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Future analytical trends for the analysis of biotherapeutic proteins

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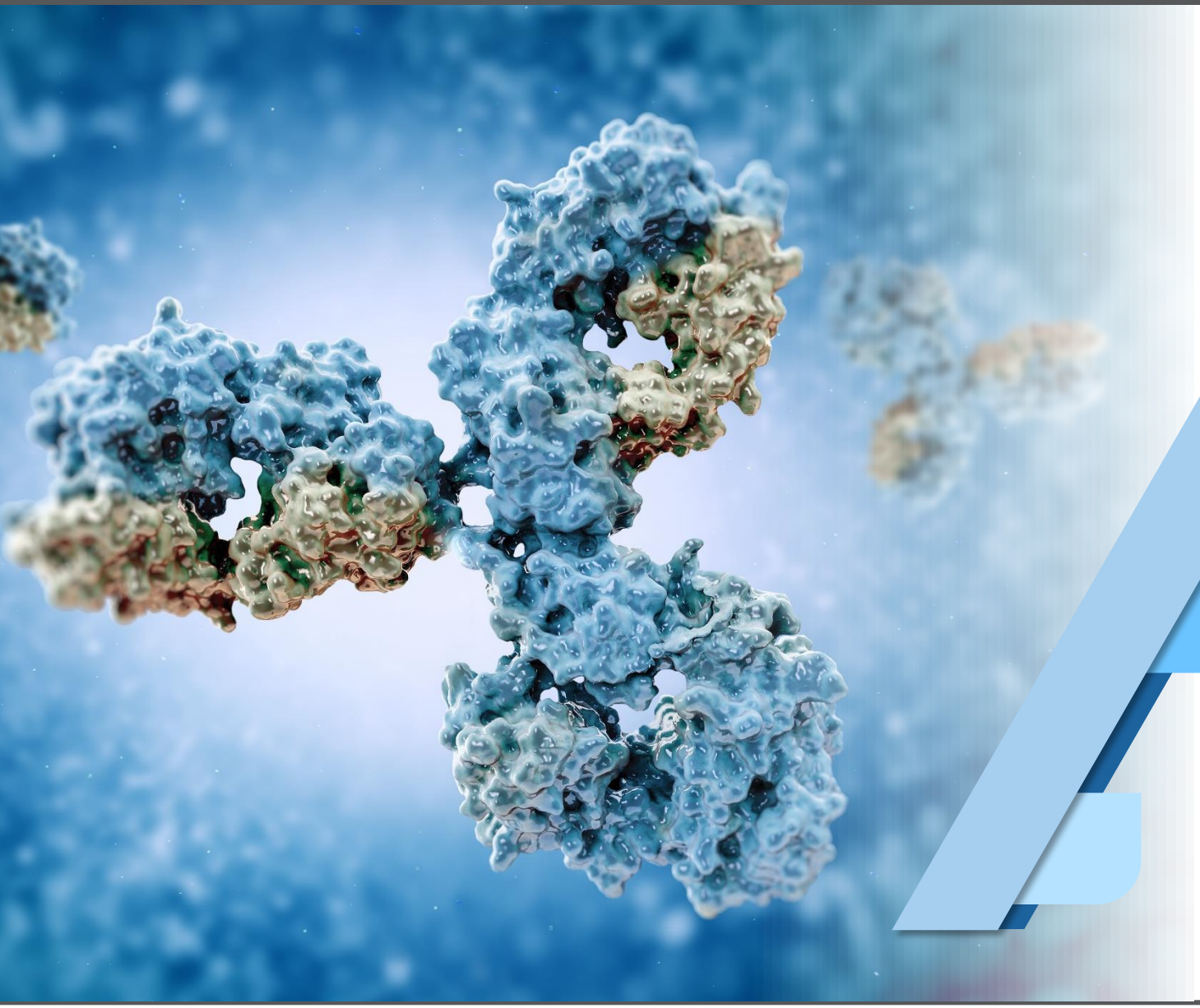
Biopharma Focus: What We Are Seeing

Industry Challenges

- Regulatory landscape is changing
- Speed, flexibility, quality and cost control: getting it right the first time and every time
- Driving towards continuous production – more production analysis
- Simple robust workflows required
- Proving bioequivalence and monitoring batch-to-batch variation
- Increasing biotherapeutic complexity is forcing new methodologies

A Complex Problem: Drug Safety and Quality



Safety
Is the product safe to use?
(e.g. Immunogenic effects?)

