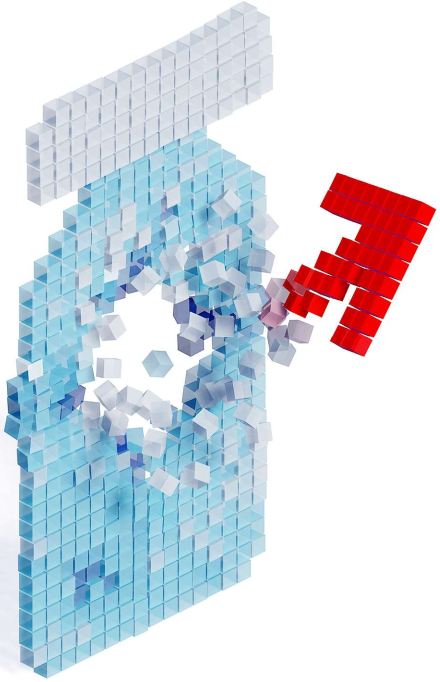
Bringing MAM to the next level:

Thermo Scientific MAM 2.0

Thermo Fisher Scientific MAM Team

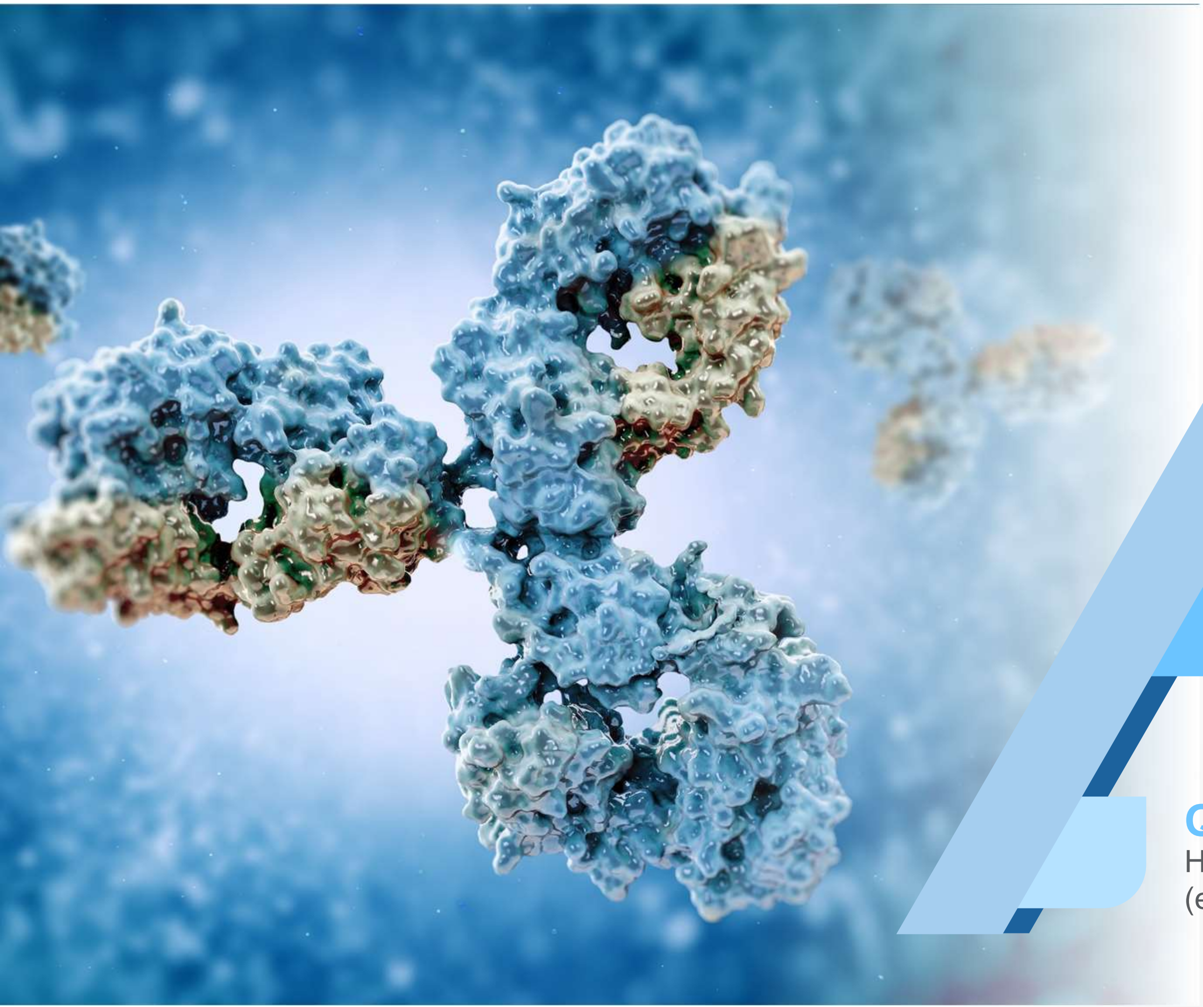
Hao Yang, Ph.D.

WCBP 2022



The world leader in serving science

A complex problem: drug quality and safety



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

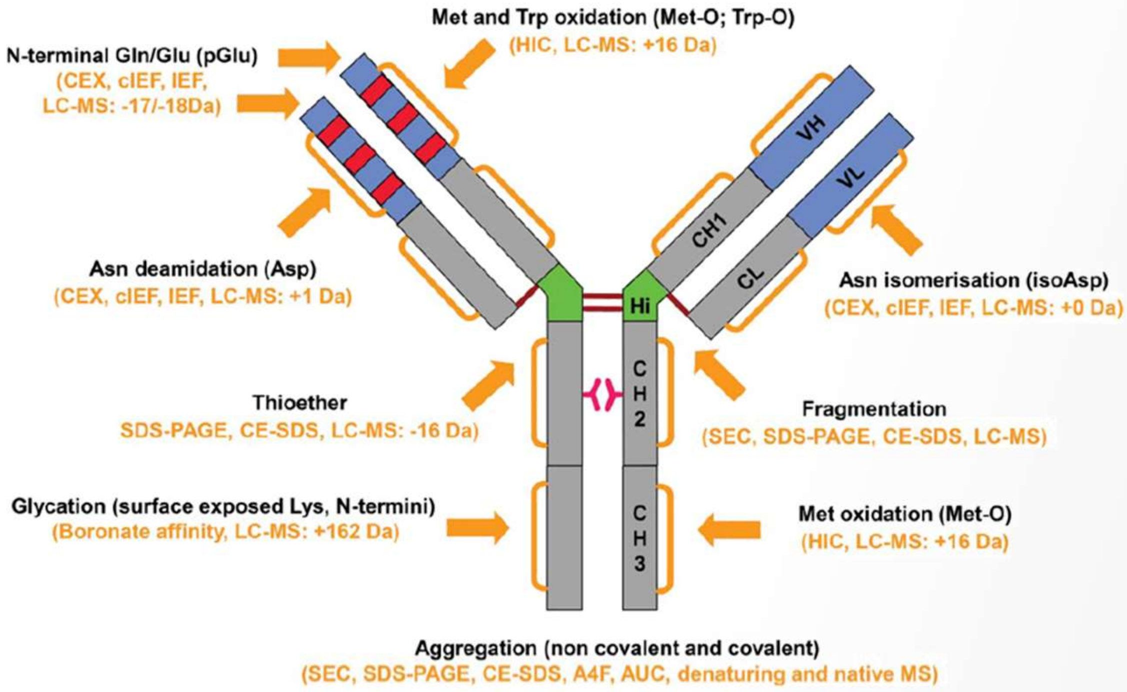
Potency
Does the drug have the expected effect?
(e.g. CDR complementation)

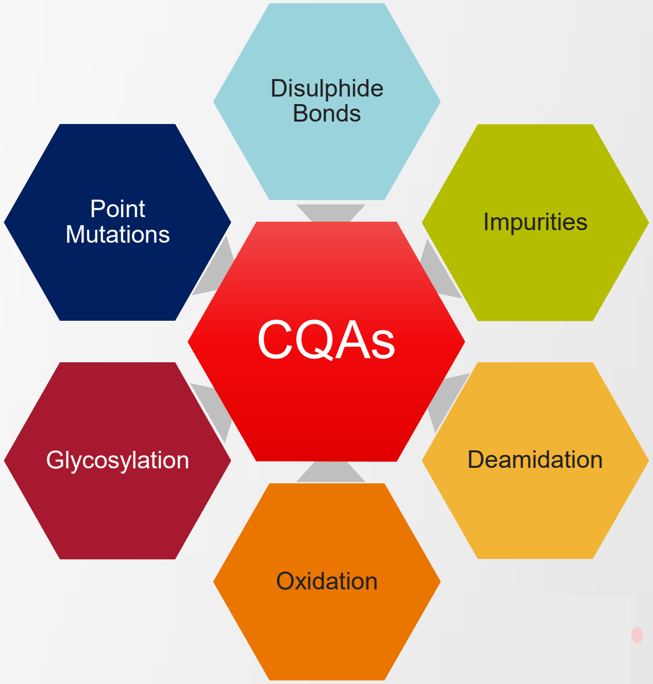
Knowledge
How do changes effect the therapeutic?
(e.g. Oxidation)

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

Definition of critical quality attribute (CQA)

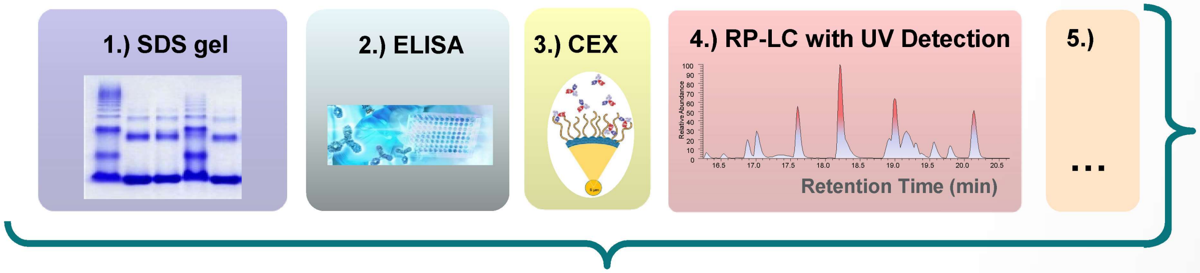
A physical, chemical or biological **property or characteristic of a drug** product that should be within an appropriate limit, range, or distribution to **ensure the desired product quality**.

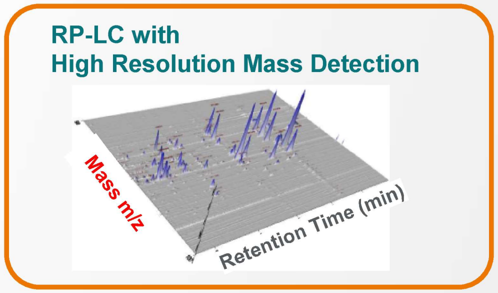




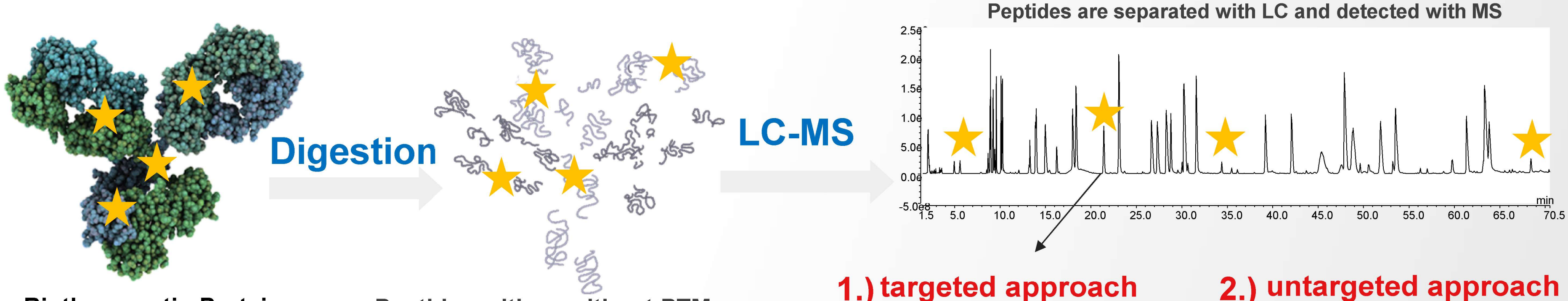
Christine Nowak, et al, mAbs, 2017; 9(8):1217-1230.

The concept of MAM – the LC-MS based multi-attribute method





Multiple workflows are required for quality control of biopharmaceuticals



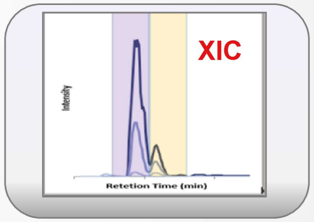
Biotherapeutic Protein

Peptides with or without PTMs

Sum of all peptides= product quality attributes (**PQA**)

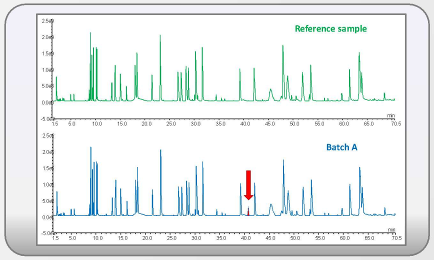
★ Set of critical peptides that need to be monitored to ensure saftey and efficay= Critical Quality Attributes (**CQA**)

1.) targeted approach



CQA Quantitation

2.) untargeted approach



New Peak Detection (NPD)

MS in QC Lab: Multi-Attribute Method

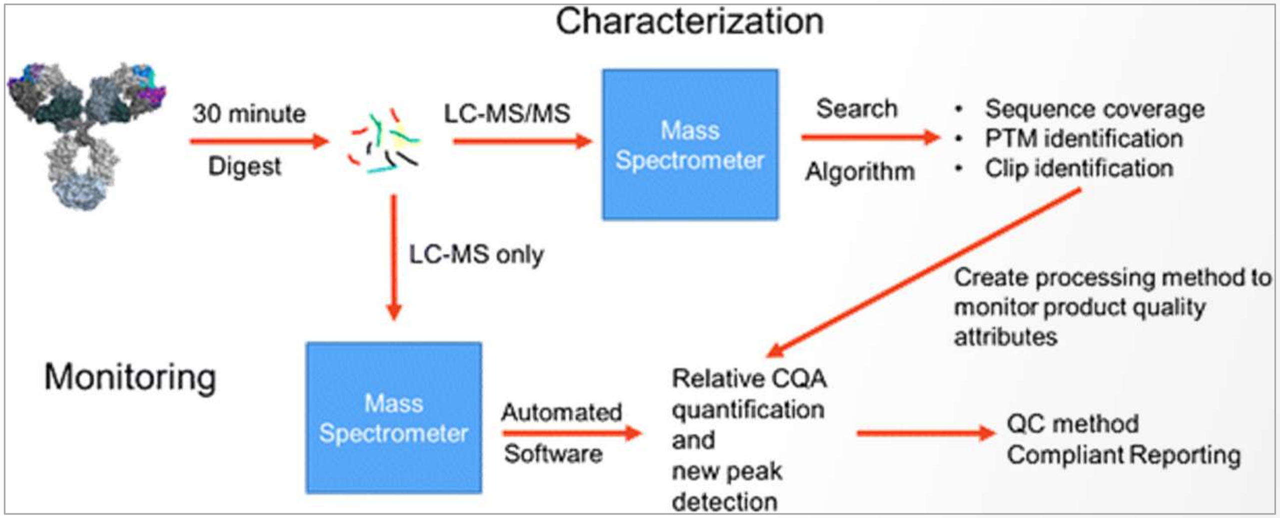
REPORT

mAbs 7:5, 881–890; September/October 2015; © 2015 Amgen Inc.

Development of a quantitative mass spectrometry multi-attribute method for characterization, quality control testing and disposition of biologics

Richard S Rogers[†,*], Nancy S Nightlinger, Brittney Livingston[†], Phil Campbell, Robert Bailey[#], and Alain Balland[§]

Analytical Sciences; Amgen Inc.; Seattle, WA USA
[†]Present affiliation: Just Biotherapeutics; Seattle, WA USA
[#]Present affiliation: Zymeworks; Seattle, WA USA
[§]Present affiliation: Boehringer Ingelheim Pharma GmbH & Co. KG; Biberach an der Riss, Germany



Characterization

30 minute Digest → LC-MS/MS → Mass Spectrometer → Search Algorithm →

- Sequence coverage
- PTM identification
- Clip identification

LC-MS only

Create processing method to monitor product quality attributes

Monitoring

Mass Spectrometer → Automated Software → Relative CQA quantification and new peak detection → QC method Compliant Reporting

>400 members

>80 companies

FDA Publications

Impact Story: Transitioning a Powerful Analytical Tool into Manufacturing to Improve the Quality of Complex Therapeutics

Regulatory Science IMPACT STORY

Content current as of:
02/05/2019

Regulated Product(s)
Drugs

Topic(s)
Research

CDER researchers are evaluating an approach that would harness the potential of mass spectrometry to ensure the quality of complex protein therapeutics to treat cancer and other diseases.

