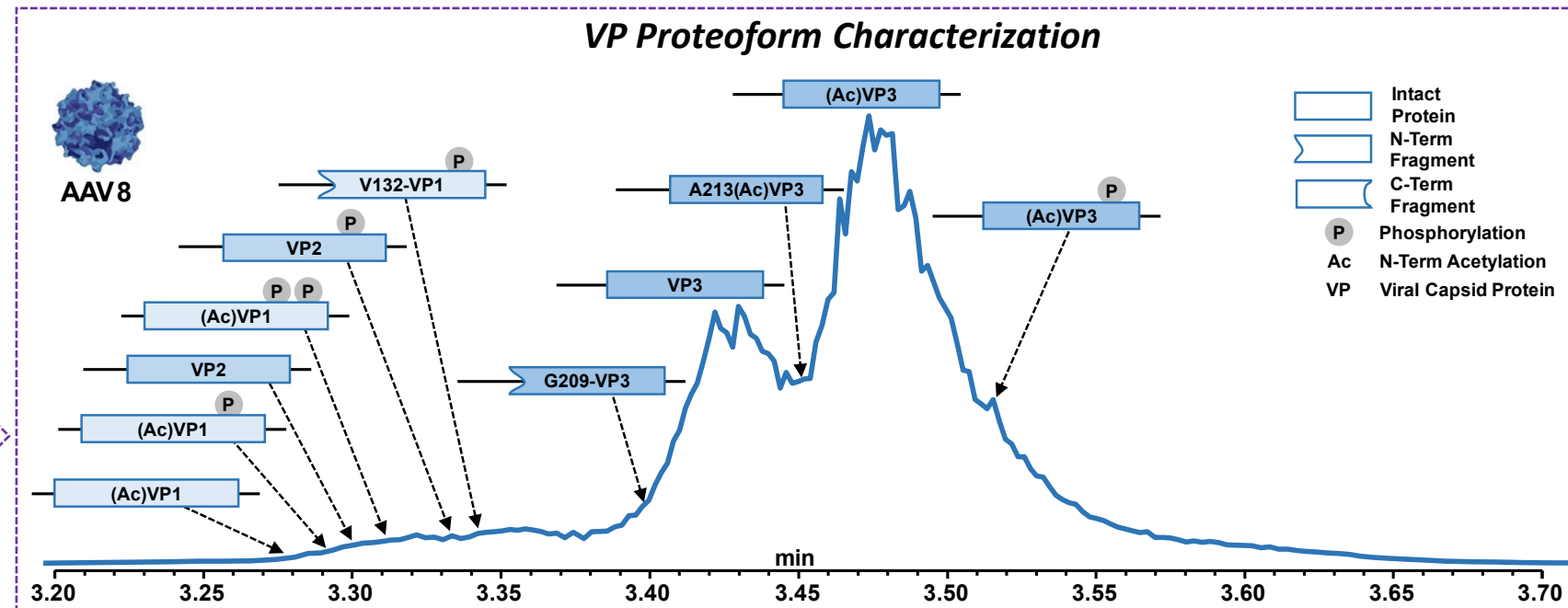


- Rapid AAV VP analysis of Full AAV8 using the ZipChip CE-MS platform.
- 5-minute runs enable increased replicate injections without sacrificing reproducibility or sensitivity
- Detection of low abundant VP proteoforms and VP fragments using BPF 5.1