Potency
Does the drug have the expected effect?
(e.g. CDR complementation)

Knowledge
How do changes effect the therapeutic?
(e.g. Oxidation)

Quality
How do changes in process effect the product?
(e.g. Glucose concentration on glycoforms)

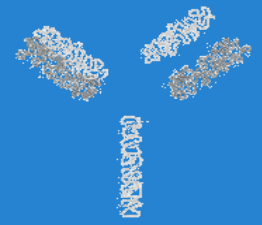
A Complex Problem: Drug Safety and Quality

- Can we reduce the analytical complexity?
- Can we get this data from fewer methods?
- Can these new methods be simple and robust enough for QC or even Process monitoring?

Multi Attribute Methods

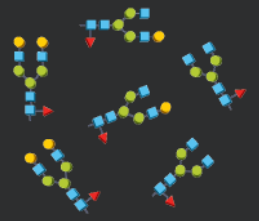
One injection?

Sub-unit Analysis

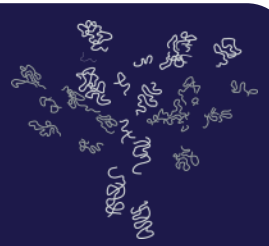


Glycan Analysis

Released Glycan Analysis



Peptide Mapping



Intact Analysis

Aggregate Screening

Charge Variant Screening

Peptide Mapping: A Complete Multi-Attribute Method

nibrt National Institute for Bioprocessing Research and Training

Method Evaluation and Advancements

Digestion
SMART Digest Kits

Separation
Acclaim Vanquish
Vanquish UHPLC

Detection
Exactive or Q Exactive Series

Processing
Chromeleon
BioPharma Finder

SMART
- Really easy to use
- Highly reproducible
- Rapid digestions

Separation
- Class leading, biocompatible
- Longer column lifetimes
- Unrivalled reproducibility

Detection
- Walk-up simplicity
- Minimal instrument contact
- Rock-steady results

Processing
- Easy to use and learn
- Enterprise & LIMS ready
- Compatible with other vendors instrumentation

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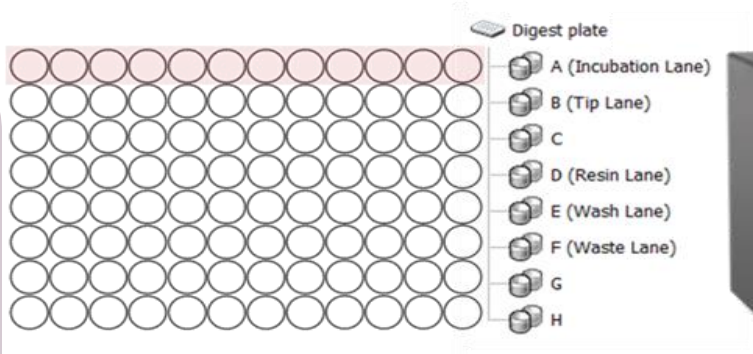
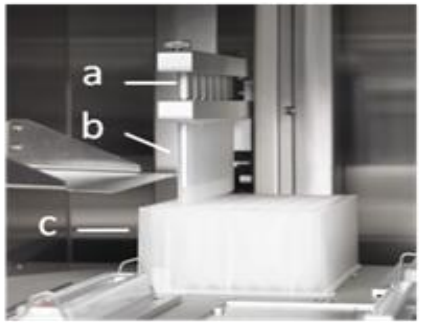
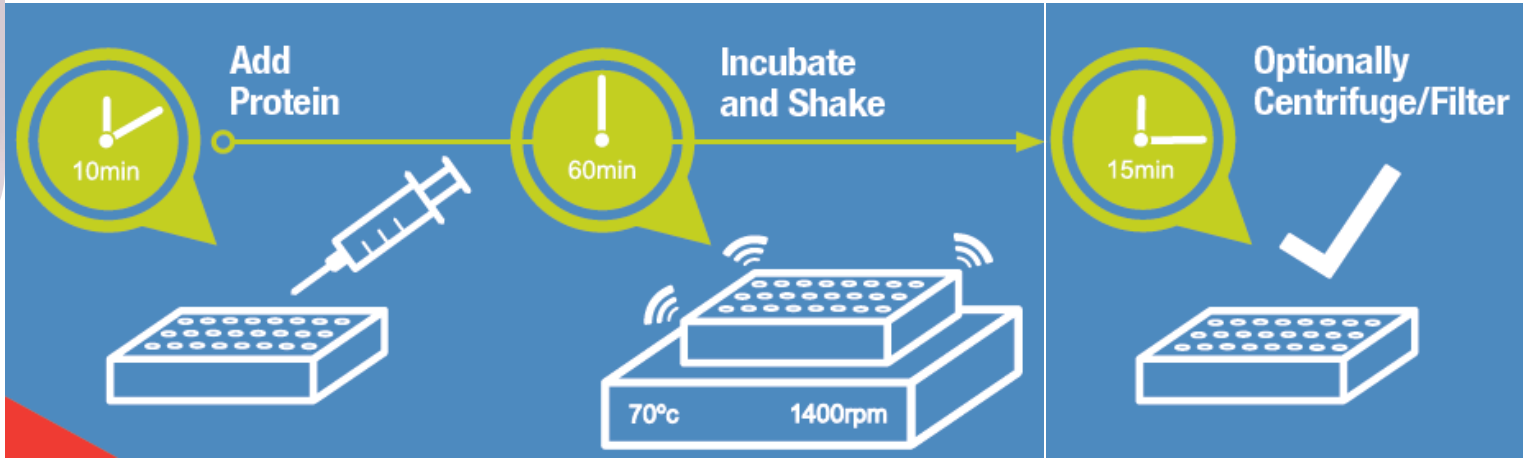
Thermo Scientific SMART Digest Kits and Automation







