

Mass Spec MAM for Product Characterization and Release

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What is MAM?

- MAM = Multi-Attribute Method
 - The name came from the MS in QC project at Amgen.
- MAM consists of 2 components.
 - Attribute Analytics (Monitoring)
 - New Peak Detection
- The main goal of implementing MAM is to replace classic QC release assays.
- We do not want to add assays to QC.
- MAM is now being applied for large molecule and cell-based therapies.

Single attribute testing used to ensure product quality

1. CE-SDS
2. CEX/cIEF
3. Glycan Map
4. ID ELISA
5. HCP ELISA
6. pA ELISA
7. Color
8. Clarity
9. Osmolality
10. pH
11. SEC
12. A280
13. qPCR
14. Endotoxins
15. Bioburden
16. Bioassay

Mass Spec based Multi-Attribute Method (MAM)

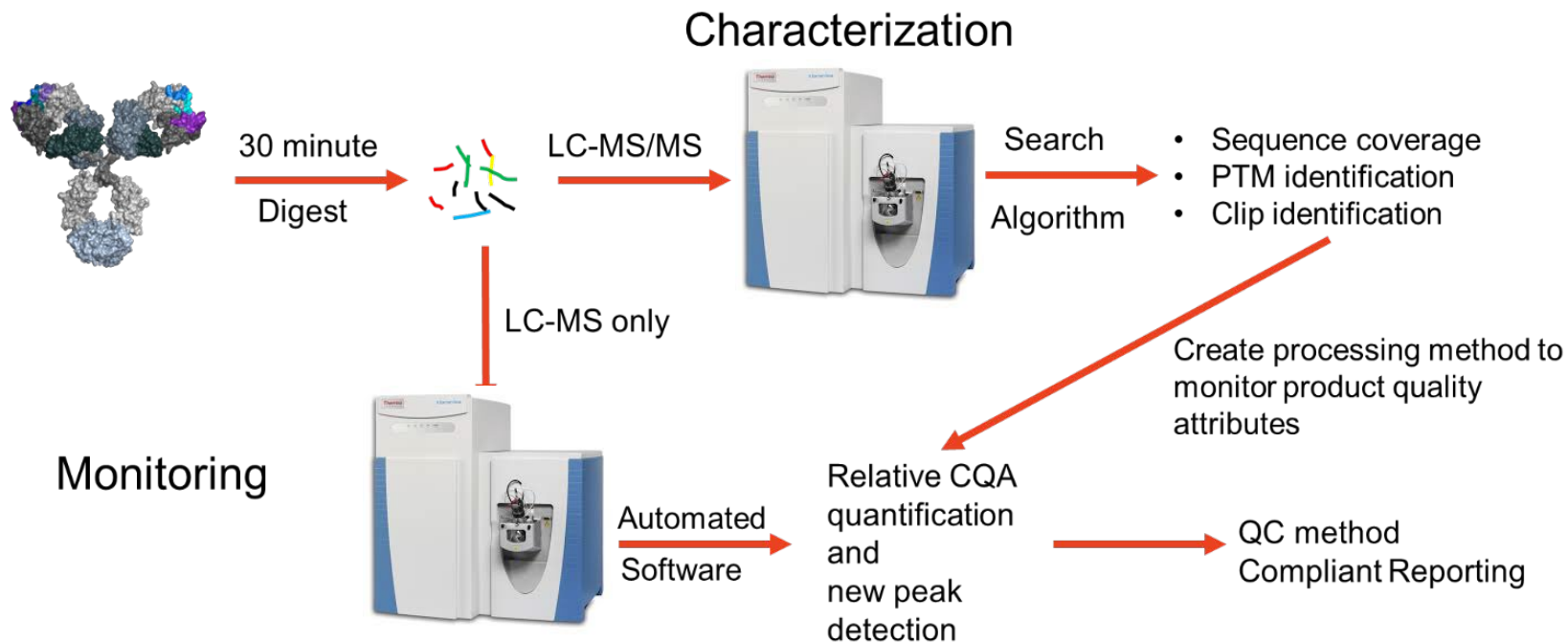
Attribute	Current Method	PAC and Release
Clips	rCE-SDS	Multi-Attribute Method
Charge Variants	CEX-HPLC	
Glycans	Glycan Map	
Identity	Immunoassay	
Process Impurities	HCP-ELISA, Prot-A-ELISA	

The Evolution of MAM



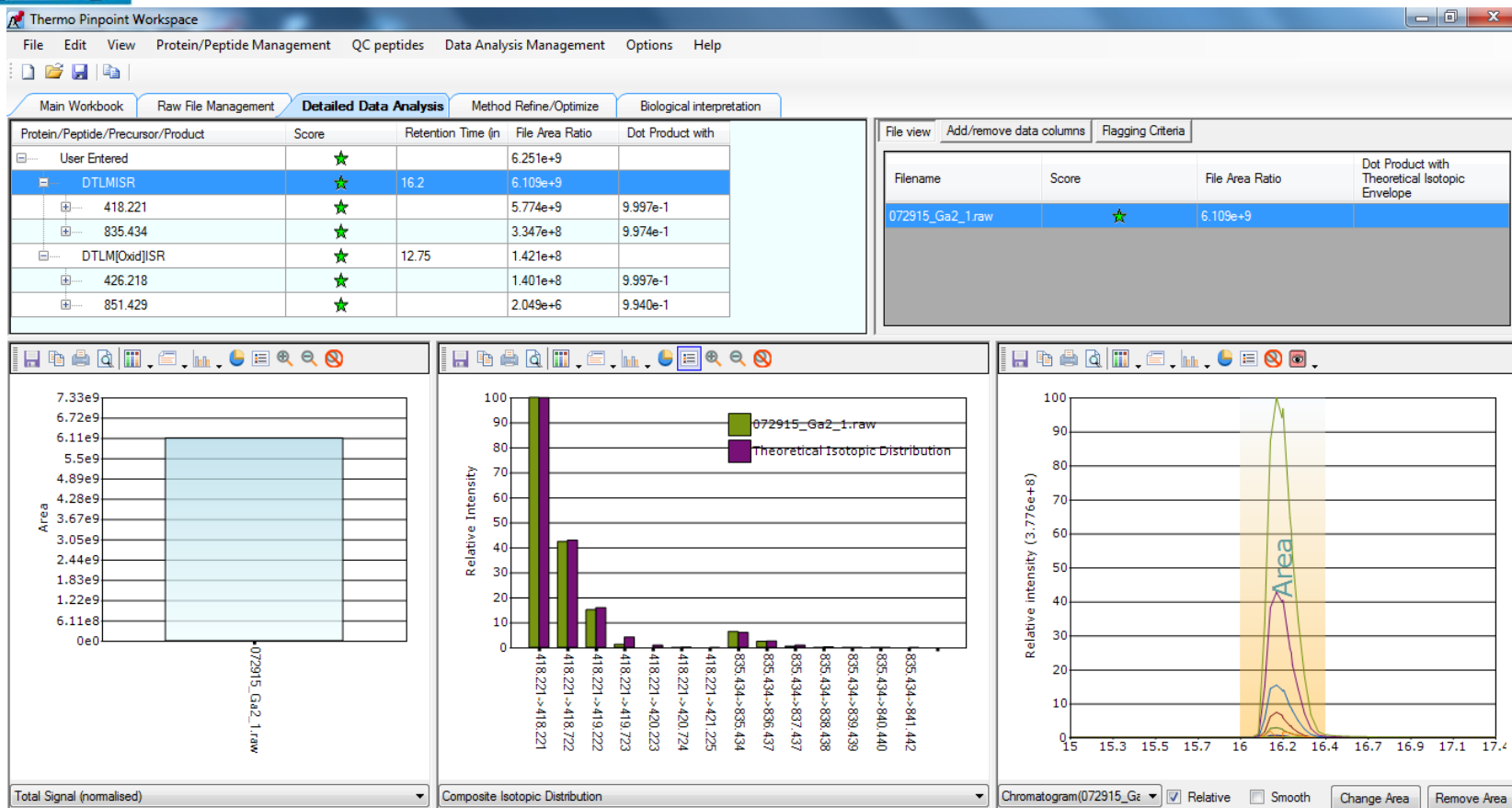
2012

Workflow for MAM (Trypsin)

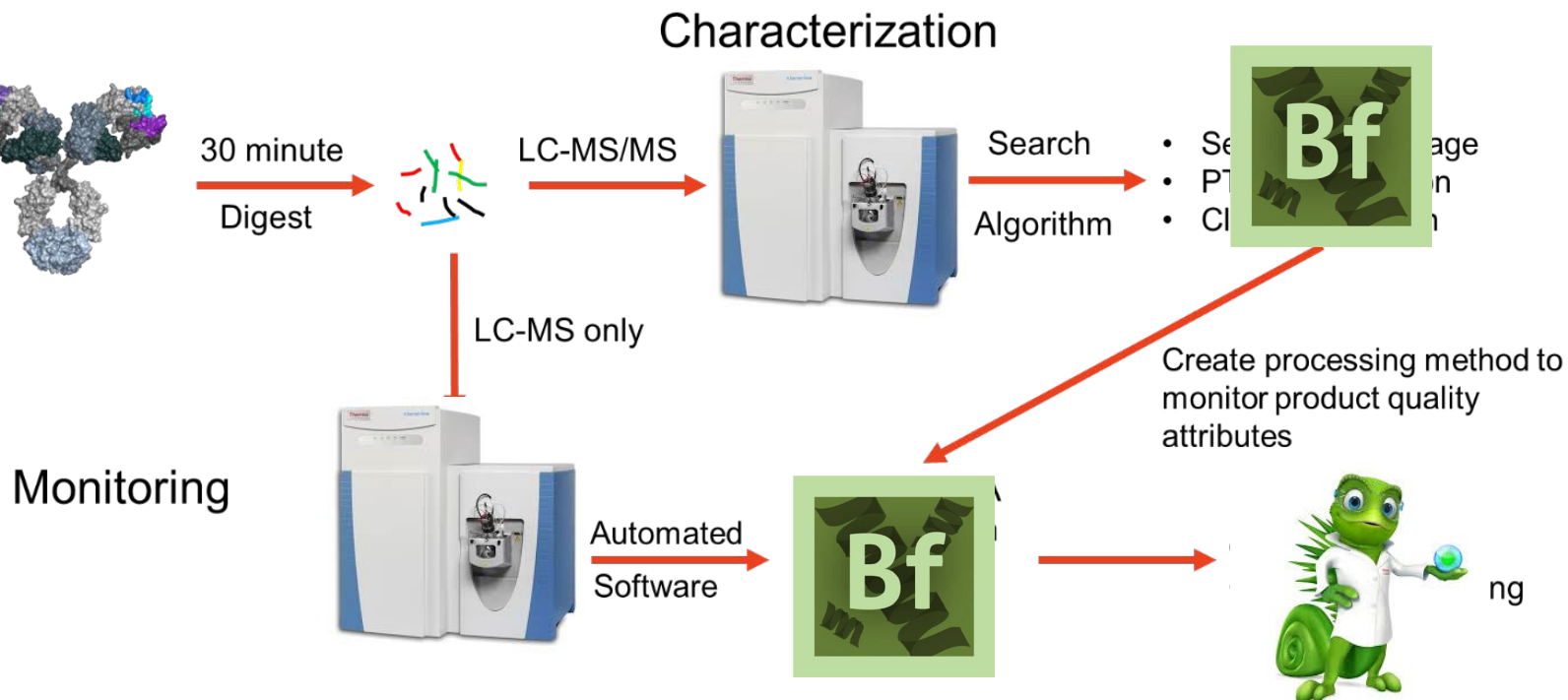




Attribute Analytics Results



Workflow for MAM (Trypsin)



Live Demo for creating an
attribute workbook



Targeted Peptide Workbook

▼ Add or Edit Target m/z

☒ Apply to All Peptides
 Charge State Range to
Number of Isotopes

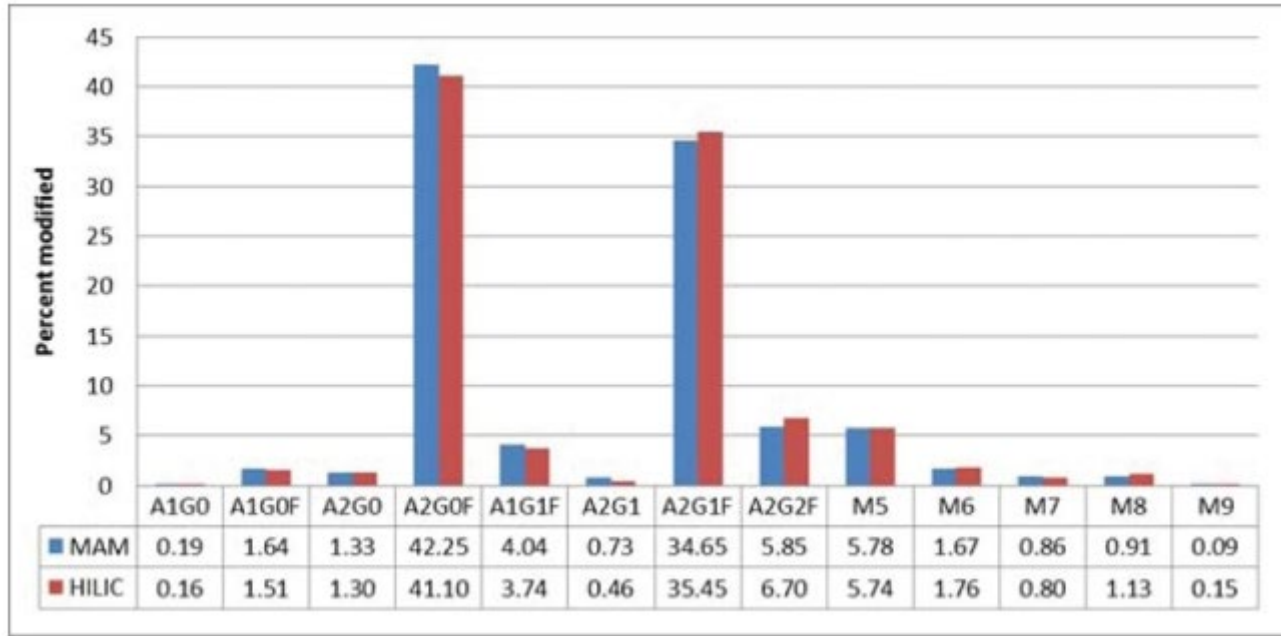
☐ Apply to Selected Peptides
 m/z Range to (The recommend number of isotopes for a peptide is between 3 and 5)

Peptide Workbook

	Level	Identification	Normalized Id	Peptide Sequence	Modification	Site	Normalized Site	Relative Quantitation Group Number	Charge State Distribution	RT (min)	RT Start (min)	RT Stop (min)	Mono Mass Theo.	Protein Name
	Aa	Aa	Aa	Aa	Aa	Aa	Aa	=	Aa	=	=	=	=	Aa
1	☐	Peptide	1:V149-K168 = 2134.... V1-K20 = 2134.9614...	VDNALQSGNSQESV...	Isomerization	D150	D2	1	3 - 3	10.94	10.85	11.05	2134.9614	NistMab_LC
2	☐	Peptide	1:V149-K168 = 2134.... V1-K20 = 2134.9614m	VDNALQSGNSQESV...	None			1	3 - 3	11.38	11.30	11.49	2134.9614	NistMab_LC
3	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	A2Ga2F	N300	N5	2	3 - 3	9.59	9.52	9.68	3281.2501	NistMab_HC
4	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	A2Ga1G1F	N300	N5	2	3 - 3	9.60	9.52	9.69	3119.1972	NistMab_HC
5	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	A2G2F	N300	N5	2	2 - 3	9.64	9.50	9.76	2957.1444	NistMab_HC
6	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	A2G1F	N300	N5	2	2 - 3	9.65	9.57	9.77	2795.0915	NistMab_HC
7	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	A1G1M4F	N300	N5	2	3 - 3	9.66	9.58	9.79	2754.0649	NistMab_HC
8	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	M5	N300	N5	2	2 - 2	9.66	9.60	9.77	2404.9277	NistMab_HC
9	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	A1G1F	N300	N5	2	2 - 3	9.67	9.59	9.76	2592.0122	NistMab_HC
10	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	A2G0F	N300	N5	2	2 - 3	9.66	9.59	9.76	2633.0387	NistMab_HC
11	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	A1G0F	N300	N5	2	2 - 3	9.68	9.60	9.76	2429.9593	NistMab_HC
12	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	A1Sg1F	N300	N5	2	3 - 3	9.73	9.65	9.82	2899.1024	NistMab_HC
13	☐	Peptide	2:E296-R304 = 1188.... E1-R9 = 1188.5047m...	EEQYNSTYR	Unglycosylated	N300	N5	2	2 - 2	9.87	9.82	9.96	1188.5048	NistMab_HC
14	☐	Peptide	G1-K22 = 2543.1241... G1-K22 = 2543.1241...	GFYPSDIAVEWESNG...	Deamidation	N14	N14	3	2 - 3	40.05	39.86	40.24	2544.1081	NistMab_HC
15	☐	Peptide	G1-K22 = 2543.1241m G1-K22 = 2543.1241m	GFYPSDIAVEWESNG...				3	2 - 3	40.48	40.35	40.76	2543.1241	NistMab_HC
16	☐	Peptide	G1-K22 = 2543.1241... G1-K22 = 2543.1241...	GFYPSDIAVEWESNG...	Deamidation	N14	N14	3	2 - 3	41.02	40.85	41.30	2544.1081	NistMab_HC
17	☐	Peptide	G1-K22 = 2543.1241... G1-K22 = 2543.1241...	GFYPSDIAVEWESNG...	Deamidation	N14	N14	3	2 - 3	41.34	41.25	41.45	2544.1081	1

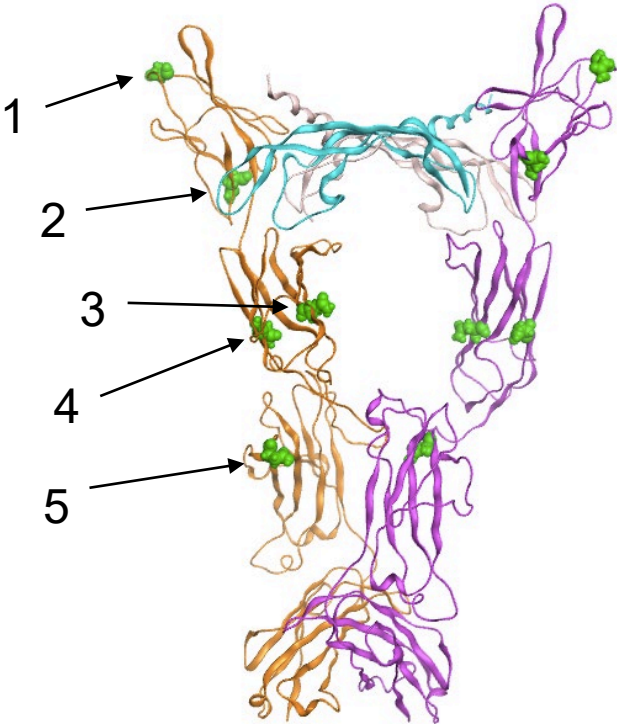
- Set the targeted workbook parameters
- Confirm group assignments

Comparison to conventional assays - Glycans



MAM provides site specific glycosylation

The HILIC glycan assay only provides a global snapshot of glycosylation

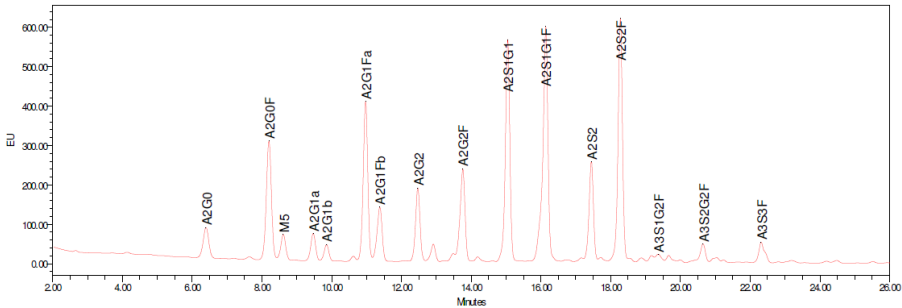


MAM

N1	N2	N3	N4	N5
A2S2F	NG	A2S1G1	A1S1	A2G1F
A2S1G1F	A2S1G1F	M5	A1G0	A2G0F
Gn	A2S2F	A1S1M4	M3	A2G2F
A3S3F	A2G2F	A1S1M5	M4	A2G0
A3S2G1F	A3S2G1F	A1S1	M5	A2G1
GnF	A3S3F	A2S1G1F	A2S1G1	A2S1G1F
A2S1Sg1F	A3S1G2F	A2G2	A1G1	A2G2
A2G2F	A2S1G1	A2S2	A2S1G1F	A1G1F
A3S1G2F	A2S1G0F	A1G1M5	M6	A1G0F
A2S1G0F	A2G1F	A1G1M4	A1G1M4	A1G1
NG	A1G1F	A2S1	A2S2	NG
A1S1F	A1S1M5F	A1G1	A2G1	A1S1F

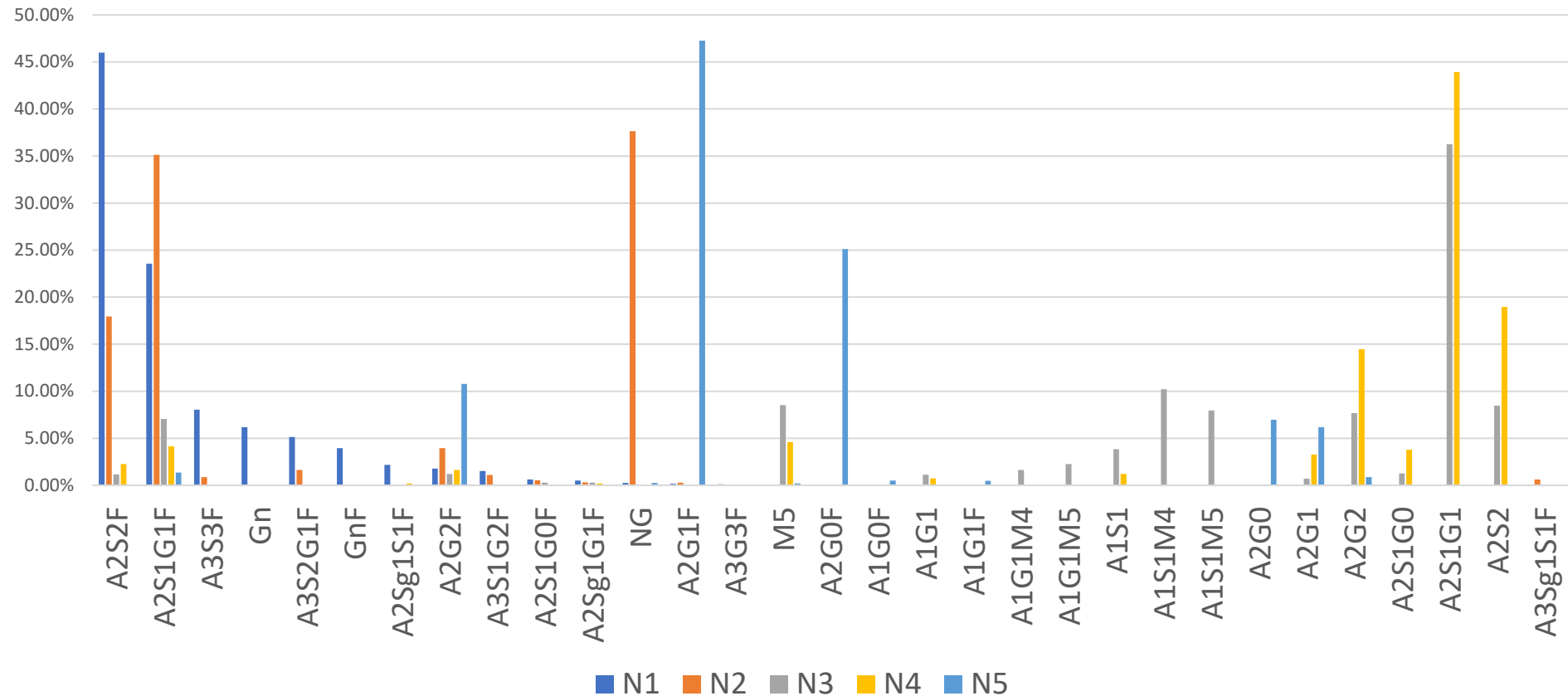
Abbreviated list of glycans on each site