Empty vs. Full Profiles

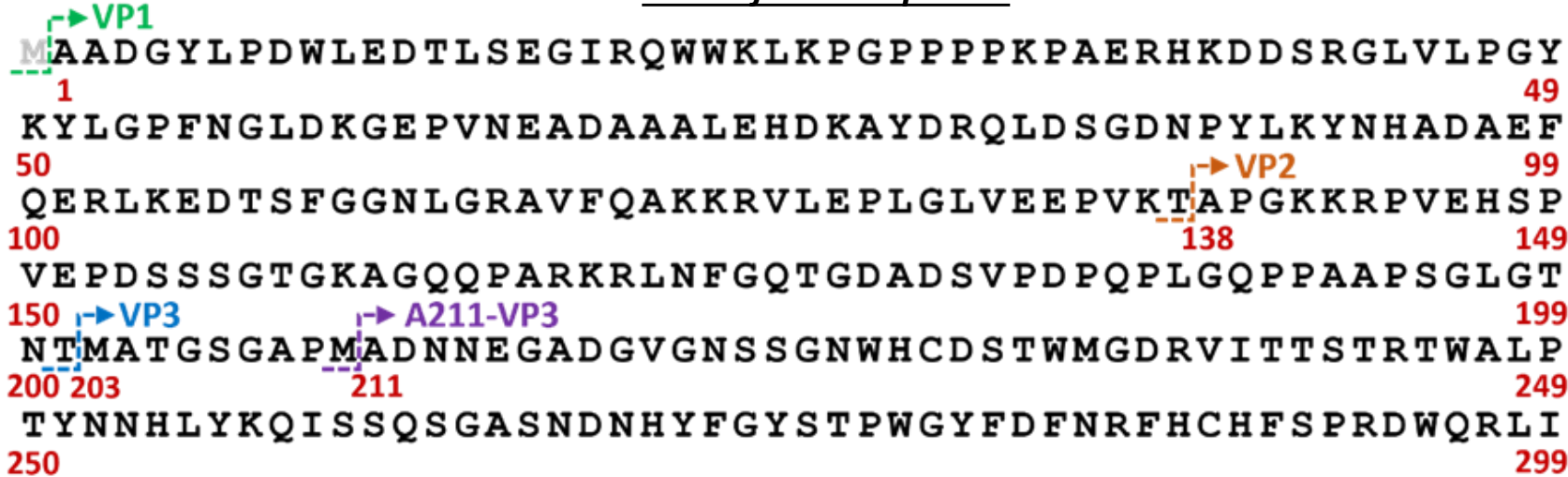


VP Proteoform Characterization

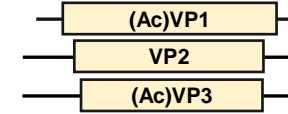


Detected VP Proteoforms and Fragments

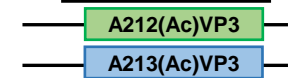
Start of AAV Sequence



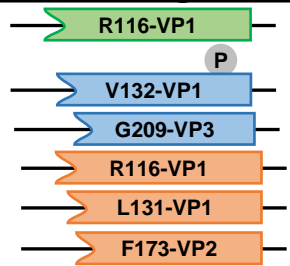
Expected VPs



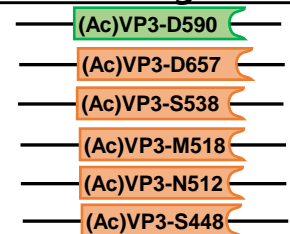
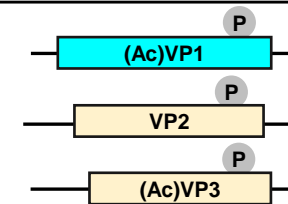
VP3 Variant



N-Term Fragments



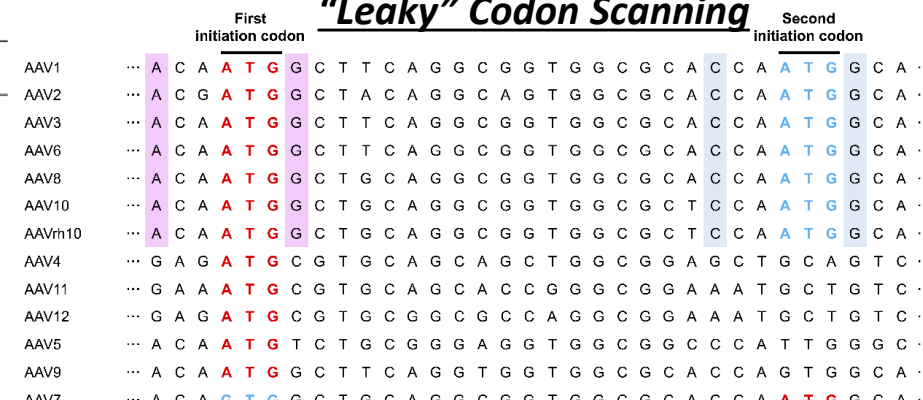
VPs with additional PTMs C-Term Fragments