Denaturation

Digestion

Clean up

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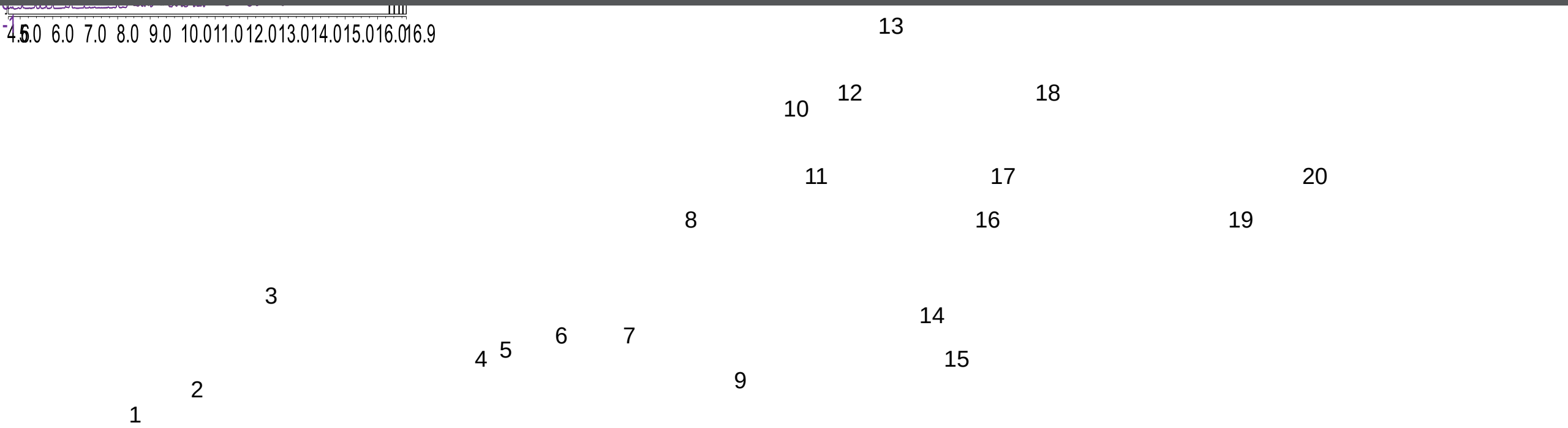
5 different Rituximab Digests by 5 different Seminar Attendees



Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10
2.54	2.41	1.89	3.39	3.53	2.16	4.41	2.10	2.10	3.65
Peak 11	**Peak 12**	**Peak 13**	**Peak 14**	**Peak 15**	**Peak 16**	**Peak 17**	**Peak 18**	**Peak 19**	**Peak 20**
1.96	3.51	3.72	2.26	2.91	1.97	3.28	2.62	3.16	1.20