Mass spectrometry (MS) is a powerful analytical tool used in nearly all protein-based

analytical chemistry

Perspective

Cite This: *Anal. Chem.* 2019, 91, 14170–14177

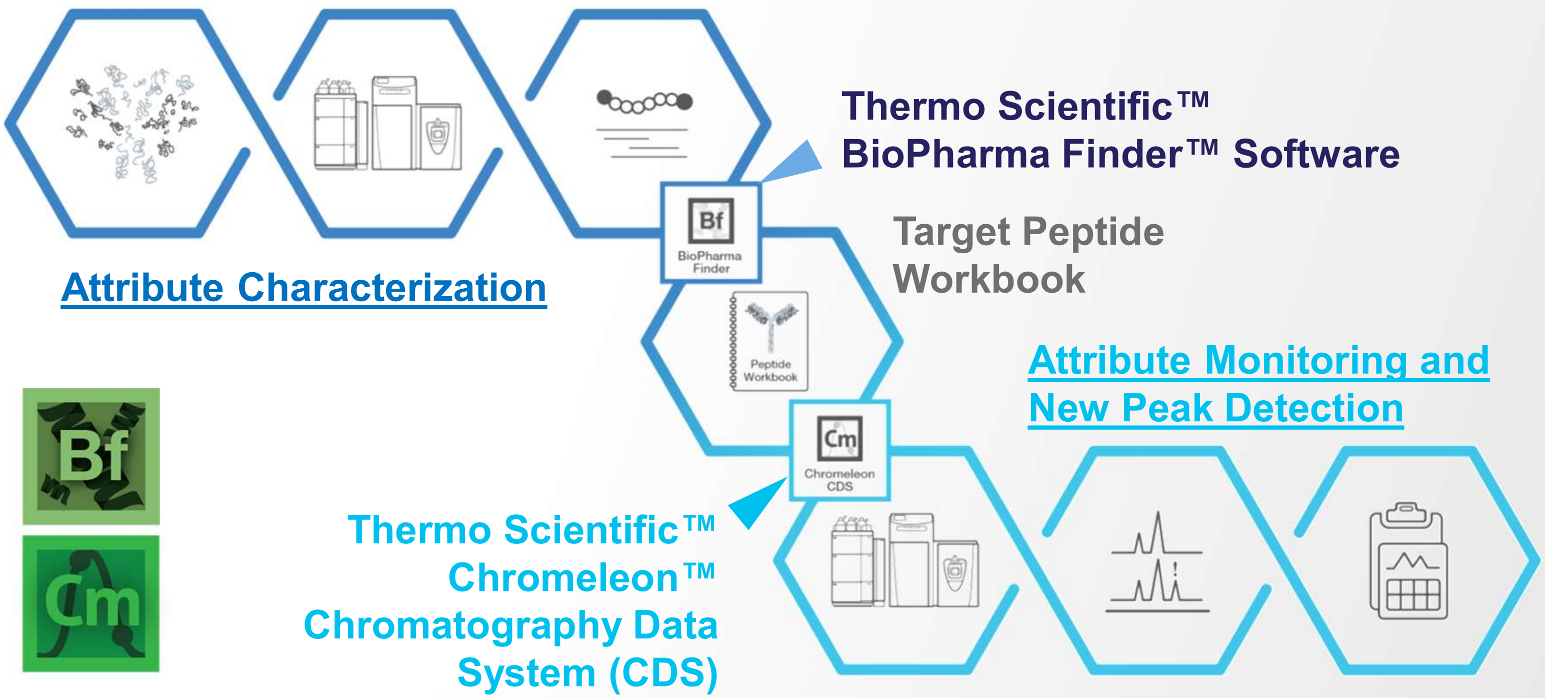
pubs.acs.org/ac

Multi-Attribute Method for Quality Control of Therapeutic Proteins

Sarah Rogstad,[*,†] Haoheng Yan,[‡] Xiaoshi Wang,[‡] David Powers,[‡] Kurt Brorson,[‡,§] Bazarragchaa Damdinsuren,[‡] and Sau Lee[†]

[†]Office of Testing and Research, Office of Pharmaceutical Quality, CDER, U.S. Food and Drug Administration, Silver Spring, Maryland 20993, United States
[‡]Office of Biotechnology Products, Office of Pharmaceutical Quality, CDER, U.S. Food and Drug Administration, Silver Spring, Maryland 20993, United States

HR MAM: First Step of a Shared MAM Journey



Sample Preparation

MS/MS Data Acquisition

Peptide Mapping

Attribute Characterization

From Research To Routine

Thermo Scientific™ BioPharma Finder™ Software

Target Peptide Workbook

Attribute Monitoring and New Peak Detection

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS)

MS1 Only Acquisition

CQA Monitoring and NPD

Reporting

Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer

Continued Conversations and Partnership

Engagement with Partners

MAM user meetings

- Identified gaps in early MAM offerings
- Provided input on future requirements
- Defined major themes for improvement

Customer-Prioritized MAM Features

Connectivity
☐ Integrated software ecosystem

Ease of Use
☐ Simplified workflow

Fit for Purpose
☐ Common HW platform from development to QC

Compliance-Ready
☐ User privilege

Training and Support
☐ Application focused

Thermo Scientific MAM 2.0: Purpose-Built MAM Solution

Fit-for- Purpose

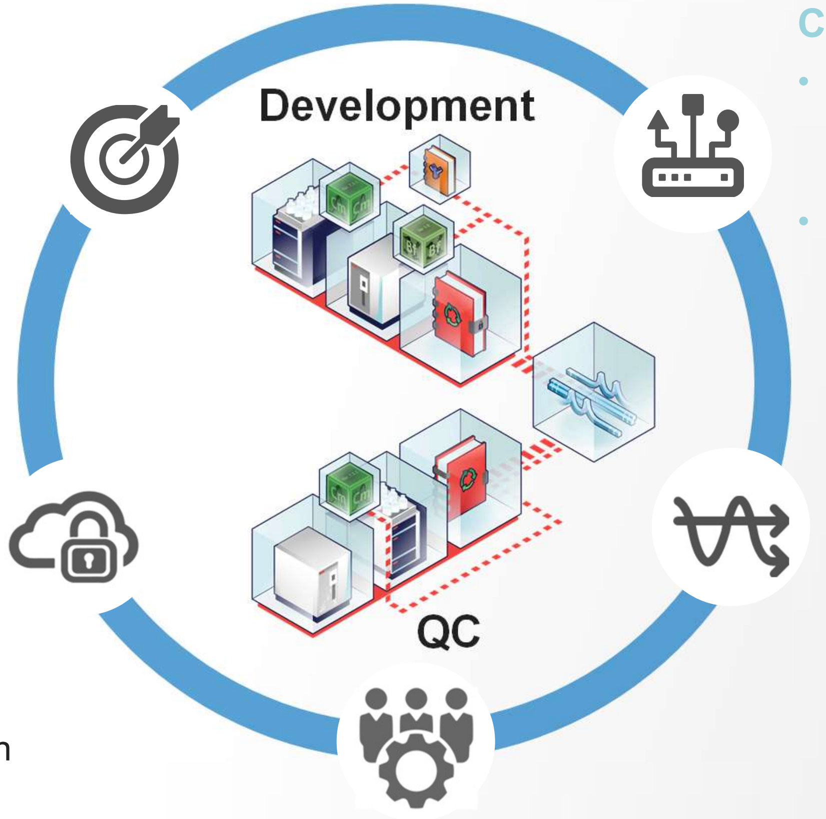
Compliance-ready

Fit-for-Purpose

- Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer for attribute characterization
- **Thermo Scientific™ Orbitrap Exploris™ MX** mass detector for attribute monitoring

Compliance-Ready

- Audit trail
- User access privilege
- Ease of administration



Connectivity

- A scalable software ecosystem for enterprise deployment at global scale
- Centralized and secure data storage for seamless knowledge sharing

Ease of Use

- Direct method transfer from development to QC
- Automatic workflow execution

Dedicated global support team of MAM experts (HW, SW, Application)

Support

Connectivity

Ease of use

MAM 2.0: Straight through to Breakthrough

An Ecosystem from Development to QC

Fit-for-Purpose

Development

Thermo Scientific™ Vanquish™ Horizon or Flex UHPLC system

Orbitrap Exploris 240 Mass Spectrometer (MS, MS/MS)

QC

Thermo Scientific™ Vanquish™ Horizon or Flex UHPLC system

Orbitrap Exploris MX Mass Detector (Full MS only)

new

- Extended Life Cycle
- Ease of Operation
- Robustness
- Cost Effective

- **Common SW and HW Platform**
- **Consistent Results**
- **Direct Method Transfer**

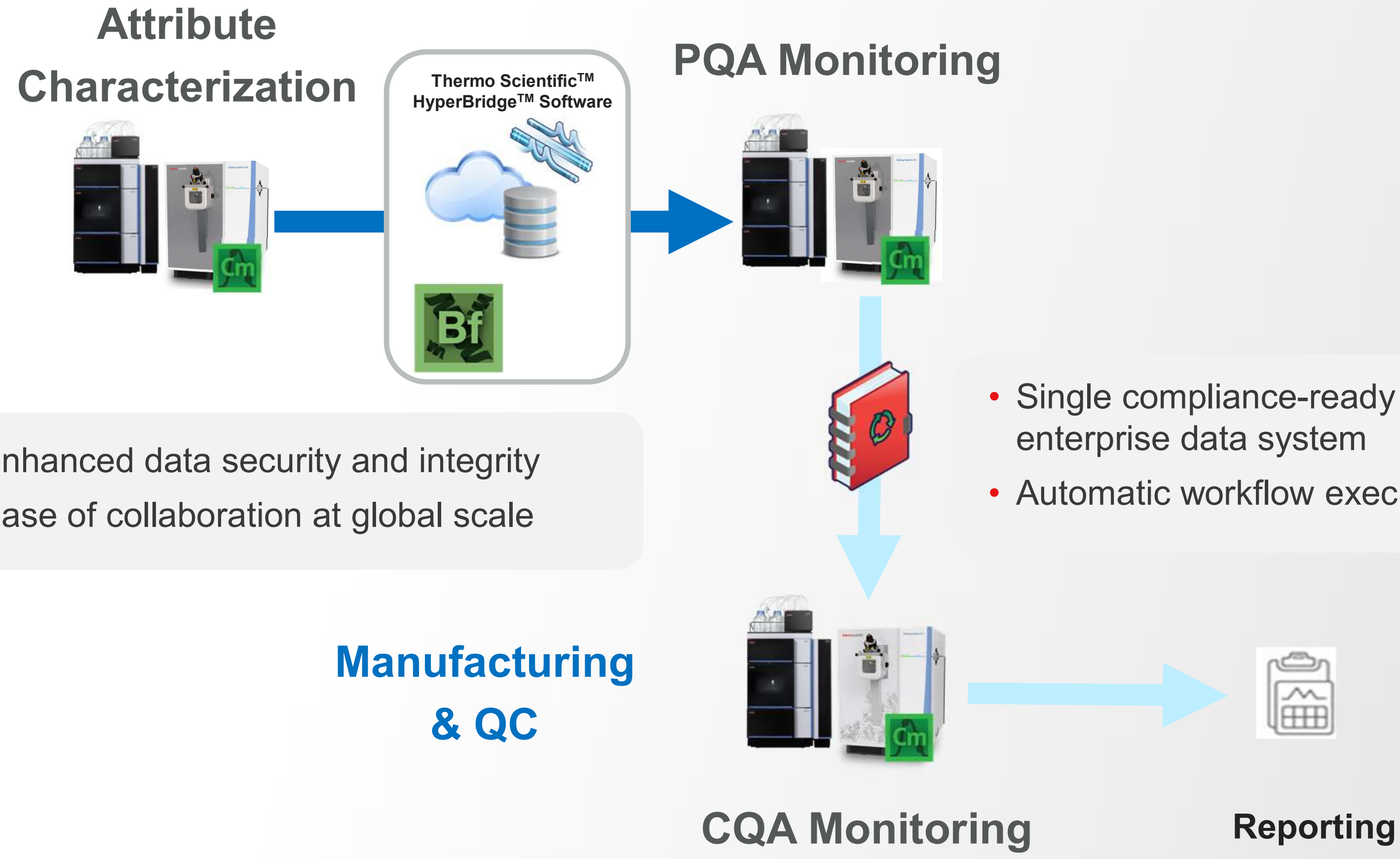
Ease of use

MAM 2.0: An Ecosystem from Development to QC

Compliance-Ready, Scalable, Fit-for-Purpose

Fit- for- Purpose

Compliance-ready



Research & Development

Attribute Characterization

Thermo Scientific™ HyperBridge™ Software

PQA Monitoring

- Enhanced data security and integrity
- Ease of collaboration at global scale

- Single compliance-ready enterprise data system
- Automatic workflow execution

Manufacturing & QC

CQA Monitoring

Reporting

Connectivity

Ease of use

MAM 2.0: Attribute Characterization

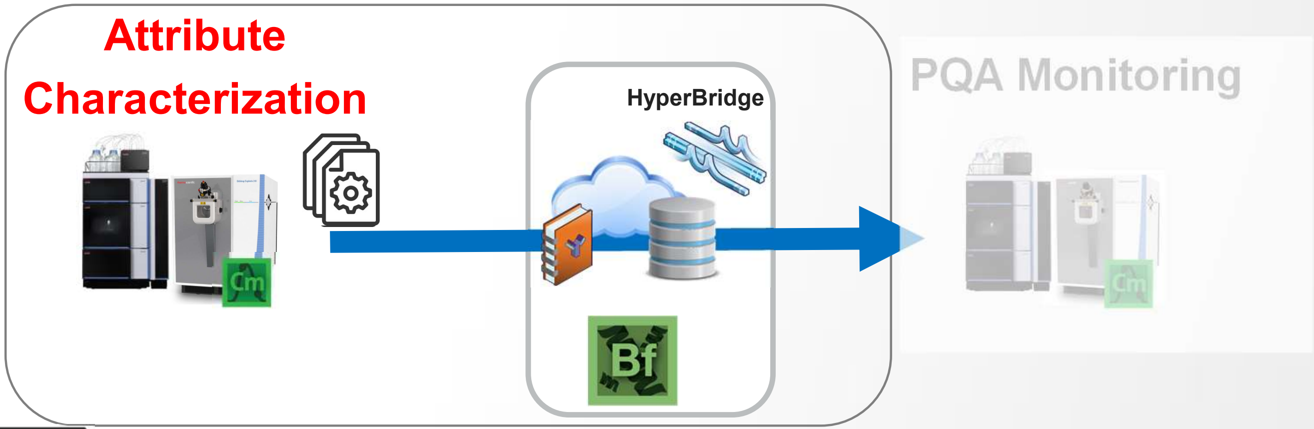
Confident attribute identification, Data security, Ease of collaboration

Fit- for- Purpose

Compliance-ready

Connectivity

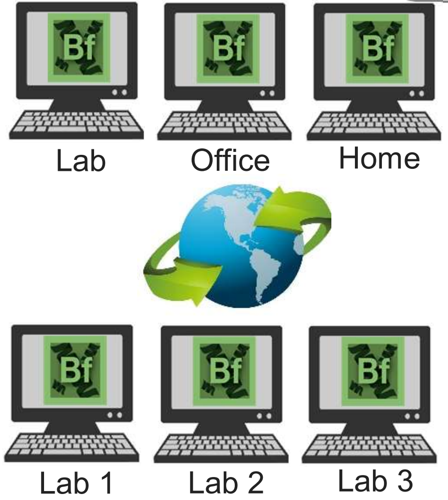
Research & Development



Attribute Characterization

HyperBridge

PQA Monitoring



- Confident and comprehensive attribute characterization
- Enhanced data security and integrity with single sign-on
- Flexible and secure data access anywhere, anytime
- Ease of collaboration at global scale