HILIC

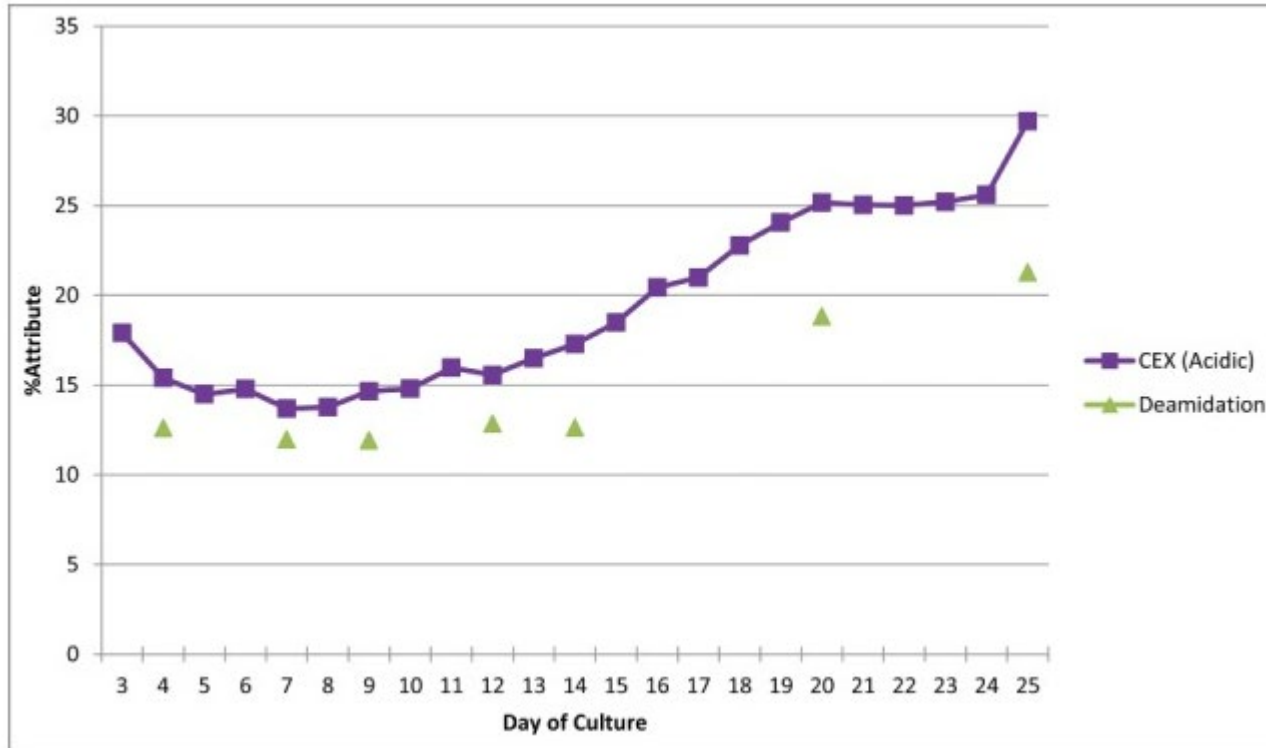


Fc-Fusion Molecule

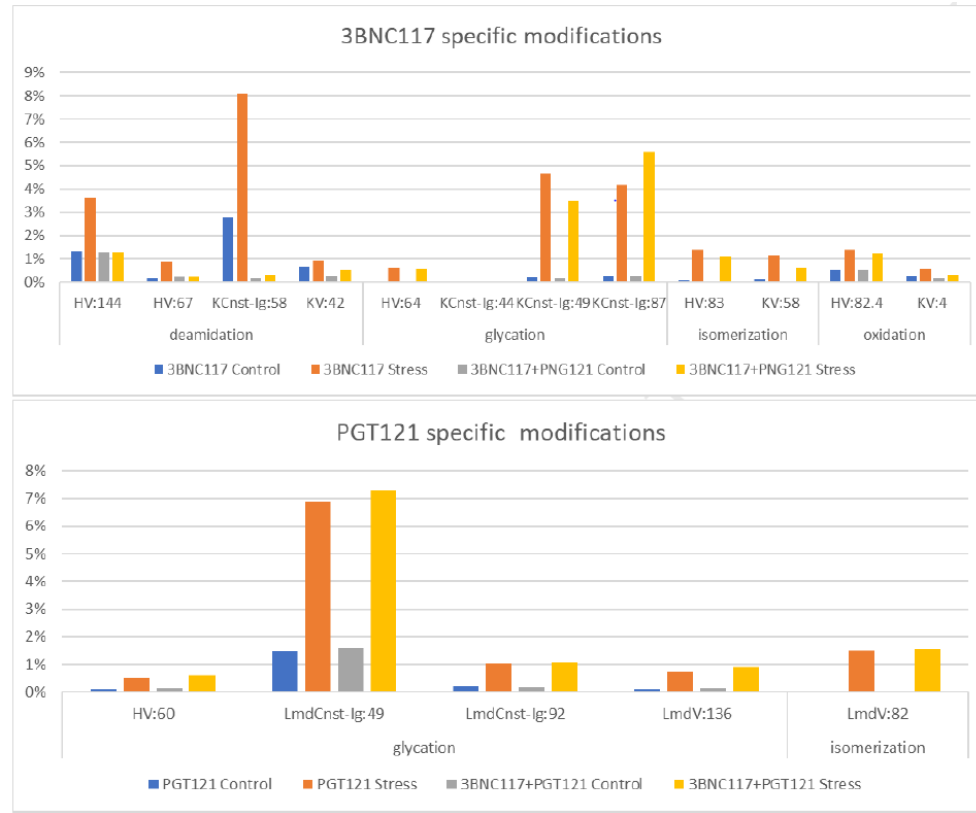
MAM Relative Glycosylation



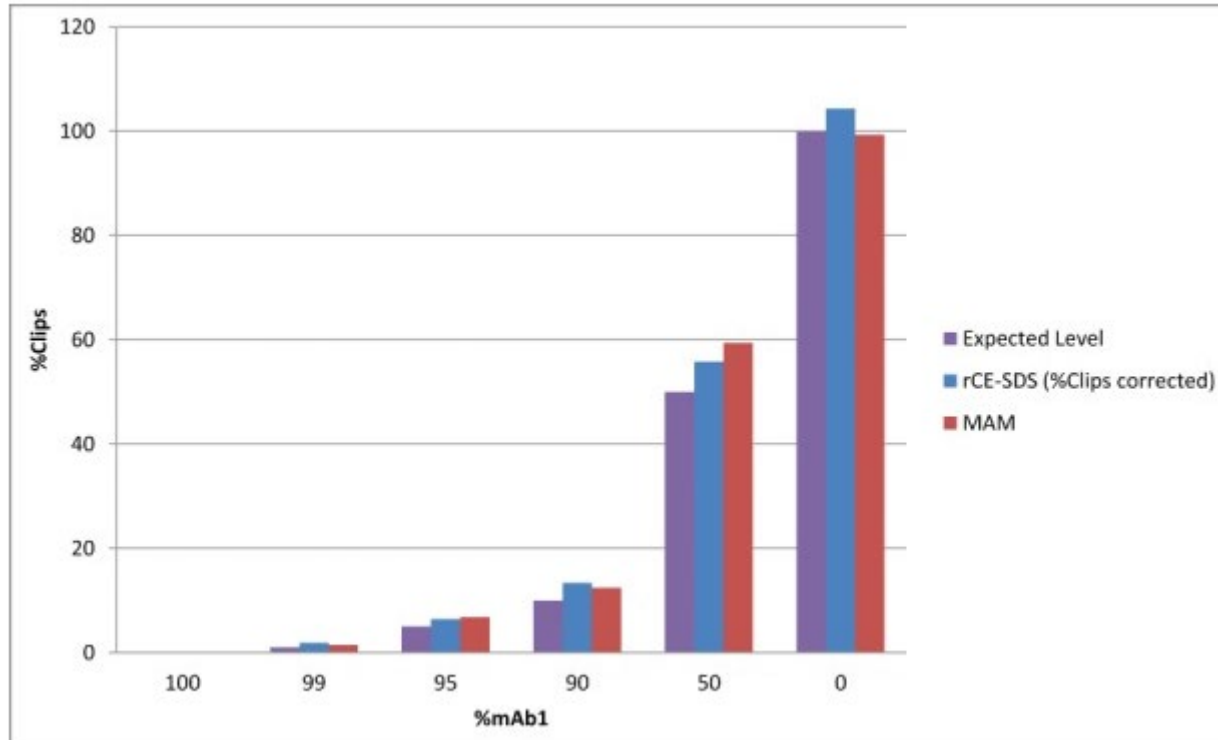
Comparison to conventional assays - Deamidation



Attribute Analytics: Co-formulated mAbs

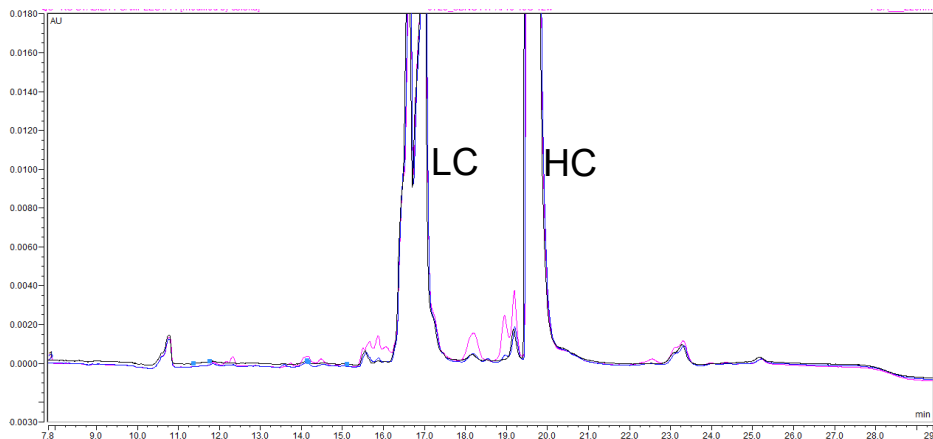


Comparison to conventional assays - Clips



New Peak Detection can identify clips

Reduced CE of Mab1 in different formulations

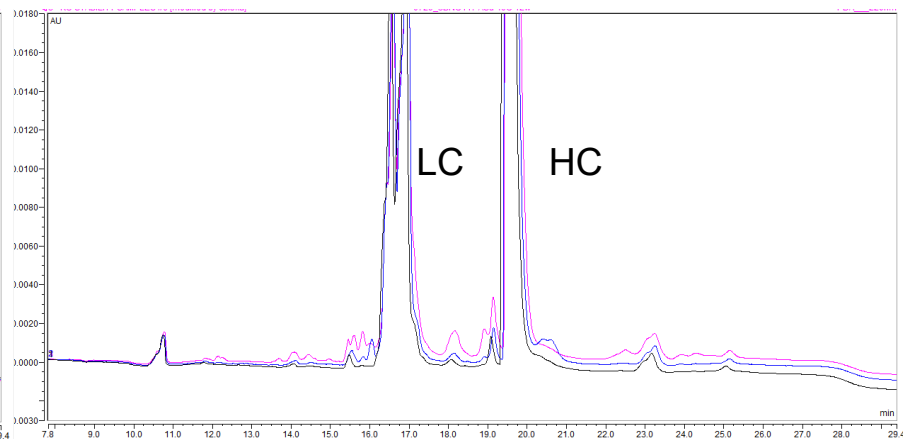


Mab1 Formulation 1

-80C

25C

40C



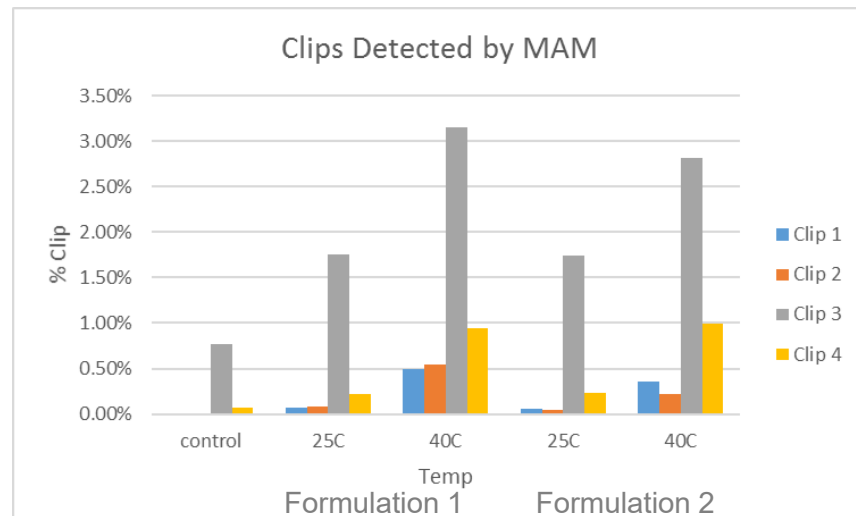
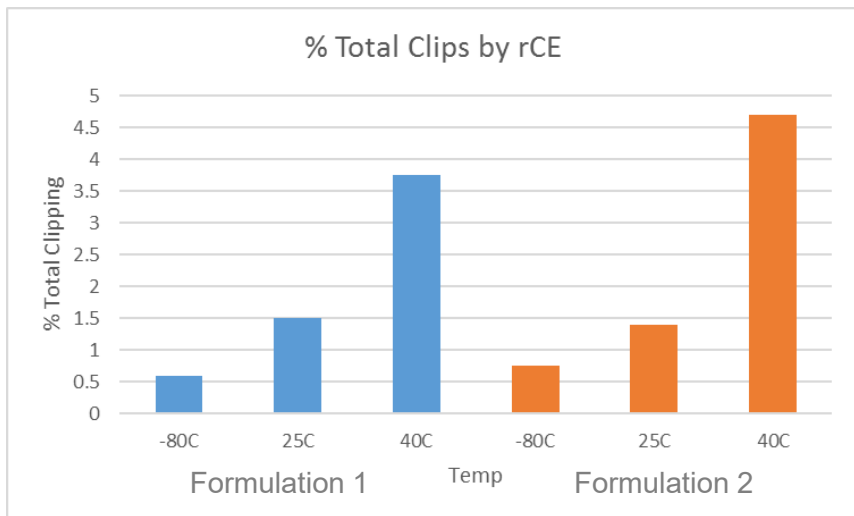
Mab1 Formulation 2

-80C

25C

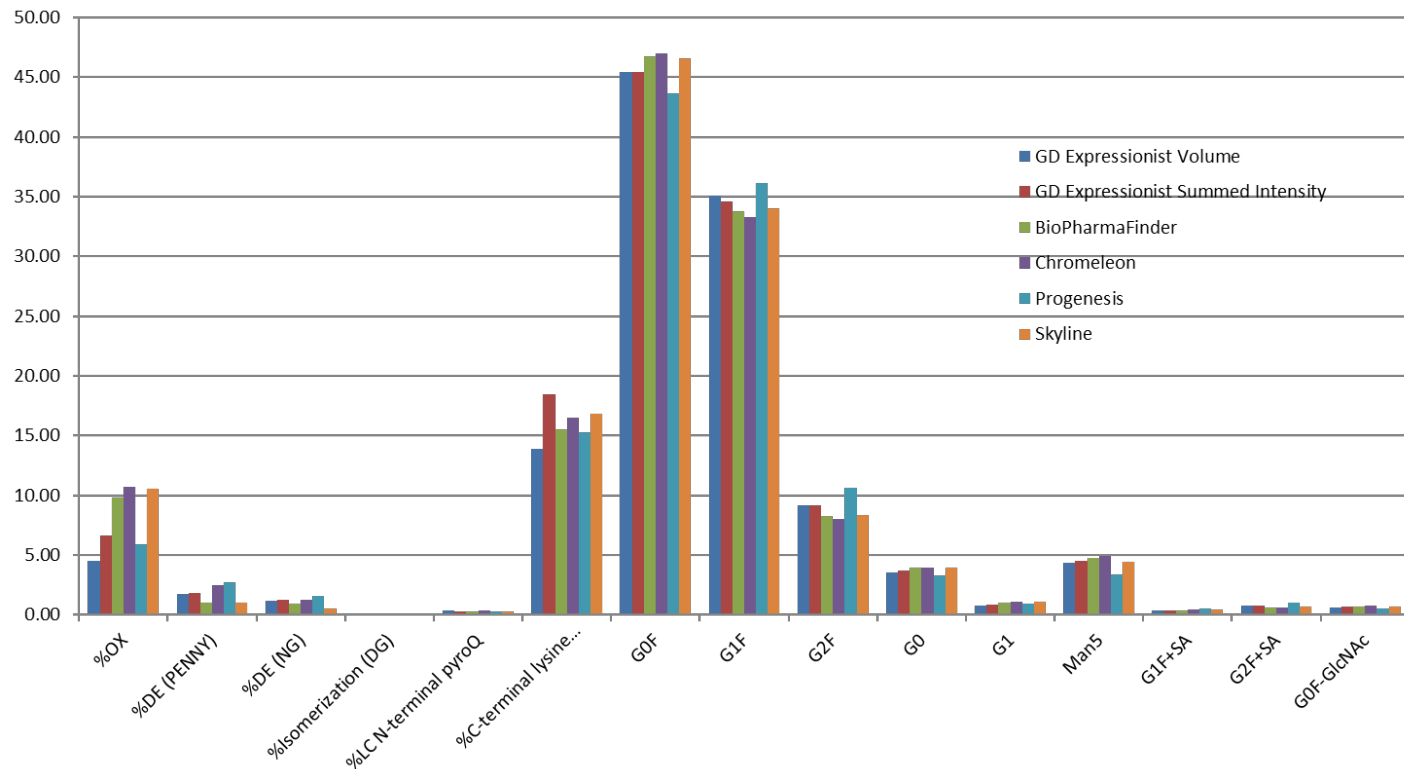
40C

Comparison of Clips Detected by rCE vs MAM



The MAM enables the identification of the site of clipping

Same Data file - Different software algorithms



Pay attention to parameters used for relative quantification

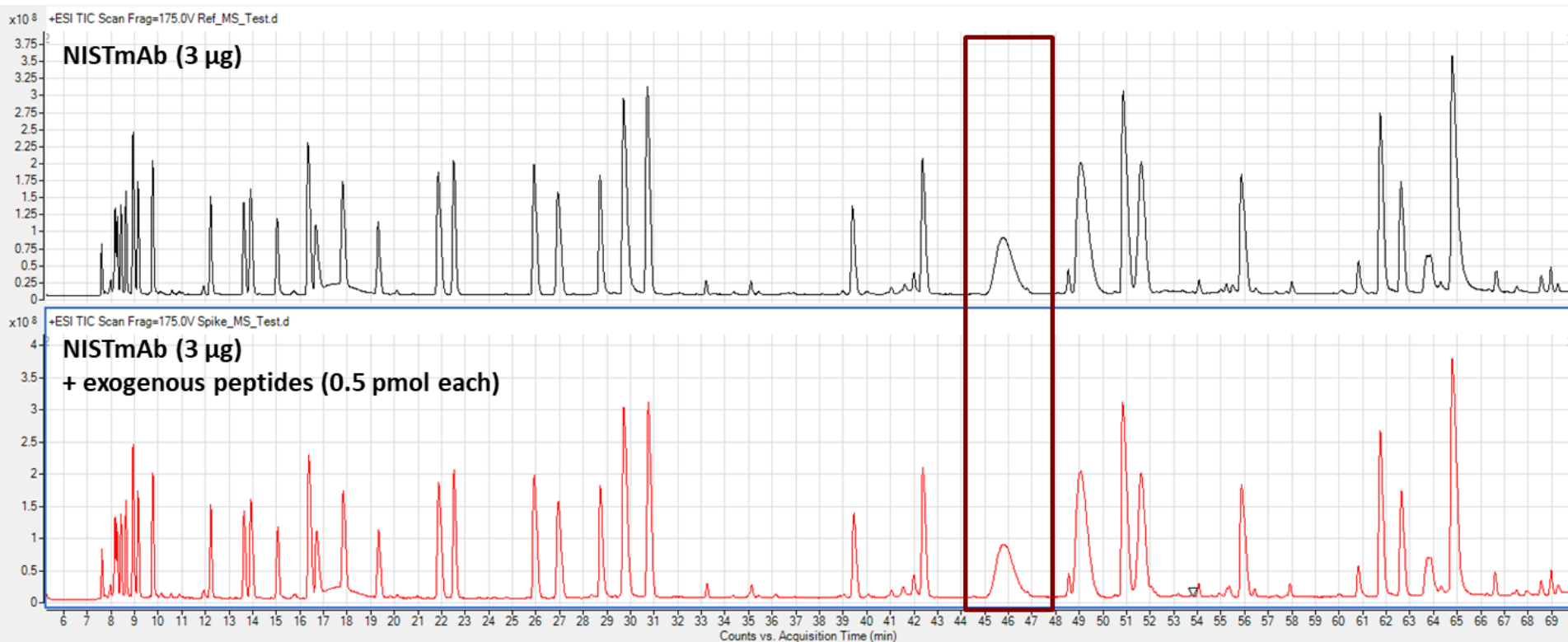
Attributes that can be monitored with the MAM

- Deamidation
- Glycation
- Glycosylation
- Oxidation
- Signal sequence
- Clips
- Unusual Glycosylation
- C-term K
- HCP and pA
- Mutations
- NGHC
- Isomerization
- Pyro-Glu

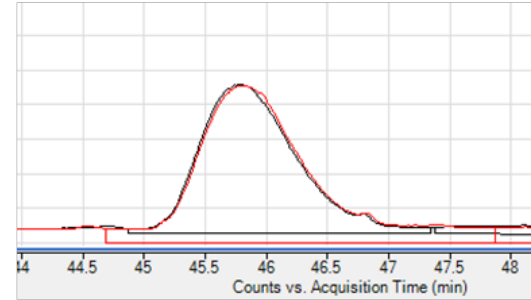
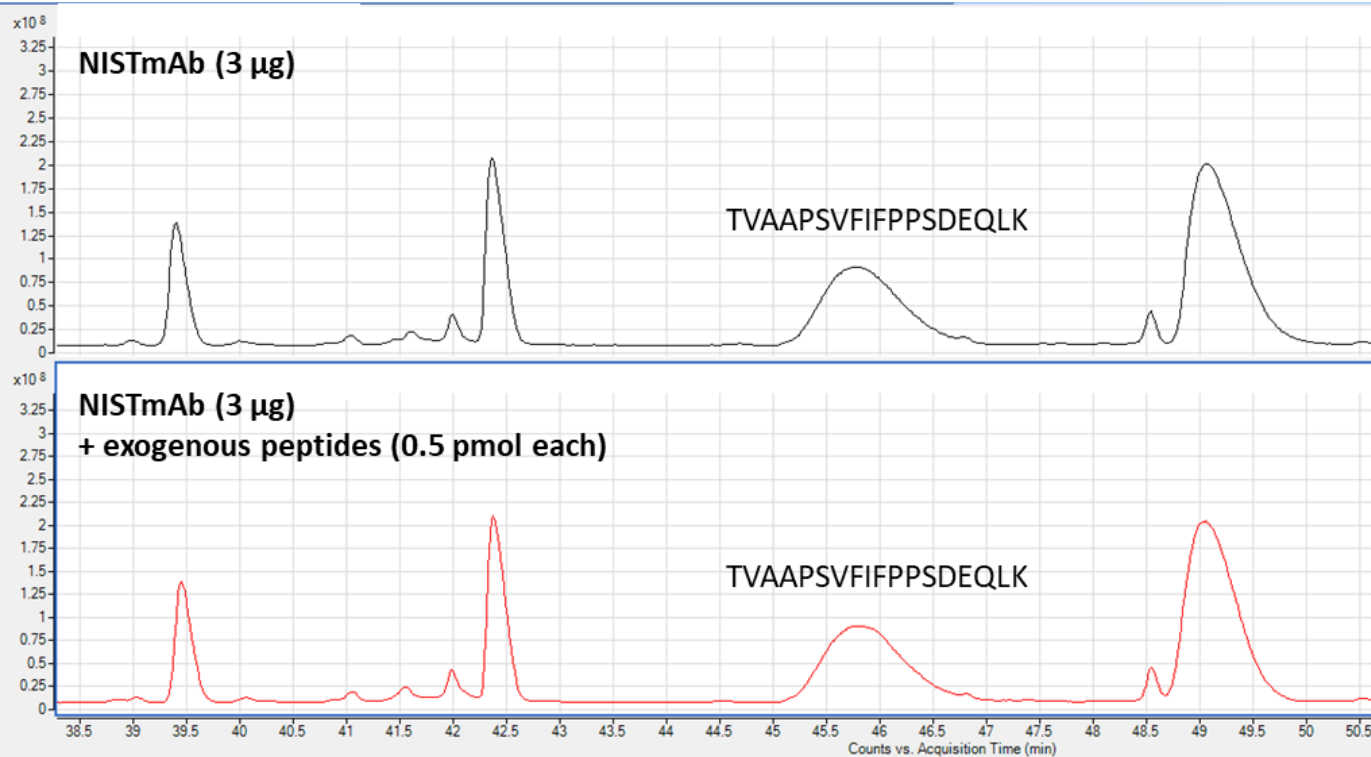
Mass Spec based new peak detection

- Essential for releasing biotherapeutics from QC
- Automated new peak detection is more sensitive than current purity tests employed by QC
- Incredibly powerful tool for detecting new critical quality attributes (revealed during stability assessment)
- Use of filters limits false positives