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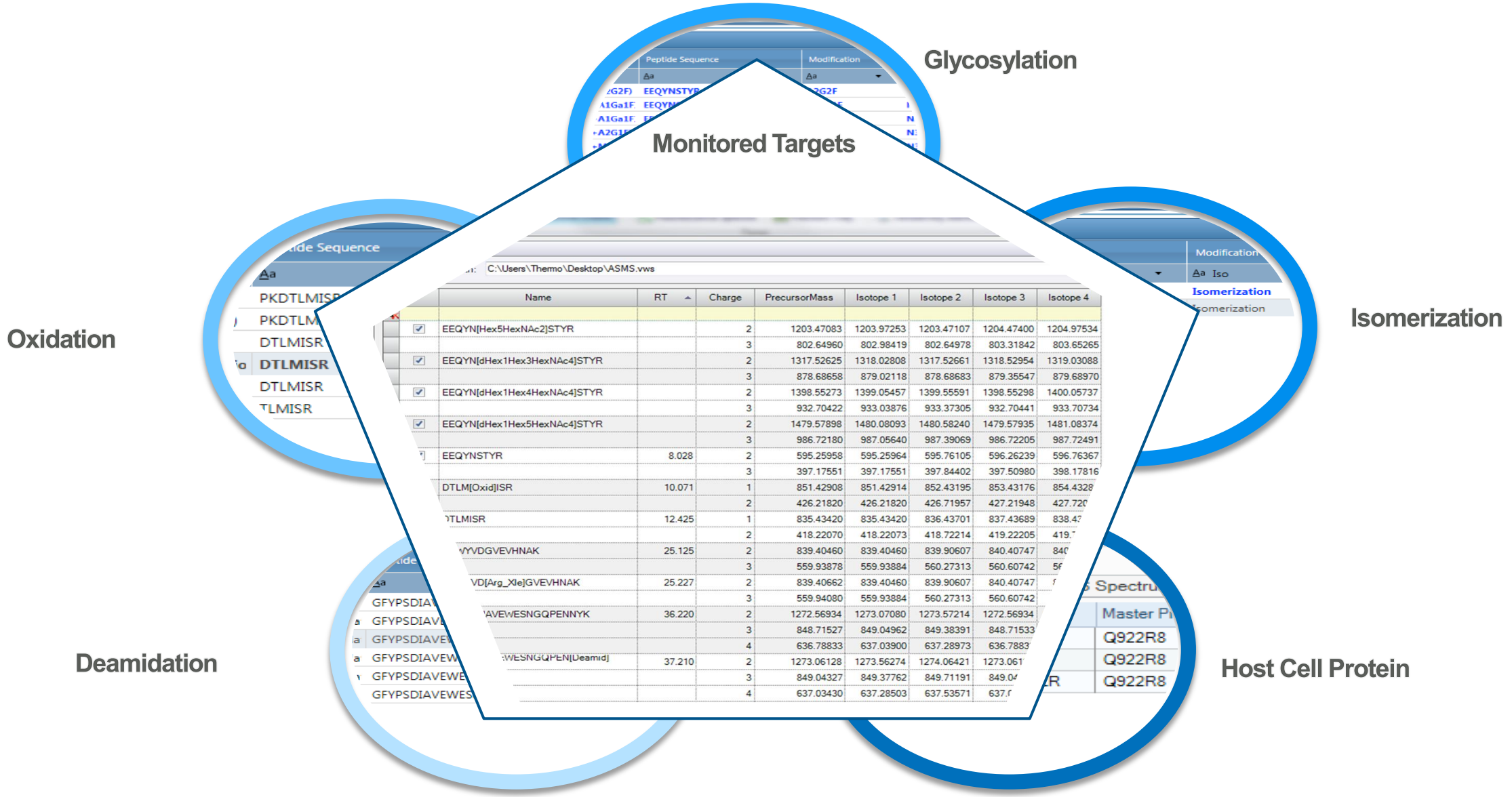
Advantages of MAM

