



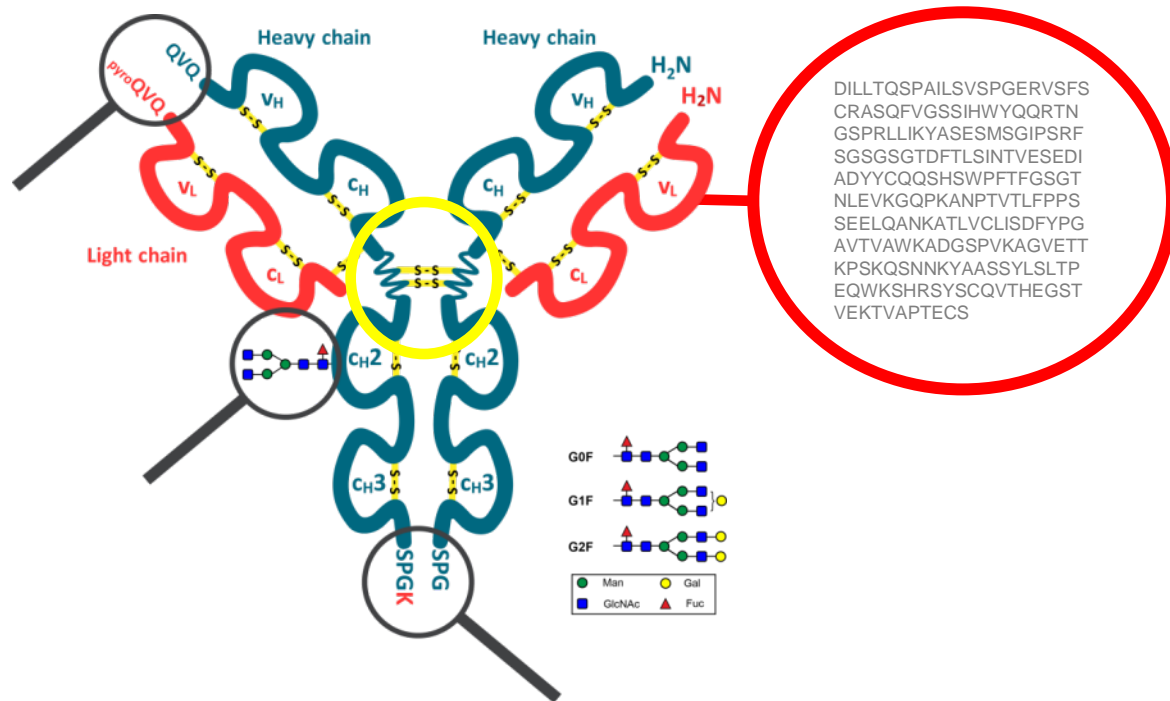
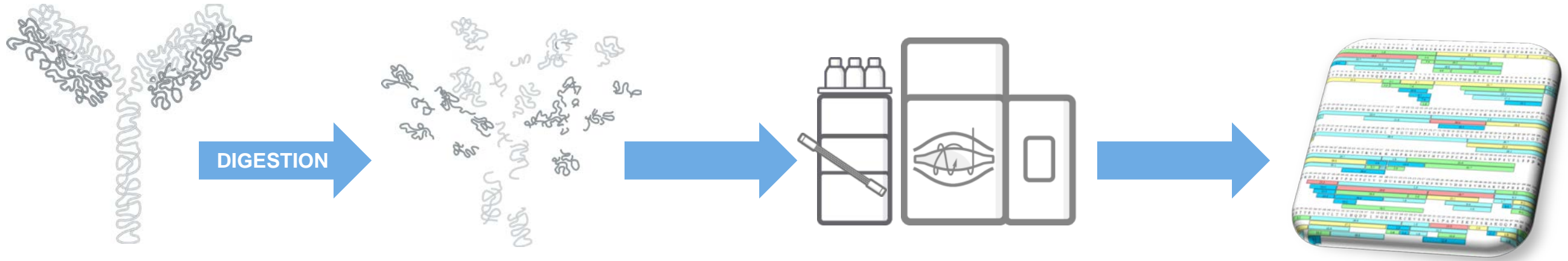
ThermoFisher
SCIENTIFIC