Attribute Characterization

PQA Identification – Confident and Comprehensive

Simplify complex processing with superior algorithms

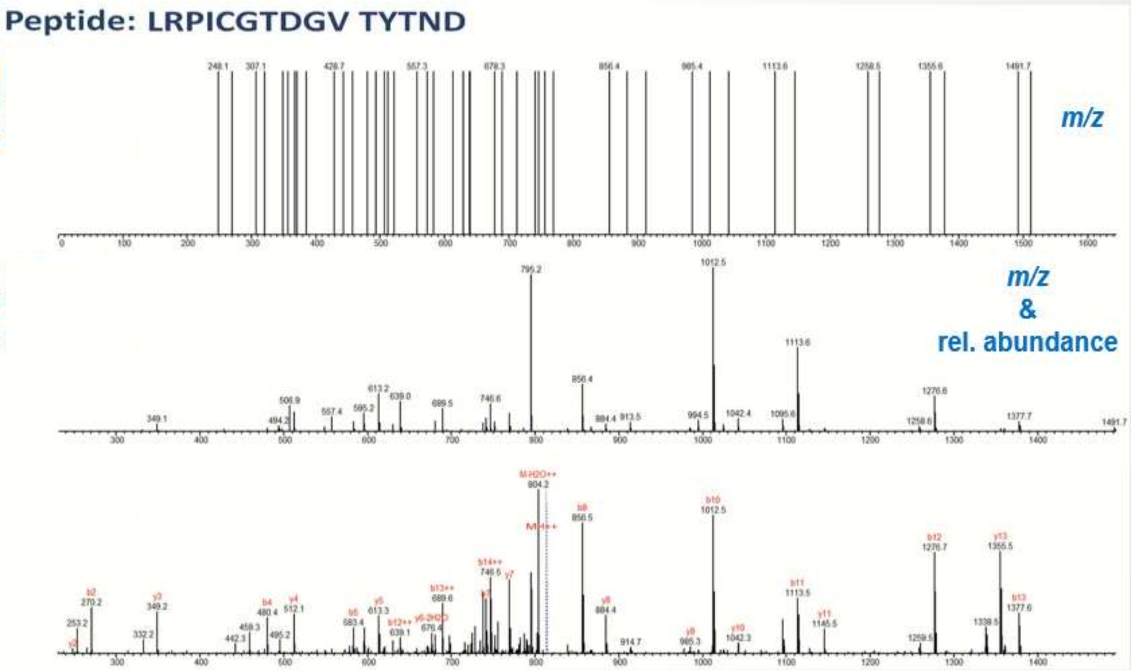
Kinetic prediction model

Fit- for- Purpose

Classic
Prediction Model

Kinetic
Prediction Model

Measured
Spectrum



Attribute Characterization

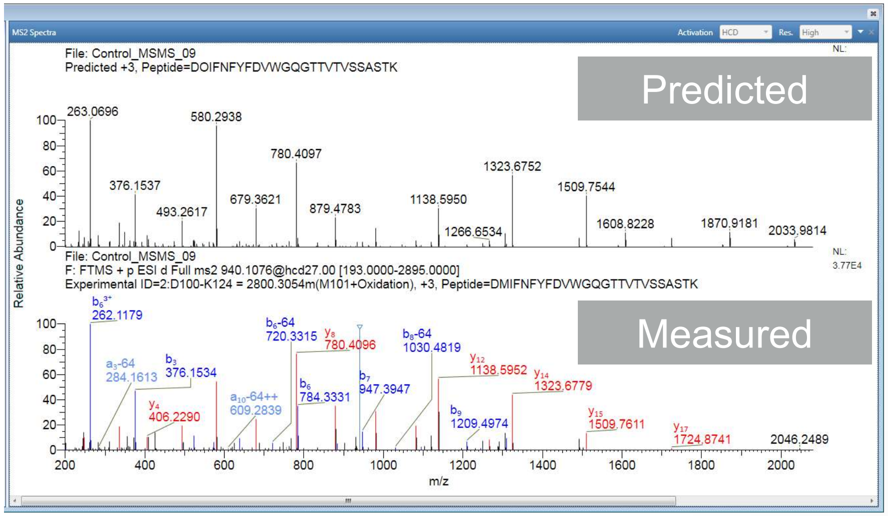
PQA Identification – Confident and Comprehensive

Prediction algorithm enables identification of expected and unspecified modifications

Kinetic prediction model

Fit- for- Purpose

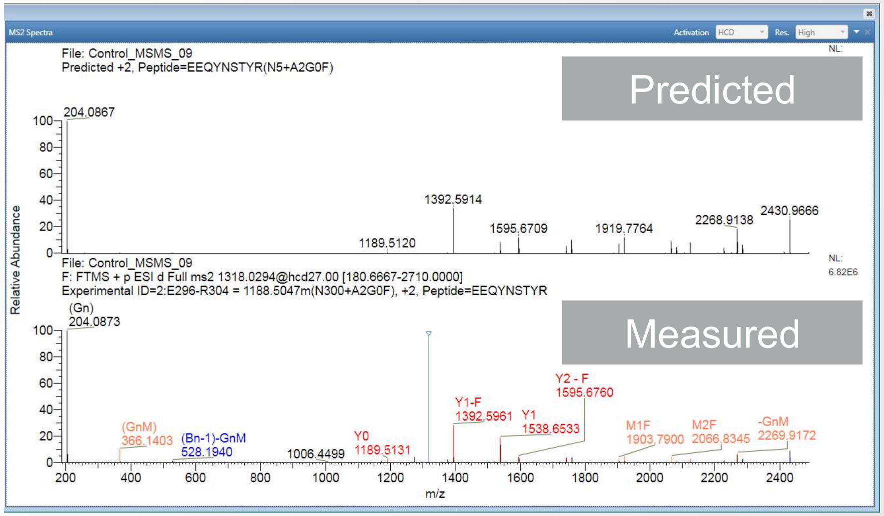
DM(ox)IFNFYFDVWGQGTTVTVSSASTK



File: Control_MSMS_09
Predicted +3, Peptide=DOIFNFYFDVWGQGTTVTVSSASTK

File: Control_MSMS_09
F: FTMS + p ESI d Full ms2 940.1076@hcd27.00 [193.0000-2895.0000]
Experimental ID=2:D100-K124 = 2800.3054m(M101+Oxidation), +3, Peptide=DMIFNFYFDVWGQGTTVTVSSASTK

EEQYN(A2G0F)STRY



File: Control_MSMS_09
Predicted +2, Peptide=EEQYNSTYR(N5+A2G0F)

File: Control_MSMS_09
F: FTMS + p ESI d Full ms2 1318.0294@hcd27.00 [180.6667-2710.0000]
Experimental ID=2:E296-R304 = 1188.5047m(N300+A2G0F), +2, Peptide=EEQYNSTYR

- **Expected and Unspecified PTMs**
- **Sequence Variants**
- **Host Cell Proteins**

Single workflow, Fast Processing!

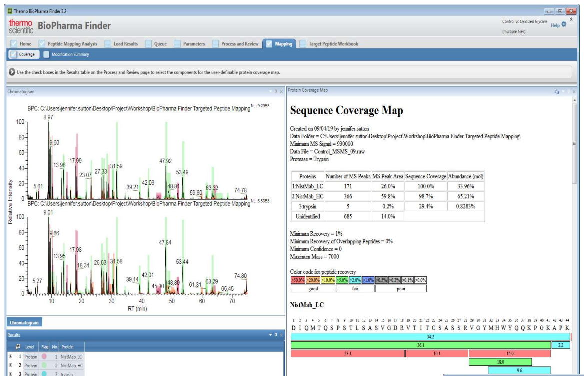
Ease of use

Attribute Characterization

PQA Review and Reporting

Intuitive Interface, Customizable Reports

Sequence Coverage Map



Sequence Coverage Map

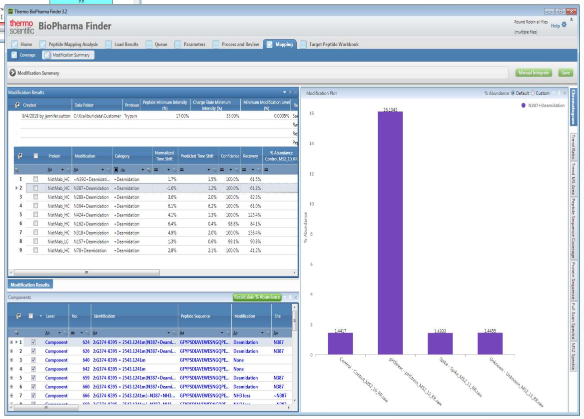
Created on 09/04/19 by jennifer.sutton
Data Folder = C:\Users\jennifer.sutton\Desktop\Project\Workshop\BioPharma Finder Targeted Peptide Mapping\
Minimum MS Signal = 930000
Data File = Control_MSMS_09.raw
Protease = Trypsin

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:NistMab_LC	171	26.0%	100.0%	33.96%
2:NistMab_HC	366	59.8%	98.7%	65.21%
3:trypsin	5	0.2%	29.4%	0.8283%
Unidentified	685	14.0%		

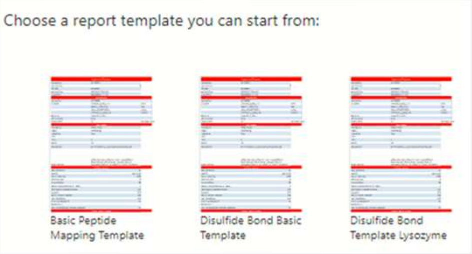
Minimum Recovery = 1%
Minimum Recovery of Overlapping Peptides = 0%
Minimum Confidence = 0
Maximum Mass = 7000

Color code for peptide recovery

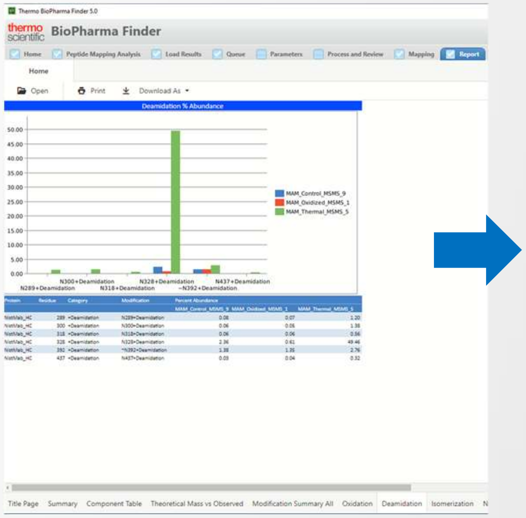
Modification Summary



Customizable Report Templates



Choose a report template you can start from:



- PDF
- Excel

Ease of use

Attribute Characterization

Enhanced Collaboration at Global Scale

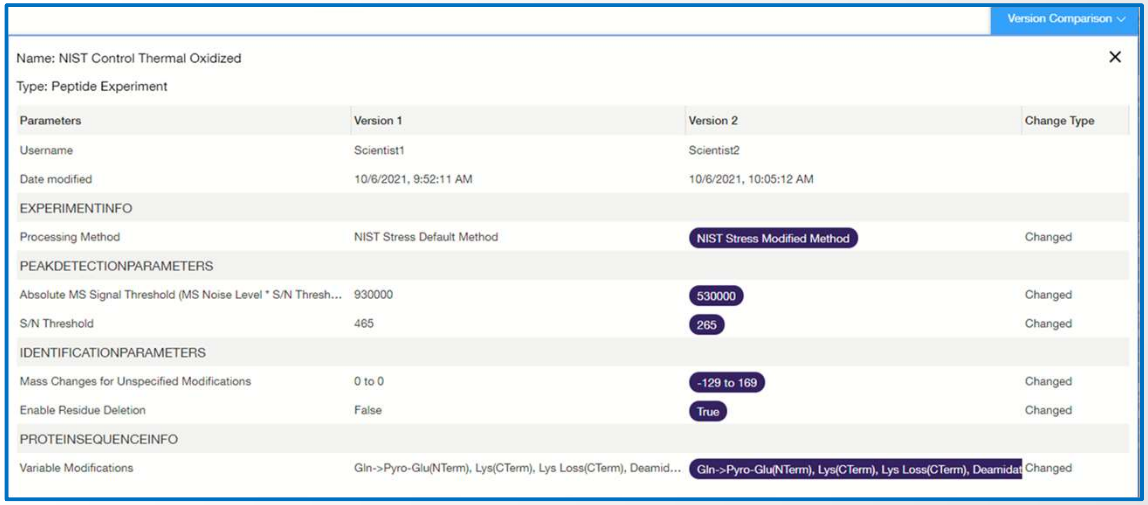
Data security with intelligent version control

Welcome
Sign into your account

Username *

☐	Experiment Name	Version	User Name	Status	Completion Time	Last Modified
☐	NIST Control Thermal Oxidized	2	Scientist2	Completed	10/6/2021, 10:05:10 AM	10/6/2021, 10:05:10 AM

☐	Version		Created On	User Name	Status	Description
☐	2	Current	10/6/2021, 10:05:12 AM	Scientist2	Completed	Modified platform method for PQA identification
☐	1	Revert	10/6/2021, 9:52:11 AM	Scientist1	Completed	Default platform method for PQA identification



Name: NIST Control Thermal Oxidized

Type: Peptide Experiment

Parameters	Version 1	Version 2	Change Type
Username	Scientist1	Scientist2	
Date modified	10/6/2021, 9:52:11 AM	10/6/2021, 10:05:12 AM	
EXPERIMENTINFO			
Processing Method	NIST Stress Default Method	NIST Stress Modified Method	Changed
PEAKDETECTIONPARAMETERS			
Absolute MS Signal Threshold (MS Noise Level * S/N Thresh...	930000	530000	Changed
S/N Threshold	465	265	Changed
IDENTIFICATIONPARAMETERS			
Mass Changes for Unspecified Modifications	0 to 0	-129 to 169	Changed
Enable Residue Deletion	False	True	Changed
PROTEINSEQUENCEINFO			
Variable Modifications	Gln->Pyro-Glu(NTerm), Lys(CTerm), Lys Loss(CTerm), Deamid...	Gln->Pyro-Glu(NTerm), Lys(CTerm), Lys Loss(CTerm), Deamidat	Changed

Connectivity

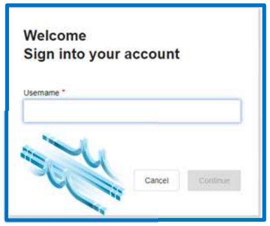
Compliance-ready

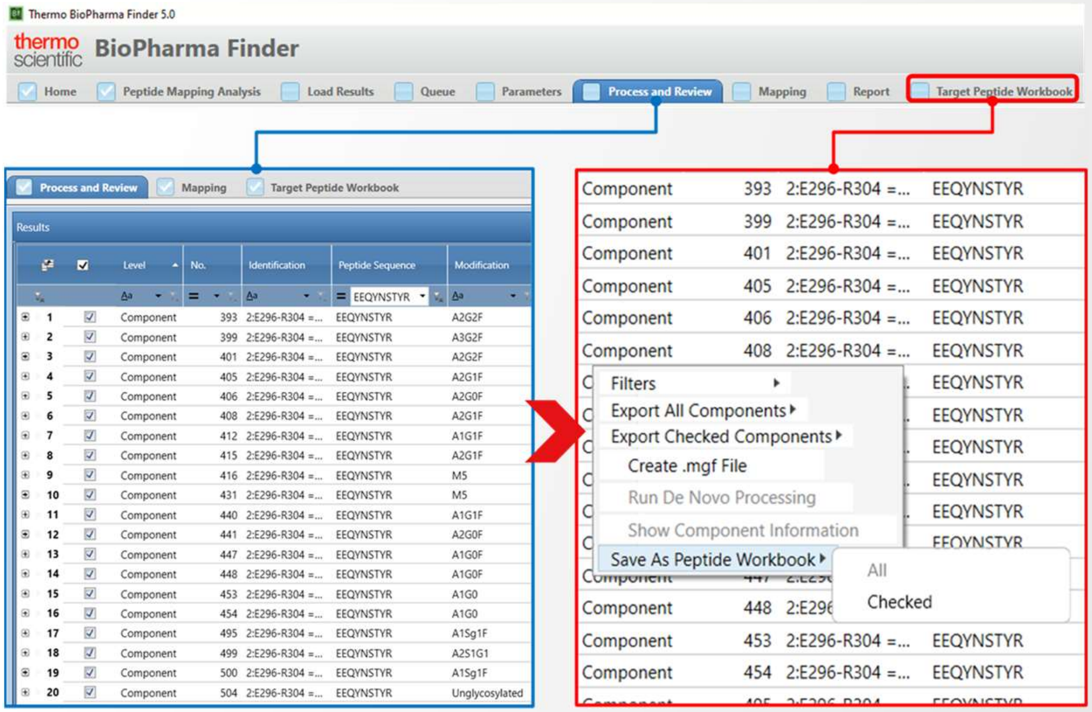
Accelerated Method Optimization!