Required CQA Characterizations	Covered by SEC MAM?	CEX	rCE-SDS	nrCE-SDS	HILIC	ID ELISA	HCP ELISA
CDR Tryptophan Degradation	Yes	No	No	No	No	No	No
C-terminal Amidation	Yes	Indirect	No	No	No	No	No
C-terminal Lysine	Yes	Yes	No	No	No	No	No
Cysteine Adducts	Maybe	Maybe	No	No	No	No	No
Deamidation	Yes	Indirect	No	No	No	No	No
Disulfide Isoforms	Maybe	Indirect	No	Yes	No	No	No
Disulfide Reduction	Maybe	No	No	Yes	No	No	No
Fragmentation (Peptide Bond)	Maybe	No	Yes	Yes	No	No	No
Fucosylation	Yes	No	No	No	No	No	No
Galactosylation	Yes	No	No	No	No	No	No
Glycation	Yes	No	Yes	Yes	No	No	No
HCP	Yes	No	No	No	No	No	Yes
High Mannose	Yes	No	No	No	Yes	No	No
Hydroxylysine	Yes	No	No	No	No	No	No
Identity	Yes	Yes	No	No	No	Yes	No
Methionine Oxidation	Yes	No	No	No	No	No	No
Mutations & Misincorporations	Yes	No	No	No	No	No	No
Non-concensus Glycosylation	Yes	No	Maybe	Maybe	No	No	No
Non-glycosylated Heavy Chain	Yes	No	No	No	No	No	No
N-terminal pyroGlutamate	Yes	Indirect	No	No	No	No	No
O-linked Glycans	Maybe	No	No	No	No	No	No
Residual Protein A	Yes	No	No	No	No	No	No
Signal Peptide	Yes	No	No	No	No	No	No
Thioether	Yes	No	No	No	No	No	No
Trisulfide	Maybe	No	No	No	No	No	No
Unusual Glycosylation	Yes	Indirect	Maybe	Maybe	Yes	No	No

Building Targeted List of Critical Quality Attributes



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CQA Profiling

CQA Overview

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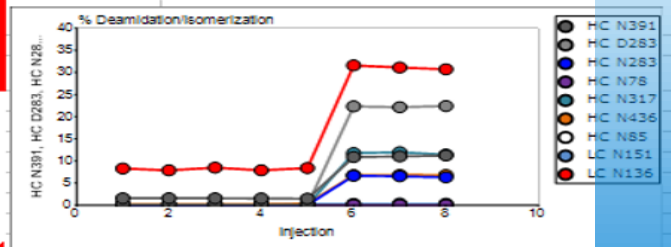
Sequence Details

Name:	HMSVictory		
Instrument:	QEHF_TheGhost	Created On:	09/Nov/16 14:49:34
Imported Data:	False	Updated On:	20/Dec/16 17:07:26
First Injection:	NISTmAb_Control1_DDA_1		
Processing Method:	SuperMAM		
MS Acquisition Time [min]	70.51		
Method Length [min]:	115.00		
Total Time [hrs]	63.25		
Data Vault:	ChromeleonLocal	Created By:	Thermo
No. of Injections:	33	Updated By:	Thermo

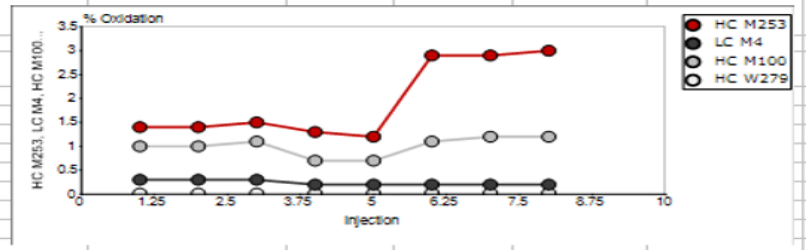
Deamidations and Isomerizations

No.	Name	PENNKY Deam HC N391 %	FNWY IsoD HC D283 %	FNWY Deam HC N283 %	NQVVLK Deam HC N78 %	VVSV Deam HC N317 %	WQQ Deam HC N436 %	YCAR Deam HC N85 %	VDNAL Deam LC N151 %	SGTAS Deam LC N136 %
11	Control_Digest1_Rep1	1.58	0.11	0.04	0.03	0.05	0.23	0.00	0.21	8.3
12	Control_Digest1_Rep2	1.59	0.11	0.04	0.03	0.05	0.24	0.00	0.30	7.9
13	Control_Digest1_Rep3	1.58	0.11	0.03	0.03	0.05	0.24	0.00	0.32	8.5
15	Control_Digest2_Rep1	1.54	0.11	0.04	0.04	0.05	0.23	0.00	0.40	7.9
18	Control_Digest3_Rep1	1.48	0.10	0.04	0.04	0.04	0.40	0.00	0.36	8.4
24	Stressed_Digest2_Rep1	10.89	22.34	6.66	0.04	11.78	6.80	0.12	1.94	31.6
25	Stressed_Digest2_Rep2	11.03	22.14	6.55	0.04	11.98	6.86	0.12	1.68	31.1
26	Stressed_Digest2_Rep3	11.25	22.41	6.31	0.04	11.45	6.84	0.12	1.65	30.7