Upgrade Your Maps: New, rapid and reproducible peptide mapping workflows

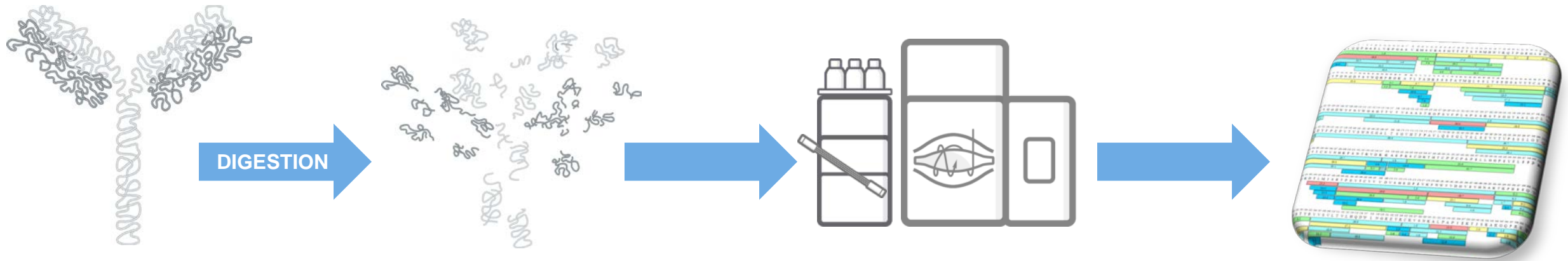
Rowan Moore, PhD
Pharma & BioPharma Marketing Manager

The world leader in serving science

Why Peptide Mapping?

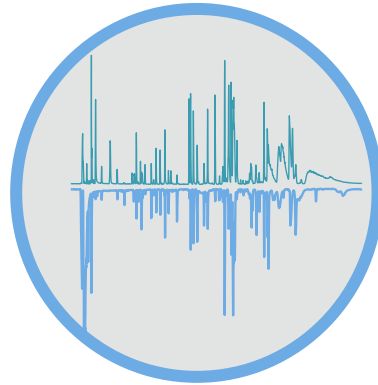


Why Peptide Mapping?



DILLTQSPAILSVPGERVVSFSC
RASQFVGSSIHWHYQQRNGSP
RLLIKYASEMSGIPSRFSGSGS
GTDFTLSINTVESEDIADYYCQQ
SHSWPFTFGSGTNLEVKGQPK
ANPTVTLFPPSSEELQANKATLV
CLISDFYPGAVTVAWKADGSPV
KAGVETTKPSKQSNNKYAASSY
LSLTPEQWKSHRSYSCQVTHE
GSTVEKTVAPTECS

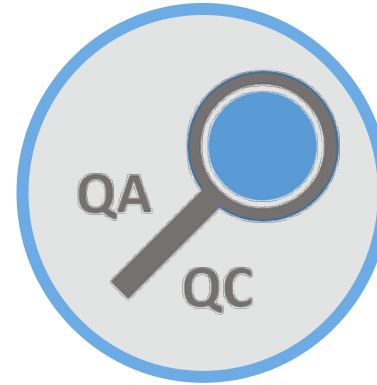
Identity & Purity



Comparability



Quantitation



Lot Release



Regulatory

Upgrade Your Maps with a Complete Peptide Mapping Solution



- Full workflow solution
- Robust and reliable
- Fast and reproducible
- Easy and convenient
- Gives you:
 - *Sequence verification*
 - *Modifications analysis*
 - *Sequence variants*
 - *Relative abundance quantification*

Why Upgrade Your Maps?

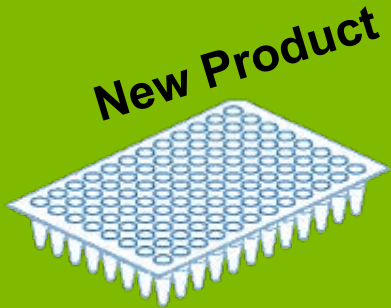
What will I gain if I upgrade my maps?

Sample preparation, separation, MS detection & data analysis of proteins.

Confirmed amino acid sequence with 100% coverage, identify and quantify modifications, identify variants.

Most Important: A simpler life! Less training required, results within a few hours.