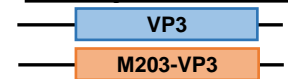
VP3 Variant Generation

Serotypes	N-terminal region		DP sequence	DG sequence	DP sequence	AAV1
	203	211	590 591	626 627	656 657	
AAV1	M A S G G G A P M A	M A	T D P A	T D G H	A N P P	... A C A A T G G C T T C A G G C G G T G G C G C A C C A A T G G C A ...
AAV2	M A T G S G A P M A	M A	R Q A A	T D G H	A N P S	... A C G A T G G C T A C A G G C A G T G G C G C A C C A A T G G C A ...
AAV3	M A S G G G A P M A	M A	T A P T	T D G H	A N P P	... A C A A T G G C T T C A G G C G G T G G C G C A C C A A T G G C A ...
AAV6	M A S G G G A P M A	M A	T D P A	T D G H	A N P P	... A C A A T G G C T T C A G G C G G T G G C G C A C C A A T G G C A ...
AAV8	M A A G G G A P M A	M A	T A P Q	T D G N	A D P P	... A C A A T G G C T G C A G G C G G T G G C G C A C C A A T G G C A ...
AAV10	M A A G G G A P M A	M A	T G P I	T D G N	A D P P	... A C A A T G G C T G C A G G C G G T G G C G C T C C A A T G G C A ...
AAVrh10	M A A G G G A P M A	M A	A A P I	T D G N	A D P P	... A C A A T G G C T G C A G G C G G T G G C G C T C C A A T G G C A ...
AAV4	M R A A A G G A A V	M A	N L P T	T D G H	A N P A	... G A G A T G C G T G C A G C A G C T G G C G G A G C T G C A G T C ...
AAV11	M R A A P G G N A V	M A	T A P I	A D G H	A N P A	... G A A A T G C G T G C A G C A C C G G G C G G A A A T G C T G T C ...
AAV12	M R A A P G G N A V	M A	T A P H	T D G H	A N P N	... G A G A T G C G T G C G G C G C C A G G C G G A A A T G C T G T C ...
AAV5	M S A G G G P L G	M A	T A P A	T G A H	G N I -	... A C A A T G T C T C G G G A G G T G G C G G C C A T T G G G C ...
AAV9	M A S G G G A P V A	M A	A Q A Q	T D G N	A D P P	... A C A A T G G C T T C A G G T G G T G G C G C A C C A G T G G C A ...
AAV7	V A A G G G A P M A	M A	T A A Q	T D G N	A N P P	... A C A G T G G C T G C A G G C G G T G G C G C A C C A A T G G C A ...