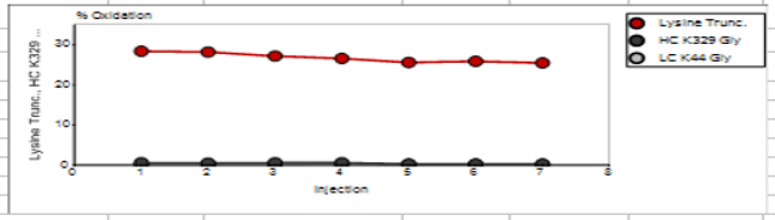
Oxidations

No.	Injection Name	DTLM Oxid HC M253 %	DIQM Oxid LC M4 %	DMIF Oxid HC M100 %	FNWY Oxid HC W279 %
11	Control_Digest1_Rep1	1.4	0.3	1.0	0.01
12	Control_Digest1_Rep2	1.4	0.3	1.0	0.01
13	Control_Digest1_Rep3	1.5	0.3	1.1	0.01
15	Control_Digest2_Rep1	1.3	0.2	0.7	0.01
18	Control_Digest3_Rep1	1.2	0.2	0.7	0.01
24	Stressed_Digest2_Rep1	2.9	0.2	1.1	0.02
25	Stressed_Digest2_Rep2	2.9	0.2	1.2	0.02
26	Stressed_Digest2_Rep3	3.0	0.2	1.2	0.02



Lysine Modifications

No.	Injection Name	SLSSPGK Lysine Trunc. %	VSNK HC K329 Gly %	APK LC K44 Gly %
11	Control_Digest1_Rep1	28.4	0.6	0.1
12	Control_Digest1_Rep2	28.2	0.5	0.1
13	Control_Digest1_Rep3	27.2	0.6	0.1
18	Control_Digest3_Rep1	26.6	0.6	0.2
24	Stressed_Digest2_Rep1	25.6	0.3	0.1
25	Stressed_Digest2_Rep2	25.9	0.3	0.1
26	Stressed_Digest2_Rep3	25.5	0.3	0.1



Sequence Overview | Glycan Profile | Sheet | Master CQA Table | Integration

Custom MS Report Template

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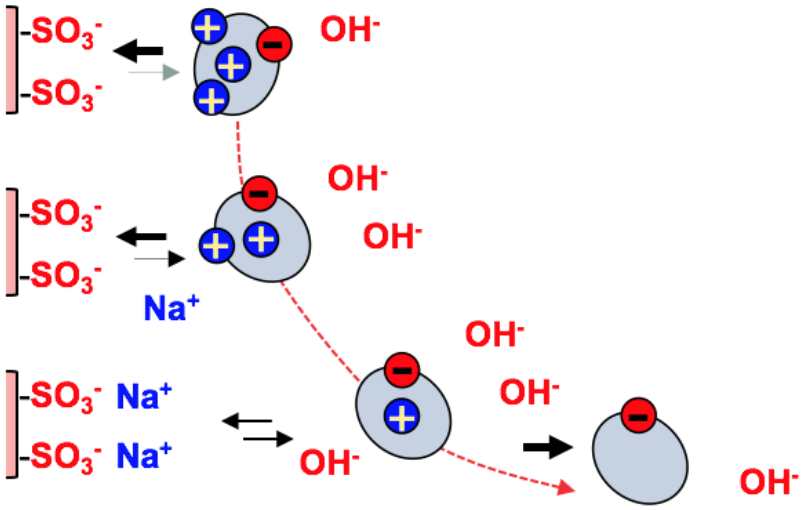
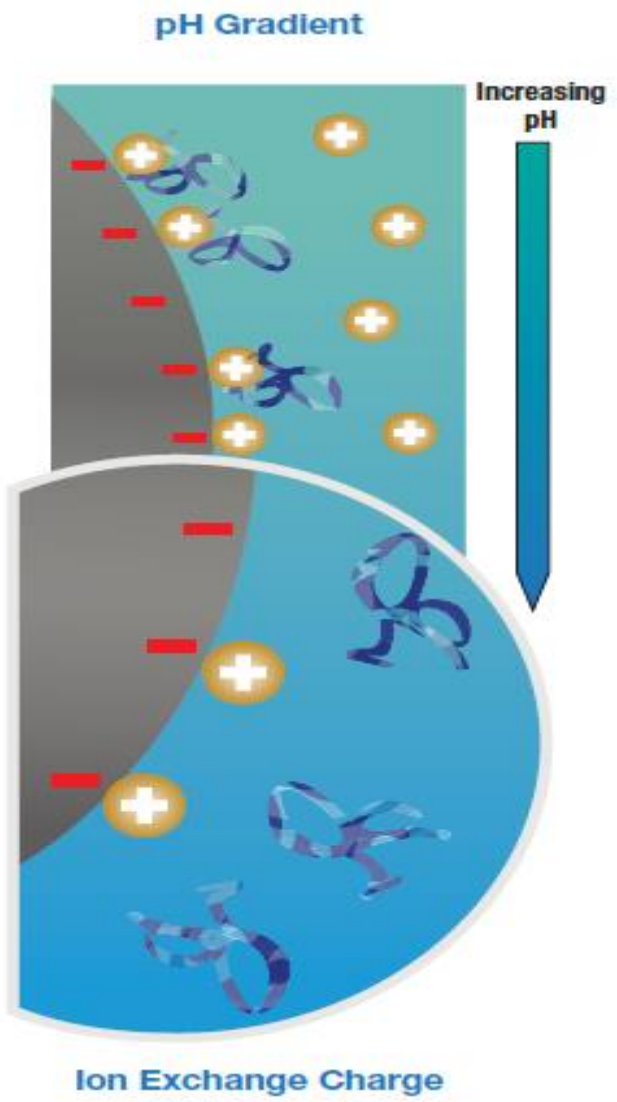
mAb Charge Variant Analysis by CEX pH Gradient Elution