Upgrade Your Maps: Our Workflow Solution



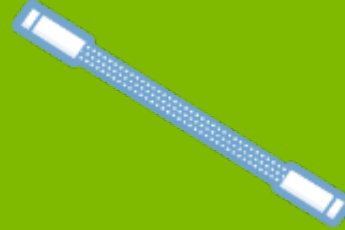
New Product

Thermo Scientific™
SMART Digest™ Kits
offers extremely
reproducible and rapid
protein digestion

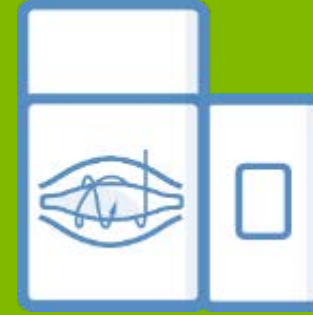


New Product

Thermo Scientific™
**Vanquish™ Flex
UHPLC Systems**
are engineered for
high resolution,
reproducible
peptide separations



Thermo Scientific™
**Acclaim™ VANQUISH™
C18 column**
is the perfect column
choice to ensure sharp
peaks during peptide
mapping



Thermo Scientific™
**Q Exactive™ Hybrid
Quadrupole-
Orbitrap™ mass
spectrometers** are the
gold standard for
accurate mass
measurements



**Brand New
Product**

Thermo Scientific™
**BioPharma
Finder™ mass informatics
platform**
is the perfect software tool
for peptide identification
and sequence mapping



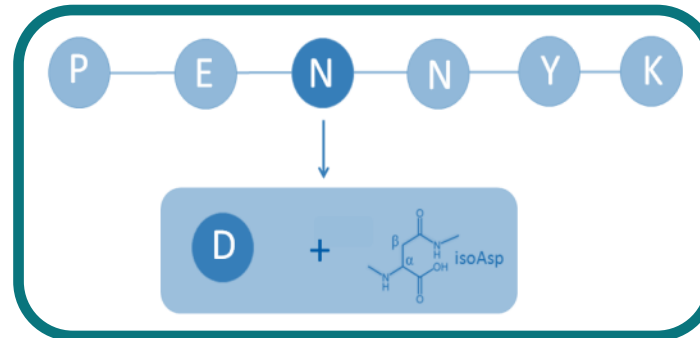
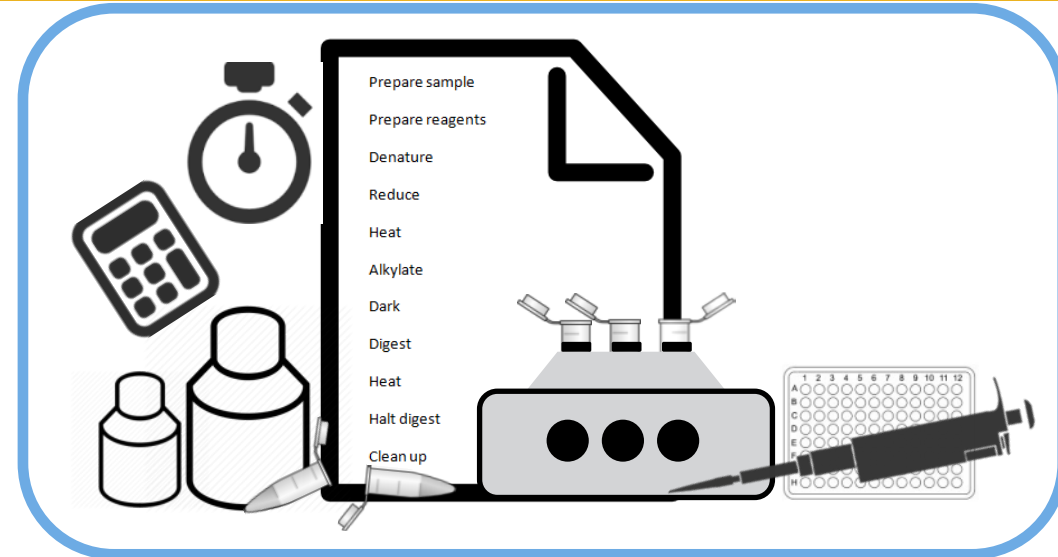
SMART Digest Kits