"Leaky" Codon Scanning



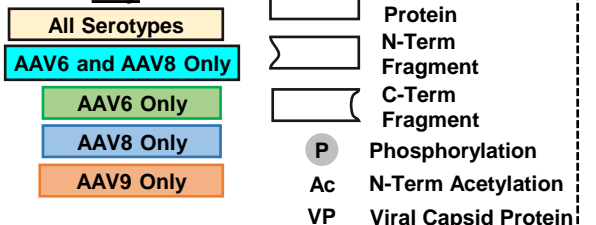
Unexpected VPs



Potential Causes of Fragments

- Baculoviral cathepsin
- Immune response
- Acidic conditions

Key



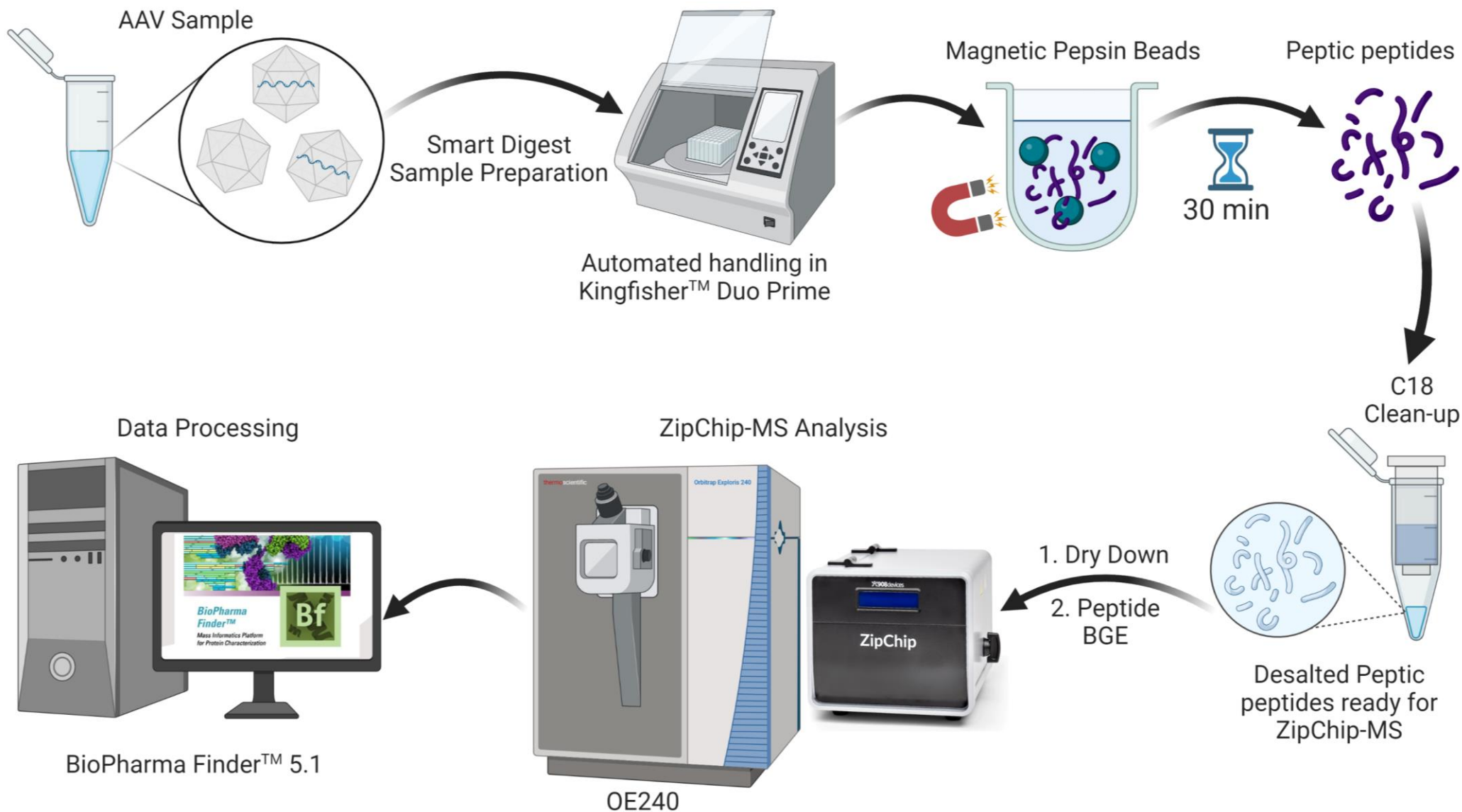
Adapted from Figure 5a of Oyama et al. (2021)

<https://www.liebertpub.com/doi/10.1089/hum.2021.009>

Adapted from Figure S4a of Oyama et al. (2021)