pH gradient elution

- Based on pI of protein
- Loss of retention with progressing pH gradient, depending on pI
- "Single" binding event, trapping at pH < pI (for CEX)

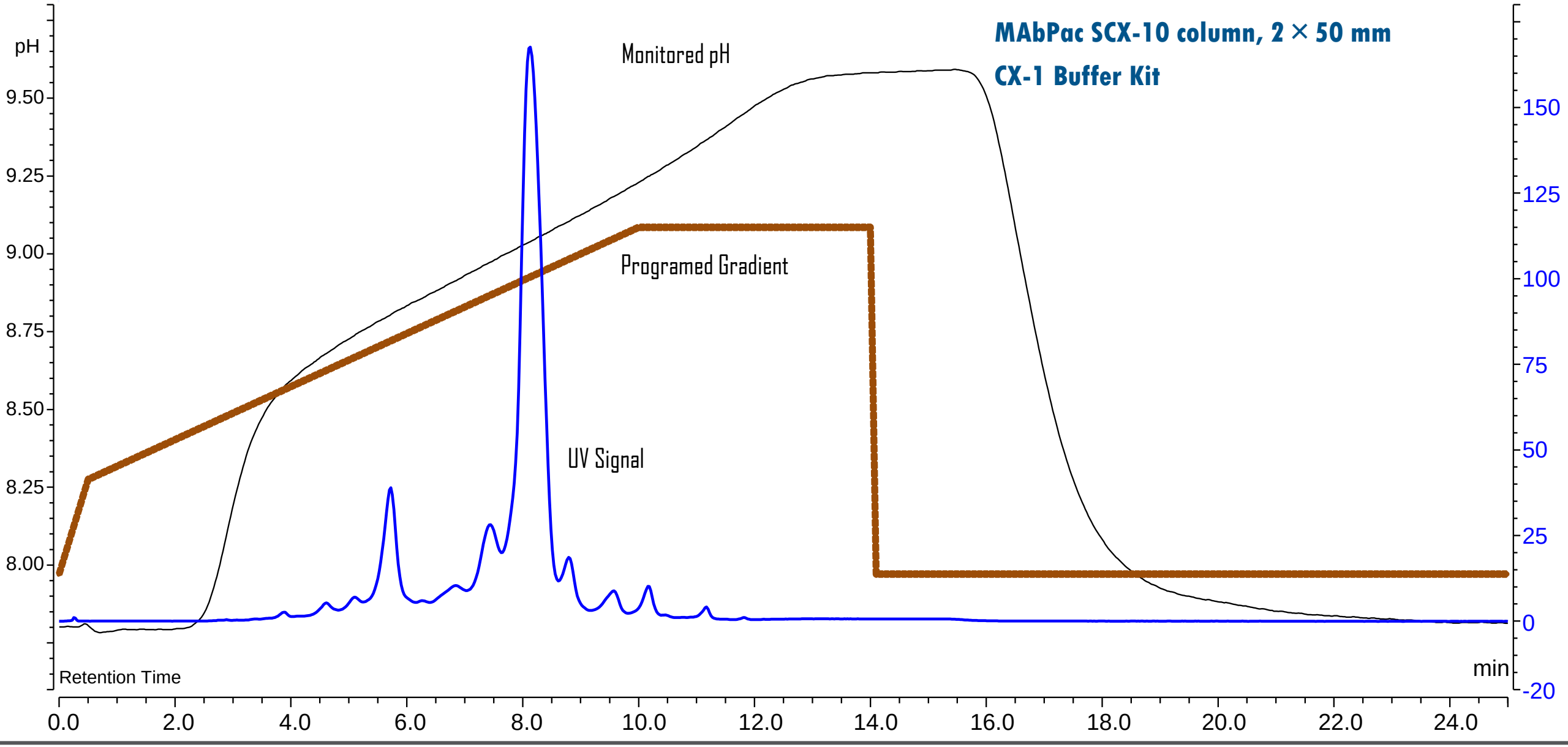




Isoelectric Focusing on a Cation Exchange Column

- mAb binds to cation exchange sites on the column
- A gradient of increasing pH is applied
- mAb is released from the exchange site when the net charge on the mAb is neutral
- This interaction happens once, then the mAb runs through the rest of the column
- Column length has little effect on the resolution
- This is a concentration technique

Trastuzumab pH Gradient with Proprietary Volatile eluents



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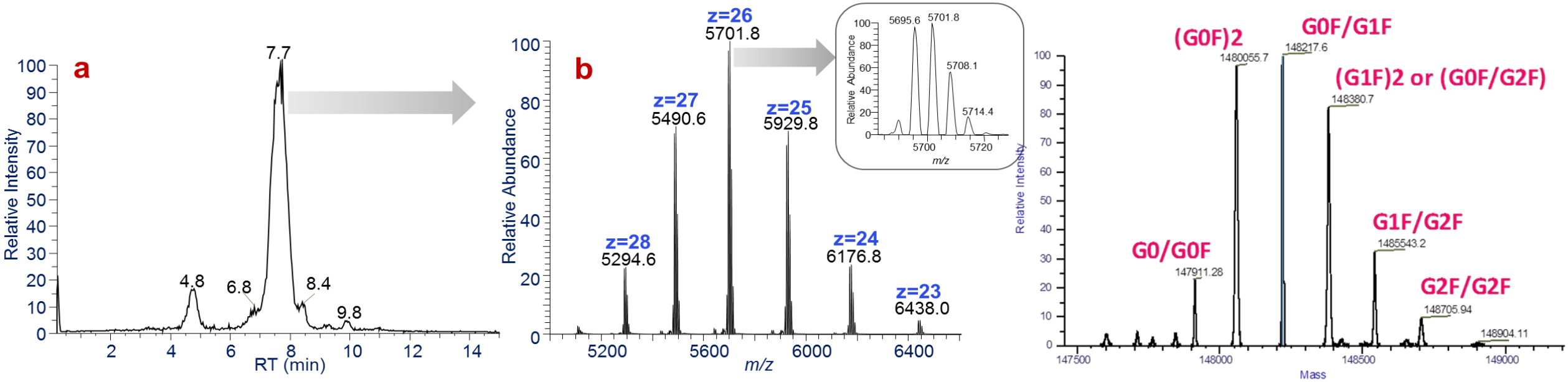
Direct MS analysis of Trastuzumab by Ion Exchange / Native Intact MS



Glycoform	Theoretical Av. Mass	Experimental Av. Mass	D Mass (ppm)
(G0F)2	148056.2	148055.7	3.4
G0F/G1F	148218.3	148217.6	5.0
G0F/G2F or (G1F)2	148380.5	148380.7	-1.5
G1F/G2F	148542.6	148543.2	-3.9

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Conclusions

- Automation of protein digestion for precision and reproducibility in a robust peptide mapping
  workflow to introduce a MAM
- Volatile pH gradient elution of proteins for CVA has several advantages; global applicability, high loading capacity, easy method transfer, native MS compatibility, simple sample preparation
- Simple parent ion MS at high resolution, allowing on-line positive identifications at intact and peptide level
- One Injection, reliable and robust:
  several critical quality attributes
- New workflows for the future enabling characterization of several attributes in one injection provides ease of use and time saving