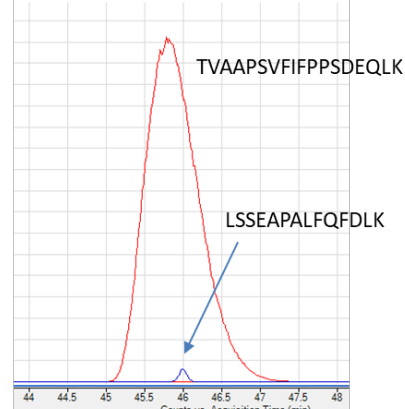
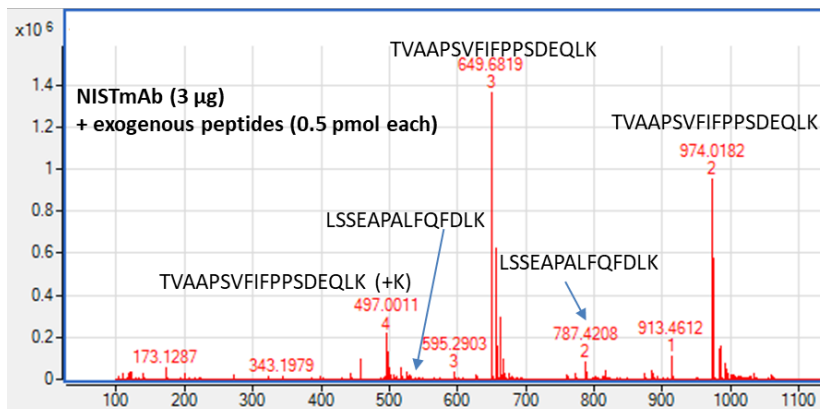
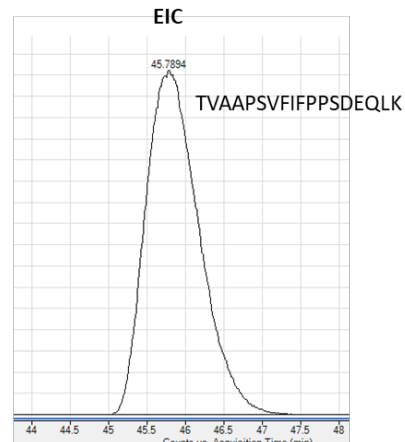
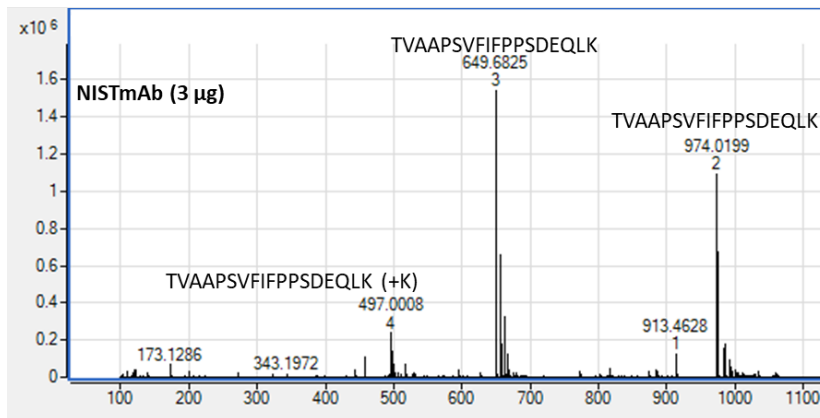
MAM: New Peak Detection



MAM: New Peak Detection



MAM: New Peak Detection



Live Demo for NPD

Set the fold change

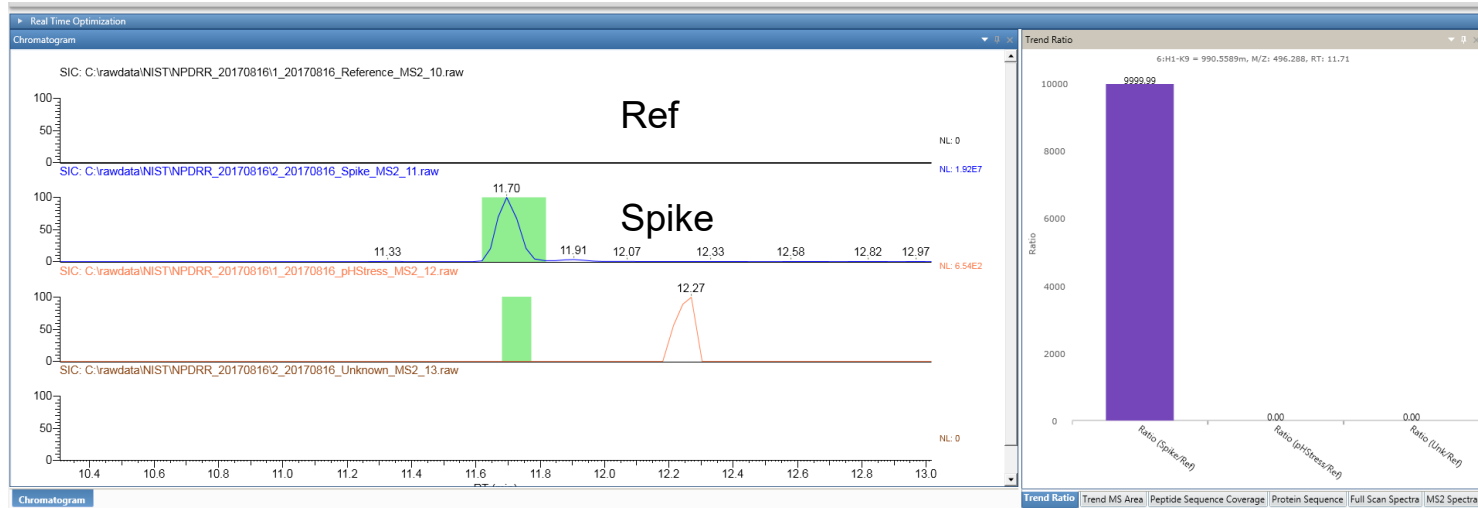
Set the intensity threshold

Set the reference file

V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}	V_{13}	V_{14}	V_{15}	V_{16}	V_{17}	V_{18}	V_{19}	V_{20}	V_{21}	V_{22}	V_{23}	V_{24}	V_{25}	V_{26}	V_{27}	V_{28}	V_{29}	V_{30}	V_{31}	V_{32}	V_{33}	V_{34}	V_{35}	V_{36}	V_{37}	V_{38}	V_{39}	V_{40}	V_{41}	V_{42}	V_{43}	V_{44}	V_{45}	V_{46}	V_{47}	V_{48}	V_{49}	V_{50}	V_{51}	V_{52}	V_{53}	V_{54}	V_{55}	V_{56}	V_{57}	V_{58}	V_{59}	V_{60}	V_{61}	V_{62}	V_{63}	V_{64}	V_{65}	V_{66}	V_{67}	V_{68}	V_{69}	V_{70}	V_{71}	V_{72}	V_{73}	V_{74}	V_{75}	V_{76}	V_{77}	V_{78}	V_{79}	V_{80}	V_{81}	V_{82}	V_{83}	V_{84}	V_{85}	V_{86}	V_{87}	V_{88}	V_{89}	V_{90}	V_{91}	V_{92}	V_{93}	V_{94}	V_{95}	V_{96}	V_{97}	V_{98}	V_{99}	V_{100}	V_{101}	V_{102}	V_{103}	V_{104}	V_{105}	V_{106}	V_{107}	V_{108}	V_{109}	V_{110}	V_{111}	V_{112}	V_{113}	V_{114}	V_{115}	V_{116}	V_{117}	V_{118}	V_{119}	V_{120}	V_{121}	V_{122}	V_{123}	V_{124}	V_{125}	V_{126}	V_{127}	V_{128}	V_{129}	V_{130}	V_{131}	V_{132}	V_{133}	V_{134}	V_{135}	V_{136}	V_{137}	V_{138}	V_{139}	V_{140}	V_{141}	V_{142}	V_{143}	V_{144}	V_{145}	V_{146}	V_{147}	V_{148}	V_{149}	V_{150}	V_{151}	V_{152}	V_{153}	V_{154}	V_{155}	V_{156}	V_{157}	V_{158}	V_{159}	V_{160}	V_{161}	V_{162}	V_{163}	V_{164}	V_{165}	V_{166}	V_{167}	V_{168}	V_{169}	V_{170}	V_{171}	V_{172}	V_{173}	V_{174}	V_{175}	V_{176}	V_{177}	V_{178}	V_{179}	V_{180}	V_{181}	V_{182}	V_{183}	V_{184}	V_{185}	V_{186}	V_{187}	V_{188}	V_{189}	V_{190}	V_{191}	V_{192}	V_{193}	V_{194}	V_{195}	V_{196}	V_{197}	V_{198}	V_{199}	V_{200}	V_{201}	V_{202}	V_{203}	V_{204}	V_{205}	V_{206}	V_{207}	V_{208}	V_{209}	V_{210}	V_{211}	V_{212}	V_{213}	V_{214}	V_{215}	V_{216}	V_{217}	V_{218}	V_{219}	V_{220}	V_{221}	V_{222}	V_{223}	V_{224}	V_{225}	V_{226}	V_{227}	V_{228}	V_{229}	V_{230}	V_{231}	V_{232}	V_{233}	V_{234}	V_{235}	V_{236}	V_{237}	V_{238}	V_{239}	V_{240}	V_{241}	V_{242}	V_{243}	V_{244}	V_{245}	V_{246}	V_{247}	V_{248}	V_{249}	V_{250}	V_{251}	V_{252}	V_{253}	V_{254}	V_{255}	V_{256}	V_{257}	V_{258}	V_{259}	V_{260}	V_{261}	V_{262}	V_{263}	V_{264}	V_{265}	V_{266}	V_{267}	V_{268}	V_{269}	V_{270}	V_{271}	V_{272}	V_{273}	V_{274}	V_{275}	V_{276}	V_{277}	V_{278}	V_{279}	V_{280}	V_{281}	V_{282}	V_{283}	V_{284}	V_{285}	V_{286}	V_{287}	V_{288}	V_{289}	V_{290}	V_{291}	V_{292}	V_{293}	V_{294}	V_{295}	V_{296}	V_{297}	V_{298}	V_{299}	V_{300}	V_{301}	V_{302}	V_{303}	V_{304}	V_{305}	V_{306}	V_{307}	V_{308}	V_{309}	V_{310}	V_{311}	V_{312}	V_{313}	V_{314}	V_{315}	V_{316}	V_{317}	V_{318}	V_{319}	V_{320}	V_{321}	V_{322}	V_{323}	V_{324}	V_{325}	V_{326}	V_{327}	V_{328}	V_{329}	V_{330}	V_{331}	V_{332}	V_{333}	V_{334}	V_{335}	V_{336}	V_{337}	V_{338}	V_{339}	V_{340}	V_{341}	V_{342}	V_{343}	V_{344}	V_{345}	V_{346}	V_{347}	V_{348}	V_{349}	V_{350}	V_{351}	V_{352}	V_{353}	V_{354}	V_{355}	V_{356}	V_{357}	V_{358}	V_{359}	V_{360}	V_{361}	V_{362}	V_{363}	V_{364}	V_{365}	V_{366}	V_{367}	V_{368}	V_{369}	V_{370}	V_{371}	V_{372}	V_{373}	V_{374}	V_{375}	V_{376}	V_{377}	V_{378}	V_{379}	V_{380}	V_{381}	V_{382}	V_{383}	V_{384}	V_{385}	V_{386}	V_{387}	V_{388}	V_{389}	V_{390}	V_{391}	V_{392}	V_{393}	V_{394}	V_{395}	V_{396}	V_{397}	V_{398}	V_{399}	V_{400}	V_{401}	V_{402}	V_{403}	V_{404}	V_{405}	V_{406}	V_{407}	V_{408}	V_{409}	V_{410}	V_{411}	V_{412}	V_{413}	V_{414}	V_{415}	V_{416}	V_{417}	V_{418}	V_{419}	V_{420}	V_{421}	V_{422}	V_{423}	V_{424}	V_{425}	V_{426}	V_{427}	V_{428}	V_{429}	V_{430}	V_{431}	V_{432}	V_{433}	V_{434}	V_{435}	V_{436}	V_{437}	V_{438}	V_{439}	V_{440}	V_{441}	V_{442}	V_{443}	V_{444}	V_{445}	V_{446}	V_{447}	V_{448}	V_{449}	V_{450}	V_{451}	V_{452}	V_{453}	V_{454}	V_{455}	V_{456}	V_{457}	V_{458}	V_{459}	V_{460}	V_{461}	V_{462}	V_{463}	V_{464}	V_{465}	V_{466}	V_{467}	V_{468}	V_{469}	V_{470}	V_{471}	V_{472}	V_{473}	V_{474}	V_{475}	V_{476}	V_{477}	V_{478}	V_{479}	V_{480}	V_{481}	V_{482}	V_{483}	V_{484}	V_{485}	V_{486}	V_{487}	V_{488}	V_{489}	V_{490}	V_{491}	V_{492}	V_{493}	V_{494}	V_{495}	V_{496}	V_{497}	V_{498}	V_{499}	V_{500}	V_{501}	V_{502}	V_{503}	V_{504}	V_{505}	V_{506}	V_{507}	V_{508}	V_{509}	V_{510}	V_{511}	V_{512}	V_{513}	V_{514}	V_{515}	V_{516}	V_{517}	V_{518}	V_{519}	V_{520}	V_{521}	V_{522}	V_{523}	V_{524}	V_{525}	V_{526}	V_{527}	V_{528}	V_{529}	V_{530}	V_{531}	V_{532}	V_{533}	V_{534}	V_{535}	V_{536}	V_{537}	V_{538}	V_{539}	V_{540}	V_{541}	V_{542}	V_{543}	V_{544}	V_{545}	V_{546}	V_{547}	V_{548}	V_{549}	V_{550}	V_{551}	V_{552}	V_{553}	V_{554}	V_{555}	V_{556}	V_{557}	V_{558}	V_{559}	V_{560}	V_{561}	V_{562}	V_{563}	V_{564}	V_{565}	V_{566}	V_{567}	V_{568}	V_{569}	V_{570}	V_{571}	V_{572}	V_{573}	V_{574}	V_{575}	V_{576}	V_{577}	V_{578}	V_{579}	V_{580}	V_{581}	V_{582}	V_{583}	V_{584}	V_{585}	V_{586}	V_{587}	V_{588}	V_{589}	V_{590}	V_{591}	V_{592}	V_{593}	V_{594}	V_{595}	V_{596}	V_{597}	V_{598}	V_{599}	V_{600}	V_{601}	V_{602}	V_{603}	V_{604}	V_{605}	V_{606}	V_{607}	V_{608}	V_{609}	V_{610}	V_{611}	V_{612}	V_{613}	V_{614}	V_{615}	V_{616}	V_{617}	V_{618}	V_{619}	V_{620}	V_{621}	V_{622}	V_{623}	V_{624}	V_{625}	V_{626}	V_{627}	V_{628}	V_{629}	V_{630}	V_{631}	V_{632}	V_{633}	V_{634}	V_{635}	V_{636}	V_{637}	V_{638}	V_{639}	V_{640}	V_{641}	V_{642}	V_{643}	V_{644}	V_{645}	V_{646}	V_{647}	V_{648}	V_{649}	V_{650}	V_{651}	V_{652}	V_{653}	V_{654}	V_{655}	V_{656}	V_{657}	V_{658}	V_{659}	V_{660}	V_{661}	V_{662}	V_{663}	V_{664}	V_{665}	V_{666}	V_{667}	V_{668}	V_{669}	V_{670}	V_{671}	V_{672}	V_{673}	V_{674}	V_{675}	V_{676}	V_{677}	V_{678}	V_{679}	V_{680}	V_{681}	V_{682}	V_{683}	V_{684}	V_{685}	V_{686}	V_{687}	V_{688}	V_{689}	V_{690}	V_{691}	V_{692}	V_{693}	V_{694}	V_{695}	V_{696}	V_{697}	V_{698}	V_{699}	V_{700}	V_{701}	V_{702}	V_{703}	V_{704}	V_{705}	V_{706}	V_{707}	V_{708}	V_{709}	V_{710}	V_{711}	V_{712}	V_{713}	V_{714}	V_{715}	V_{716}	V_{717}	V_{718}	V_{719}	V_{720}	V_{721}	V_{722}	V_{723}	V_{724}	V_{725}	V_{726}	V_{727}	V_{728}	V_{729}	V_{730}	V_{731}	V_{732}	V_{733}	V_{734}	V_{735}	V_{736}	V_{737}	V_{738}	V_{739}	V_{740}	V_{741}	V_{742}	V_{743}	V_{744}	V_{745}	V_{746}	V_{747}	V_{748}	V_{749}	V_{750}	V_{751}	V_{752}	V_{753}	V_{754}	V_{755}	V_{756}	V_{757}	V_{758}	V_{759}	V_{760}	V_{761}	V_{762}	V_{763}	V_{764}	V_{765}	V_{766}	V_{767}	V_{768}	V_{769}	V_{770}	V_{771}	V_{772}	V_{773}	V_{774}	V_{775}	V_{776}	V_{777}	V_{778}	V_{779}	V_{780}	V_{781}	V_{782}	V_{783}	V_{784}	V_{785}	V_{786}	V_{787}	V_{788}	V_{789}	V_{790}	V_{791}	V_{792}	V_{793}	V_{794}	V_{795}	V_{796}	V_{797}	V_{798}	V_{799}	V_{800}	V_{801}	V_{802}	V_{803}	V_{804}	V_{805}	V_{806}	V_{807}	V_{808}	V_{809}	V_{810}	V_{811}	V_{812}	V_{813}	V_{814}	V_{815}	V_{816}	V_{817}	V_{818}	V_{819}	V_{820}	V_{821}	V_{822}	V_{823}	V_{824}	V_{825}	V_{826}	V_{827}	V_{828}	V_{829}	V_{830}	V_{831}	V_{832}	V_{833}	V_{834}	V_{835}	V_{836}	V_{837}	V_{838}	V_{839}	V_{840}	V_{841}	V_{842}	V_{843}	V_{844}	V_{845}	V_{846}	V_{847}	V_{848}	V_{849}	V_{850}	V_{851}	V_{852}	V_{853}	V_{854}	V_{855}	V_{856}	V_{857}	V_{858}	V_{859}	V_{860}	V_{861}	V_{862}	V_{863}	V_{864}	V_{865}	V_{866}	V_{867}	V_{868}	V_{869}	V_{870}	V_{871}	V_{872}	V_{873}	V_{874}	V_{875}	V_{876}	V_{877}	V_{878}	V_{879}	V_{880}	V_{881}	V_{882}	V_{883}	V_{884}	V_{885}	V_{886}	V_{887}	V_{888}	V_{889}	V_{890}	V_{891}	V_{892}	V_{893}	V_{894}	V_{895}	V_{896}	V_{897}	V_{898}	V_{899}	V_{900}	V_{901}	V_{902}	V_{903}	V_{904}	V_{905}	V_{906}	V_{907}	V_{908}	V_{909}	V_{910}	V_{911}	V_{912}	V_{913}	V_{914}	V_{915}	V_{916}	V_{917}	V_{918}	V_{919}	V_{920}	V_{921}	V_{922}	V_{923}	V_{924}	V_{925}	V_{926}	V_{927}	V_{928}	V_{929}	V_{930}	V_{931}	V_{932}	V_{933}	V_{934}	V_{935}	V_{936}	V_{937}	V_{938}	V_{939}	V_{940}	V_{941}	V_{942}	V_{943}	V_{944}	V_{945}	V_{946}	V_{947}	V_{948}	V_{949}	V_{950}	V_{951}	V_{952}	V_{953}	V_{954}	V_{955}	V_{956}	V_{957}	V_{958}	V_{959}	V_{960}	V_{961
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Ref vs Spike

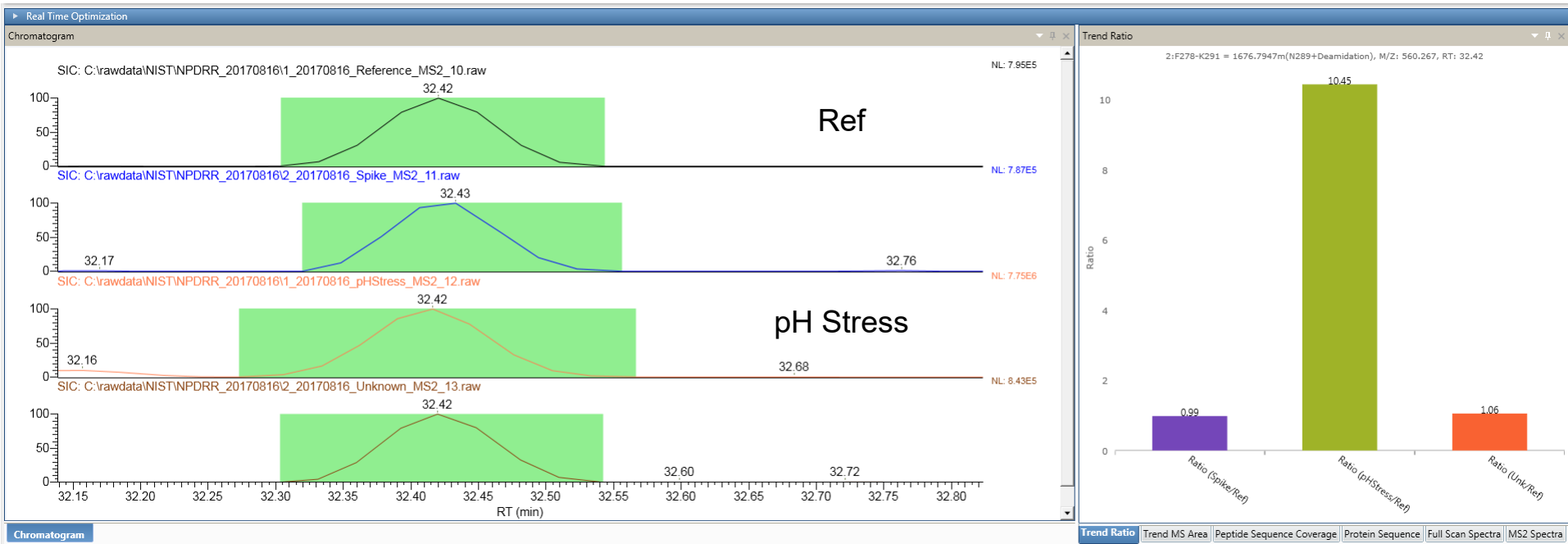


NPD detected 15 of 15 peptides

- 4 peptides were visible in the base peak chromatogram
- 6 peptides co-eluted with the IgG1 peptides
- 5 peptides below base peak threshold when visually inspected
- 0 false positives
- 0 false negatives
- **NPD can detect peaks that co-elute or are below the visible base peak threshold.**



Ref vs pH Stress



N289 Deamidation

Compliant software package for QC



Chromeleon 7.2 Attribute Report

Component Sequence Summary

Sequence Details

Name:	Test20150909	Created On:	09/Sep/15 16:23:34
Directory:	Test20150909	Created By:	rrogers
Data Vault:	ChromeleonLocal	Updated On:	09/Sep/15 17:27:39
No. of Injections:	2	Updated By:	rrogers

Component: DTLM[Oxid]ISR

Quantitation Ion: - / 851.42914, 852.43195, 853.43176, 426.21820, 426.71957, 427.21948

No.	Ret.Time min MS Quantitation Peak DTLM[Oxid]ISR	Area counts*min MS Quantitation Peak DTLM[Oxid]ISR	Height counts MS Quantitation Peak DTLM[Oxid]ISR	Rel.Area (DTLMISR) % MS Quantitation Peak DTLM[Oxid]ISR	MS Quantitation Peak DTLM[Oxid]ISR
1	12.804	1416260.904	22710740.186	2.09	Pass
2	12.647	1038306.529	17325777.260	1.40	Pass

Both samples pass oxidation specification!