Achieve reproducible protein digestion

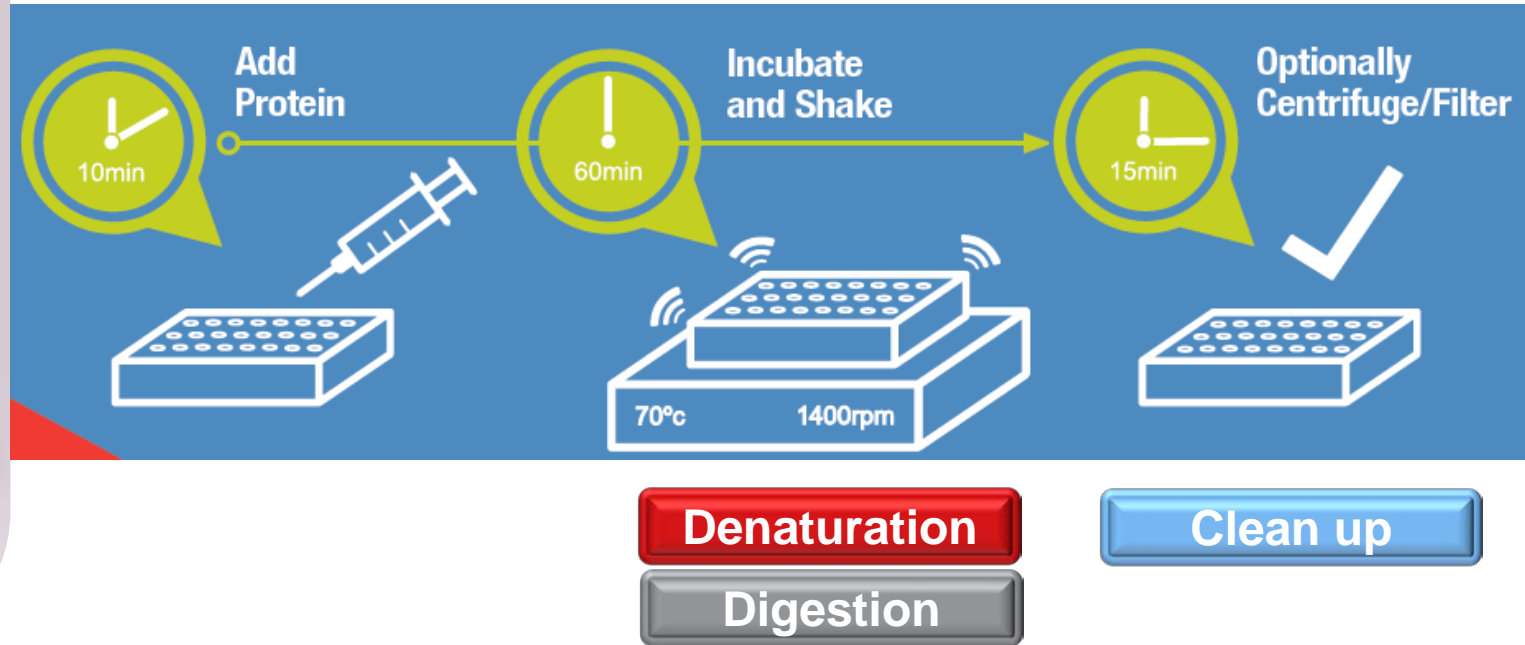
- within minutes
- with a simple 3-step protocol

What is the Problem with Protein Digestion?

- Lengthy multi-step protocols
- Process-induced PTMs
- Reproducibility
- Throughput/speed
- Method development ease



Thermo Scientific™ SMART Digest™ Kits



Let's Immobilize Trypsin...and Make it Heat Stable!