Attribute Characterization

Enhanced Collaboration at Global Scale

PQA selection for targeted monitoring





	☑	Level	No.	Identification	Peptide Sequence	Modification
		Aa	=	Aa	= EEQYNSTYR	Aa
1	☑	Component	393	2:E296-R304 =...	EEQYNSTYR	A2G2F
2	☑	Component	399	2:E296-R304 =...	EEQYNSTYR	A3G2F
3	☑	Component	401	2:E296-R304 =...	EEQYNSTYR	A2G2F
4	☑	Component	405	2:E296-R304 =...	EEQYNSTYR	A2G1F
5	☑	Component	406	2:E296-R304 =...	EEQYNSTYR	A2G0F
6	☑	Component	408	2:E296-R304 =...	EEQYNSTYR	A2G1F
7	☑	Component	412	2:E296-R304 =...	EEQYNSTYR	A1G1F
8	☑	Component	415	2:E296-R304 =...	EEQYNSTYR	A2G1F
9	☑	Component	416	2:E296-R304 =...	EEQYNSTYR	M5
10	☑	Component	431	2:E296-R304 =...	EEQYNSTYR	M5
11	☑	Component	440	2:E296-R304 =...	EEQYNSTYR	A1G1F
12	☑	Component	441	2:E296-R304 =...	EEQYNSTYR	A2G0F
13	☑	Component	447	2:E296-R304 =...	EEQYNSTYR	A1G0F
14	☑	Component	448	2:E296-R304 =...	EEQYNSTYR	A1G0F
15	☑	Component	453	2:E296-R304 =...	EEQYNSTYR	A1G0
16	☑	Component	454	2:E296-R304 =...	EEQYNSTYR	A1G0
17	☑	Component	495	2:E296-R304 =...	EEQYNSTYR	A1Sg1F
18	☑	Component	499	2:E296-R304 =...	EEQYNSTYR	A2S1G1
19	☑	Component	500	2:E296-R304 =...	EEQYNSTYR	A1Sg1F
20	☑	Component	504	2:E296-R304 =...	EEQYNSTYR	Unglycosylated

Component	393	2:E296-R304 =...	EEQYNSTYR
Component	399	2:E296-R304 =...	EEQYNSTYR
Component	401	2:E296-R304 =...	EEQYNSTYR
Component	405	2:E296-R304 =...	EEQYNSTYR
Component	406	2:E296-R304 =...	EEQYNSTYR
Component	408	2:E296-R304 =...	EEQYNSTYR
Component	448	2:E296	
Component	453	2:E296-R304 =...	EEQYNSTYR
Component	454	2:E296-R304 =...	EEQYNSTYR

PQA Selection

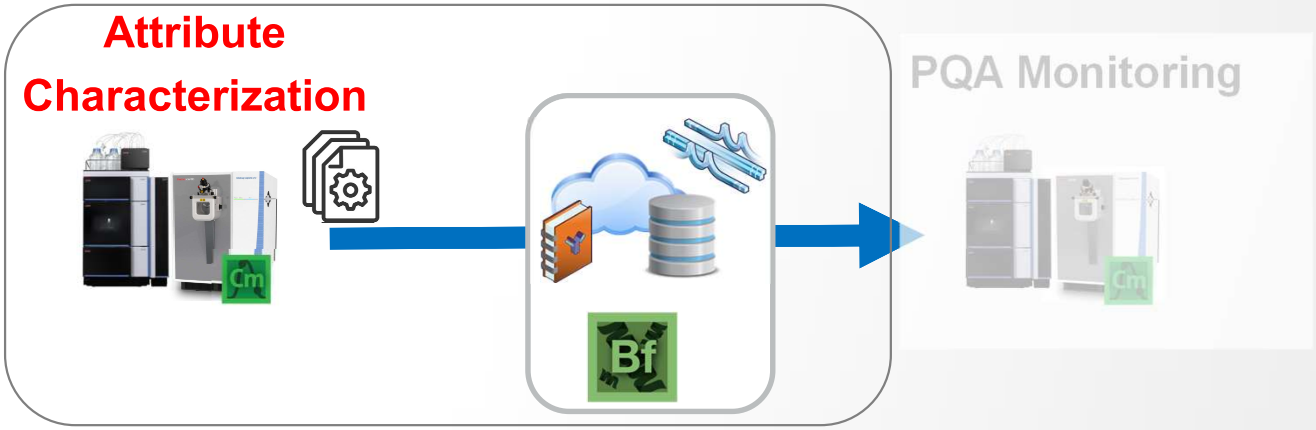
Workbook Creation

Connectivity

Ease of use

MAM 2.0: Attribute Characterization

Research & Development



Attribute Characterization

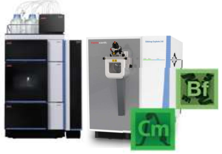
PQA Monitoring

Fit- for- Purpose

Compliance-ready

Connectivity

Ease of use



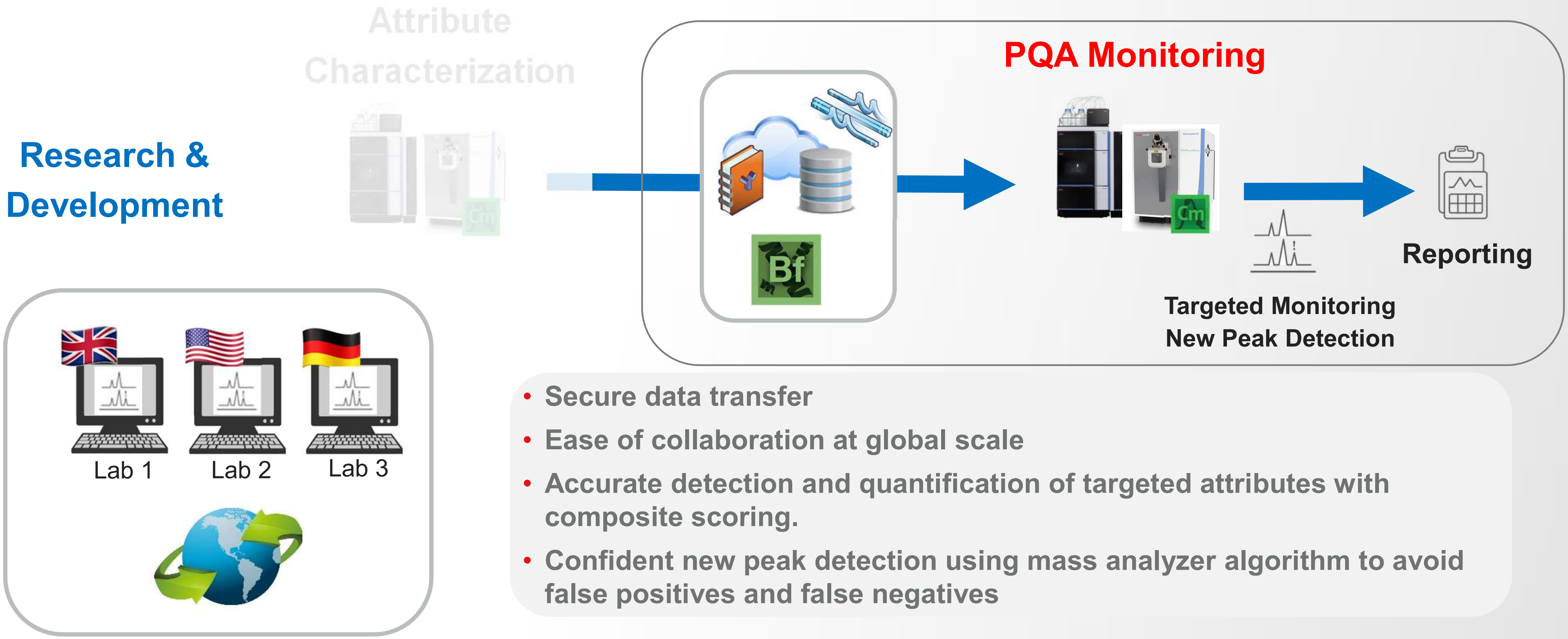
Confident and comprehensive attribute characterization with HRAM MS/MS

A software ecosystem with centralized data storage

Flexible and secure data access at global scale for ease of collaboration

MAM 2.0: Attribute Monitoring and New Peak Detection

Accurate, confident detection and quantification of targeted attributes and new peaks



Attribute Characterization

Research & Development

PQA Monitoring

Reporting

Targeted Monitoring
New Peak Detection

Lab 1
Lab 2
Lab 3

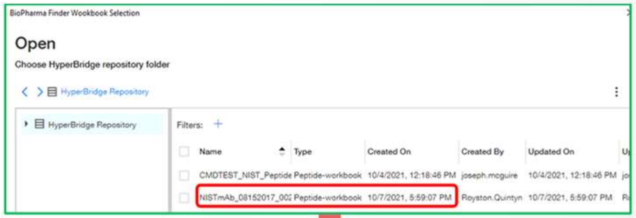
- Secure data transfer
- Ease of collaboration at global scale
- Accurate detection and quantification of targeted attributes with composite scoring.
- Confident new peak detection using mass analyzer algorithm to avoid false positives and false negatives

PQA Monitoring – Made more connected

Fit- for- Purpose

- ➢ Target Peptide Workbook
- Accurate Component Detection
- Intelligent Peak Integration
- PQA Quantitation
- Customized Reporting
- New Peak Detection

Import target peptide workbook
from HyperBridge
to Chromeleon

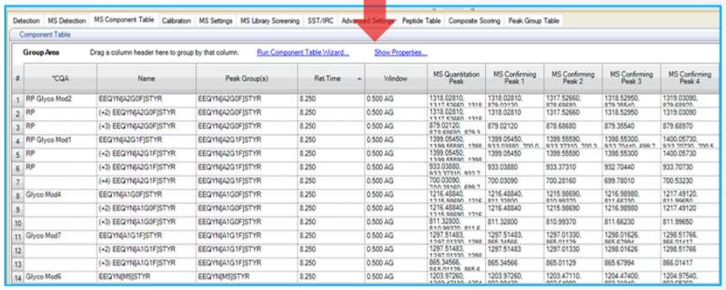


BioPharma Finder Wookbook Selection

Open

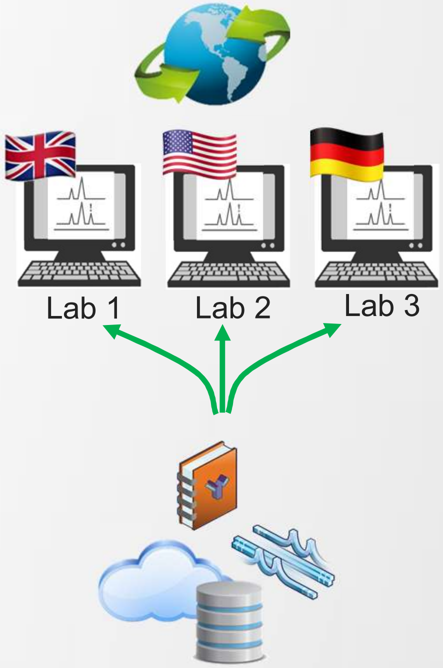
Choose HyperBridge repository folder

☐	Name	Type	Created On	Created By	Updated On	U
☐	CMDTEST_NIST_Peptide	Peptide-workbook	10/4/2021, 12:18:46 PM	joseph.mcguire	10/4/2021, 12:18:46 PM	jo
☐	NISTmAb_08152017_00	Peptide-workbook	10/7/2021, 5:59:07 PM	Royston.Quintyn	10/7/2021, 5:59:07 PM	R



Group Area Drag a column header here to group by that column.

#	*CQA	Name	Peak Group(s)	Ret.Time	Window	MS Quantitation Peak	MS Confirming Peak 1	MS Confirming Peak 2	MS Confirming Peak 3	MS Confirming Peak 4
1	RP Glyco Mod2	EEQYN[A2G0F]STYR	EEQYN[A2G0F]STYR	8.250	0.500 AG	1318.02810,	1318.02810,	1317.52660,	1318.52950,	1319.03090,
2	RP	(+2) EEQYN[A2G0F]STYR	EEQYN[A2G0F]STYR	8.250	0.500 AG	1318.02810,	1318.02810	1317.52660	1318.52950	1319.03090
3	RP	(+3) EEQYN[A2G0F]STYR	EEQYN[A2G0F]STYR	8.250	0.500 AG	879.02120,	879.02120	878.68680	879.35540	879.68970
4	RP Glyco Mod1	EEQYN[A2G1F]STYR	EEQYN[A2G1F]STYR	8.250	0.500 AG	1399.05450,	1399.05450,	1399.55590,	1398.55300,	1400.05730,
5	RP	(+2) EEQYN[A2G1F]STYR	EEQYN[A2G1F]STYR	8.250	0.500 AG	1399.05450,	1399.05450	1399.55590	1398.55300	1400.05730
6	RP	(+3) EEQYN[A2G1F]STYR	EEQYN[A2G1F]STYR	8.250	0.500 AG	933.03880,	933.03880	933.37310	932.70440	933.70730
7		(+4) EEQYN[A2G1F]STYR	EEQYN[A2G1F]STYR	8.250	0.500 AG	700.03090,	700.03090	700.28160	699.78010	700.53230
8	Glyco Mod4	EEQYN[A1G0F]STYR	EEQYN[A1G0F]STYR	8.250	0.500 AG	1216.48840,	1216.48840,	1215.98690,	1216.98980,	1217.49120,
9		(+2) EEQYN[A1G0F]STYR	EEQYN[A1G0F]STYR	8.250	0.500 AG	1216.48840,	1216.48840	1215.98690	1216.98980	1217.49120
10		(+3) EEQYN[A1G0F]STYR	EEQYN[A1G0F]STYR	8.250	0.500 AG	811.32800,	811.32800	810.99370	811.66230	811.99650
11	Glyco Mod7	EEQYN[A1G1F]STYR	EEQYN[A1G1F]STYR	8.250	0.500 AG	1297.51483,	1297.51483,	1297.01330,	1298.01626,	1298.51766,
12		(+2) EEQYN[A1G1F]STYR	EEQYN[A1G1F]STYR	8.250	0.500 AG	1297.51483,	1297.51483	1297.01330	1298.01626	1298.51766
13		(+3) EEQYN[A1G1F]STYR	EEQYN[A1G1F]STYR	8.250	0.500 AG	865.34566,	865.34566	865.01129	865.67994	866.01417
14	Glyco Mod6	EEQYN[M5]STYR	EEQYN[M5]STYR	8.250	0.500 AG	1203.97260,	1203.97260,	1203.47110,	1204.47400,	1204.97540,



Thermo Scientific
HyperBridge

Connectivity

Ease of use

- Seamless transfer of target peptide workbook
- Data sharing at global scale

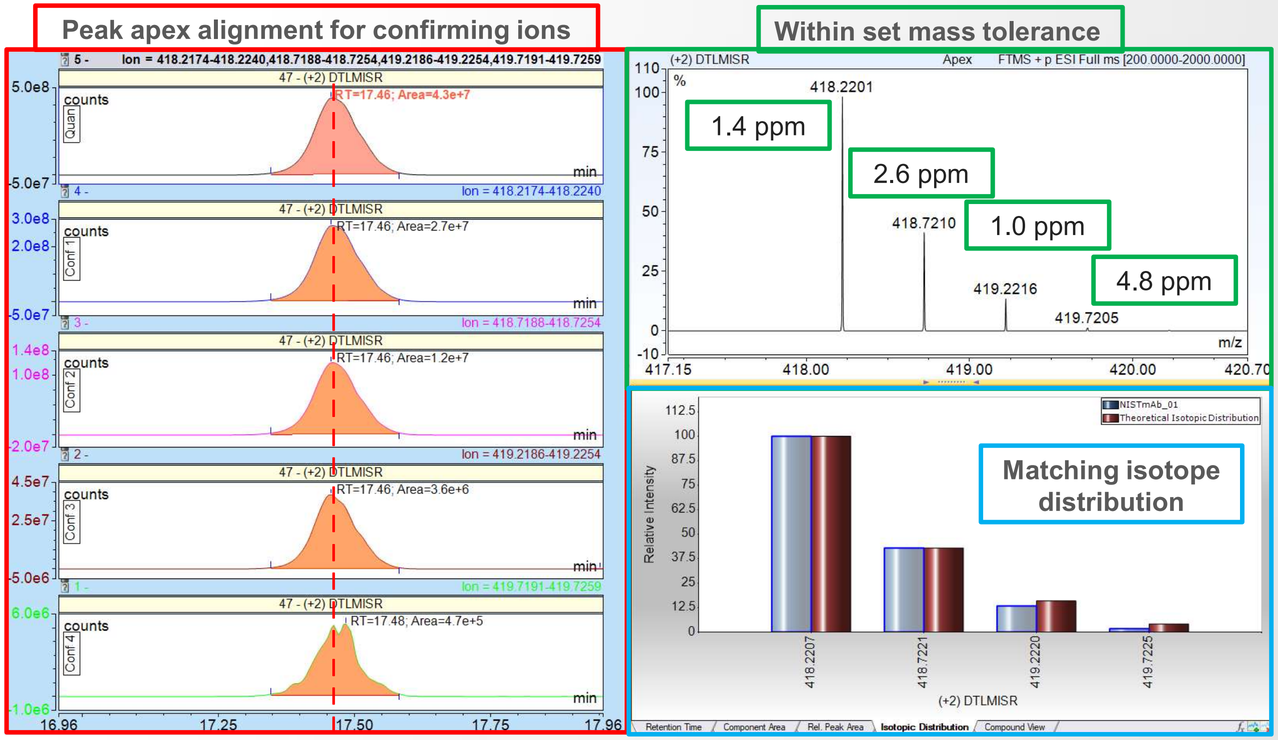
Attribute Monitoring

PQA Monitoring – Higher confidence

Fit- for- Purpose

- Target Peptide Workbook
- ➢ Accurate Component Detection
- Intelligent Peak Integration
- PQA Quantitation
- Customized Reporting
- New Peak Detection