Non-Targeted MS Processing

Interactive Result Tables Test20150909 (Sequence) - Chromeleon Chromatography Studio

Home Tables Frame Table Format

Paste Injection Processing Chromatogram Plot Start
Channel Frame Plot Start All
Frame Frame Report Stop
Clipboard Navigation Panes Processing

Non-Targeted MS Processi... <<

Setup Preparation Alignment Detection

☐ Use Default Parameters

Reference Injection
☐ Relative 2 ☒ Fixed Reference

Frame Parameters

m/z Min:	300.00
m/z Max:	1,800.00
m/z Width [ppm]:	10.00
Retention Time Start [min]:	5.00
Retention Time Stop [min]:	80.02
Frame Time Width [min]:	0.50

No frame selected.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	(
	Frame ID	Component ID	Mass m/z	RT min	Ratio	Base Component	PR Element	PR Root	PR Size	Charge	Molecular Weight m/z				
1															
2															
5															
6															

Frame Filter (0 of 2,889) with rule "Frame.PatternRecognitionElement = 0 and Frame.PatternRecognitionSize > 1 and Frame.Charge > 1 and Frame.Charge < 5 and Frame.Ratio > 10.00000"

Match ALL of the following rules:

Charge

No New Peaks!

rrrogers

The Evolution of MAM

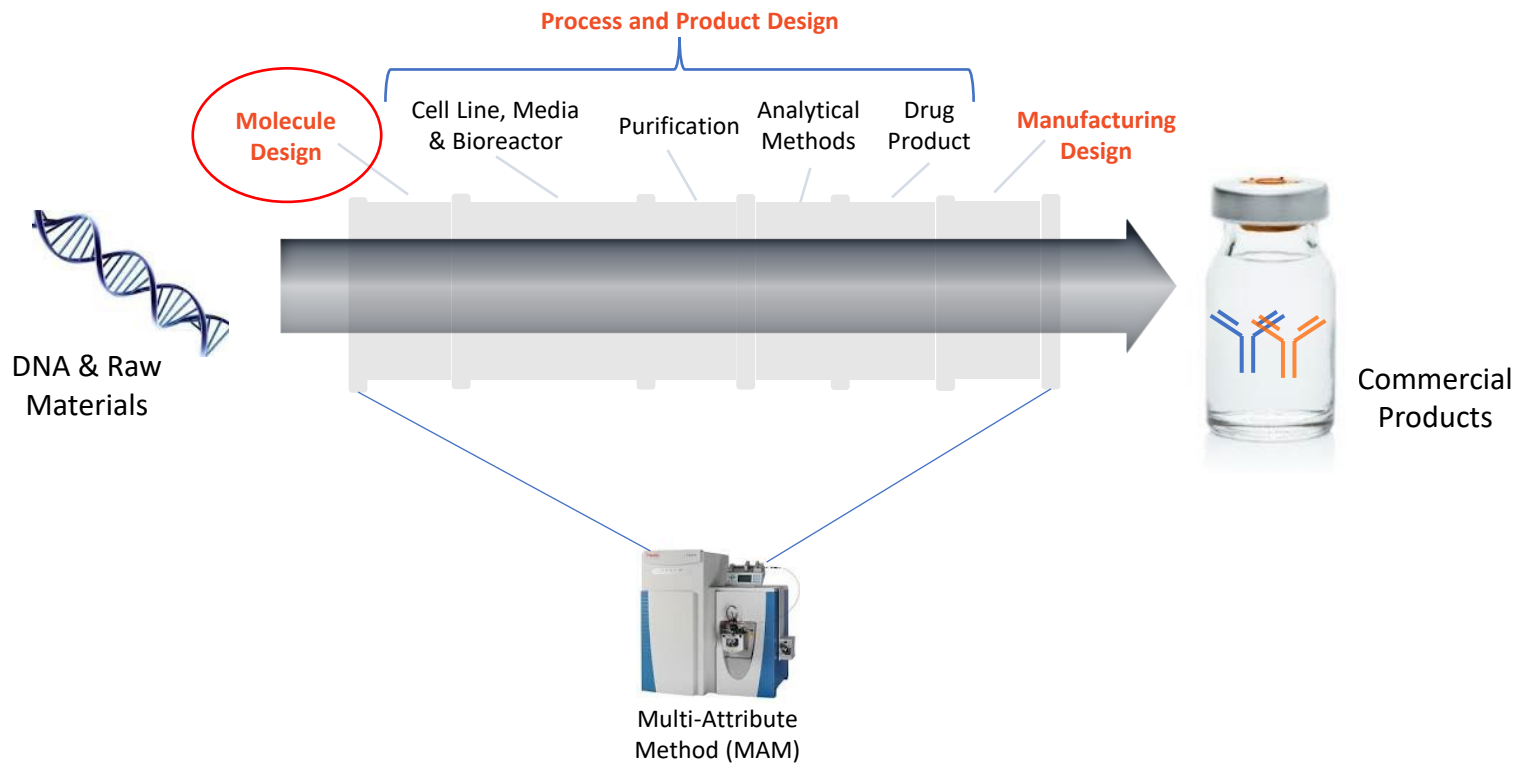


2012

2015

Amgen submitted MAM data to FDA for the first time in 2015

MAM is used at all stages of Process Development

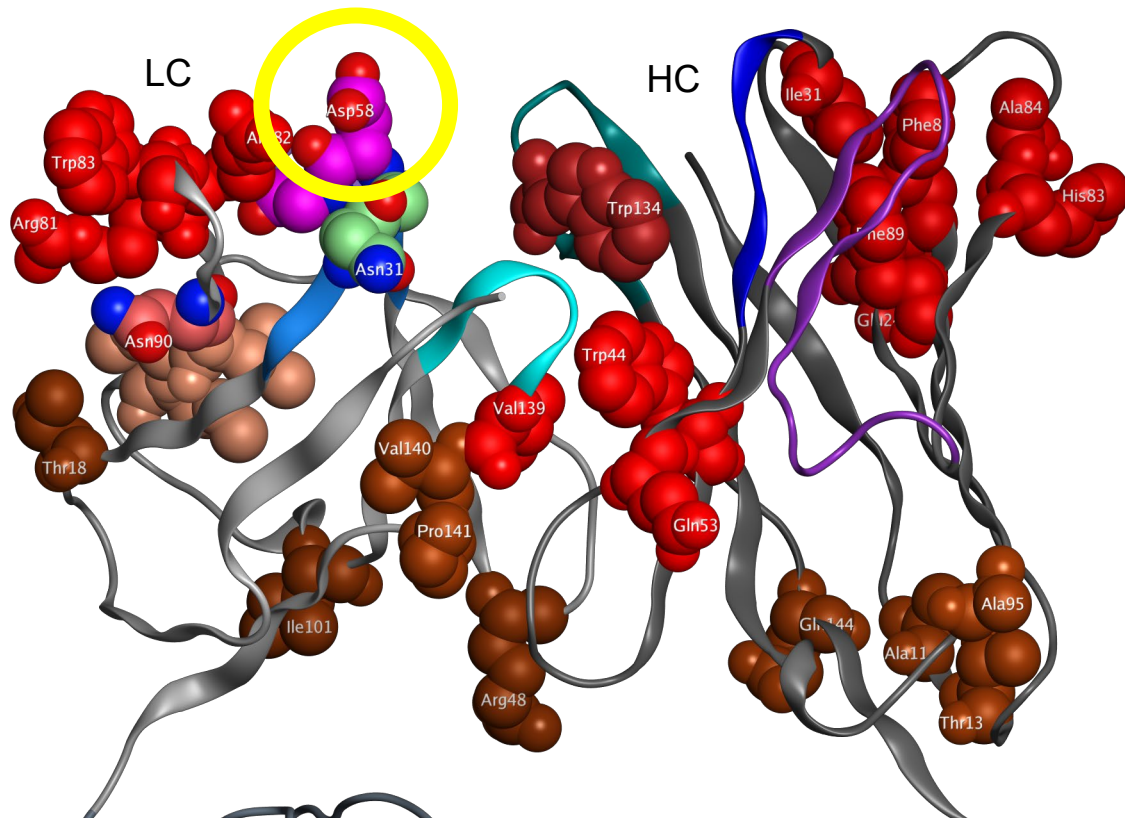


Molecular Design

Illustration of *in silico* predicted versus MAM-determined PTMs for three different antibodies, showing the disparity between them and number of variant design differences.

	mAb1			mAb2			mAb3		
	PTM (in silico)	PTM (MAM ≥ 1%)	MAM Range %	PTM (in silico)	PTM (MAM ≥ 1%)	MAM Range %	PTM (in silico)	PTM (MAM ≥ 1%)	MAM Range %
Deamidation	1	0	0.85%	2	3	0% - 19.78%	0	0	0.00%
Glycation	0	0	0.05% - 0.33%	0	0	0.00%	0	0	0.00%
Isomerization	3	0	0% - 0.22%	2	0	0% - 0.39%	4	1	0% - 1.7%
Met Oxidation	1	1	1.14%	0	1	0.58% - 1.7%	1	0	0.00%
N-Link Glycosylation	1	1	88.70%	0	0	0.00%	1	0	0.00%
Trp Oxidation	5	0	0% - 0.61%	2	0	0.00%	4	0	0.00%
Total	11	2		6	4		10	1	
Combinatorial Variants	2047	3		63	15		1023	1	
Combinatorial Variants less Oxidation	31	1		15	7		31	1	

MAM and molecular optimization



Slide courtesy of Randy Ketchem

Table 1: Color Scheme Used for Antibody Region Coloring.

Region/Feature	Color	
Light Chain	SILVER	
Heavy Chain	DIM_GRAY	
LC-CDR1	DODGER_BLUE	
LC-CDR2	MEDIUM_PURPLE	
LC-CDR3	CYAN	
HC-CDR1	BLUE	
HC-CDR2	DARK_ORCHID	
HC-CDR3	DARK_CYAN	
CL	LIGHT_SLATE_GRAY	
CH1	DARK_SLATE_GRAY	
Hinge	GRAY	
CH2	LIGHT_GRAY	
CH3	LIGHT_STEEL_BLUE	
Post-CH3	SLATE_GRAY	

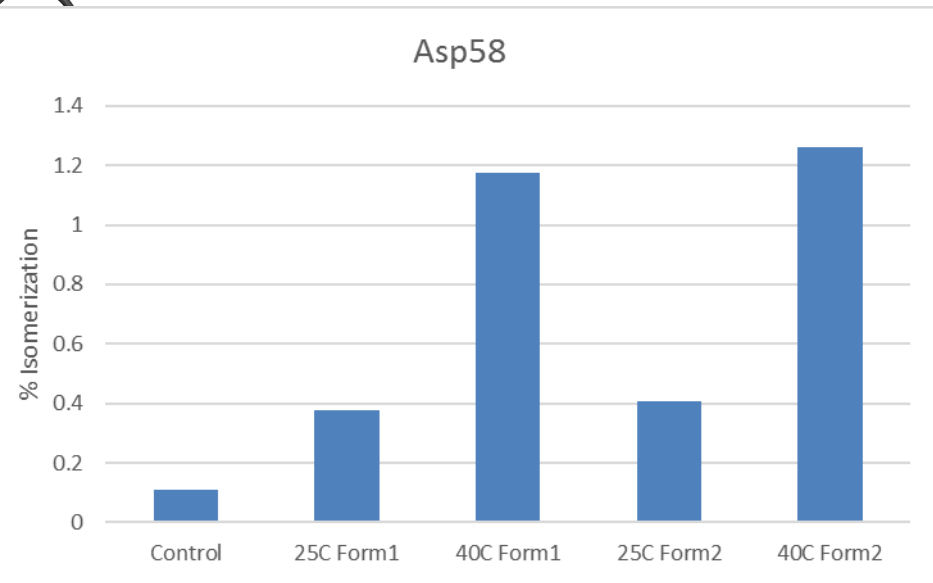
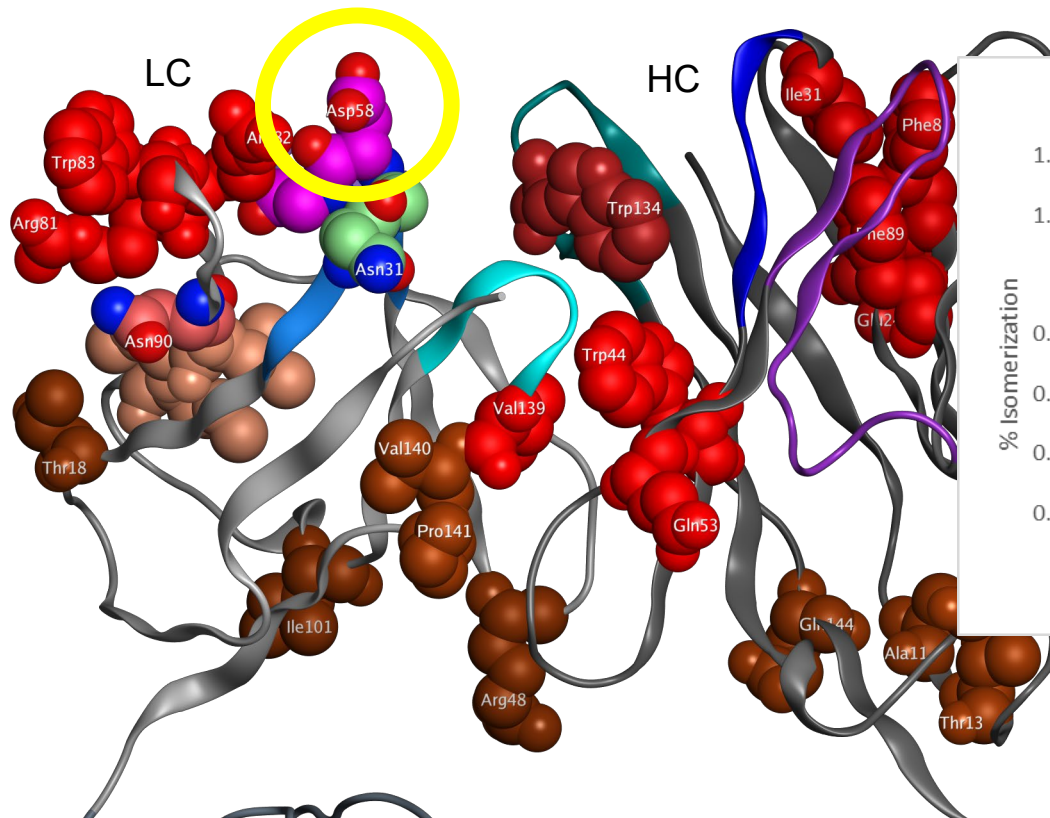
Table 2: Color Scheme Used for Hot Spot Coloring.

Hot Spot	Color	
Non-Standard Cys	YELLOW	
Potential N-linked glycosylation site	LIGHT_SALMON	
Covariance Site	RED	
Potential Isomerization Site (CDR)	FUCHSIA	
Potential Deamidation Site (CDR)	LIGHT_GREEN	
Potential Trp Oxidation Site (CDR3)	FIREBRICK	
Humanization	GREEN	
Tier 3 Hot Spots	CHOCOLATE	

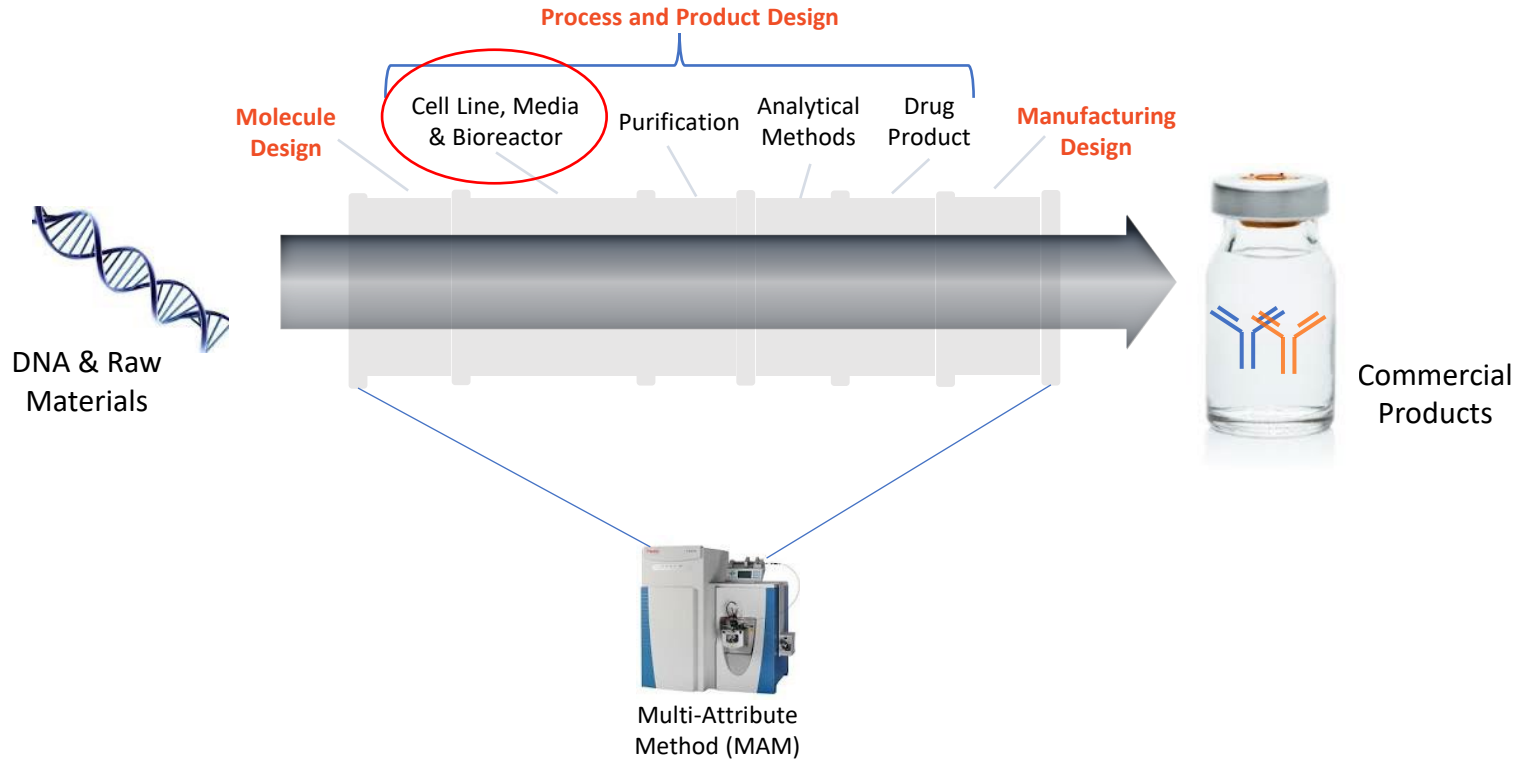
Table 3: ABHAND Residue Coloring by Type.

Type	Residues	Color	
Acidic	DE	RED	
Basic	KRH	BLUE	
Hydrophobic	ALIMCVP	GREEN	
Aromatic	FWY	VIOLET	
Neutral	NQSTBZ	GOLD	
Polar			
Deletion	G*X	BLACK	

MAM and molecular optimization



MAM is used at all stages of Process Development



Upstream monitoring



- Ammonia
- Lactate
- Ammonia
- pH
- Cell density
- Viability
- others

What about product quality attributes?

Upstream

Site 1			Site 2			Site 3			Site 4 (Fc)		
Host-1	Host-2	Host-3	Host-1	Host-2	Host-3	Host-1	Host-2	Host-3	Host-1	Host-2	Host-3

Variation in glycan profile of Fc-A produced from different CHO host cell lines. Individual lines represent glycans from different pools. Afuco is % afucosylated glycan; % SA is percent sialic acid; %HM is percent total high mannose. This Fc-fusion has 4 glycosylation sites with site 4 present on the Fc portion of the molecule. Sample were taken on day 8 (D8) and day 10 (D10).

Upstream

Impact of pH on Fc-B
glycans. The innovator
molecule is compared to Fc-
B biosimilar run in
bioreactors run at different
pH and a previous process
(TS6). % sialic acid is NANA;
%B-gal is % exposed beta-
galactose; % Mannose is %
of all high mannose species;
% Afuco is % afucosylated
species.

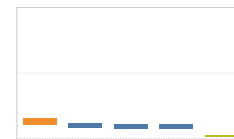
Site 1

Site 2

Site 3

Site 4

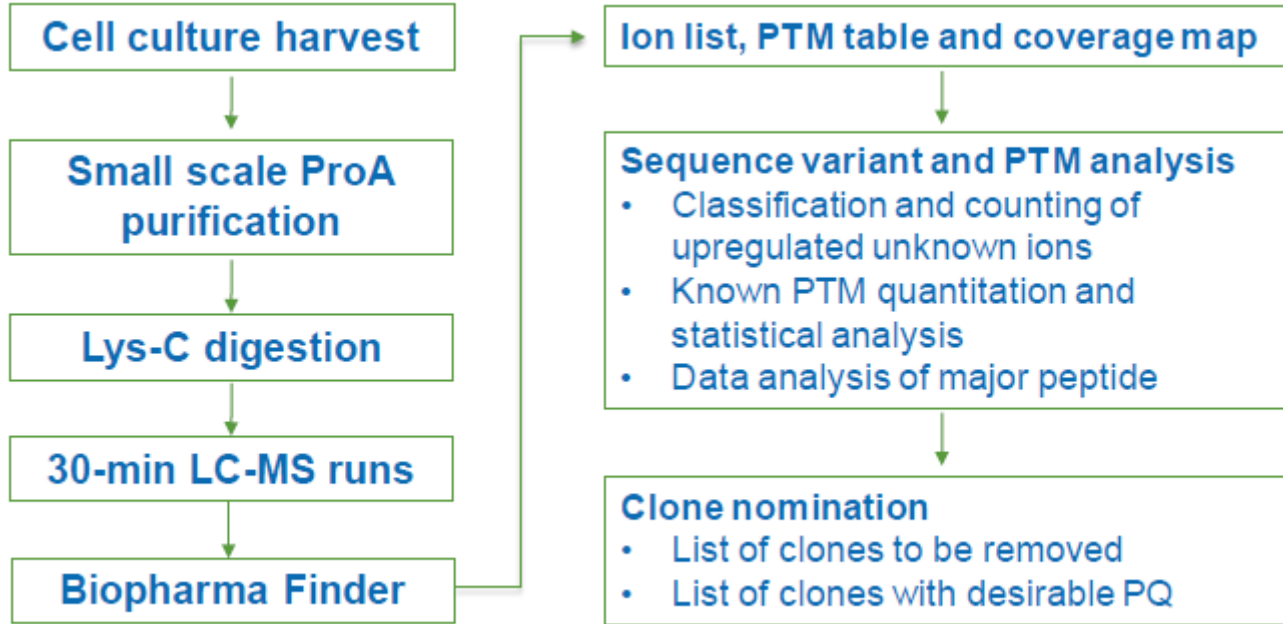
Site 5 (Fc)



Upstream

- Sequence Variant and Posttranslational Modification Analysis During Cell Line Selection via High-Throughput Peptide Mapping.
- Xu CF, Wang Y, Bryngelson P, Sosic Z, Zang L.
- Adv Exp Med Biol. 2019;1140:225-236.
- Selection of high-producing lead and backup cell lines with high-fidelity primary structure is a major goal of cell line development of protein therapeutics. Conventional techniques for sequence variant analysis, such as mass spectrometry (MS) and next-generation sequencing (NGS) have limitations on the sample number and turnaround time, thus often are only applied at the final stages of development, where an undesired lead or backup clone could cause a significant delay in project timeline. Here we presented a high-throughput (HT) peptide mapping workflow which can be applied at early stages of cell line selection for testing of a batch of 30-40 clones within 2-week turnaround while reporting valuable information on sequence variants and posttranslational modifications (PTMs). The successful application of this workflow was demonstrated for two mAb programs. Multiple clones were removed from a total of 33 mAb-1 clones using various criteria: nine clones contained at least one >1% upregulated unknown peptide ions, 11 clones contained at least eight >0.1% upregulated unknowns, and six clones contained upregulated critical PTMs. For mAb-2, light chain (LC) sequence extension of approximately 30 amino acids were detected in 6 out of 36 clones at levels up to 11%. Besides, a Q to H mutation at ~30% was detected in the heavy chain (HC) of a single clone. Q to H mutation has mass change of 9.00 Da and failed to be detected by intact mass analysis. Rapid PTM quantitation also facilitated the selection of clones with desirable quality attributes, such as N-glycan profile. Hence, we demonstrated a risk-reducing strategy where abnormal clones could be detected at earlier stages of cell line selection, which should result in reduced and more predictable timeline of cell line development.