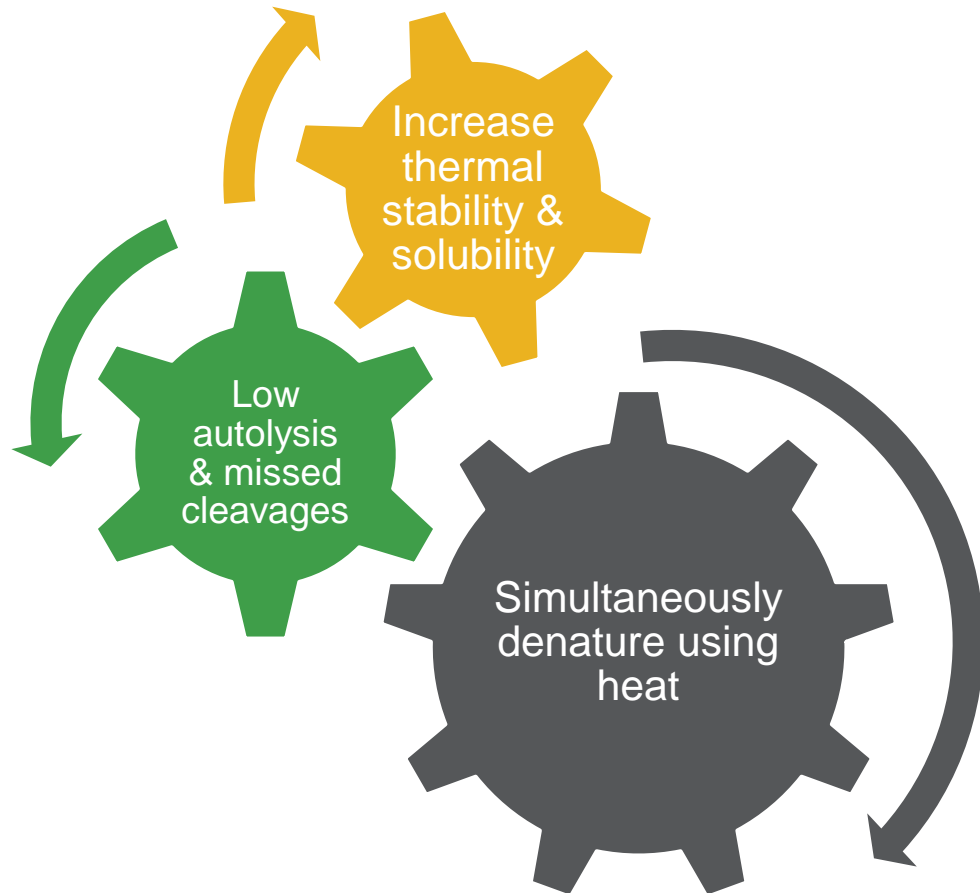
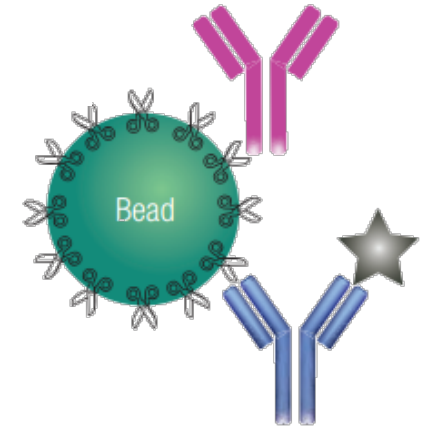


Enzyme

Solubility

Denaturation

Diffusion



Yellowstone

- **Save time**
 - Reagent prep/denaturation, reduction/alkylation
- **Simplify**
 - low autolysis
- **Increase sensitivity**
- **Increase robustness**
 - Stable, reproducible activity

Let's Optimize Heat Denaturation!