Composite scoring to ensure accurate component detection



Peak apex alignment for confirming ions

Within set mass tolerance

1.4 ppm

2.6 ppm

1.0 ppm

4.8 ppm

Matching isotope distribution

Ease of use

PQA Monitoring – Made simpler

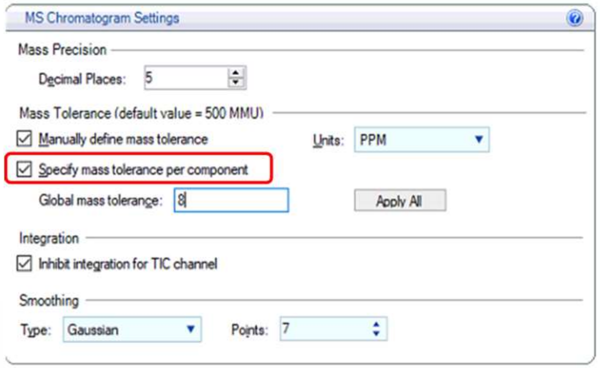
Fit- for- Purpose

- Target Peptide Workbook
- Accurate Component Detection
- ➢ Intelligent Peak Integration
- PQA Quantitation
- Customized Reporting
- New Peak Detection

Select detection reference for consistent start and stop peak integration

Define mass tolerance threshold per component

- Ability to mass resolve peaks that are difficult to resolve chromatographically
- N387 deamidation (PENNYK peptide)



MS Chromatogram Settings

Mass Precision

Decimal Places: 5

Mass Tolerance (default value = 500 MMU)

☑ Manually define mass tolerance

Units: PPM

☑ Specify mass tolerance per component

Global mass tolerance: 8

Apply All

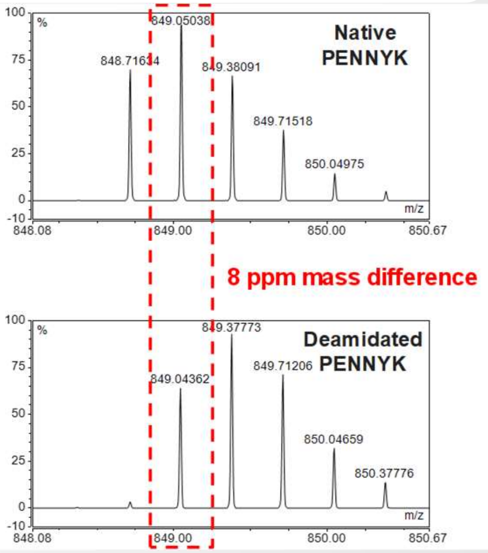
Integration

☑ Inhibit integration for TIC channel

Smoothing

Type: Gaussian

Points: 7



Ease of use

Attribute Monitoring

PQA Monitoring – Made simpler

Fit- for- Purpose

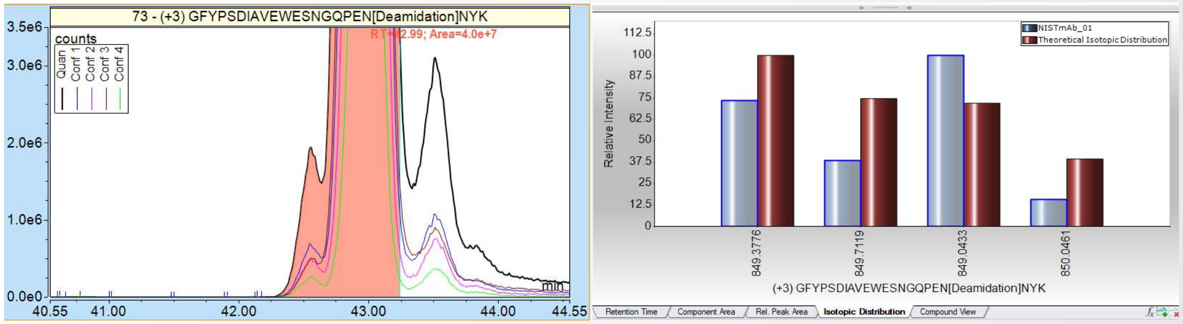
- Target Peptide Workbook
- Accurate Component Detection
- ➢ Intelligent Peak Integration
- PQA Quantitation
- Customized Reporting
- New Peak Detection

Select detection reference for consistent start and stop peak integration

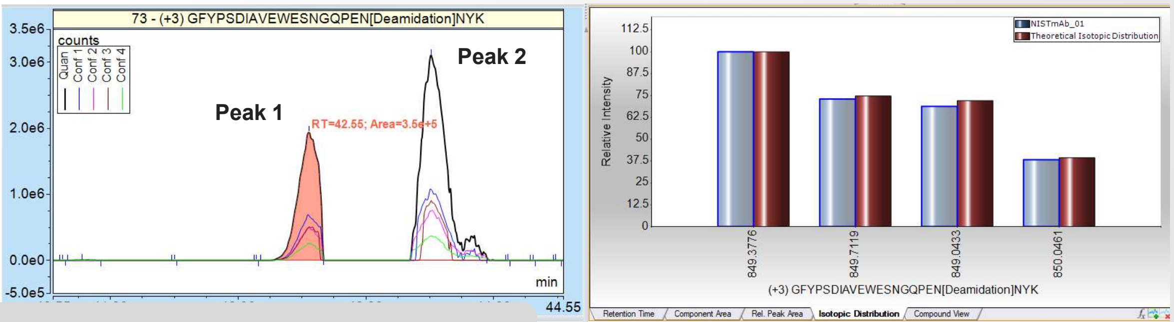
Define mass tolerance threshold per component

- Ability to mass resolve peaks that are difficult to resolve chromatographically
- N387 deamidation (PENNYK peptide)

Set mass tolerance to 8ppm



Set mass tolerance to 3ppm



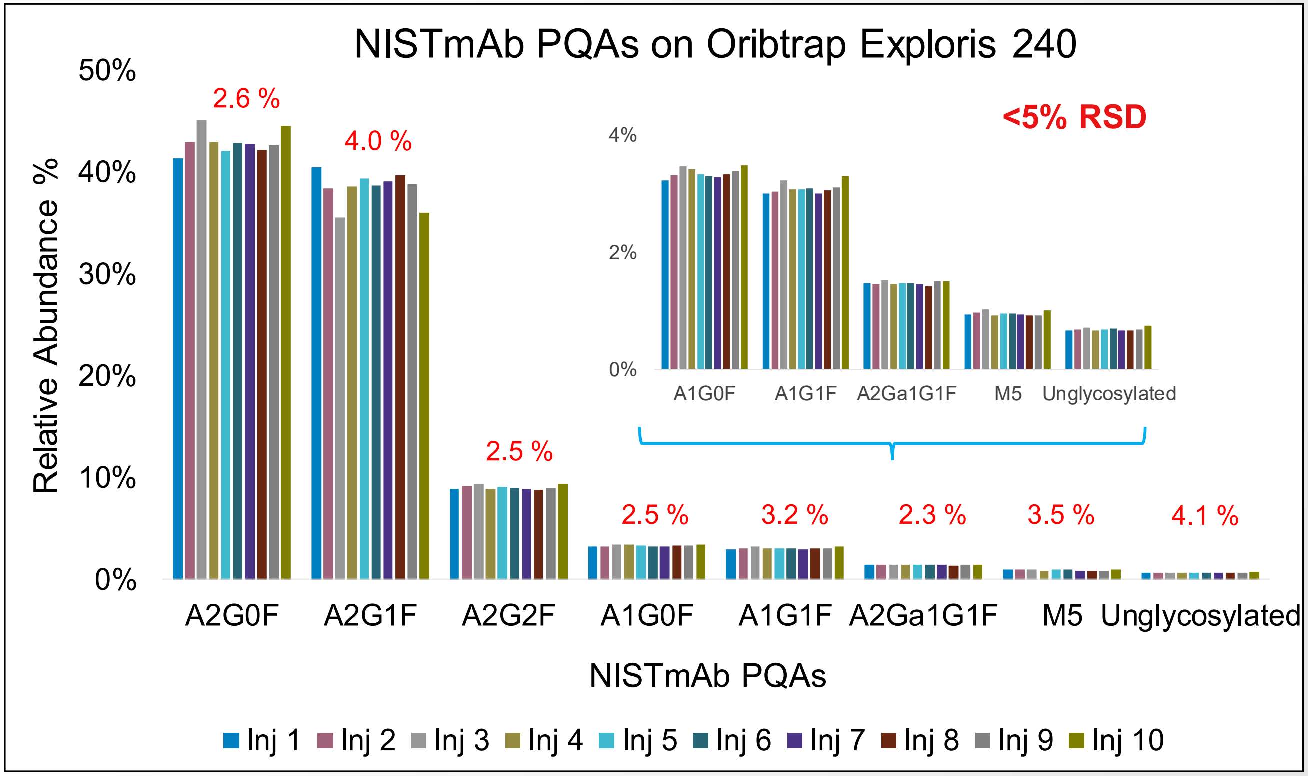
Attribute Monitoring

Ease of use

PQA Monitoring – Accurate Quantitation

Fit- for- Purpose

- Target Peptide Workbook
- Accurate Component Detection
- Intelligent Peak Integration
- ➢ PQA Quantitation
- Customized Reporting
- New Peak Detection



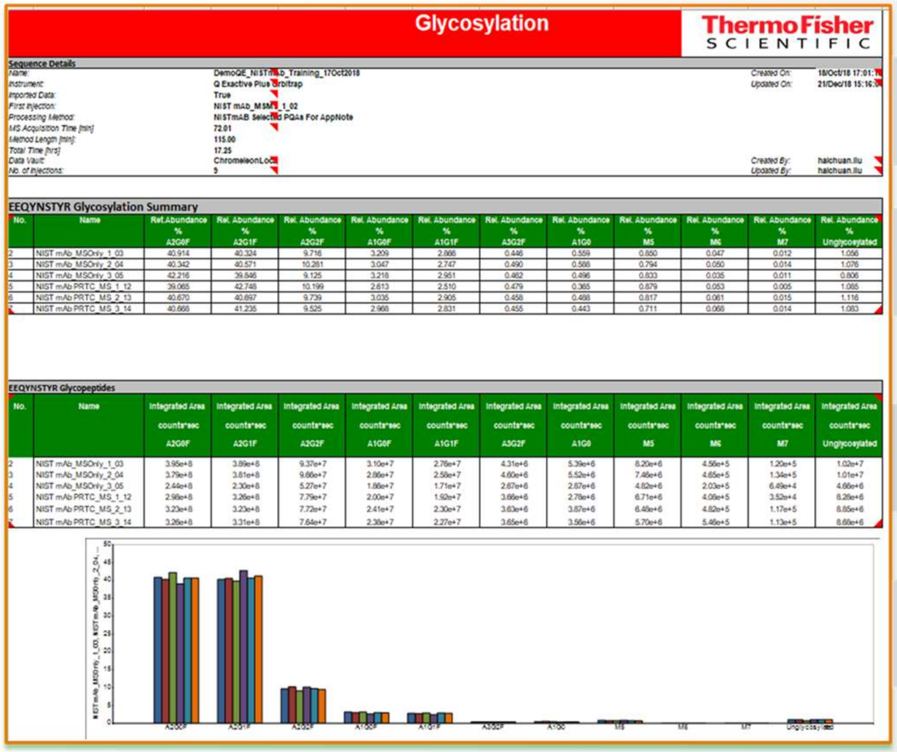
Ease of use

Attribute Monitoring

PQA Monitoring – Customizable

Fit- for- Purpose

- Target Peptide Workbook
- Accurate Component Detection
- Intelligent Peak Integration
- PQA Quantitation
- ➢ Customized Reporting
- New Peak Detection



Glycosylation

Sequence Details

Name: DemoQE_NISTmAb_Training_17Oct2018
Instrument: Q Exactive Plus Orbitrap
Imported Data: True
First Injection: NIST mAb_MSMS_1_02
Processing Method: NISTmAB Selected PQAs For AppNote
MS Acquisition Time [min]: 72.01
Method Length [min]: 115.00
Total Time [hrs]: 17.25
Data Vault: ChromeleonLocal
No. of Injections: 9

Created On: 18/Oct/18 17:01:1
Updated On: 21/Dec/18 15:16:0
Created By: haichuan.liu
Updated By: haichuan.liu

EEQYNSTYR Glycosylation Summary

No.	Name	Rel.Abundance % A2G0F	Rel. Abundance % A2G1F	Rel. Abundance % A2G2F	Rel. Abundance % A1G0F	Rel. Abundance % A1G1F	Rel. Abundance % A3G2F	Rel. Abundance % A1G0	Rel. Abundance % M5	Rel. Abundance % M6	Rel. Abundance % M7	Rel. Abundance % Unglycosylated
2	NIST mAb_MSOnly_1_03	40.914	40.324	9.716	3.209	2.866	0.446	0.559	0.850	0.047	0.012	1.056
3	NIST mAb_MSOnly_2_04	40.342	40.571	10.281	3.047	2.747	0.490	0.586	0.794	0.050	0.014	1.076
4	NIST mAb_MSOnly_3_05	42.216	39.846	9.125	3.218	2.951	0.482	0.496	0.833	0.035	0.011	0.806
5	NIST mAb PRTC_MS_1_12	39.065	42.748	10.199	2.613	2.510	0.479	0.365	0.879	0.053	0.005	1.085
6	NIST mAb PRTC_MS_2_13	40.670	40.697	9.739	3.035	2.905	0.458	0.488	0.817	0.061	0.015	1.116
7	NIST mAb PRTC_MS_3_14	40.666	41.235	9.525	2.968	2.831	0.455	0.443	0.711	0.068	0.014	1.083

EEQYNSTYR Glycopeptides

No.	Name	Integrated Area counts*sec A2G0F	Integrated Area counts*sec A2G1F	Integrated Area counts*sec A2G2F	Integrated Area counts*sec A1G0F	Integrated Area counts*sec A1G1F	Integrated Area counts*sec A3G2F	Integrated Area counts*sec A1G0	Integrated Area counts*sec M5	Integrated Area counts*sec M6	Integrated Area counts*sec M7	Integrated Area counts*sec Unglycosylated
2	NIST mAb_MSOnly_1_03	3.95e+8	3.89e+8	9.37e+7	3.10e+7	2.76e+7	4.31e+6	5.39e+6	8.20e+6	4.56e+5	1.20e+5	1.02e+7
3	NIST mAb_MSOnly_2_04	3.79e+8	3.81e+8	9.66e+7	2.86e+7	2.58e+7	4.60e+6	5.52e+6	7.46e+6	4.65e+5	1.34e+5	1.01e+7
4	NIST mAb_MSOnly_3_05	2.44e+8	2.30e+8	5.27e+7	1.86e+7	1.71e+7	2.67e+6	2.87e+6	4.82e+6	2.03e+5	6.49e+4	4.66e+6
5	NIST mAb PRTC_MS_1_12	2.96e+8	3.26e+8	7.79e+7	2.00e+7	1.92e+7	3.66e+6	2.78e+6	6.71e+6	4.06e+5	3.52e+4	8.26e+6
6	NIST mAb PRTC_MS_2_13	3.23e+8	3.23e+8	7.72e+7	2.41e+7	2.30e+7	3.63e+6	3.87e+6	6.48e+6	4.82e+5	1.17e+5	8.85e+6
7	NIST mAb PRTC_MS_3_14	3.26e+8	3.31e+8	7.64e+7	2.38e+7	2.27e+7	3.65e+6	3.56e+6	5.70e+6	5.46e+5	1.13e+5	8.68e+6