Cell line selection workflow

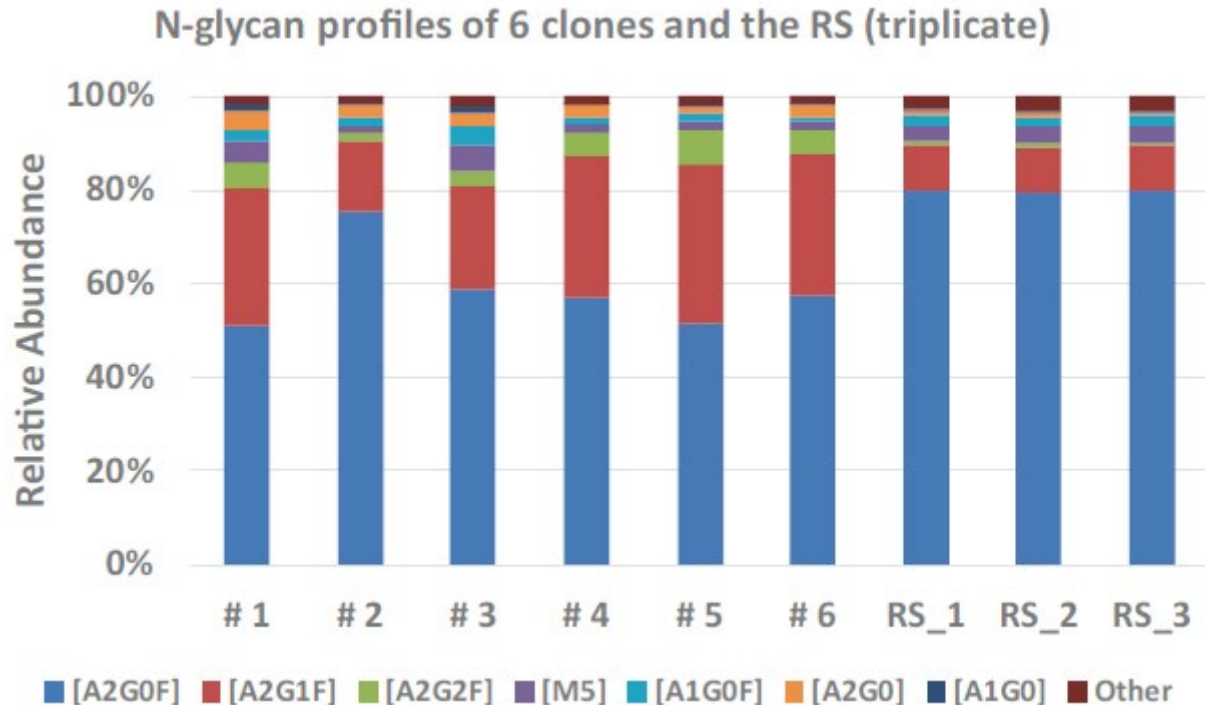


Parameters for SVA and Variable Mass Shifts

Advanced Search

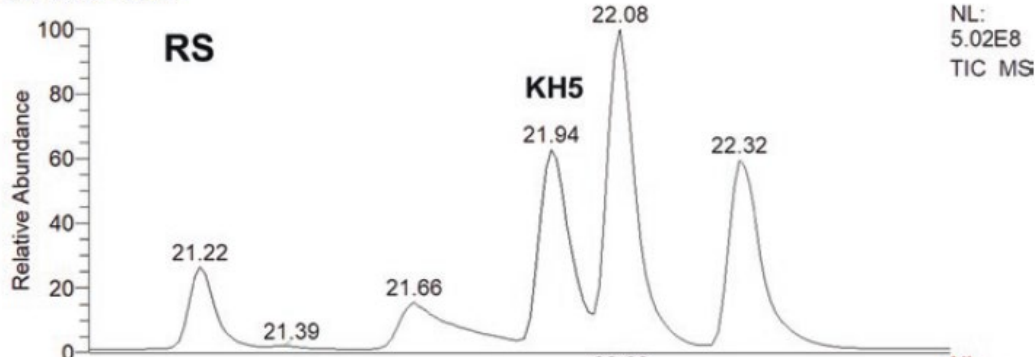
Enable Mass Search for Unspecified Modifications	☐
Mass Changes for Unspecified Modifications	0 to 0
Glycosylation	CHO
Search for Amino Acid Substitutions	Single Base Change
Enable Residue Deletion	☐

Glycans-Optimize to match RS



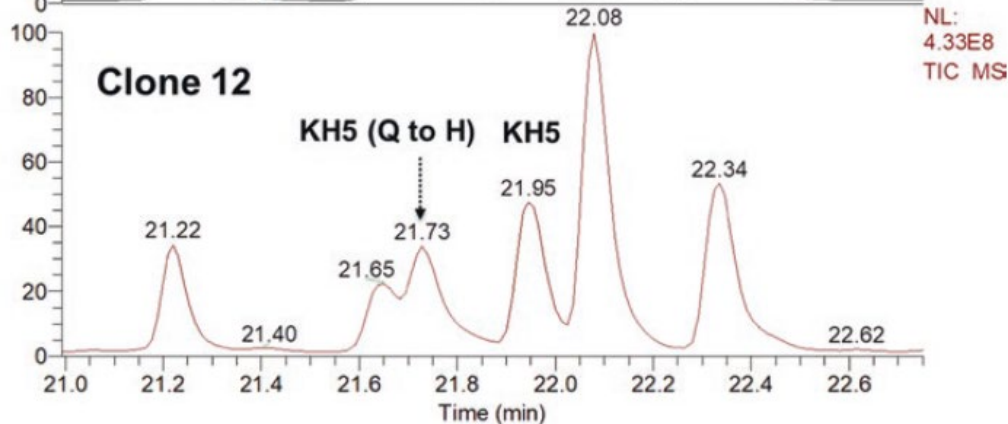
Sequence Variant Analysis

RT: 20.99 - 22.75



SV can be:

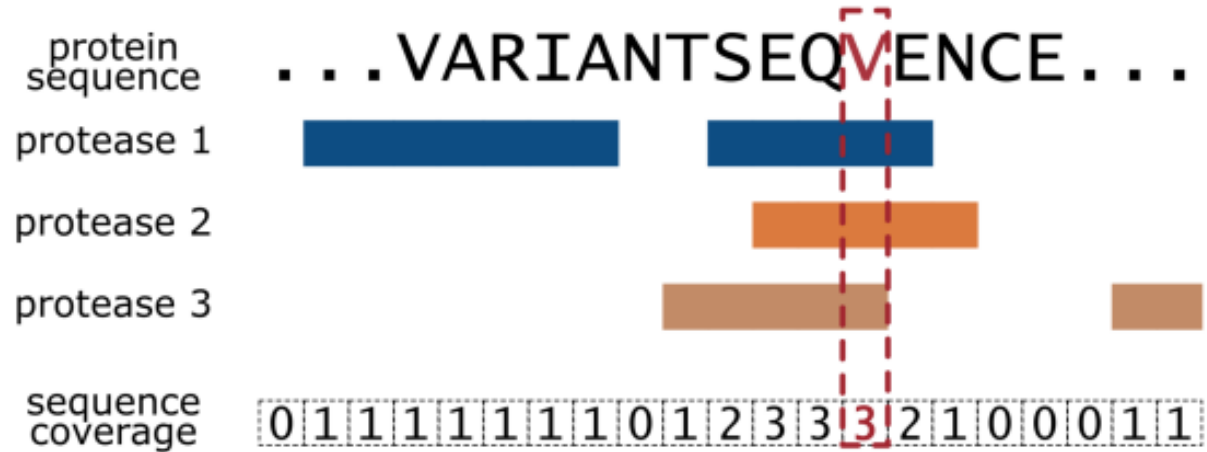
- Amino Acid Misincorporations
- DNA mutations



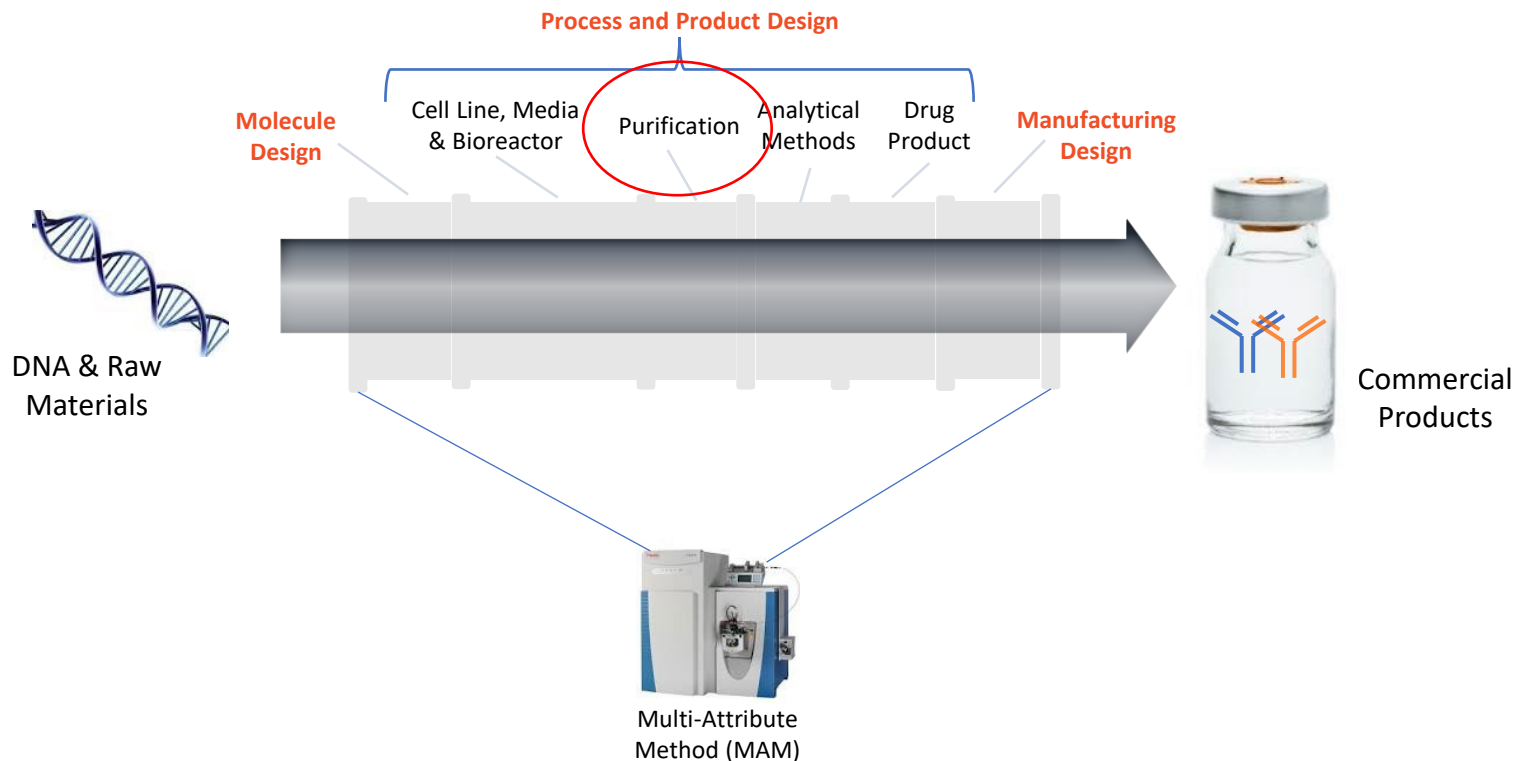
SV mitigations

- Remove clones that have mutations
- Optimize media to control misincorporations

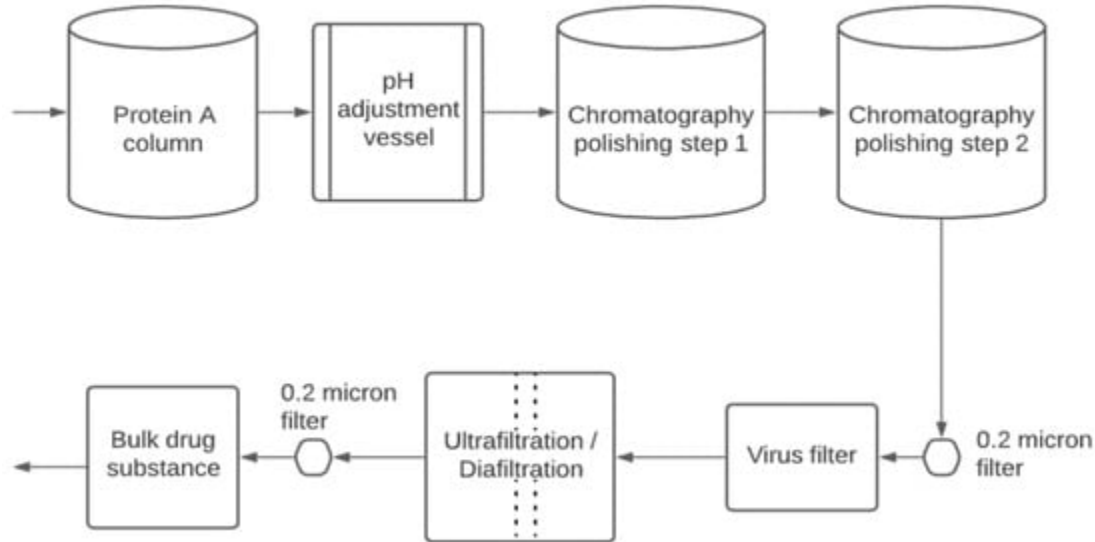
Confirm SV with Multiple Enzymes



MAM is used at all stages of Process Development

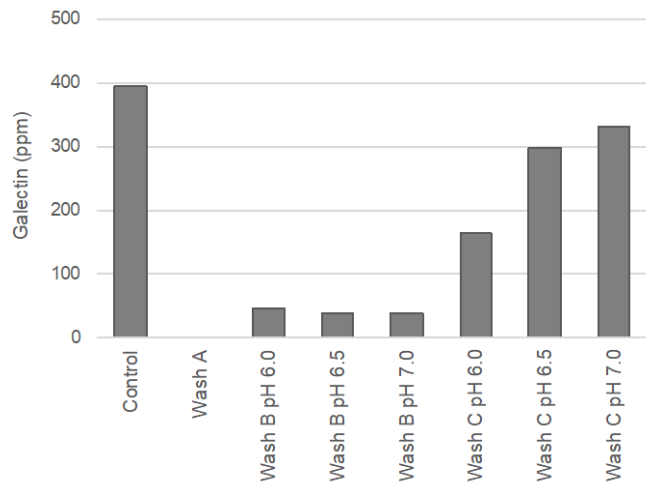


Downstream Purification

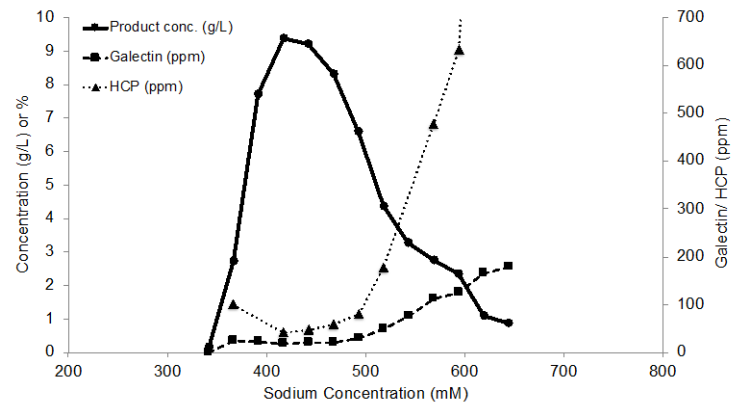


Major goals are to remove process related impurities and enrich the desired product profile

Evaluating Galectin-3 Removal by Different Chromatography Steps Using MAM



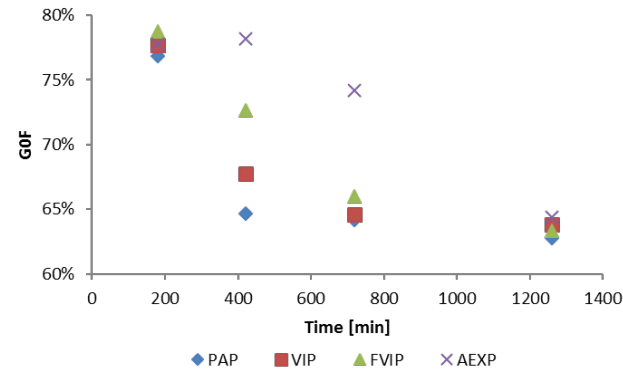
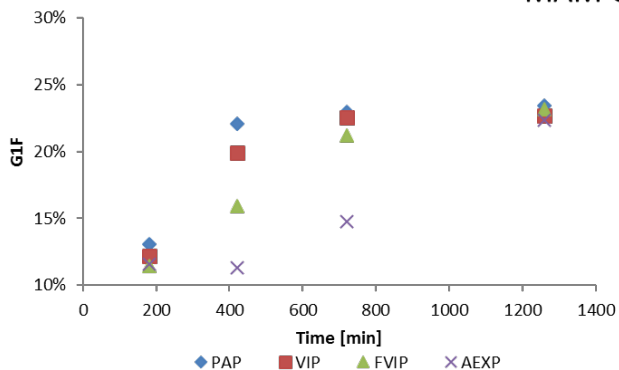
Levels of galectin-3 observed in a Protein A chromatography wash screen



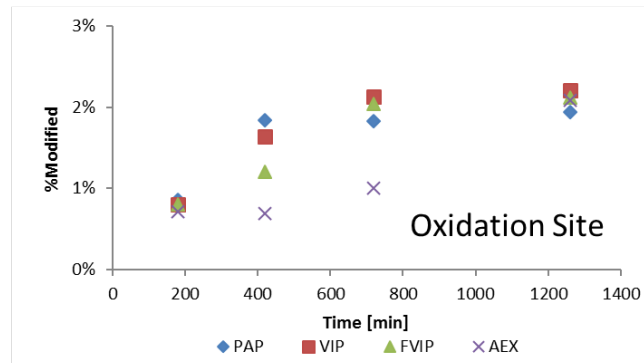
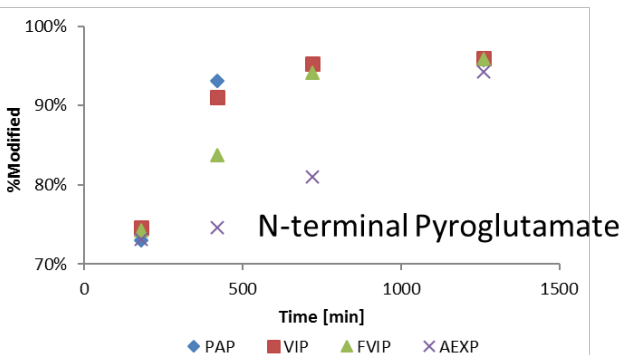
Levels of galectin-3 and HCP observed in NaCl gradient elution fractions for a CEX chromatography step

MAM for Continuous Process Monitoring

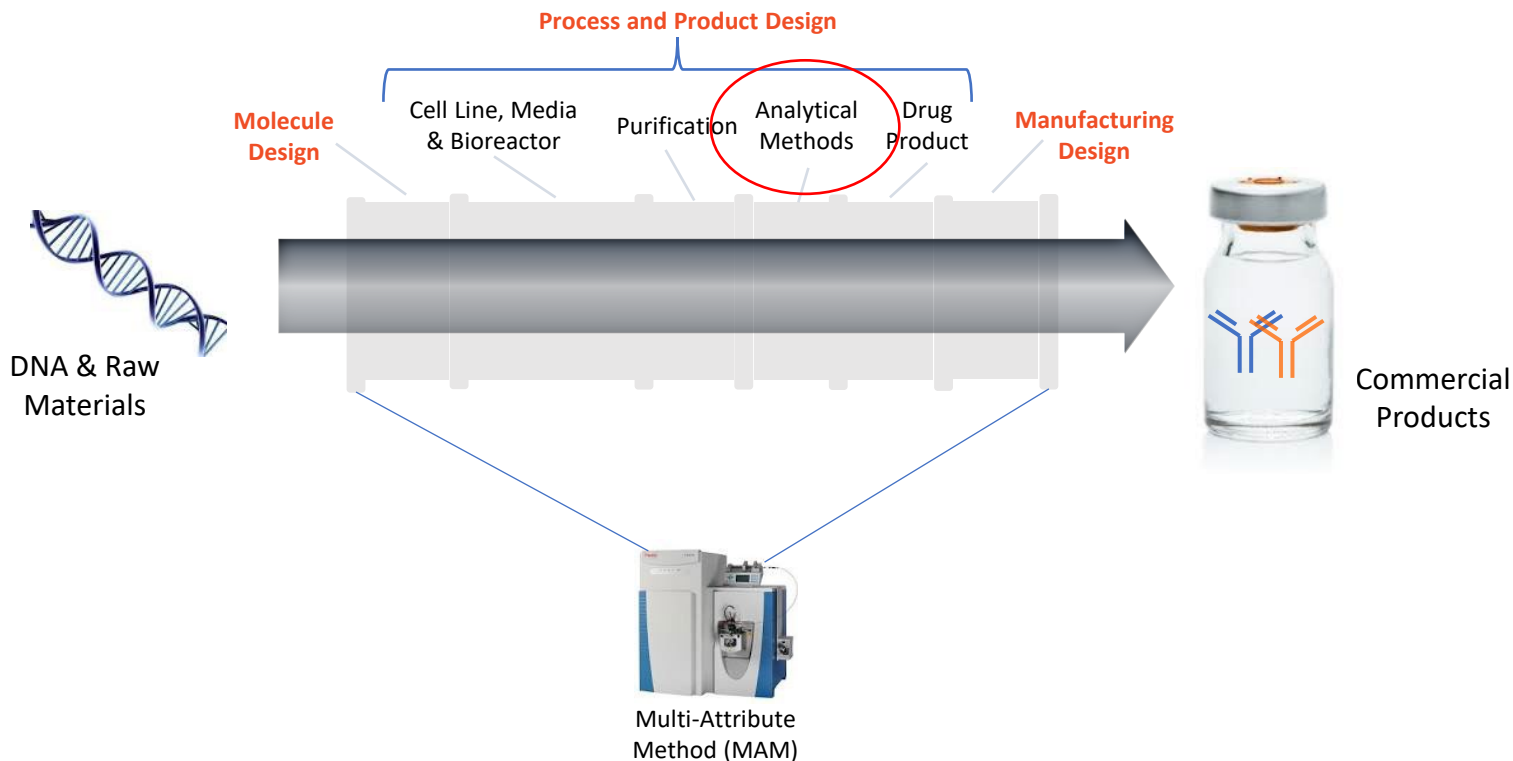
MAM Glycan Analysis



Other MAM Data

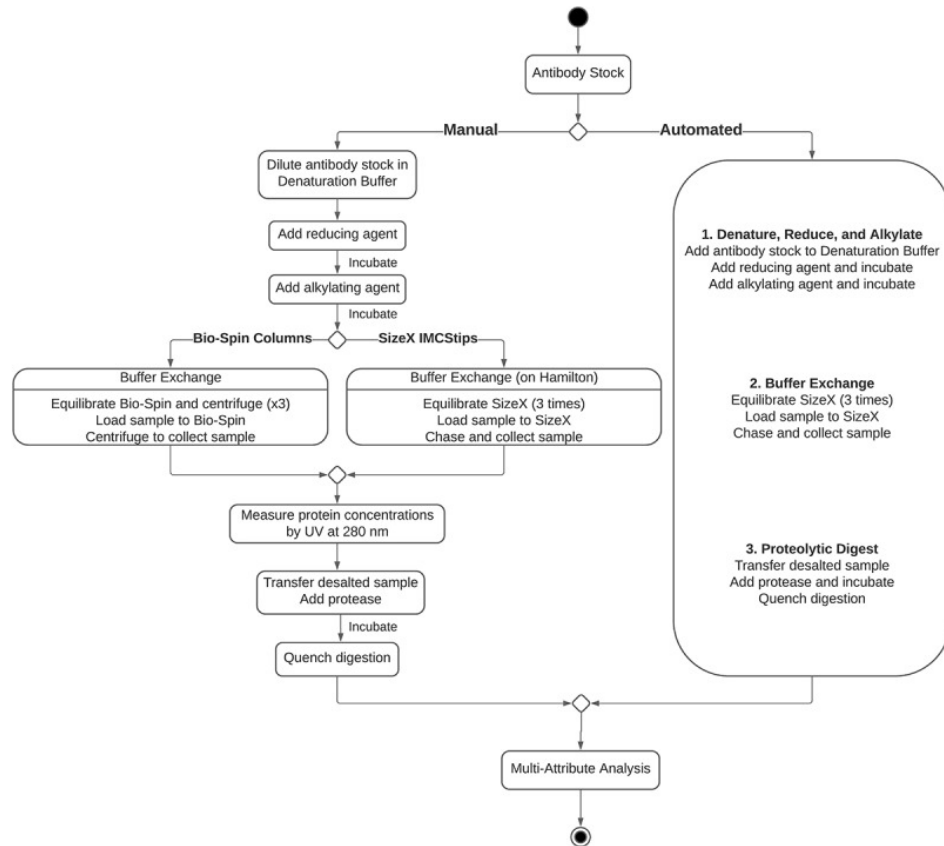


MAM is used at all stages of Process Development



How can the Analytical team handle the new sample submissions?