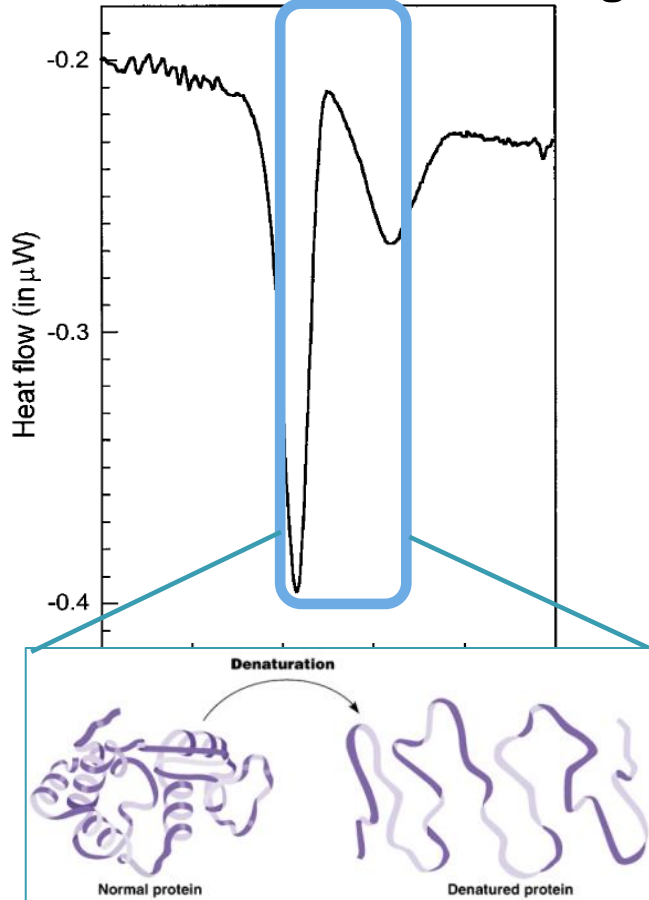
Enzyme

Solubility

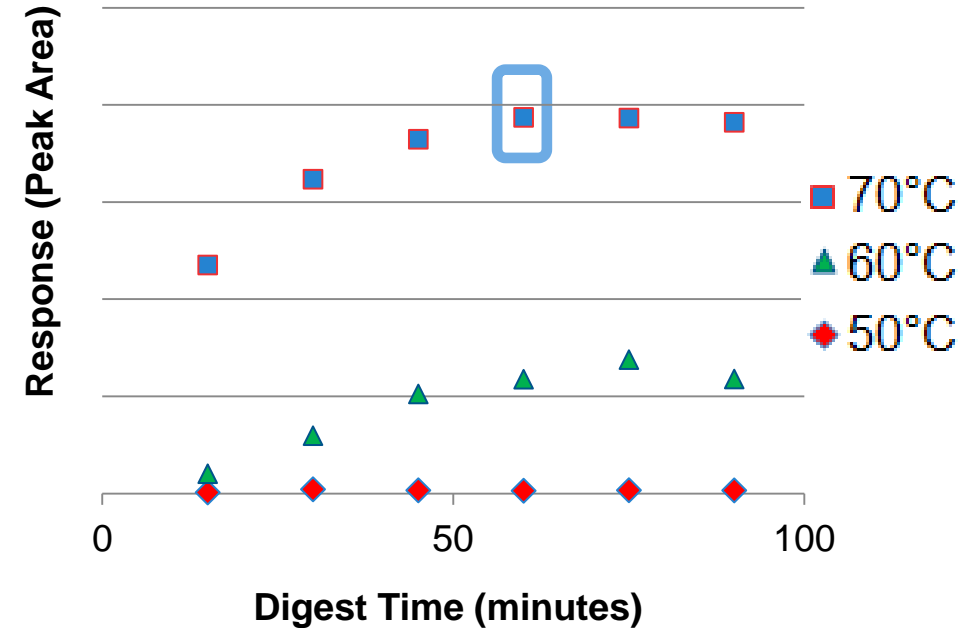
Denaturation

Diffusion

Thermal denaturation of IgG



Native IgG Digest Profile monitoring VSVLTVLHQDWLNGK



394

Biophysical Journal Volume 78 January 2000 394–404

The Thermal Stability of Immunoglobulin: Unfolding and Aggregation of a Multi-Domain Protein

Anal. Chem. 2000, 72, 2667–2670

Thermal Denaturation: A Useful Technique in Peptide Mass Mapping

thermo scientific

APPLICATION NOTE

SMART Digest compared to classic in-solution digestion of rituximab for in-depth peptide mapping characterization

Authors: Martin Samonig¹, Alexander Schwahn², Ken Cook³, Mike Oliver⁴, and Remco Swart¹

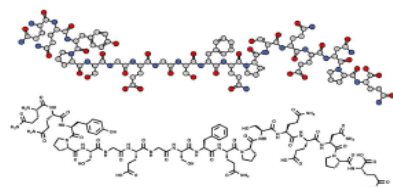
¹Thermo Fisher Scientific, Germering, Germany; ²Thermo Fisher Scientific, Basel, Switzerland; ³Thermo Fisher Scientific, Hemel Hempstead, United Kingdom; ⁴Thermo Fisher Scientific, Runcorn, United Kingdom