Sequence Info

Ratio Table

Peak Area Table

Graphical View of the Result

Ease of use

Attribute Monitoring

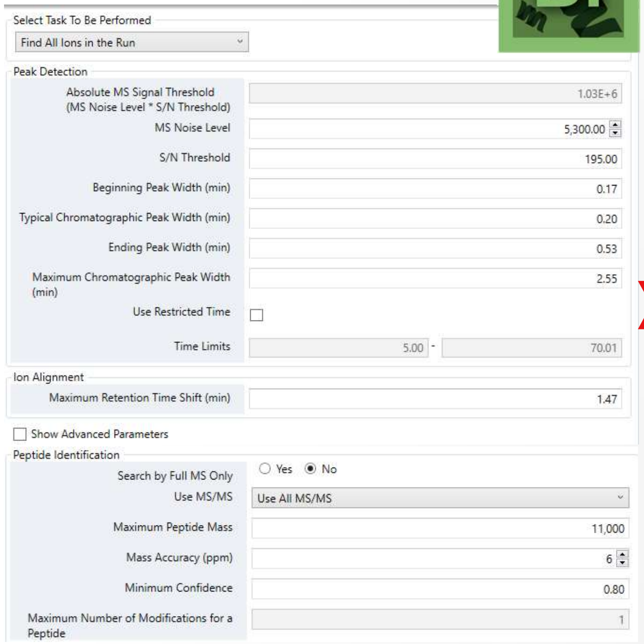
PQA Monitoring – No missed information

Mass Analyzer algorithm harmonization for new peak detection

Non-targeted MS processing

Fit- for- Purpose

- Target Peptide Workbook
- Accurate Component Detection
- Intelligent Peak Integration
- PQA Quantitation
- Customized Reporting
- ➢ New Peak Detection



Select Task To Be Performed
Find All Ions in the Run

Peak Detection

Absolute MS Signal Threshold (MS Noise Level * S/N Threshold)	1.03E+6
MS Noise Level	5,300.00
S/N Threshold	195.00
Beginning Peak Width (min)	0.17
Typical Chromatographic Peak Width (min)	0.20
Ending Peak Width (min)	0.53
Maximum Chromatographic Peak Width (min)	2.55
Use Restricted Time	☐
Time Limits	5.00 - 70.01

Ion Alignment

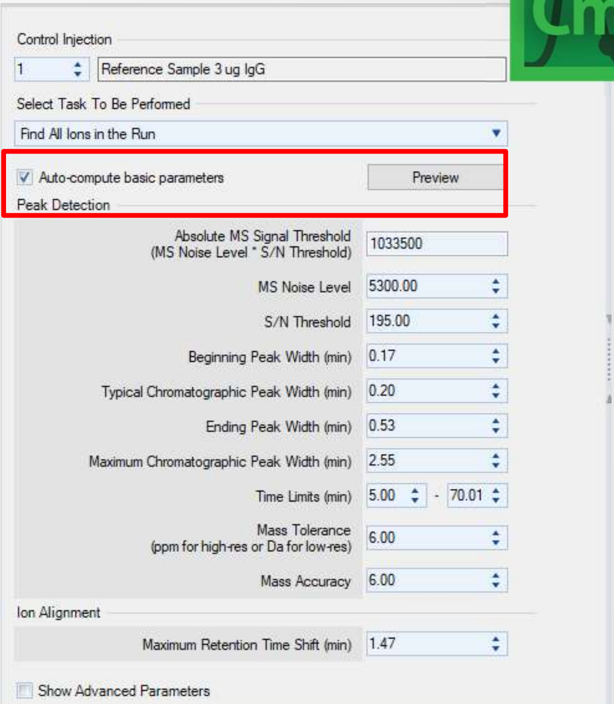
Maximum Retention Time Shift (min)	1.47

☐ Show Advanced Parameters

Peptide Identification

Search by Full MS Only	○ Yes ● No
Use MS/MS	Use All MS/MS
Maximum Peptide Mass	11,000
Mass Accuracy (ppm)	6
Minimum Confidence	0.80
Maximum Number of Modifications for a Peptide	1

26



Control Injection
1 | Reference Sample 3 ug IgG

Select Task To Be Performed
Find All Ions in the Run

☑ Auto-compute basic parameters

Peak Detection

Absolute MS Signal Threshold (MS Noise Level * S/N Threshold)	1033500
MS Noise Level	5300.00
S/N Threshold	195.00
Beginning Peak Width (min)	0.17
Typical Chromatographic Peak Width (min)	0.20
Ending Peak Width (min)	0.53
Maximum Chromatographic Peak Width (min)	2.55
Time Limits (min)	5.00 - 70.01
Mass Tolerance (ppm for high-res or Da for low-res)	6.00
Mass Accuracy	6.00

Ion Alignment

Maximum Retention Time Shift (min)	1.47

☐ Show Advanced Parameters

Identical parameter settings used for NPD

Ease of use

PQA Monitoring – No missed information

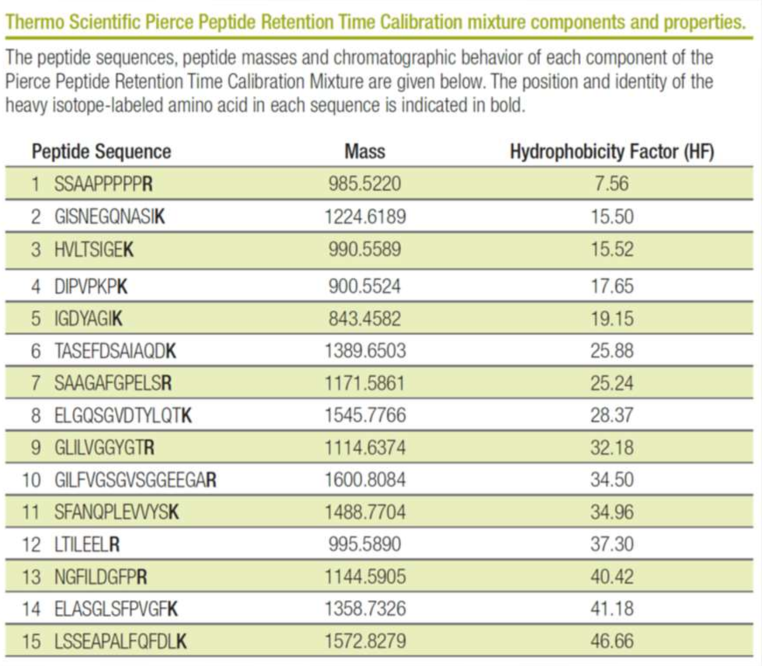
Mass Analyzer algorithm harmonization for new peak detection

Non-targeted MS processing

Positive control: 15 new peaks from PRTC spiked into NISTmAb digest

Fit- for- Purpose

- Target Peptide Workbook
- Accurate Component Detection
- Intelligent Peak Integration
- PQA Quantitation
- Customized Reporting
- ➢ New Peak Detection



Thermo Scientific Pierce Peptide Retention Time Calibration mixture components and properties.

The peptide sequences, peptide masses and chromatographic behavior of each component of the Pierce Peptide Retention Time Calibration Mixture are given below. The position and identity of the heavy isotope-labeled amino acid in each sequence is indicated in bold.

	Peptide Sequence	Mass	Hydrophobicity Factor (HF)
1	SSAAPPPPP**R**	985.5220	7.56
2	GISNEGQNASI**K**	1224.6189	15.50
3	HVLTSIGE**K**	990.5589	15.52
4	DIPVPKP**K**	900.5524	17.65
5	IGDYAGI**K**	843.4582	19.15
6	TASEFDSAIAQD**K**	1389.6503	25.88
7	SAAGAFGPELS**R**	1171.5861	25.24
8	ELGQSGVDTYLQT**K**	1545.7766	28.37
9	GLILVGGYGT**R**	1114.6374	32.18
10	GILFVGSGVSGGEEGA**R**	1600.8084	34.50
11	SFANQPLEVVYS**K**	1488.7704	34.96
12	LTILEEL**R**	995.5890	37.30
13	NGFILDGFP**R**	1144.5905	40.42
14	ELASGLSFPVGF**K**	1358.7326	41.18
15	LSSEAPALFQFDL**K**	1572.8279	46.66

Ease of use

Attribute Monitoring

PQA Monitoring – No missed information

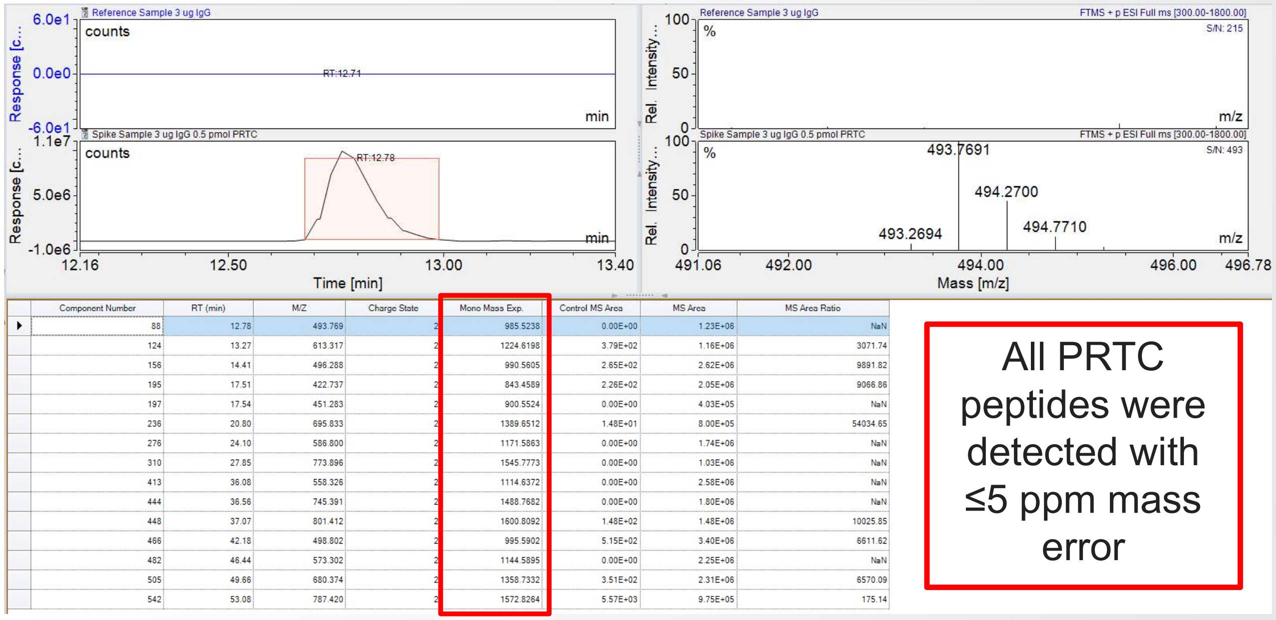
Mass Analyzer algorithm harmonization for new peak detection

Non-targeted MS processing

Positive control: 15 new peaks from PRTC spiked into NISTmAb digest

Fit- for- Purpose

- Target Peptide Workbook
- Accurate Component Detection
- Intelligent Peak Integration
- PQA Quantitation
- Customized Reporting
- ➢ New Peak Detection



Component Number	RT (min)	M/Z	Charge State	Mono Mass Exp.	Control MS Area	MS Area	MS Area Ratio
88	12.78	493.769	2	985.5238	0.00E+00	1.23E+06	NaN
124	13.27	613.317	2	1224.6198	3.79E+02	1.16E+06	3071.74
156	14.41	496.288	2	990.5605	2.65E+02	2.62E+06	9891.82
195	17.51	422.737	2	843.4589	2.26E+02	2.05E+06	9066.86
197	17.54	451.283	2	900.5524	0.00E+00	4.03E+05	NaN
236	20.80	695.833	2	1389.6512	1.48E+01	8.00E+05	54034.65
276	24.10	586.800	2	1171.5863	0.00E+00	1.74E+06	NaN
310	27.85	773.896	2	1545.7773	0.00E+00	1.03E+06	NaN
413	36.08	558.326	2	1114.6372	0.00E+00	2.58E+06	NaN
444	36.56	745.391	2	1488.7682	0.00E+00	1.80E+06	NaN
448	37.07	801.412	2	1600.8092	1.48E+02	1.48E+06	10025.85
466	42.18	498.802	2	995.5902	5.15E+02	3.40E+06	6611.62
482	46.44	573.302	2	1144.5895	0.00E+00	2.25E+06	NaN
505	49.66	680.374	2	1358.7332	3.51E+02	2.31E+06	6570.09
542	53.08	787.420	2	1572.8264	5.57E+03	9.75E+05	175.14

All PRTC peptides were detected with ≤5 ppm mass error

Ease of use

Attribute Monitoring

PQA Monitoring – No missed information

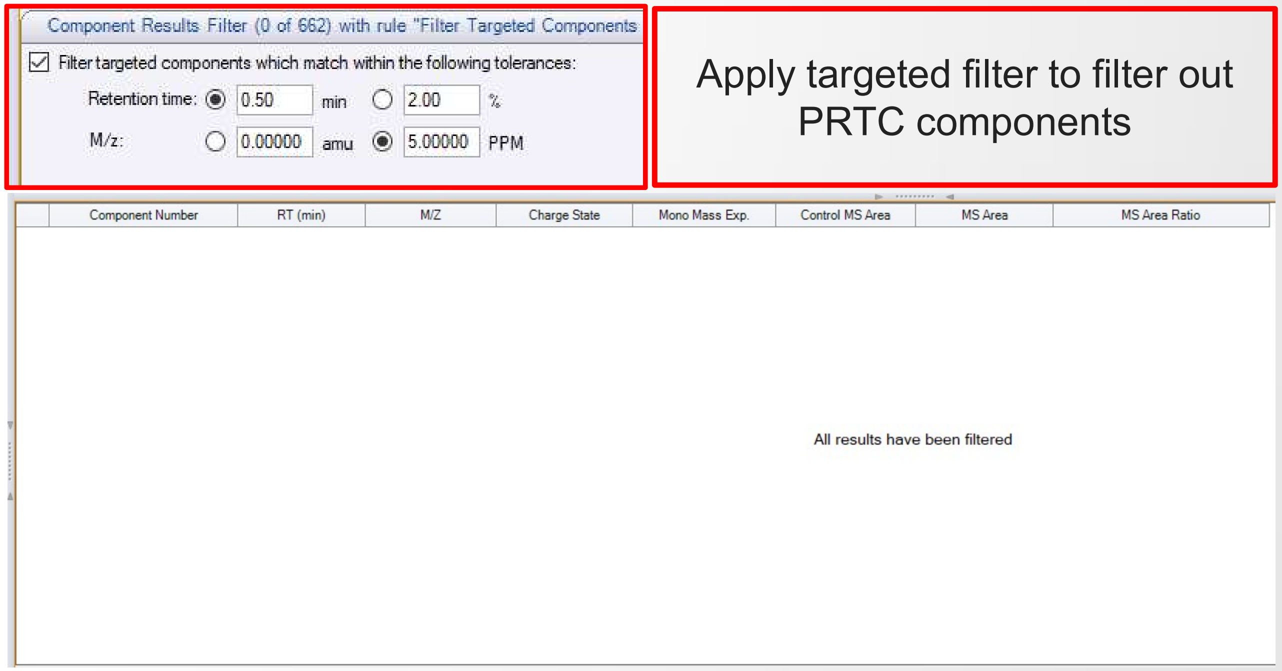
Non-targeted MS processing

Mass Analyzer algorithm harmonization for new peak detection

Removing targeted components to avoid false positives

Fit- for- Purpose

- Target Peptide Workbook
- Accurate Component Detection
- Intelligent Peak Integration
- PQA Quantitation
- Customized Reporting
- ➢ New Peak Detection



Component Results Filter (0 of 662) with rule "Filter Targeted Components"

☑ Filter targeted components which match within the following tolerances:

Retention time: ● 0.50 min ○ 2.00 %

M/z: ○ 0.00000 amu ● 5.00000 PPM

Component Number	RT (min)	M/Z	Charge State	Mono Mass Exp.	Control MS Area	MS Area	MS Area Ratio

All results have been filtered

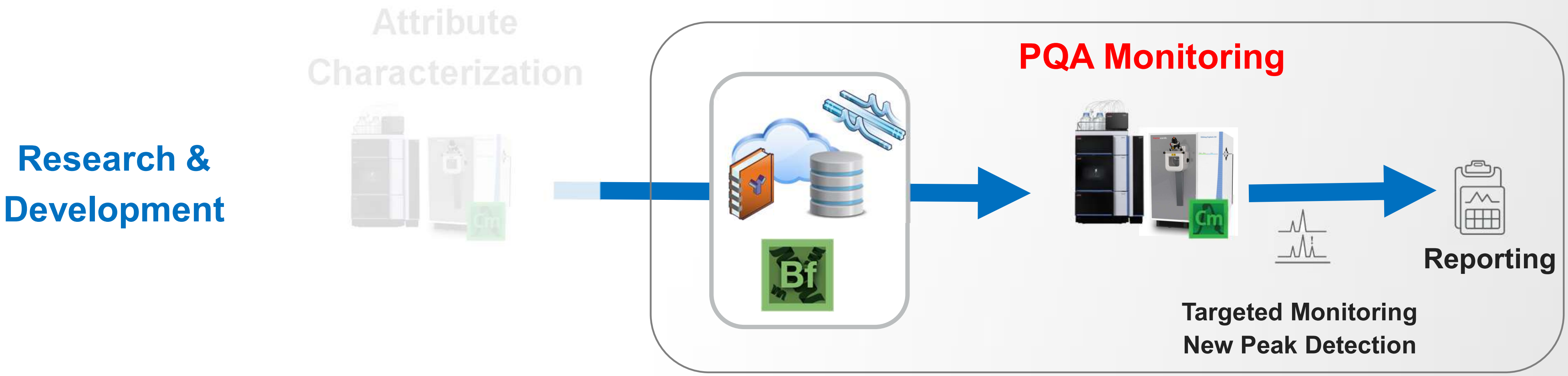
Apply targeted filter to filter out PRTC components