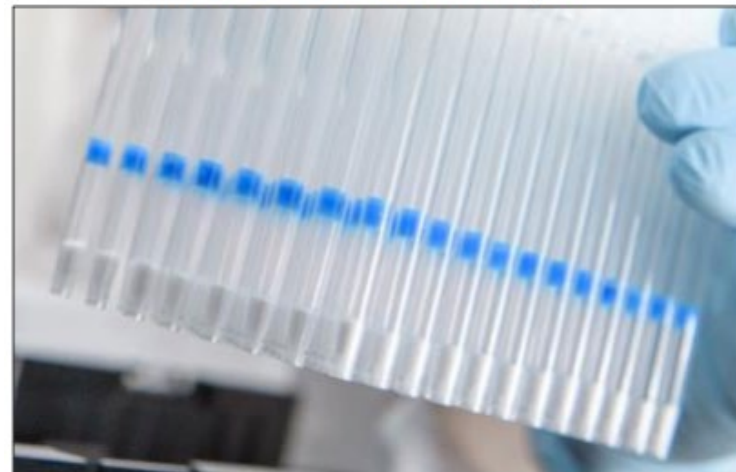
Sample Prep Automation



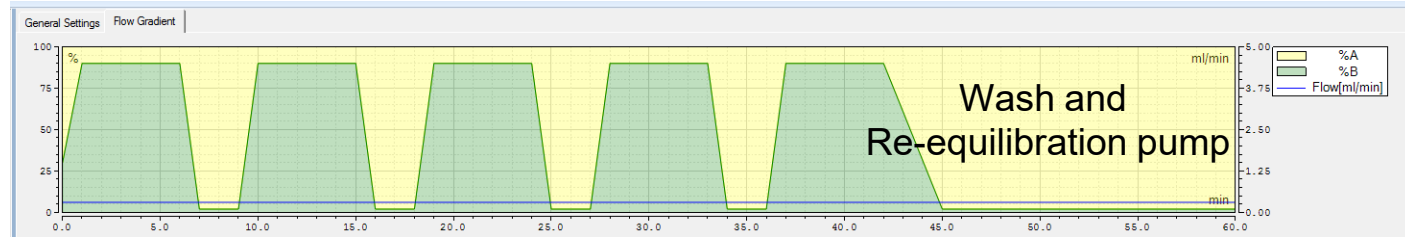
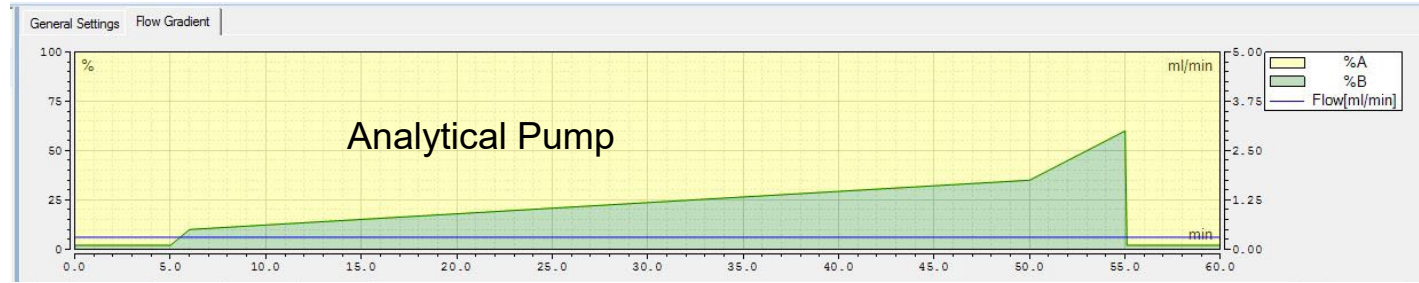
Hamilton Microlab STAR



IMCS SizeX 100 Tips



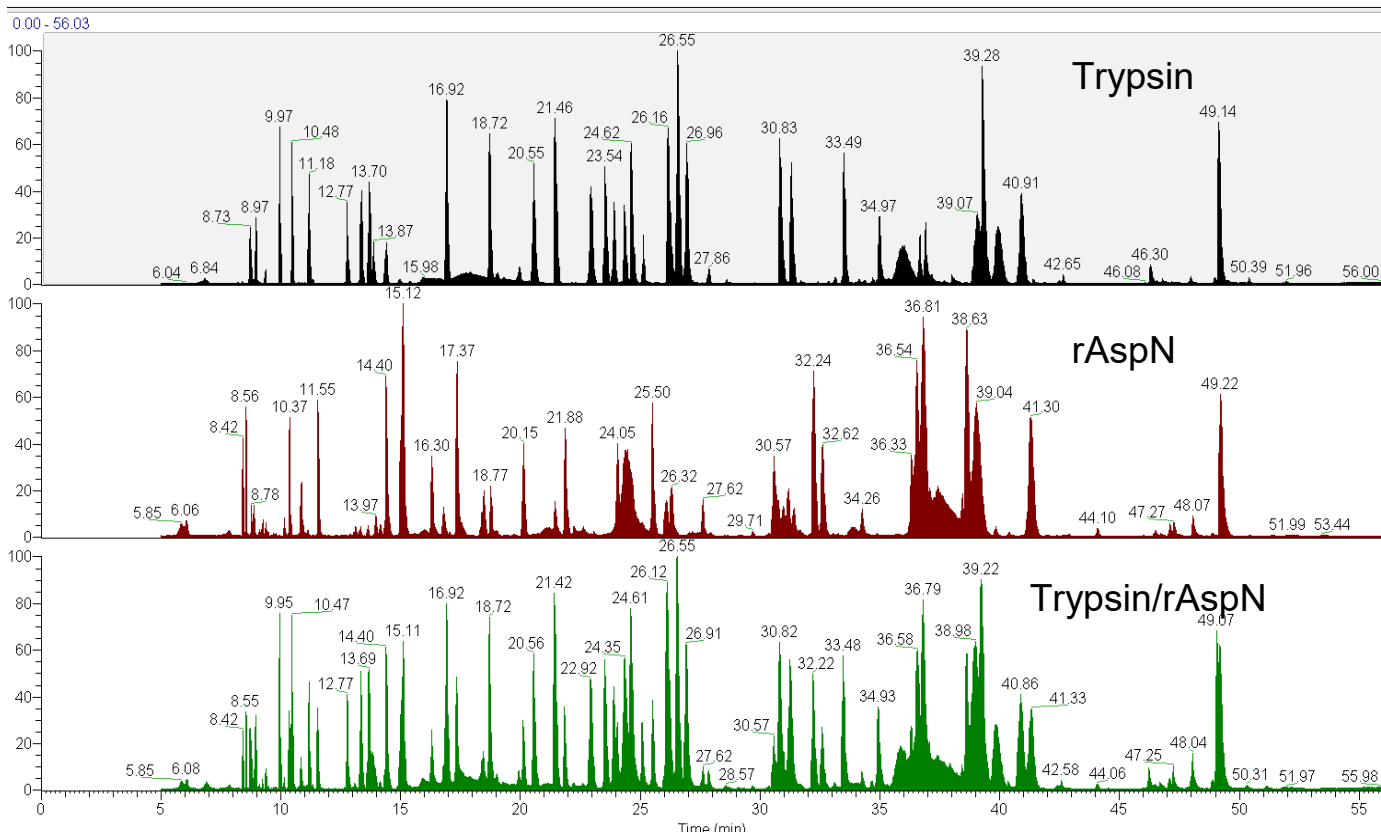
Increase throughput with a dual column UPLC system



Saw tooth gradient reduces/eliminates carryover

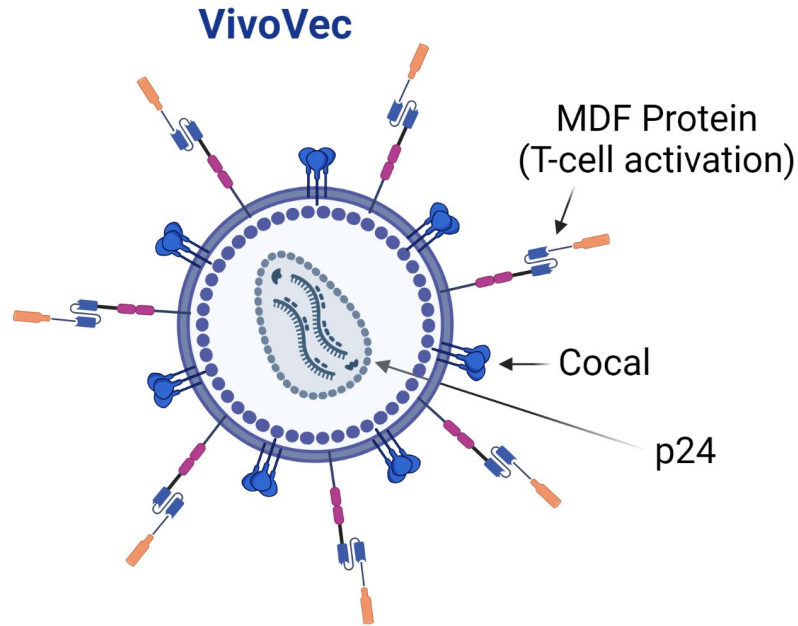
Dual column UPLC

Multi-Enzyme MAM ensures complete coverage of a molecule



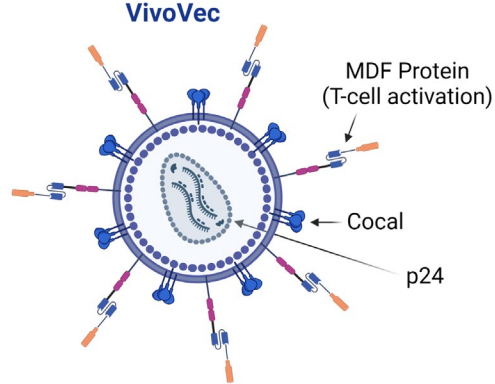
- The Multi-Enzyme MAM can increase the coverage of CQAs in a single injection.
- The Multi-Enzyme MAM saves 3 hours in instrument time.
- The Multi-Enzyme MAM can yield 100% sequence confirmation.

Using MAM for Cell and Gene Therapies

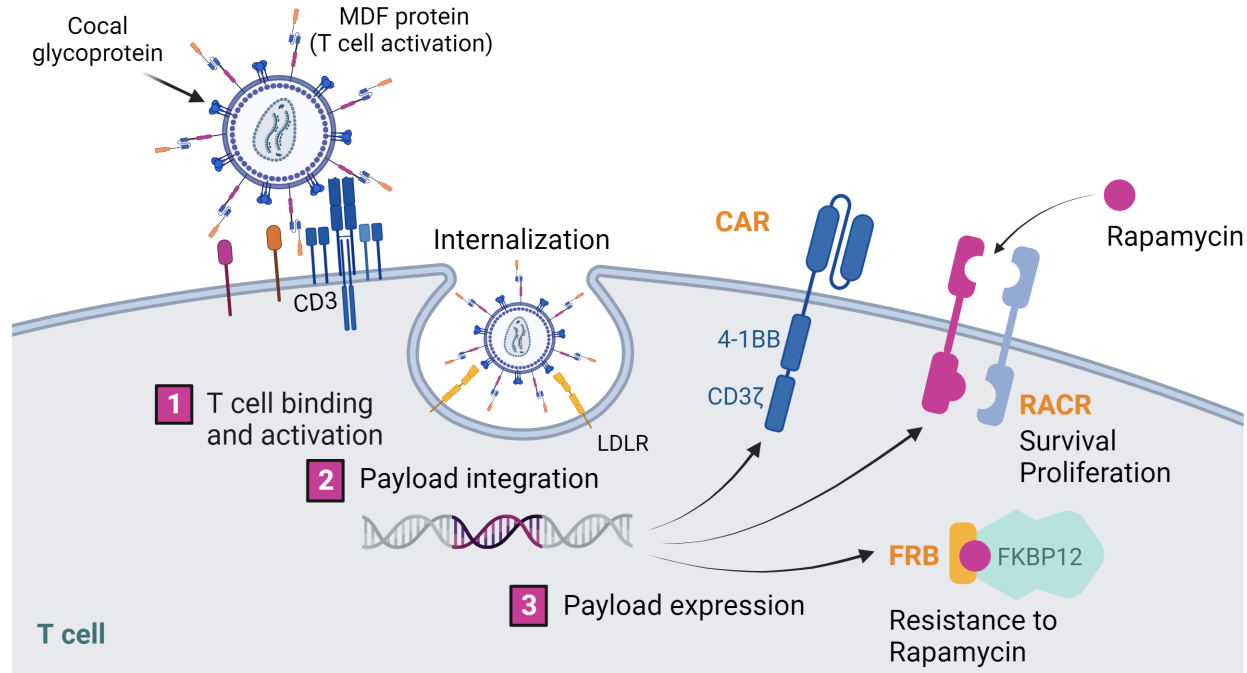


Lentiviral Vector for in vivo CAR-T

Composition and Mechanism of Action of VivoVec



- MDF=Multi-Domain Fusion
- VivoVec surface engineering enables efficient T cell activation and transduction in vivo
- MDF protein targets T cells
- Cocal reduces potential for immunogenicity (relative to VSV-G)



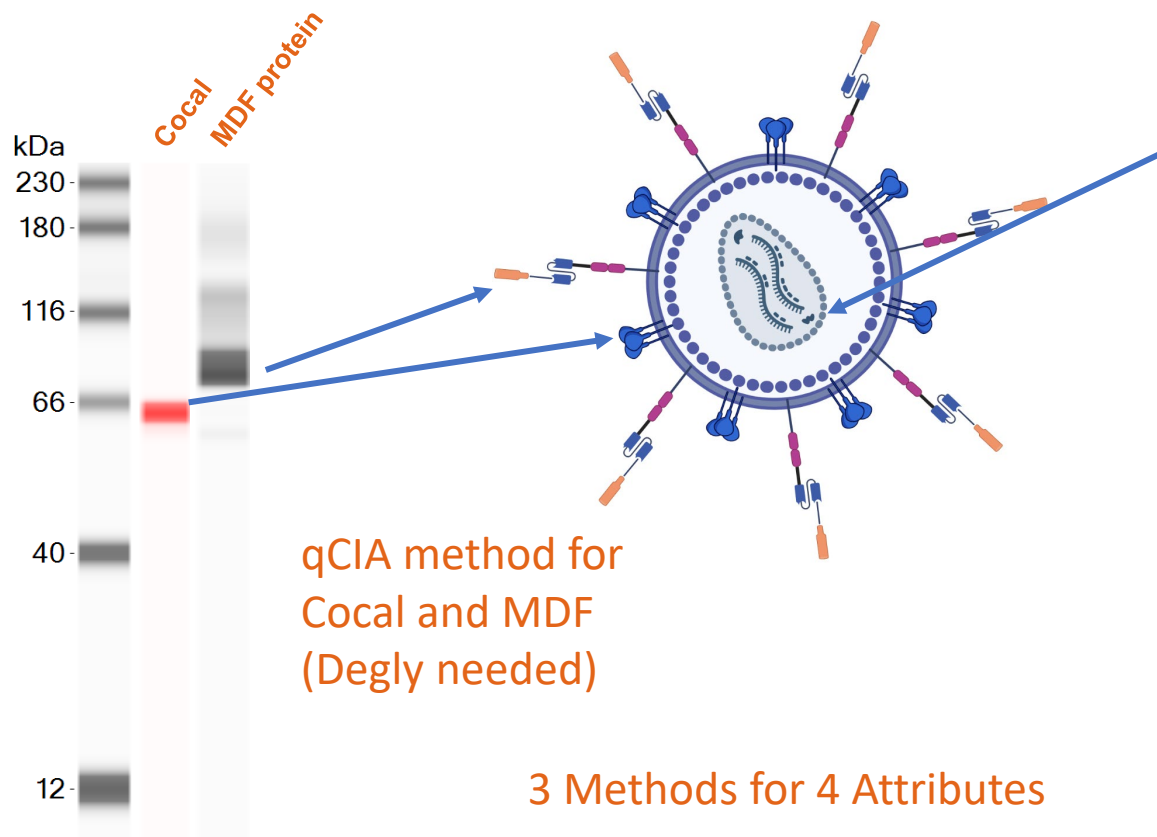
Single attribute testing used to ensure product quality

1. CAR expression
2. p24 expression
3. MDF expression
4. Cocal expression
5. RACR expression
6. HCP ELISA
7. Sanger Sequencing
8. Color
9. Clarity
10. Osmolality
11. pH
12. Sterility
13. Endotoxin
14. Mycoplasma
15. RCL
16. Adventitious virus
17. DNA based Process Impurities

Single attribute testing used to ensure product quality

1. CAR expression
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12. Sterility
13. Endotoxin
14. Mycoplasma
15. RCL
16. Adventitious virus
17. DNA based Process Impurities

Current State: Quantification of MDF, Cocal, and p24



Workflow for Gene Therapy MAM



1 Method for 4 Attributes

The Evolution of MAM

The Amgen logo consists of the word "AMGEN" in a bold, blue, sans-serif font.

2012

The Just logo features the word "Just" in a large, bold, black sans-serif font. Below it, the tagline "biotherapeutics for all" is written in a smaller, black, sans-serif font. A small orange circle is positioned to the right of the word "Just".

2015



2015-2016

The MAM Consortium logo features the word "MAM" in a large, bold, blue, sans-serif font. Below it, the word "Consortium" is written in a smaller, blue, sans-serif font.

2016

MAM Consortium

- The purpose of the consortium is to enable the BioPharma community to implement a robust mass spec based method for biotherapeutic characterization and release of biotherapeutics from QC.
- A goal of the MAM consortium is to reduce the number assays required for QC release.
- We have used the NISTmAb to evaluate similarity between companies and vendors (focusing on new peak detection). The round robin is led by Trina Mouchahoir.
- The MAM Consortium has members from companies spanning government agencies, biopharma, mass spec vendors, software vendors, reagent vendors, and CDMOs.



Round Robin New Peak Detection Study

MAM Consortium Round Robin New Peak Detection Study

- Evaluate interlaboratory reproducibility of MAM
- Understand qualitative and quantitative capabilities of the platform
- Establish performance metrics
- Assess the state-of-the-industry regarding MAM

MAM Consortium Round Robin New Peak Detection Study Sample Kit



System Suitability Peptide Mix



Reference



pH Stress



Spiked



Unknown

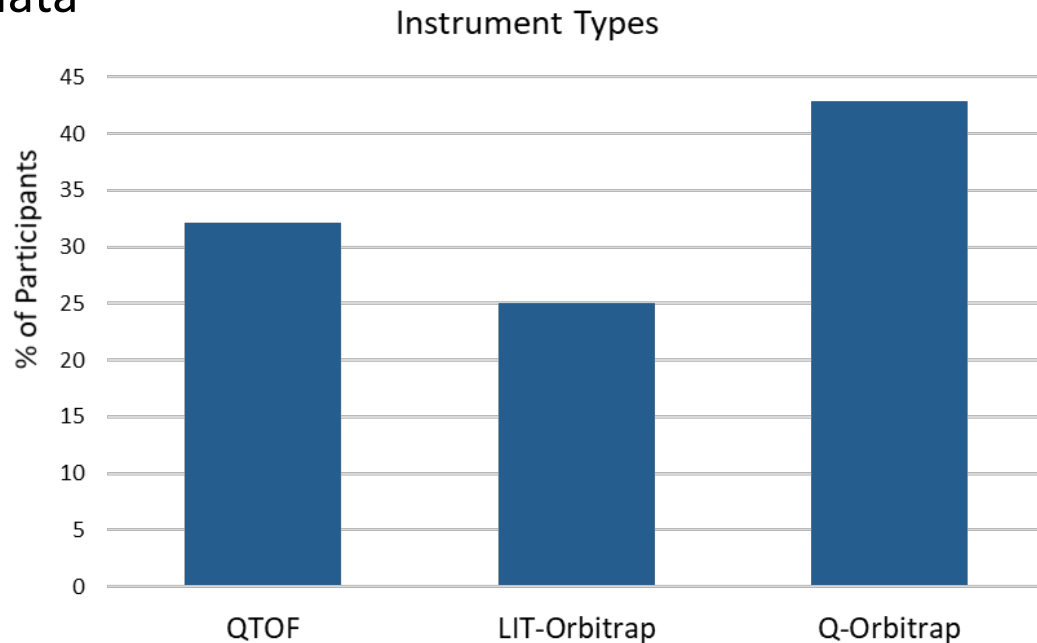
NISTmAb Digests

MAM Consortium Round Robin New Peak Detection Study Protocol

- Samples analyzed using same:
 - Column
 - Gradient
 - Injection sequence
- LC and MS instruments vary with each participant
- Participants use preferred data analysis software and platforms
- Returned data regarding System Suitability Peptide Mix
- Reported data regarding identification and quantification of:
 - Pre-defined peptides and quality attributes (reference digest)
 - New, missing or changed peaks (reference vs sample)
- Datasets are anonymized

Preliminary Numbers

- 28 sets of processed data
- 16 sets of raw data



Outlier: More than 1.5x above the upper quartile,
shape coded by participant

Maximum: Greatest value, excluding outliers

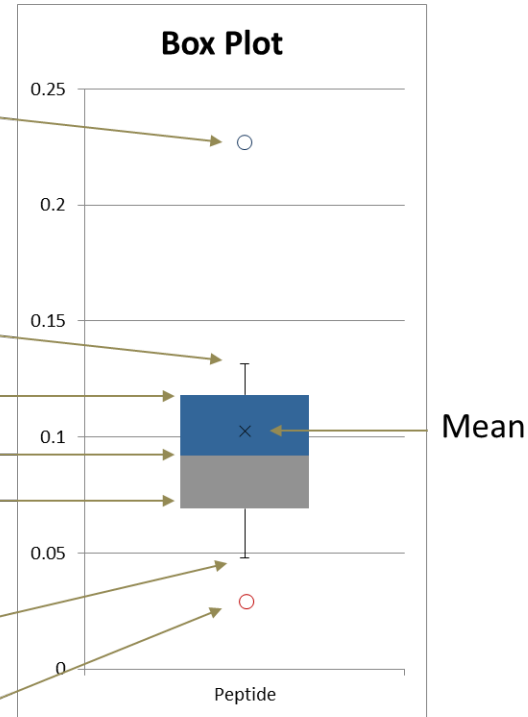
Upper Quartile

Median

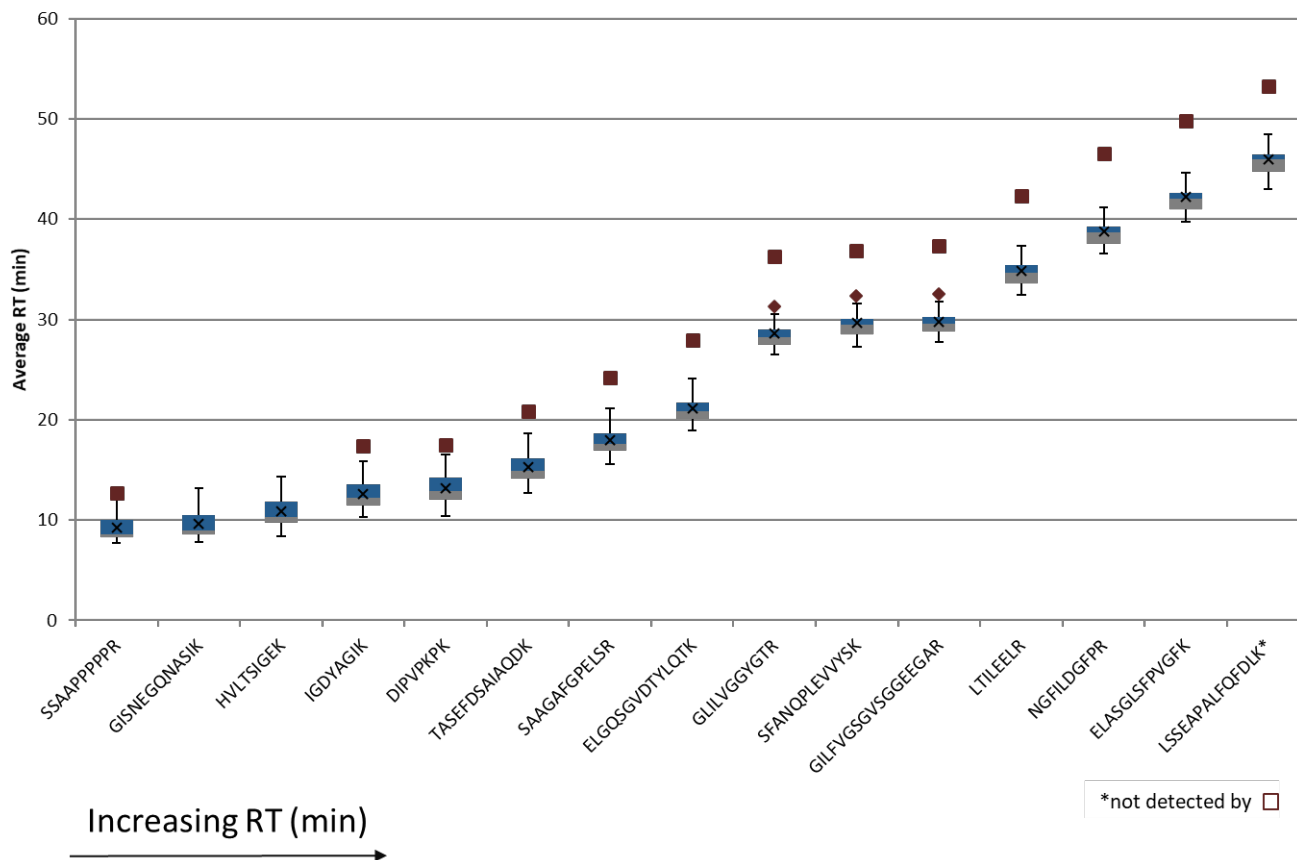
Lower Quartile

Minimum: Least value, excluding outliers

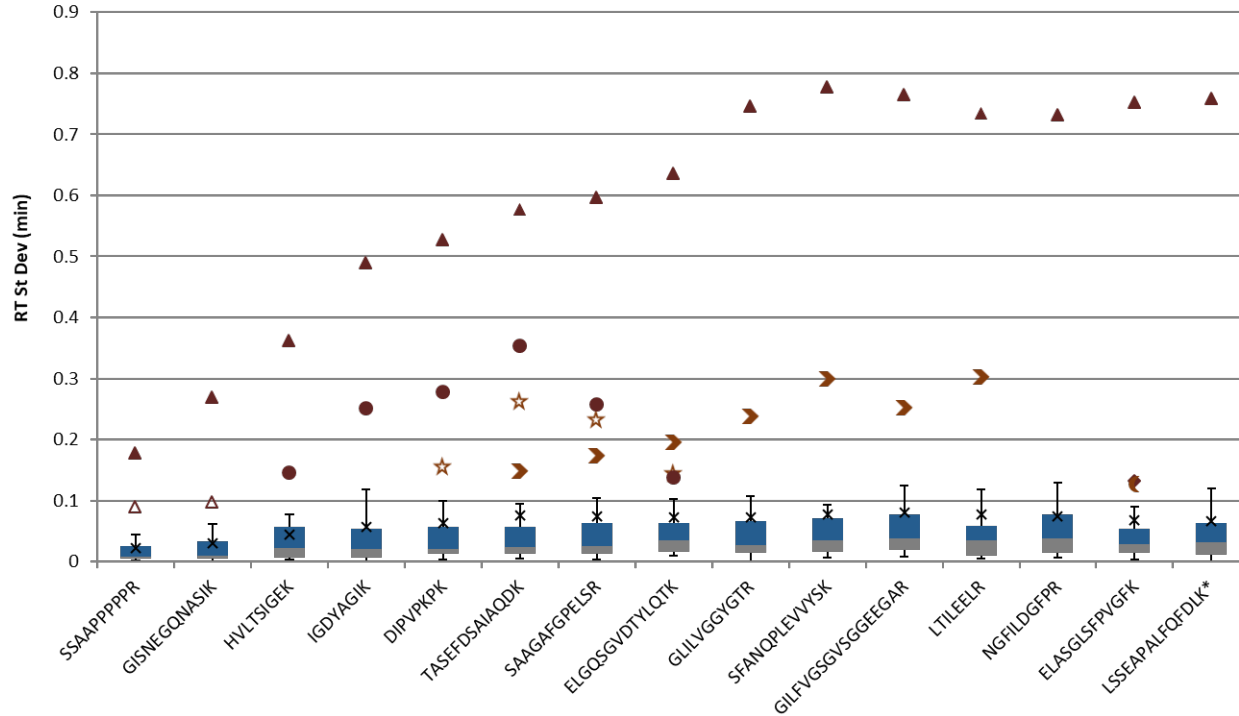
Outlier: More than 1.5x below the lower quartile,
shape coded by participant