Key words

SMART Digest, tryptic digestion, in-solution protein digestion, monoclonal antibody, mAb, Vanquish, reversed phase, mass spectrometry, Q Exactive, Orbitrap, biopharmaceutical, biomolecules, peptide mapping

Goal

To compare the results achieved by using the newly developed Thermo Scientific™ SMART Digest™ kit to those obtained from classic in-solution protein digestion methods, focusing on protein sequence coverage and identified post-translational modifications (PTMs), including deamidation, oxidation, and glycosylation. A Thermo Scientific™ Acclaim™ VANQUISH™ C18 column with conventional water/acetonitrile-based gradients and



the Thermo Scientific™ Vanquish™ Flex UHPLC system were used for separation in combination with the Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer.

Introduction

Peptide mapping is a common technique in the biopharmaceutical industry to characterize monoclonal antibodies (mAbs) for the determination of product identity and stability. Many conventional sample preparation methods are time consuming with digestion times of several hours and can introduce modifications such as deamidation, oxidation, and carbamylation.¹ In this study, two classic in-solution digestion approaches were compared to the recently developed SMART Digest kit method to quantify the extent of post-translational and chemical modifications of a therapeutic recombinant mAb. The critical requirements for each

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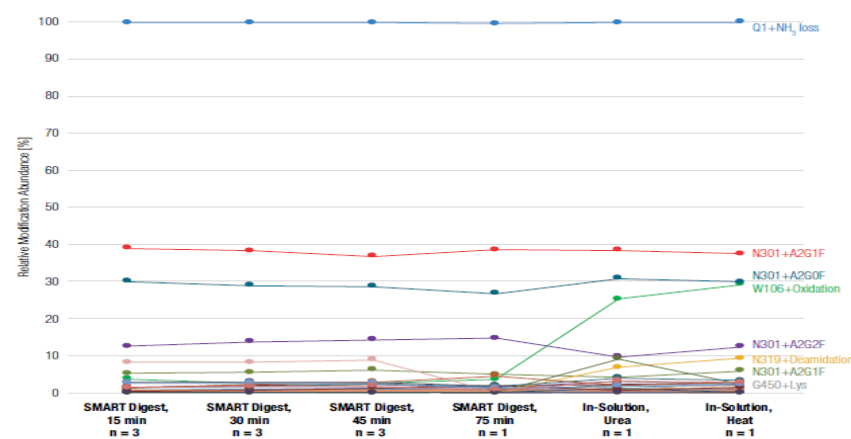


Figure 5. Relative abundance of 85 identified modifications including oxidation, double oxidation, glycation, glycosylation, NH₃ loss, isomerization, lysine truncation, methylation, dimethylation, and carbamylation.

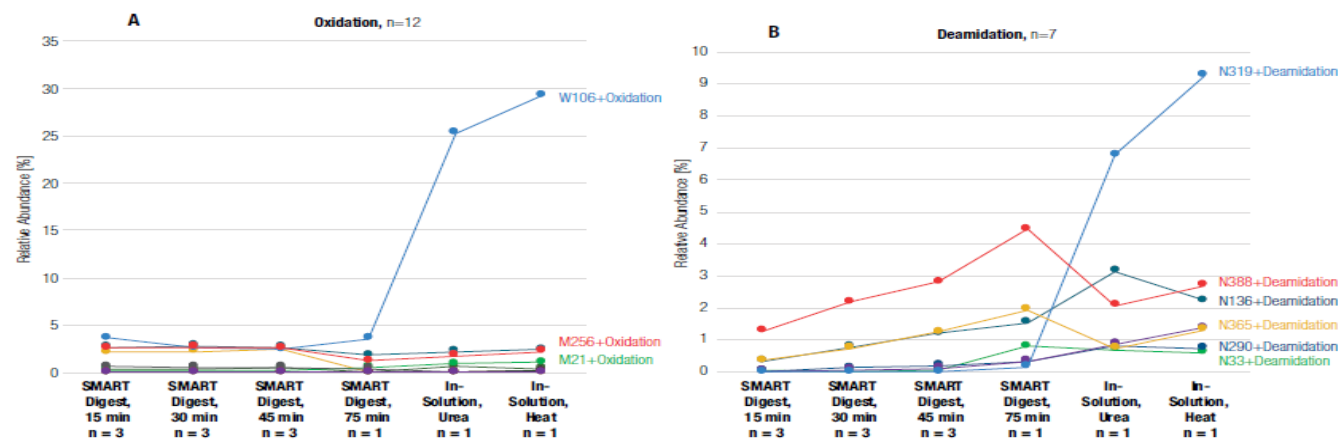


Figure 6. Relative abundance of 12 identified oxidations (A) and 7 deamidations (B) in different runs with various digestion methods.