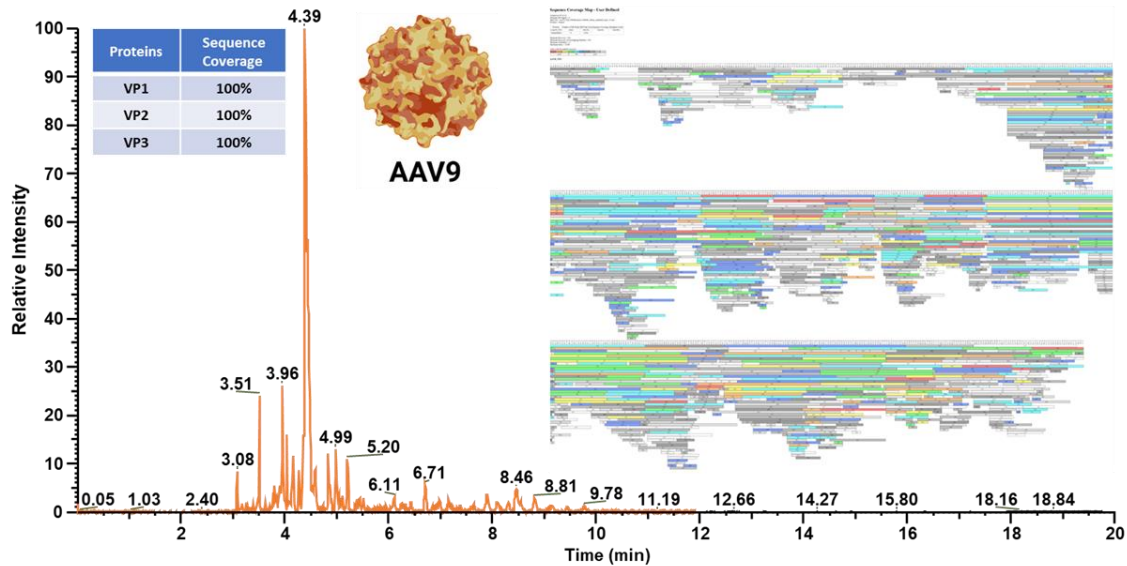
<https://www.liebertpub.com/doi/10.1089/hum.2021.009>

AAV Peptide Mapping



AAV Sequence Coverage and Post-translational Modifications

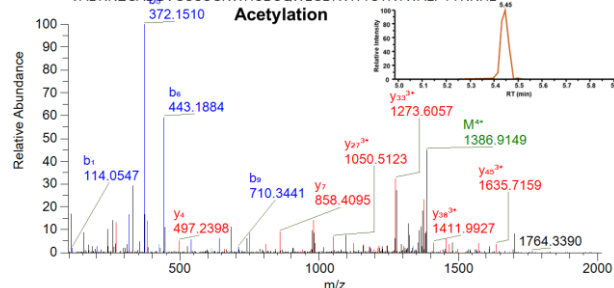
- Rapid performance with analysis in under 20 minutes
- 100% sequence coverage for all VPs including VP3 variants
- Identification of commonly monitored post-translational modifications (PTMs)



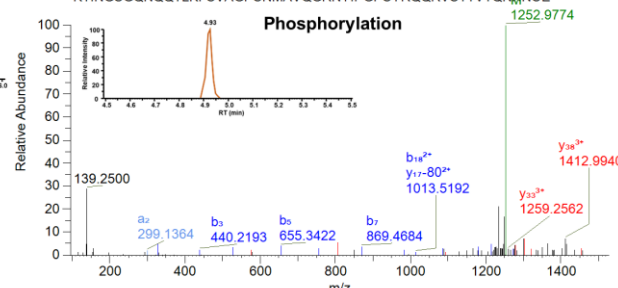
Protein	Modification	Peptide Sequence	Average % Abundance	STDEV N=2
AAV9_VP1	Q119+NH3 loss_Glutarimide	QAKKRLLEPLGL	57.21	3.52
AAV9_VP1	A203+Acetylation	ASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRTVTTSTRTRWALPTYNNHL ASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRTVTTSTR	98.25	0.08
AAV9_VP1	M372+Oxidation	FMPQYGYLTLNDGSQAVGRSSF	1.31	0.12
AAV9_VP1	D383+Succinimide D	LTLDGSQAVGRSSF MIPQYGYLTLNDGSQAVGRSSF FMPQYGYLTLNDGSQAVGRSSF	1.03	0.04
AAV9_VP1	M403+Oxidation	EYFPSQMLRTGNNFQF	1.09	0.04
AAV9_VP1	M435+Oxidation	DRLMNLIDQYL FENVPFHSSYAHSQLDRLMNLIDQ	1.49	0.13
AAV9_VP1	N451+Deamidation	YYLSKTINGSGQNQQTLKFSVAGPSNM	1.23	0.25
AAV9_VP1	~Y483+Phosphorylation	SVAGPSNMAVQGRNYPGPSYRQQRVSTTVTQNNNSE AVQGRNYPGPSYRQQRVSTTVTQNNNSE	2.57	0.08
AAV9_VP1	M523+Oxidation	ALNGRNSLMNPGPAMASHKEGEDRFFPLSGSL FAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSL	6.55	0.77
AAV9_VP1	~Q607+NH3 loss_Glutarimide	QDRDVYLQGPW	19.68	11.22
AAV9_VP1	Q614+NH3 loss_Glutarimide	QGPIWAKIPHTDGNFHPSPLMGGFGMKHPPQIL QGPIWAKIPHTDGNFHPSPLMGGF	34.76	4.88
AAV9_VP1	D625+Succinimide D	AKIPHTDGNFHPSPLMGGFGMKHPPQIL	1.88	0.25