Ease of use

Attribute Monitoring

Summary: Attribute Monitoring and New Peak Detection

Fit-for-Purpose



Research & Development

Attribute Characterization

PQA Monitoring

Reporting

Targeted Monitoring
New Peak Detection

Connectivity

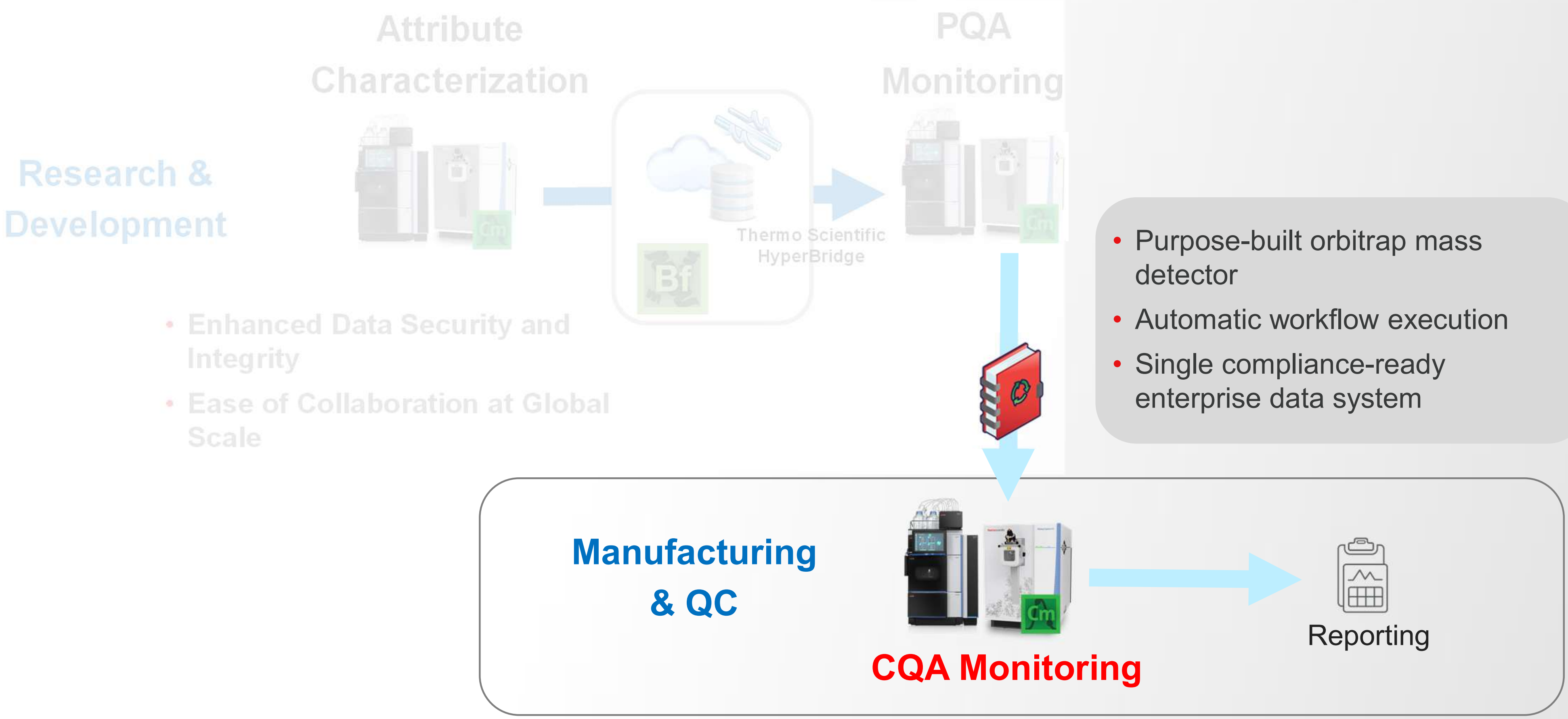
Ease of use

- **A software ecosystem** to facilitate **collaboration at global scale**
- **Composite scoring** to ensure **accurate detection of targeted peptides**
- **Intelligent peak integration** to ensure **accurate quantification**
- **Improved new peak detection (NPD)**

Attribute Monitoring

MAM 2.0: CQA Monitoring in QC

Compliance-Ready, Built-for-Purpose, Simplicity



- Enhanced Data Security and Integrity
- Ease of Collaboration at Global Scale

- Purpose-built orbitrap mass detector
- Automatic workflow execution
- Single compliance-ready enterprise data system

CQA Monitoring in QC

Orbitrap Exploris MX: the mass detector built for QC

- **Built on the same Orbitrap technology**
  - Full MS scans only
  - Resolution: up to 180K @ m/z 200
  - Mass accuracy: 1-point calibration achieves <3 ppm RMS drift over at least 4 weeks
  - m/z range: 40-3000 (up to 8000 with BioPharma option)
- **Consistent unit-to-unit performance**
- **Compliance-ready**
- **Ease of operation**
  - Direct method transfer from Orbitrap Exploris 240
  - Automatic workflow execution
- **Extended life cycle**
- **Cost effective**

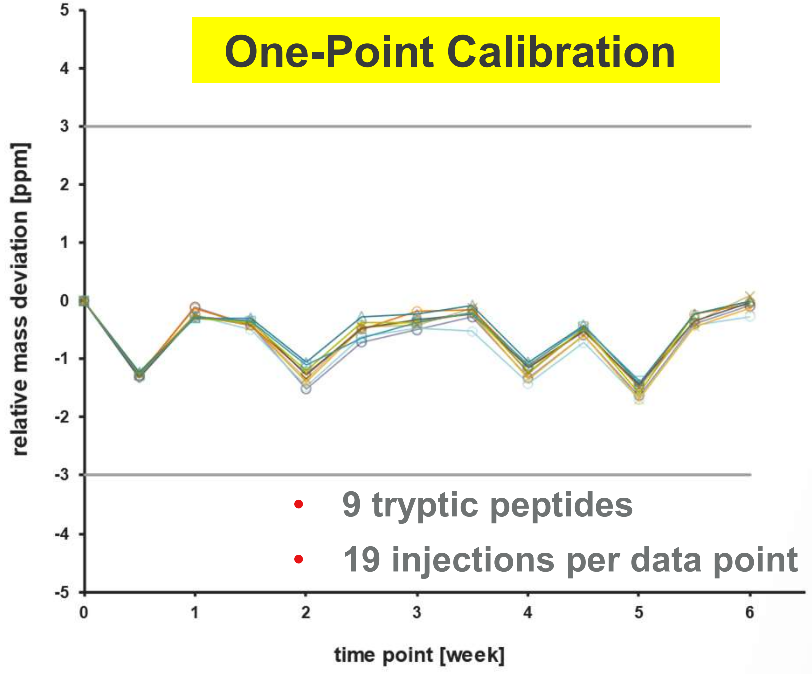
Fit- for- Purpose

Compliance-ready

Ease of use

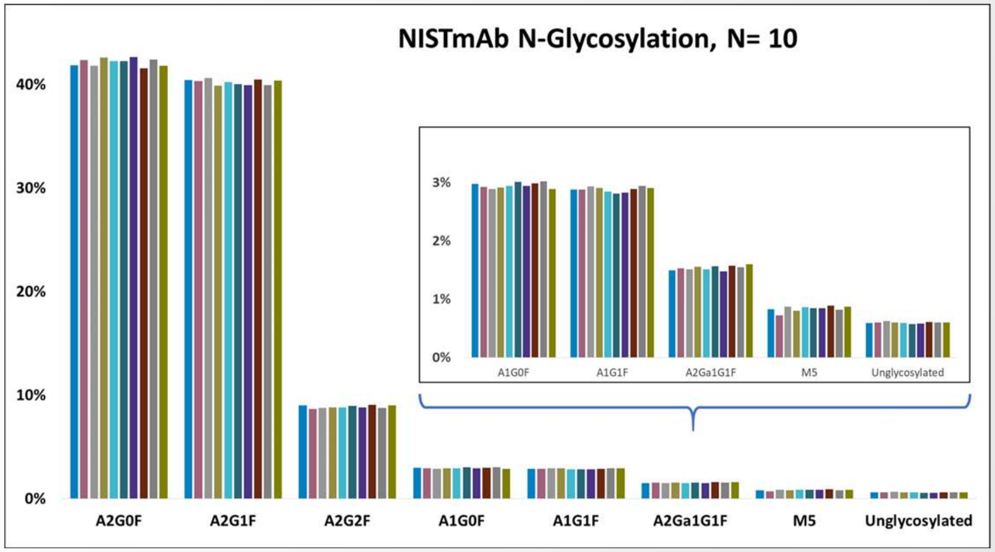
Orbitrap Exploris MX: Consistent Performance

Fit- for- Purpose



- 9 tryptic peptides
- 19 injections per data point

< 3ppm mass deviation over 6 weeks



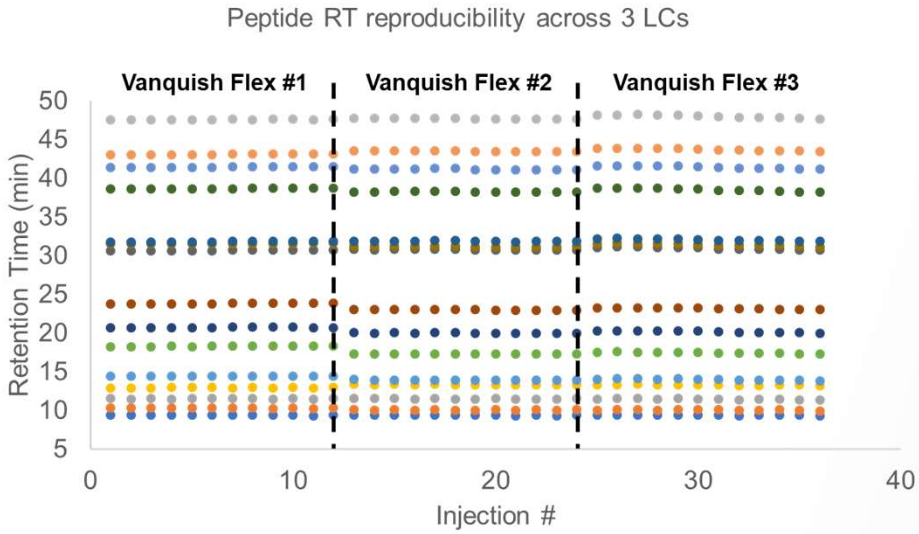
Consistent CQA measurement with RSD< 4%

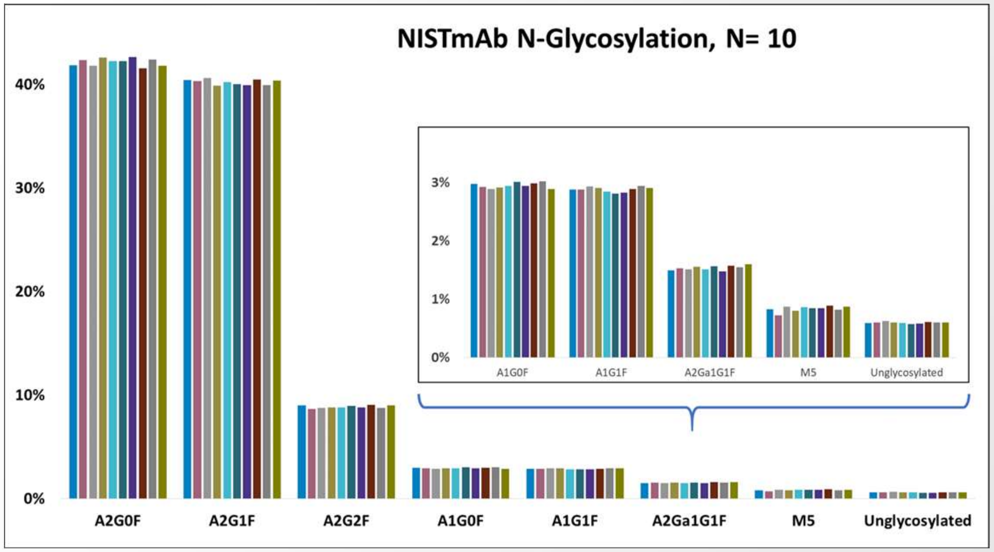
Ease of use

CQA Monitoring in QC

Orbitrap Exploris MX: Consistent Performance

Fit- for- Purpose





15 peptides, RT across gradient
%CV between 0.13% to 2.89%

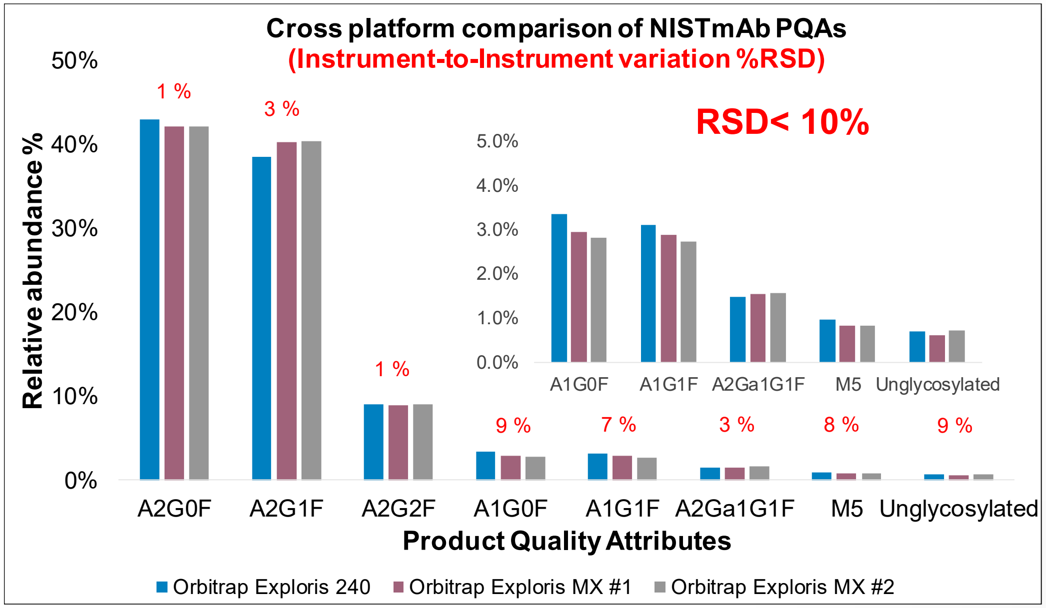
Consistent CQA measurement with RSD< 4%

Ease of use

CQA Monitoring in QC

Consistency with Easy Method Transfer to QC

Seamless method migration from development to QC



Orbitrap Exploris 240
Mass Spectrometer

MAM eWorkflow

Orbitrap Exploris MX
Mass Detector

Fit- for- Purpose

Compliance-ready

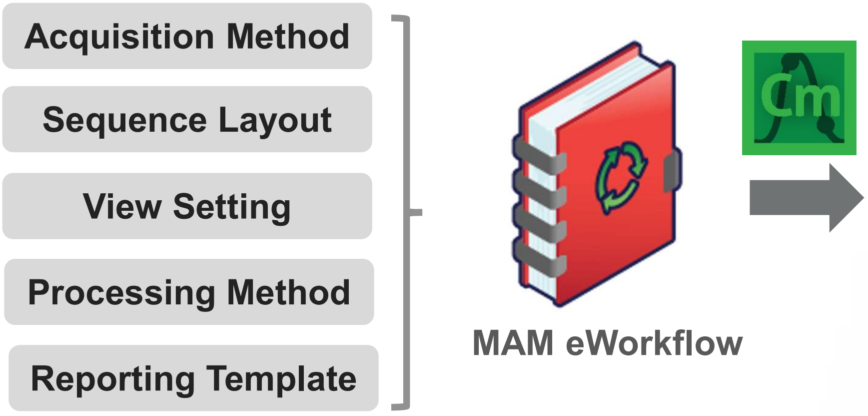
Connectivity

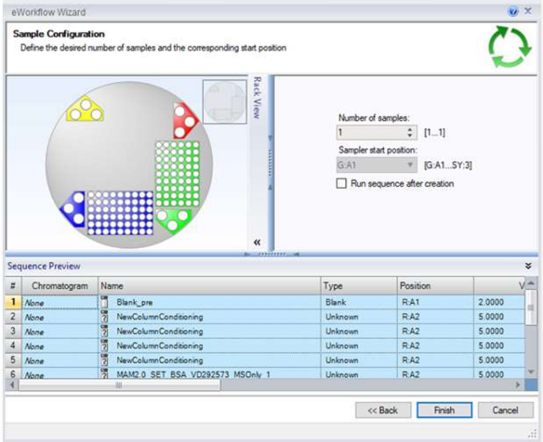
Ease of use

CQA Monitoring in QC

Automatic Workflow Execution

ThermoFisher SCIENTIFIC





Sample Configuration

Define the desired number of samples and the corresponding start position

Number of samples: 1 [1...1]