System Suitability Peptide Retention Time



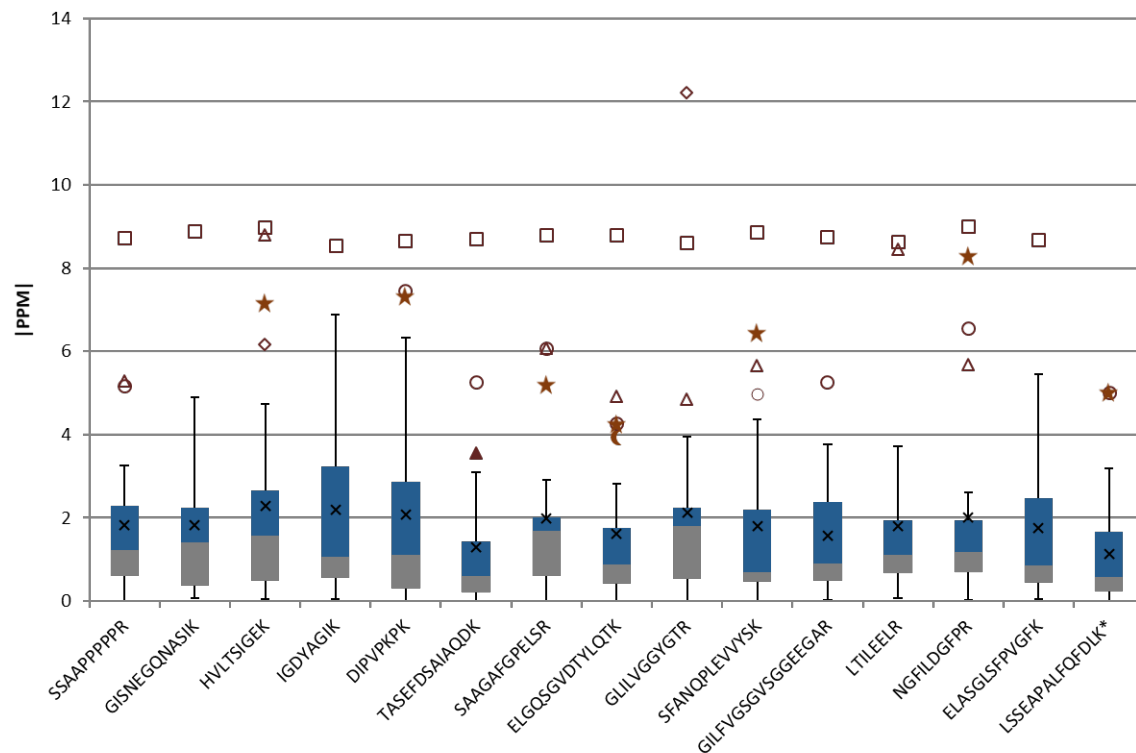
System Suitability Peptide Retention Time Variation (Within-Lab)



*not detected by

Not included due to aligned RT values:

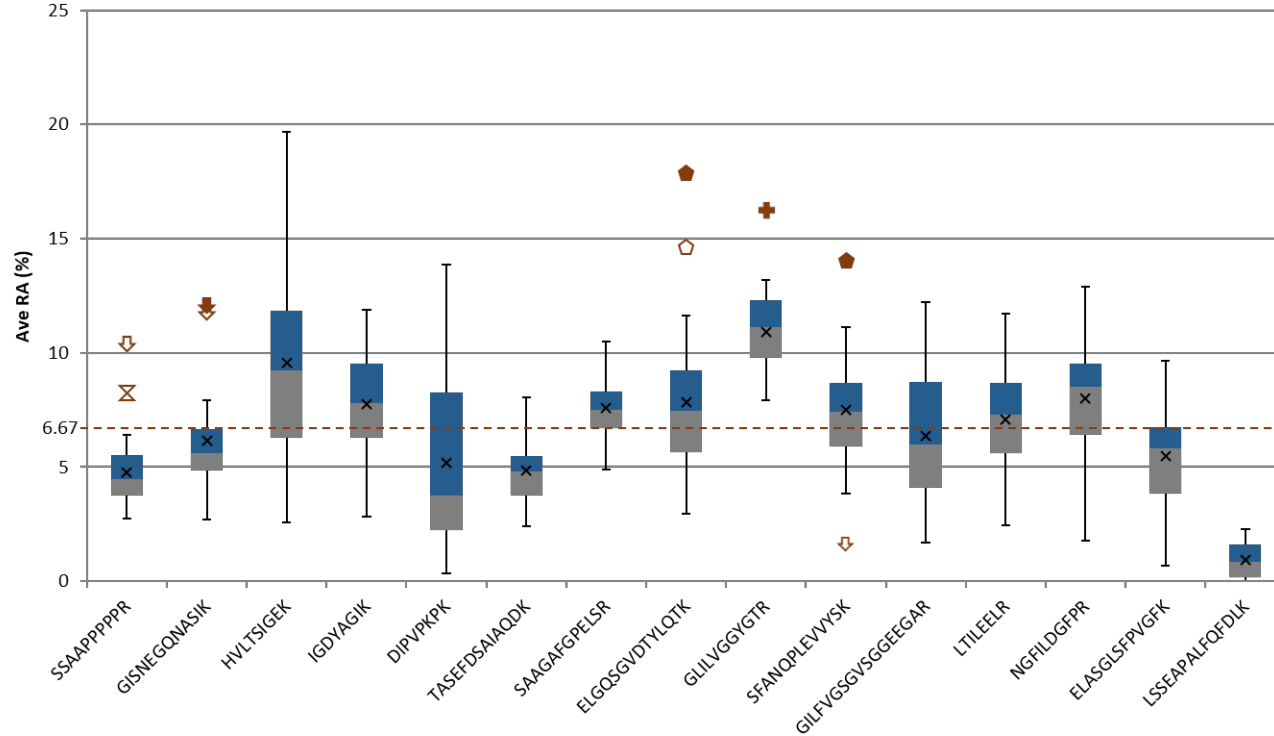
System Suitability Peptide Mass Accuracy



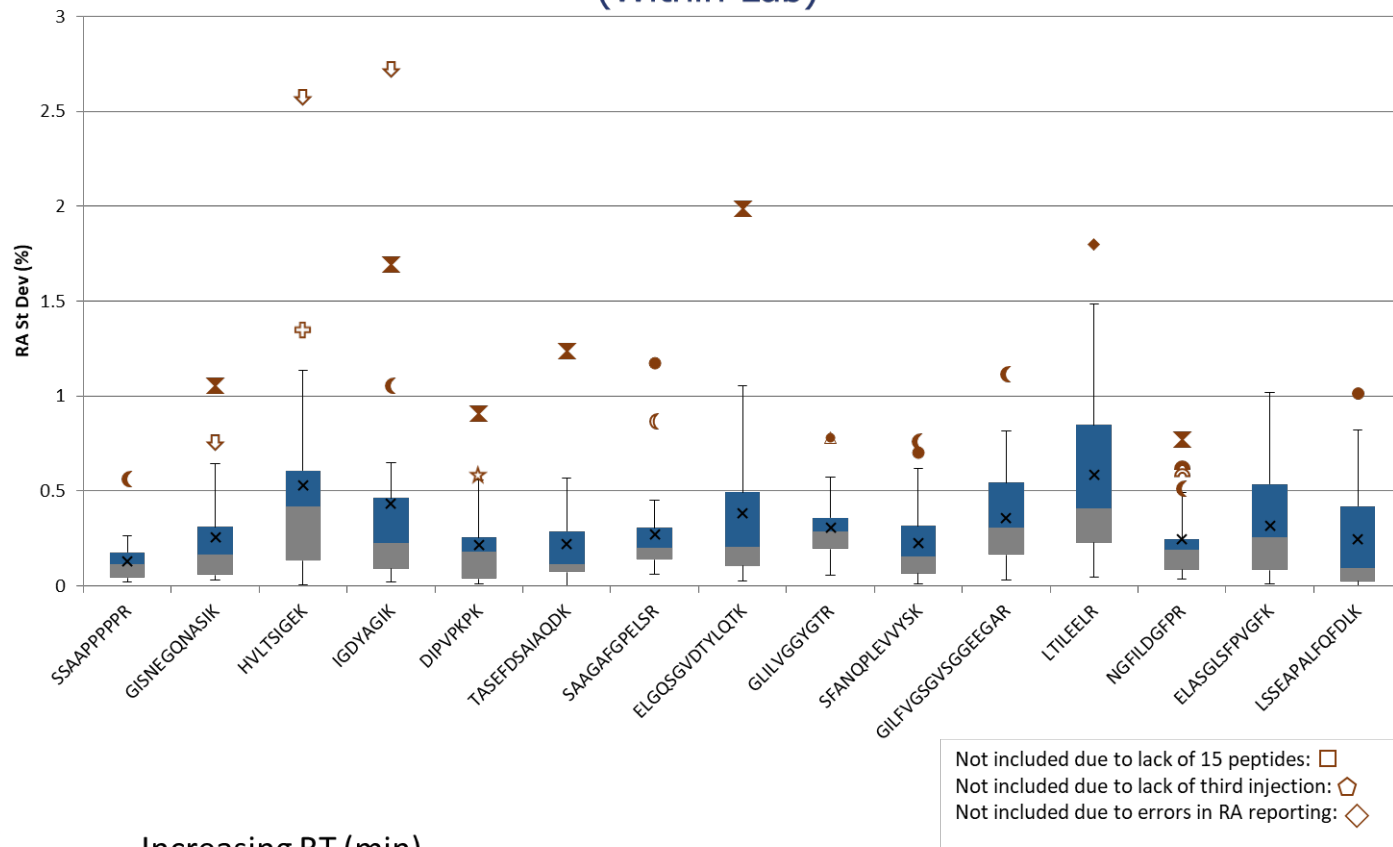
*not detected by

Increasing RT (min)
→

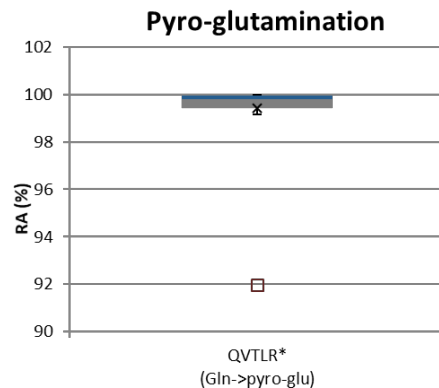
System Suitability Peptide Relative Abundance



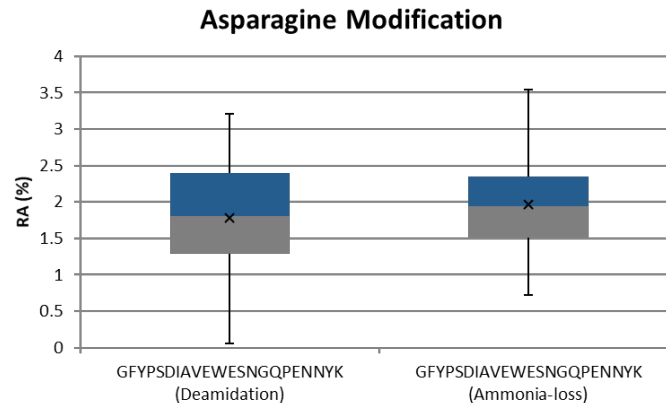
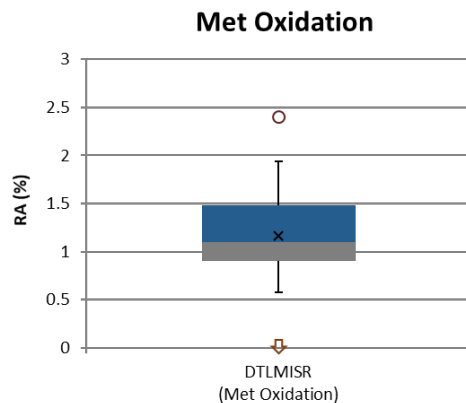
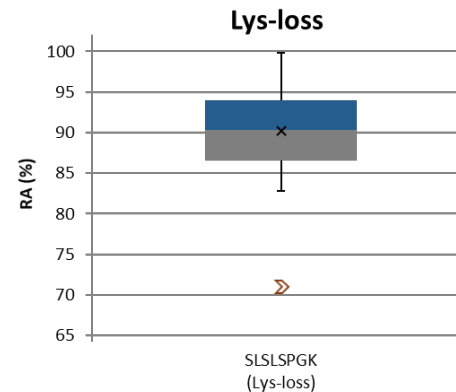
System Suitability Peptide Relative Abundance Variation (Within-Lab)



NISTmAb Reference Peptide Relative Abundance

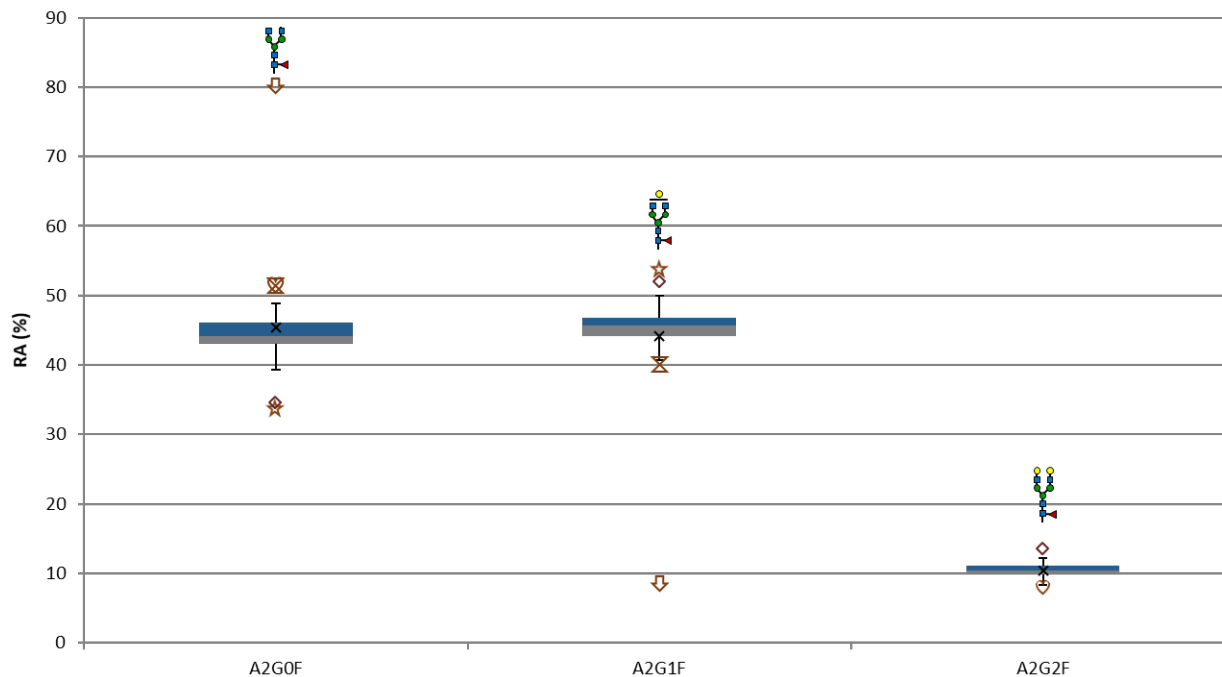


*neither modified nor unmodified detected by ▲



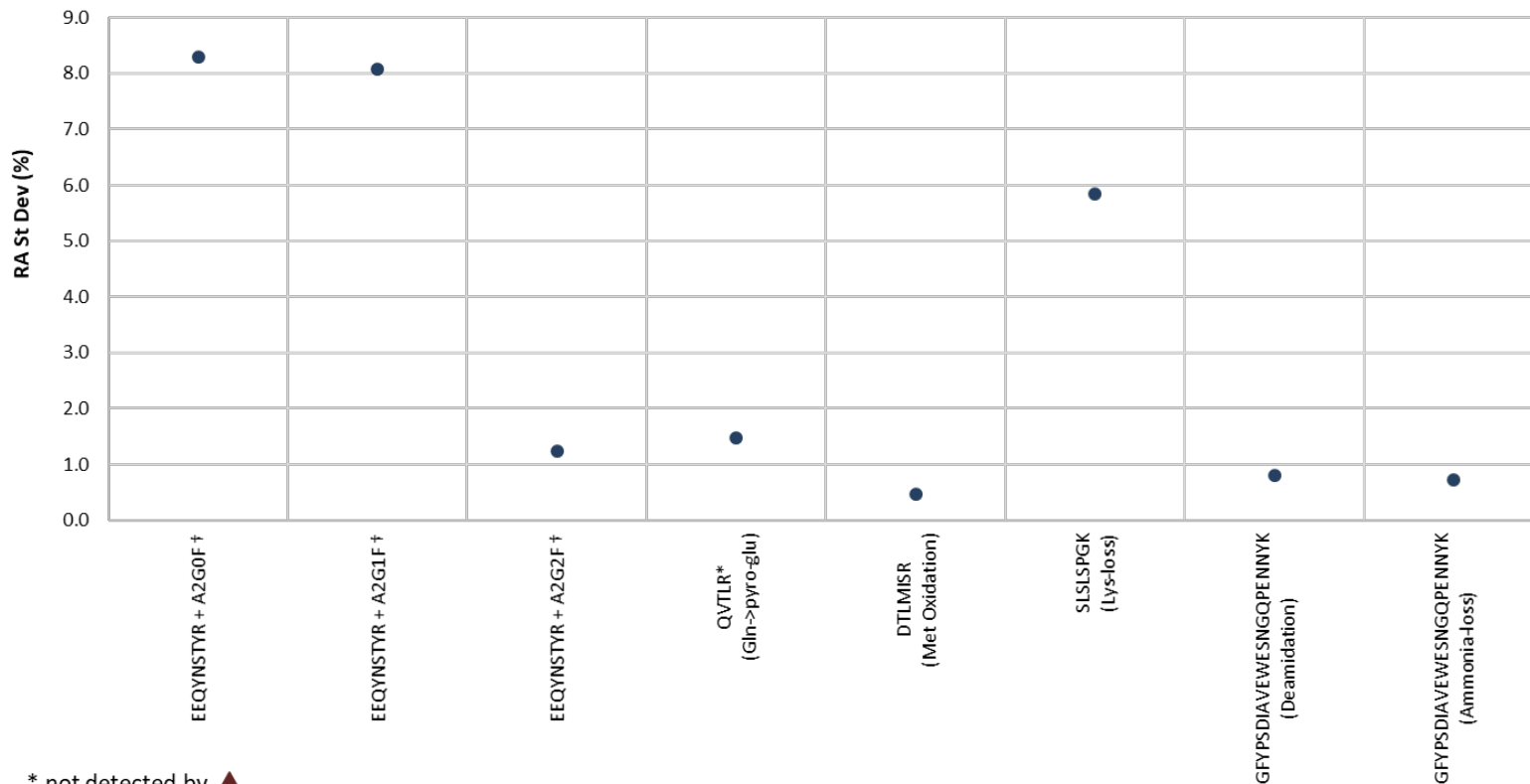
NISTmAb Reference Peptide Relative Abundance

Major EEQYNSTYR Glycopeptides



- not included due to lack of detection of A2G1F and A2G2F
- and ◇ not included due to lack of detection of A2G2F
- ♥ not included due to lack of detection of A2G0F, A2G1F and A2G2F

NISTmAb Reference Peptide Modification Relative Abundance Variation (Between-Lab)



* not detected by ▲

† not included due to lack of detection of A2G2F1F and A2G2F:○

not included due to lack of detection of A2G:◻ and ◻

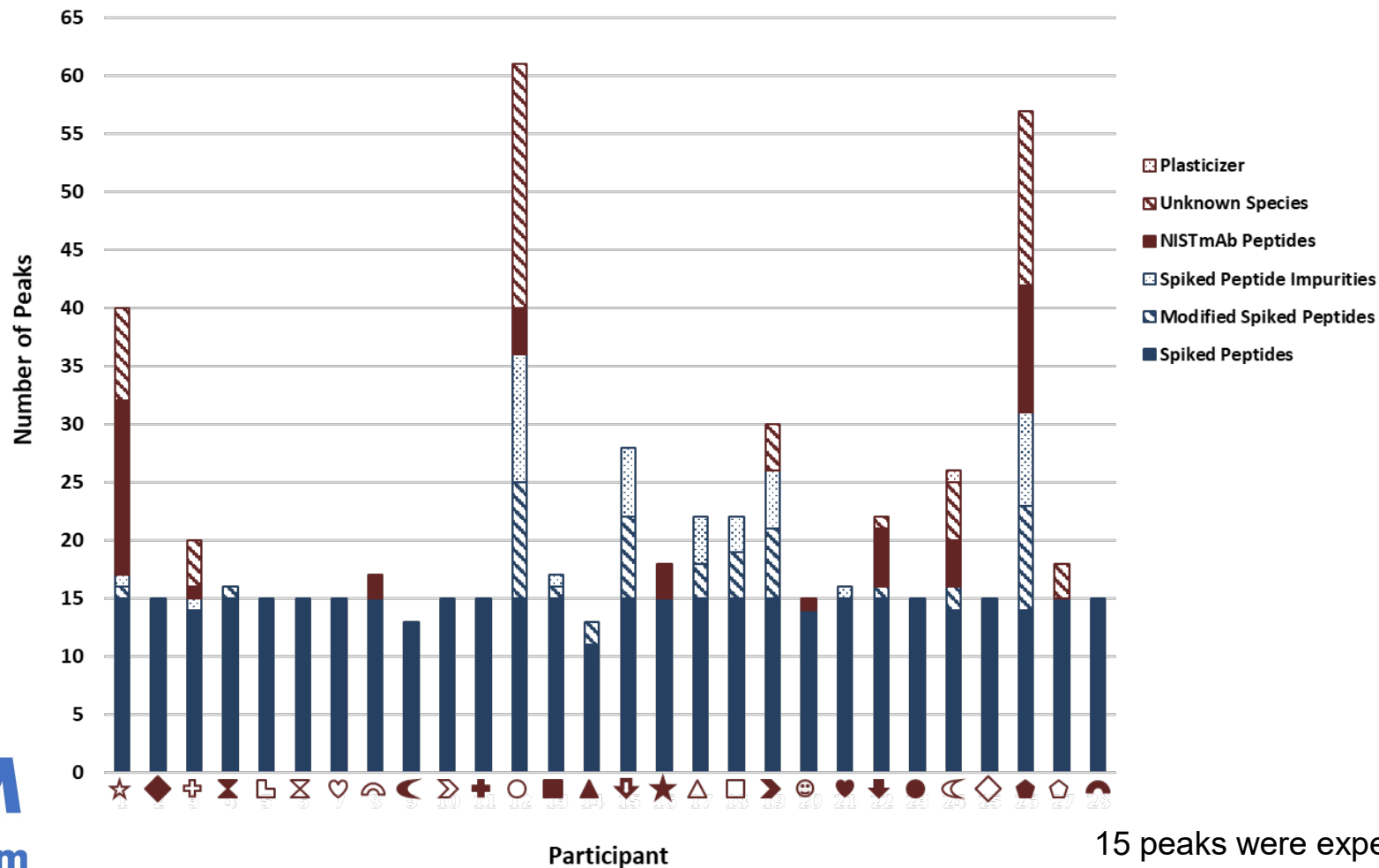
not included due to lack of detection of A2G0F, A2G1F and A2G2F:♥