~ Indicates exact location of PTM not determined by BPF

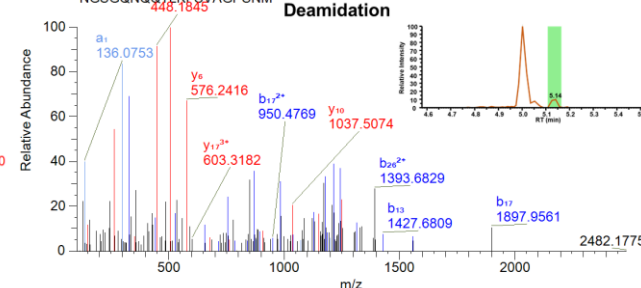
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F: FTMS + p ESI d Full ms2 1386.8885@hcd28.00 [95.0000-5682.5854]
Experimental ID=1:A203-L255 = 5498.4995m(A203+Acetylation), +4, Peptide=ASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRTVTTSTRTRWALPTYNNHL



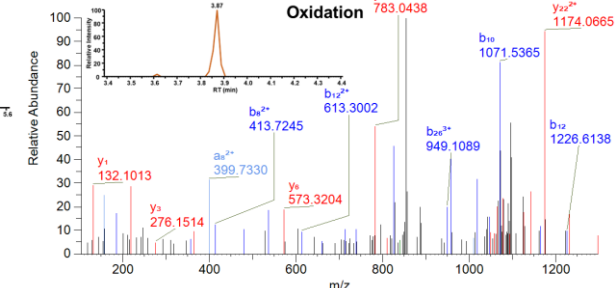
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F: FTMS + p ESI d Full ms2 1253.2086@hcd28.00 [95.0000-6416.4839]
Experimental ID=1:Y444-E499 = 6178.0315m(~Y483+Phosphorylation), +5, Peptide=YYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYPGPSYRQQRVSTTVTQNNNSE



File: AAV9_Empty_NibrProtocol_908MS_20min_combined_rep1_15
F: FTMS + p ESI d Full ms2 979.1473@hcd28.00 [95.0000-3019.2507]
Experimental ID=1:Y444-M470 = 2932.4389m(N451+Deamidation), +3, Peptide=YYLSKTINGSGQNQQTLKFSVAGPSNM



File: AAV9_Empty_NibrProtocol_908MS_20min_combined_rep1_15
F: FTMS + p ESI d Full ms2 855.1663@hcd28.00 [95.0000-3513.1584]
Experimental ID=1:A509-L540 = 3399.6452m(M523+Oxidation), +4, Peptide=ALNGRNSLMNPGPAMASHKEGEDRFFPLSGSL



Summary

- ❑ ZipChip coupled to MS detection offers a versatile platform for analysis of biopharmaceuticals on multiple different levels, just pick the background electrolyte and chip format for your application of interest.
- ❑ Overall method performance is excellent, high sensitivity from low sample amounts and excellent data depth when using Orbitrap MS detection.
- ❑ Different applications demonstrated, from intact to peptide mapping analysis.
- ❑ All these methods can be considered platform and ready to go out of the box. Application to various molecular formats shown, including viral particle analysis for gene therapy products.
- ❑ ZipChip's versatility make it an attractive technology for labs where throughput and time are important.



Acknowledgements

NIBRT:

Florian Füssl, Sara Carillo, Silvia Millan-Martín, Craig Jakes, Josh Smith, Rachel Ronan, Patrice Knightly.

908 Devices:

Kate Yu, Aditya Kulkarni, Erin Redman, Ashley Bell, Graziella Piras.