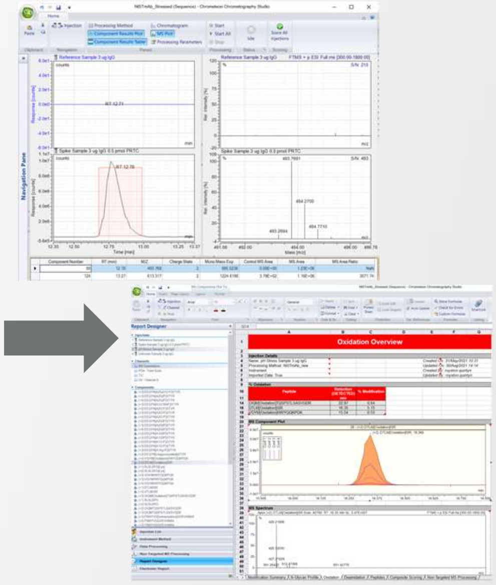
Sampler start position: G:A1 [G:A1...SY:3]

☐ Run sequence after creation

Sequence Preview

#	Chromatogram	Name	Type	Position	V
1	*None*	Blank_pre	Blank	R:A1	2.0000
2	*None*	NewColumnConditioning	Unknown	R:A2	5.0000
3	*None*	NewColumnConditioning	Unknown	R:A2	5.0000
4	*None*	NewColumnConditioning	Unknown	R:A2	5.0000
5	*None*	NewColumnConditioning	Unknown	R:A2	5.0000
6	*None*	MAM2.0_SET_BSA_VD292573_MSOnly_1	Unknown	R:A2	5.0000

Sequence Setup



Report

- Seamless method migration from development to QC
- Secure method transfer
- Easy workflow setup and execution for users at all levels

Compliance-ready

Ease of use

CQA Monitoring in QC

MAM 2.0: Built for Compliance End-to-End

Compliance-ready

eWorkflows to automate sequence creation

Aspirin - Content Uniformity

Type: RegulatoryAnalysis

Status: Approved for Use

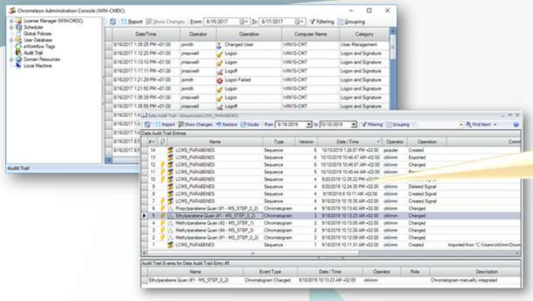
Secure login with role-based permissions

Enter Chromeleon User Name And Password

Chromeleon 7
Chromeleon Console

User Name: Frank.totala@thermofisher.com
Password:
Role: Lab Manager

Audit trails automatically record all activity



Chromeleon Administration Console (WIN-CMDC)

Date/Time	Operator	Operation	Computer Name	Category
8/16/2017 1:35:29 PM +01:00	jsmith	Changed User	WIN10-CM7	User Management
8/16/2017 1:12:20 PM +01:00	jmaxwell	Logon	WIN10-CM7	Logon and Signature
8/16/2017 1:13:10 PM +01:00	jmaxwell	Logon	WIN10-CM7	Logon and Signature
8/16/2017 1:17:11 PM +01:00	jmaxwell	Logoff	WIN10-CM7	Logon and Signature
8/16/2017 1:21:29 PM +01:00	jsmith	Logon Failed	WIN10-CM7	Logon and Signature
8/16/2017 1:21:50 PM +01:00	jsmith	Logon	WIN10-CM7	Logon and Signature
8/16/2017 1:35:39 PM +01:00	jmaxwell	Logon	WIN10-CM7	Logon and Signature
8/16/2017 1:35:55 PM +01:00	jmaxwell	Logoff	WIN10-CM7	Logon and Signature
8/16/2017 1:4				
8/16/2017 1:4				
8/16/2017 1:4				
8/16/2017 8:5				
8/16/2017 8:5				

Data Audit Trail - Sequence/LCMS_PARABENES

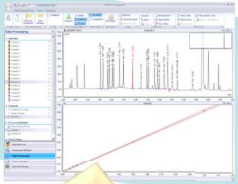
Data Audit Trail Entries

#	Name	Type	Version	Date / Time	Operator	Operation	Comm
14	LCMS_PARABENES	Sequence	6	10/10/2019 1:26:07 PM +02:00	psauter	Created	
13	LCMS_PARABENES	Sequence	6	10/10/2019 10:46:47 AM +02:00	cklimm	Exported	
12	LCMS_PARABENES	Sequence	6	10/10/2019 10:46:07 AM +02:00	cklimm	Changed	
11	LCMS_PARABENES	Sequence	5	10/10/2019 10:45:44 AM +02:00	cklimm	R	
10	LCMS_PARABENES	Sequence	4	9/20/2019 12:25:22 PM			
9	LCMS_PARABENES	Sequence	4	9/20/2019 12:24:30 PM +02:00	cklimm	Deleted Signal	
8	LCMS_PARABENES	Sequence	4	9/19/2019 8:10:11 AM +02:00	cklimm	Created Signal	
7	LCMS_PARABENES	Sequence	4	9/18/2019 10:15:35 AM +02:00	cklimm	Created Signal	
6	Propylparabene Quan (#1 - MS_STEP_0_2)	Chromatogram	4	9/18/2019 10:13:40 AM +02:00	cklimm	Changed	
5	Ethylparabene Quan (#1 - MS_STEP_0_2)	Chromatogram	3	9/18/2019 10:13:23 AM +02:00	cklimm	Changed	
4	Methylparabene Quan (#2 - MS_STEP_1)	Chromatogram	2	9/18/2019 10:13:08 AM +02:00	cklimm	Changed	
3	Methylparabene Quan (#4 - MS_STEP_3)	Chromatogram	2	9/18/2019 10:12:26 AM +02:00	cklimm	Changed	
2	Methylparabene Quan (#1 - MS_STEP_0_2)	Chromatogram	2	9/18/2019 10:12:09 AM +02:00	cklimm	Changed	
1	LCMS_PARABENES	Sequence	1	9/18/2019 10:11:31 AM +02:00	cklimm	Created	Imported from "C:\Users\cklimm\Down

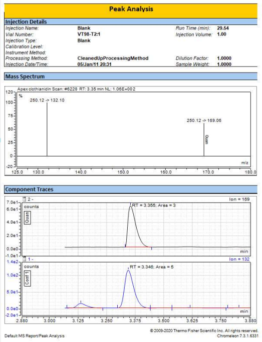
Audit Trail Events for Data Audit Trail Entry #5

Name	Event Type	Date / Time	Operator	Role	Description
Ethylparabene Quan (#1 - MS_STEP_0_2)	Chromatogram Changed	9/18/2019 10:13:23 AM +02:00	cklimm		Chromatogram manually integrated

Managed Access to Instruments with full event logging



Automatic data processing and comprehensive reporting



Peak Analysis

Injection Details

Injection Name:	Blank	*Run Time (min):*	29.54
Vial Number:	VT98-T2:1	*Injection Volume:*	1.00
Injection Type:	Blank		
Calibration Level:			
Instrument Method:			
Processing Method:	CleanedUpProcessingMethod	*Dilution Factor:*	1.0000
Injection Date/Time:	05/Jan/11 20:31	*Sample Weight:*	1.0000

Mass Spectrum

Component Traces

Default MS Report/Peak Analysis

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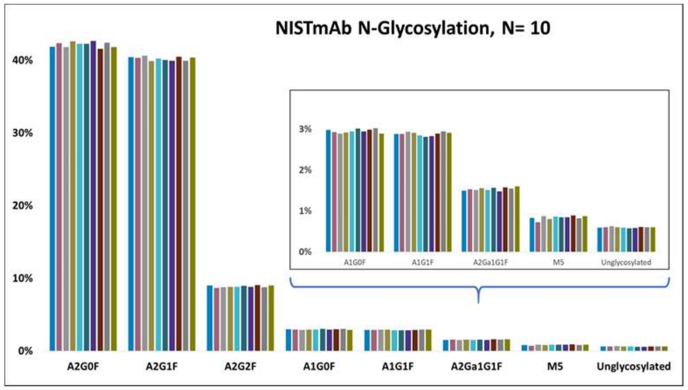
Chromeleon 7.3.1.6331

CQA Monitoring in QC

Summary : CQA Monitoring in QC

Compliance-Ready, Built-for-Purpose, Simplicity

- **Orbitrap Exploris MX delivers consistent and robust performance**
  - ✓ Same platform as Orbitrap Exploris 240
  - ✓ Same high-quality results with same instrument parameters.
  - ✓ Mitigates method re-development for QC transfer
- Automatic workflow execution
- Chromeleon CDS - complete suite of compliant tools



Fit-for- Purpose

Compliance-ready

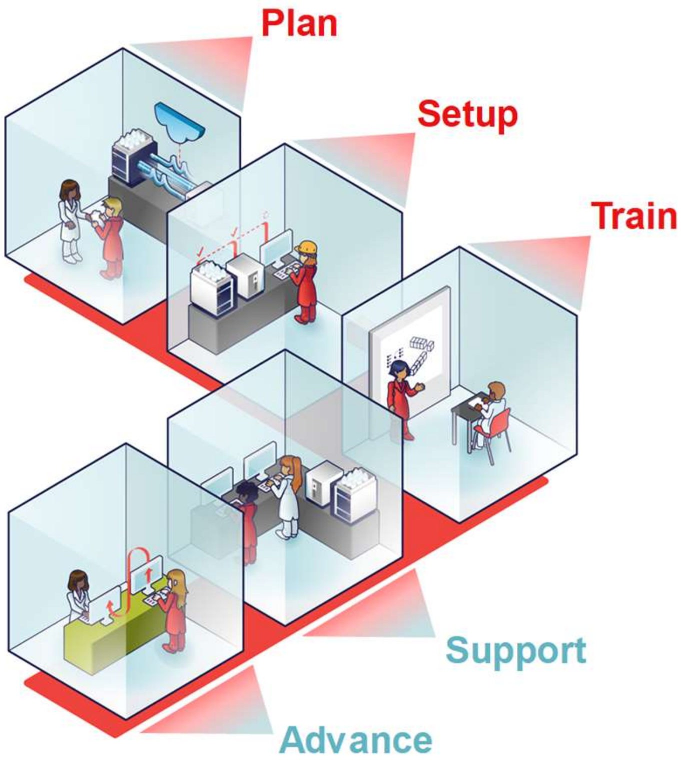
Ease of use

Manufacturing & QC

CQA Monitoring

Reporting

MAM Expert Support Team



- **Supported by a dedicated MAM focused team.**
  - Application scientists
  - Service engineers
  - Software experts

- **Combination of on-site and remote training customizable to meet your needs.**

- **A dedicated support process to quickly connect you with the right MAM expert.**

Support

MAM 2.0: System Performance Evaluation Tests (SET)

- **Instrument performance tested for MAM application requirements**
- An LC-MS test run by MAM support engineer to evaluate system performance against a comprehensive set of acceptance criteria that are relevant for peptide mapping and monitoring

Sample → **SET eWorkflow** → **Process and Report**

Thermo Scientific Pierce™ BSA protein digest

Thermo Scientific™ Hypersil GOLD™ Vanquish C18 column

Vanquish Horizon or Flex UHPLC

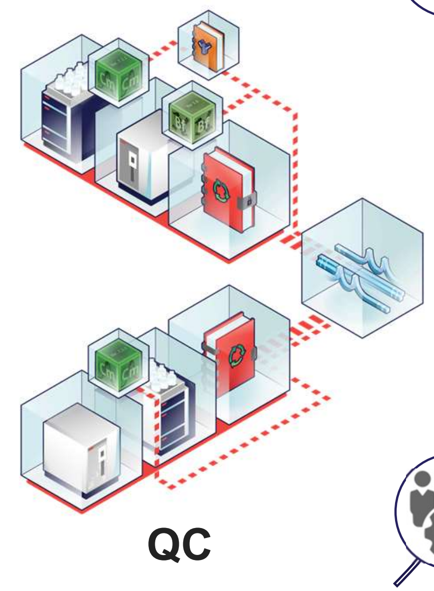
Orbitrap Exploris 240 or Orbitrap Exploris MX Mass Spectrometer

Chromeleon CDS for data acquisition, processing, and reporting

https://www.thermofisher.com/us/en/home/industrial/pharma-biopharma/biopharmaceutical-analytical-testing/multi-attribute-method.html

Summary: Thermo Scientific MAM 2.0

End-to-end, compliance-ready platform for deployment from development to QC

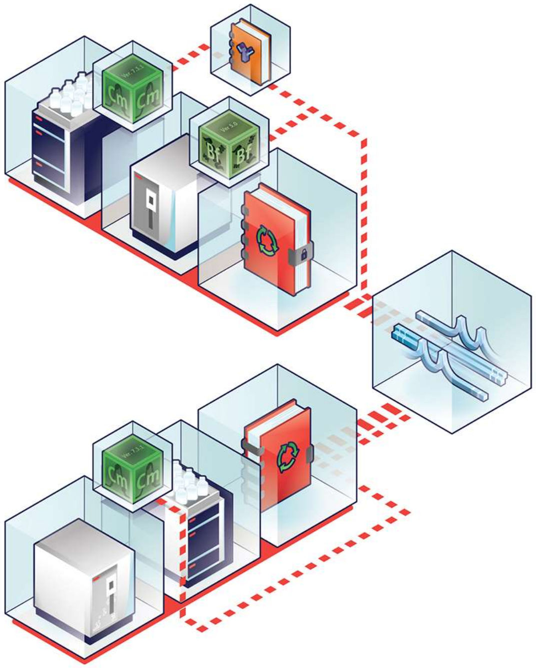


Connectivity	**A software ecosystem for connectivity at global scale**
Ease of Use	Seamless method migration from development to QC Automatic execution of entire MAM workflow in QC
Fit for Purpose	Same Orbitrap platform - Direct method transferability **Orbitrap Exploris MX is purposely built for QC**
Compliance-Ready	**End-to-end compliant tools in Chromeleon CDS**
Training and Support	**Dedicated MAM expert support team**

Attribute Characterization | **Attribute Monitoring** | **CQA Monitoring in QC**

Thermo Scientific MAM 2.0

DEVELOPMENT



QC

Where

development

&

quality control

come together

Fit- for- Purpose

Compliance-ready

Connectivity

Ease of use

Support

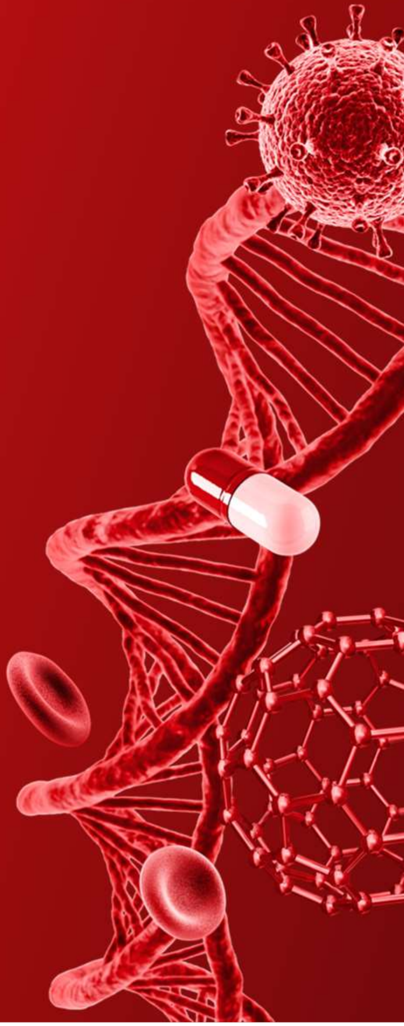
Acknowledgement

>200 Thermo Fisher Scientific Colleagues

- **Digital Transformation team**
- **Orbitrap Exploris MX team**
- **HPLC team**
- **Chromatography Consumables team**
- **Application team**
- **Marketing team**

Various industry partnerships

Thank you



Questions