Increasing RT (min)

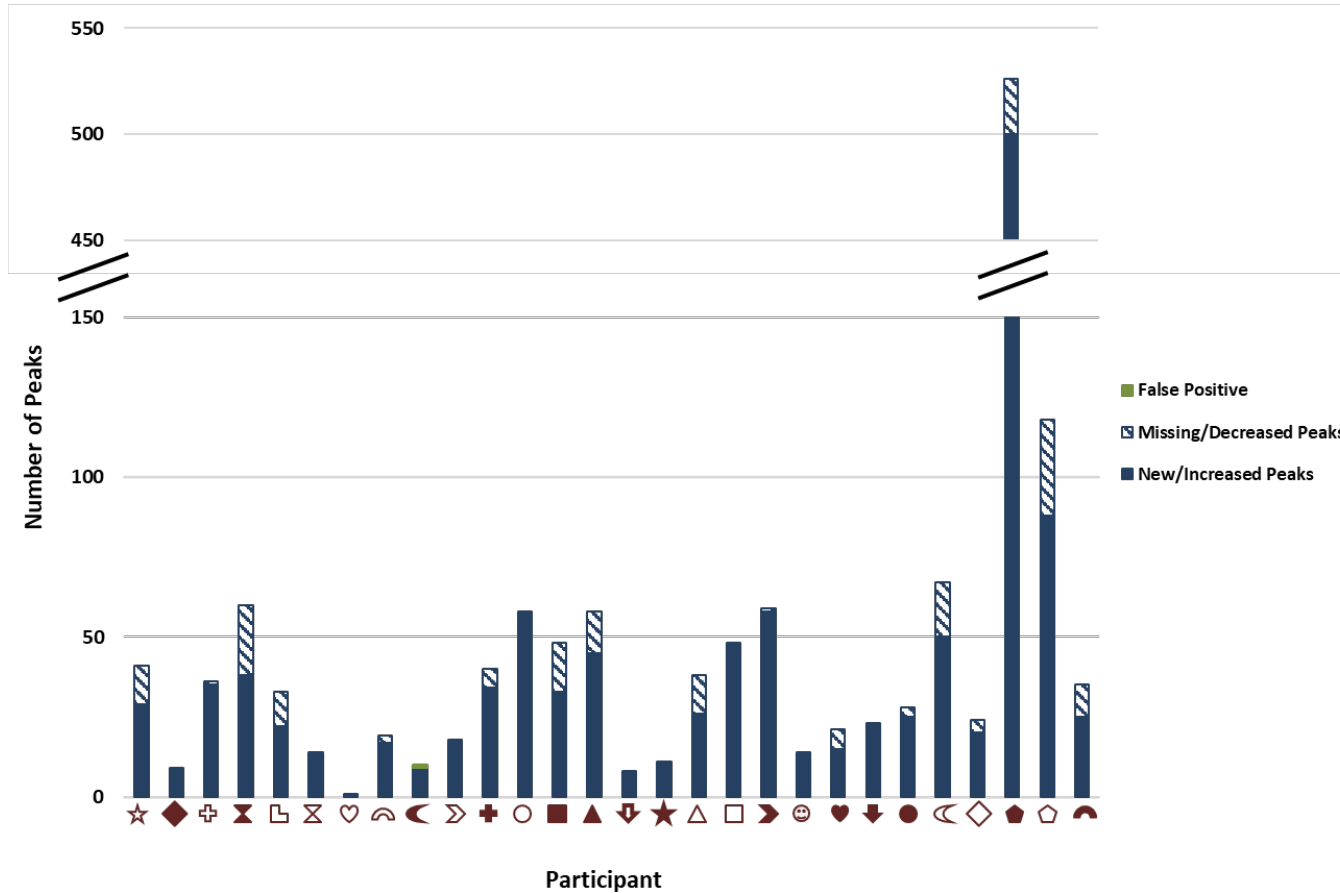


Now for the NPDRR results!

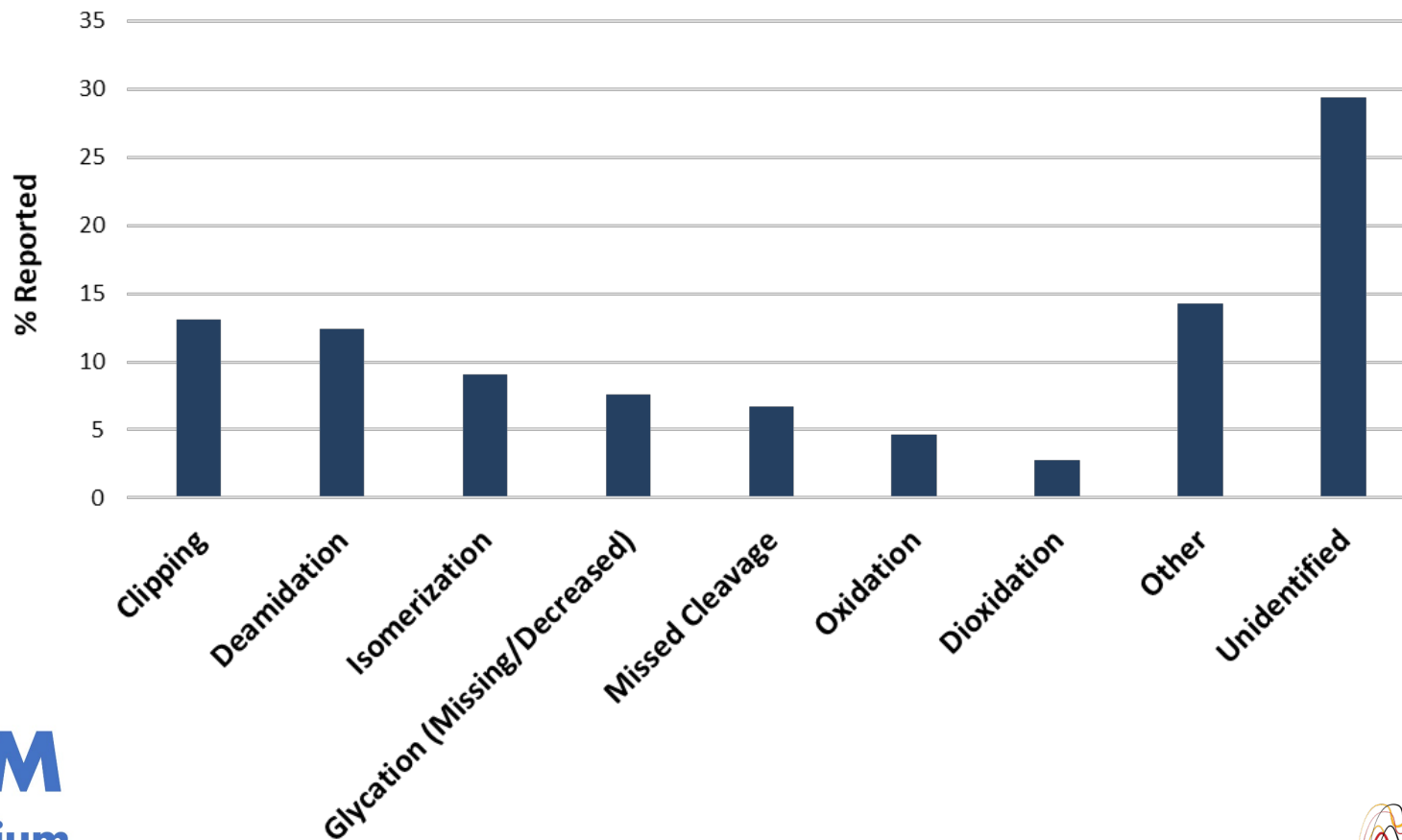
Spiked Digest New Peak Detection



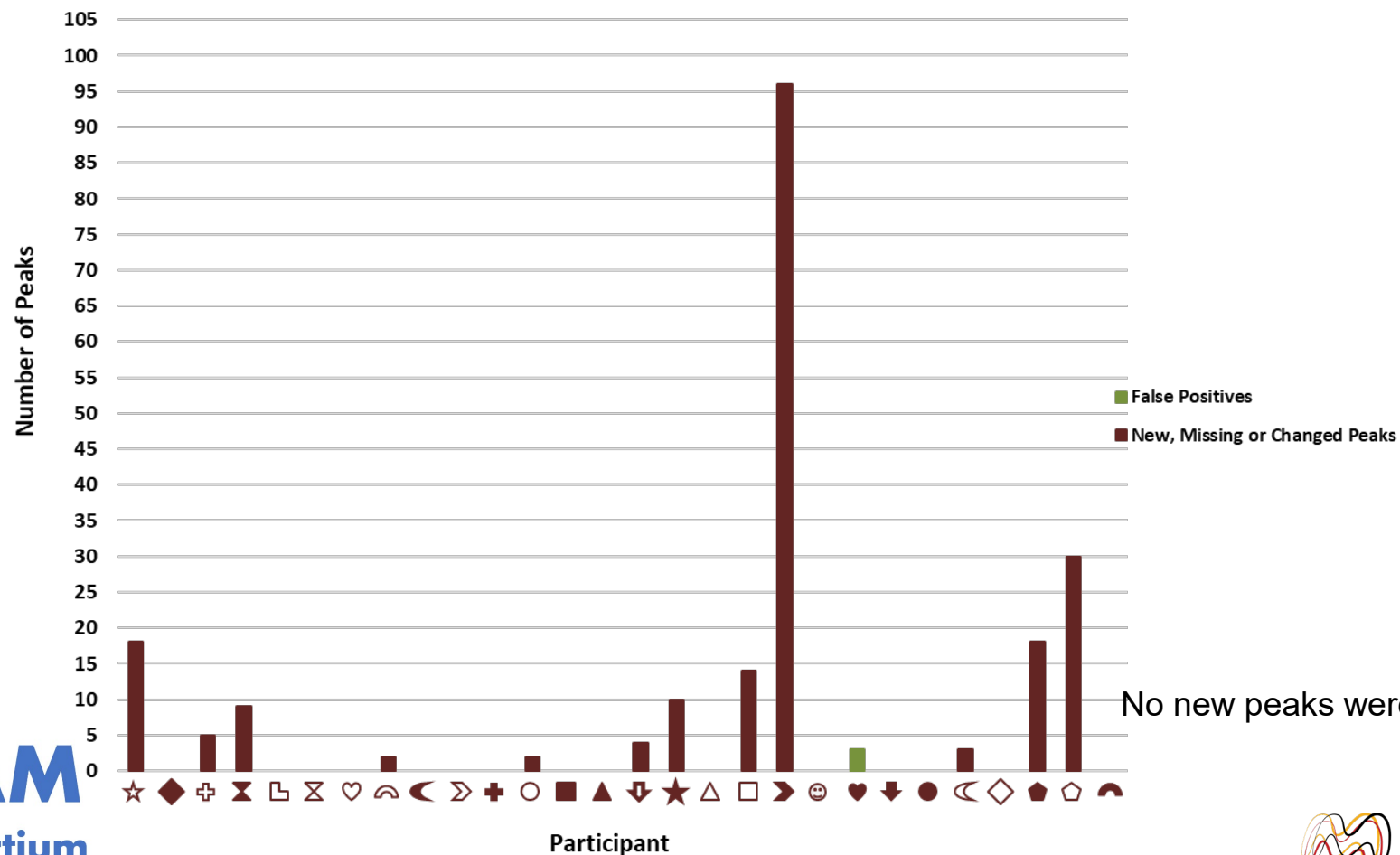
pH Stress Digest New Peak Detection



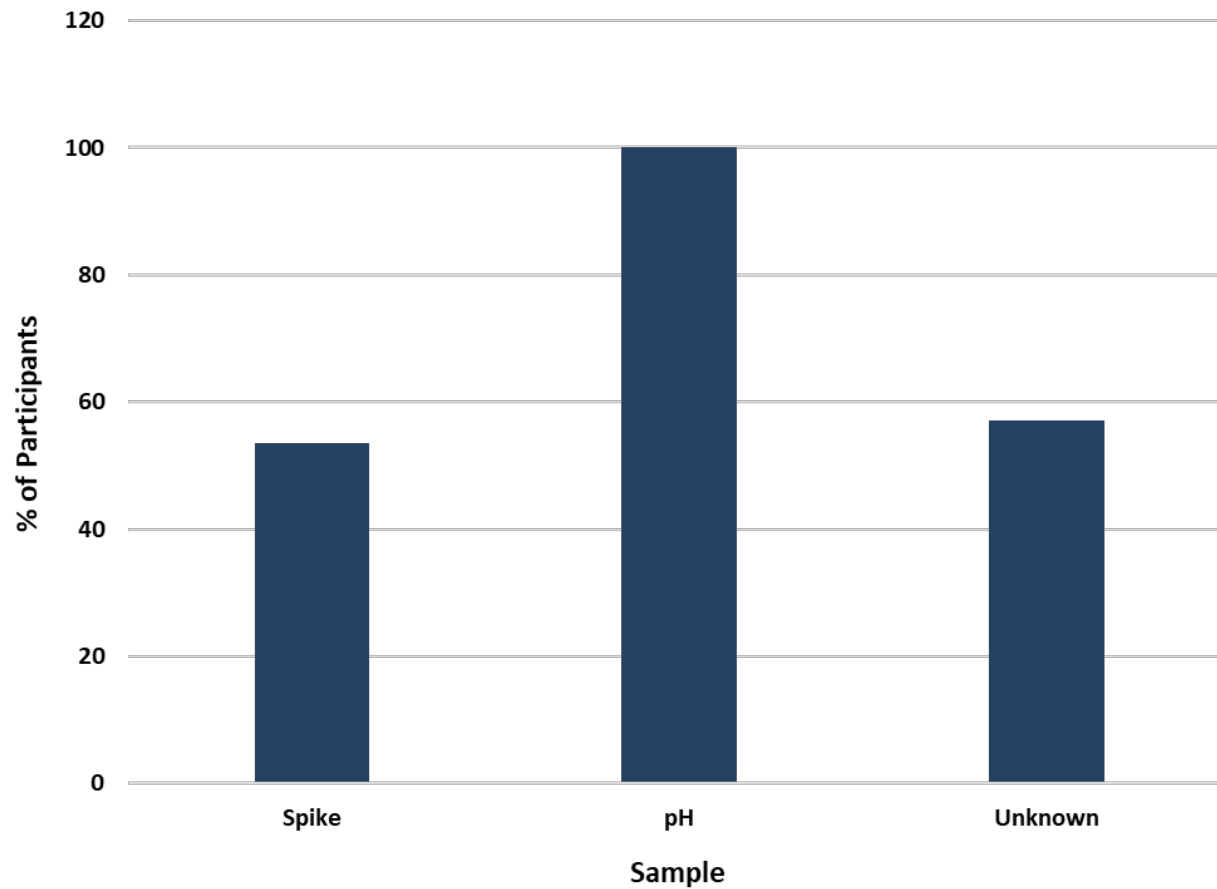
Survey of New, Missing, Changed Peak Modifications (pH Stress Digest)



Unknown Digest New Peak Detection



"Passing" Results



Individual "Pass/Fail" Status

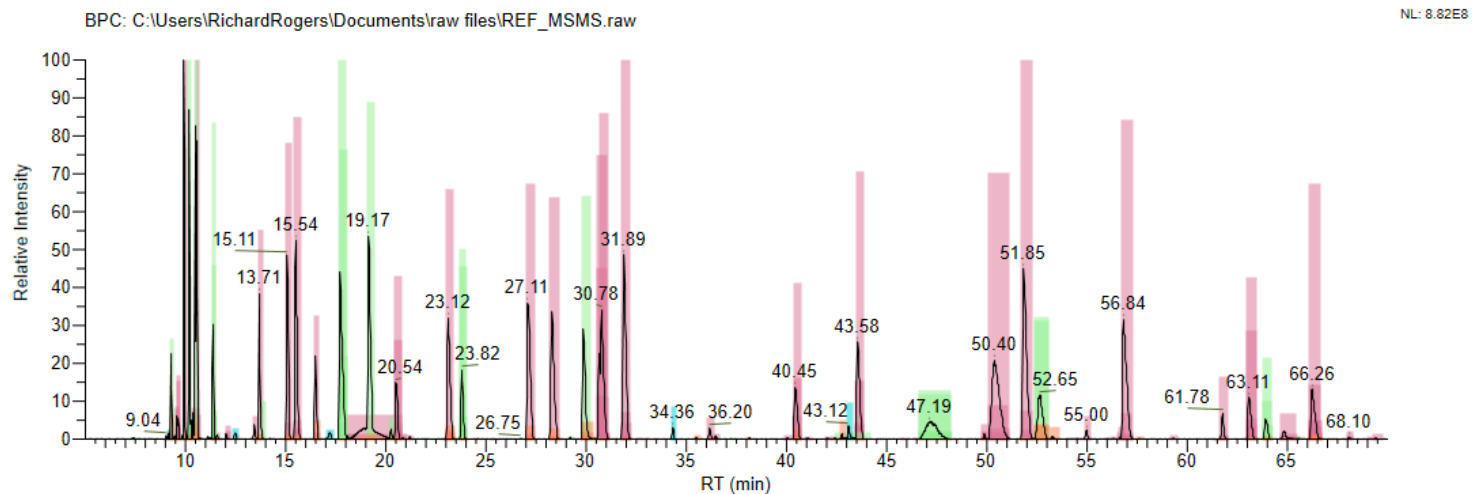
	Spike	pH	Unknown			
☆						
◆						
+						
✂						
📄						
✂						
♡						
☒						
☾						
☾						
➤						
+						
○						
■						
▲						
↓						
★						
△						
□						
➤						
😊						
♥						
↓						
●						
☾						
◇						
🏠						
🏠						
☾						
	Spike	pH	Unknown			

12 datasets “pass” for all three analyses

Conclusions and Future Directions

- Out of 28 datasets, 12 were considered “passing”
- Identify sources of false positives
- Identify sources of false negatives
- Identify ways to improve reproducibility
 - Community specifications
 - System suitability criteria for MAM platform
- Analyze 16 raw data sets with harmonized software platforms

Ensure major peaks are identified

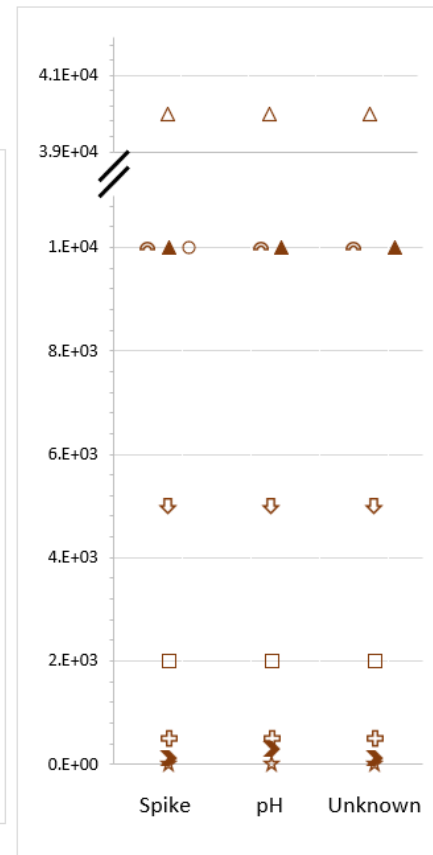
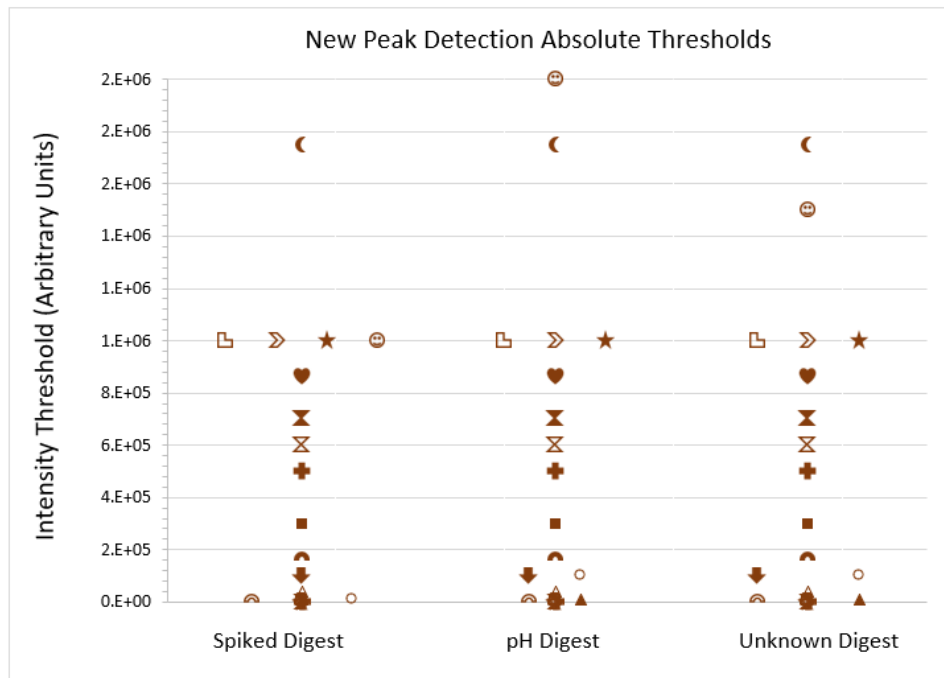


Chromatogram

Results

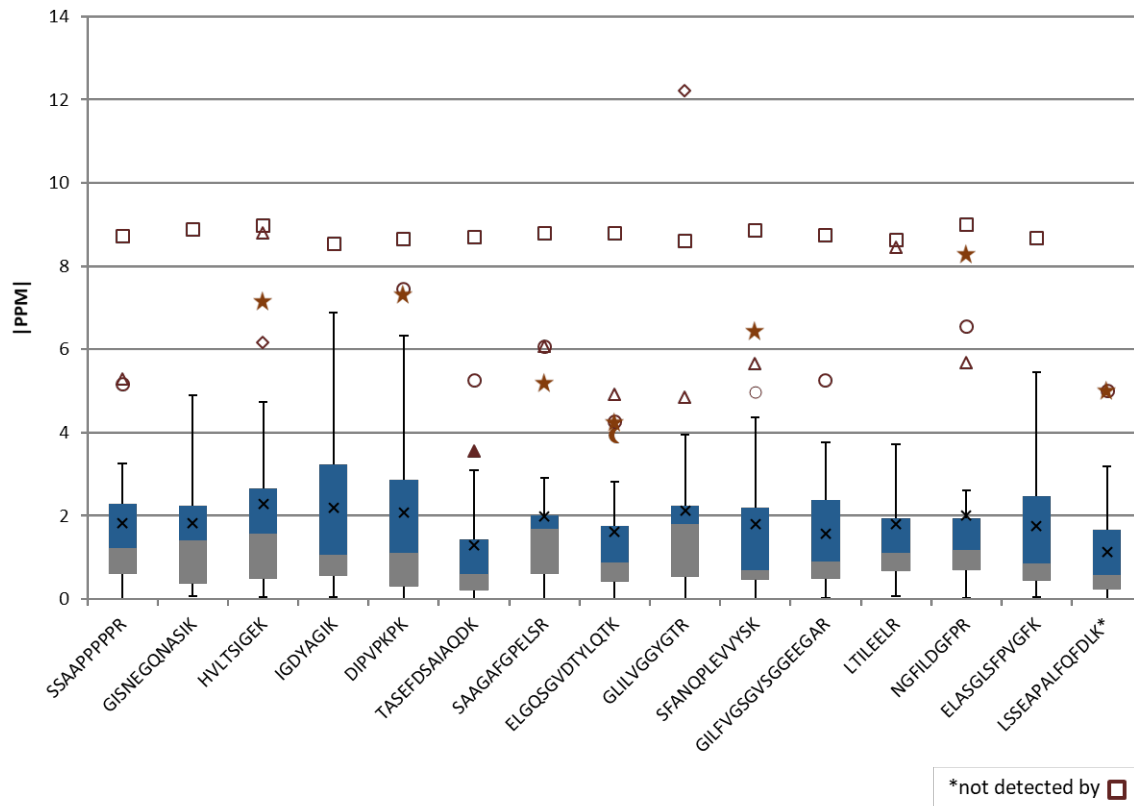
	Level	Flag	No.	Protein
+	1	Protein	1	Nist_mab_HC
+	2	Protein	2	Nist_mab_LC
+	3	Protein	3	Trypsin
+	4	Protein	4	Unidentified

Potential false positives



Potential false negatives

System Suitability Peptide Mass Accuracy



*not detected by

Summary

- The MAM is precise and can track attribute trends similar to conventional assays.
- The MAM can directly monitor attributes (Man5, c-terminal K, NGHC, deamidation, and Iso D).
- Automated new peak detection using the MAM is more sensitive and robust than the conventional purity assays.
- Automated new peak detection can identify new modifications.
- Multi-Enzyme MAM can save 3 hours per molecule and achieve complete coverage of a molecule.
- The MAM can be used for molecular optimization, PAC and real-time release of biotherapeutics.