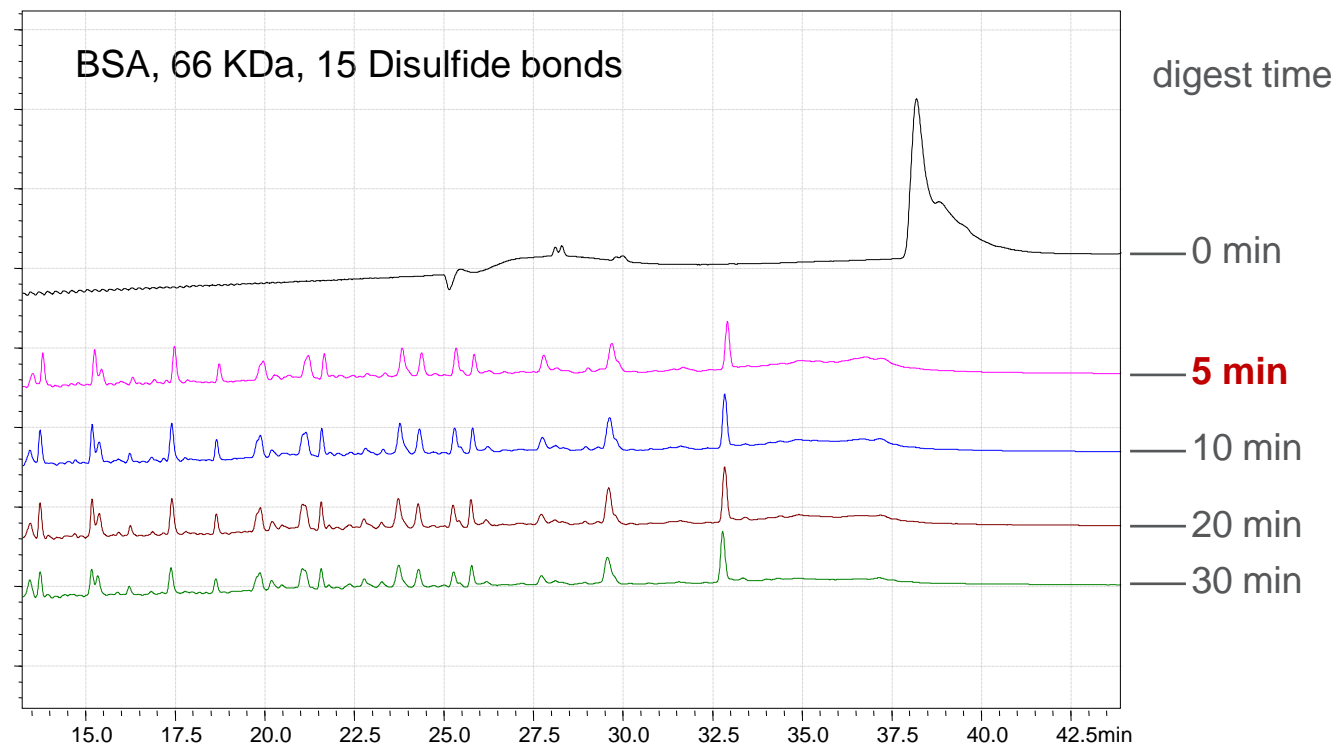
SMART Digest Kits: Complete Digestion in Minutes

Recommended digestion starting conditions for known proteins*	
Protein	Digest Time (min)
Insulin	4
BSA	< 5
Carbonic anhydrase	< 5
Lysozyme	< 5
Apo-B	30
IgG	45
IgG in 50 µL plasma	75
Ribonuclease A	150
Thyroglobulin	240
C-reactive protein	240

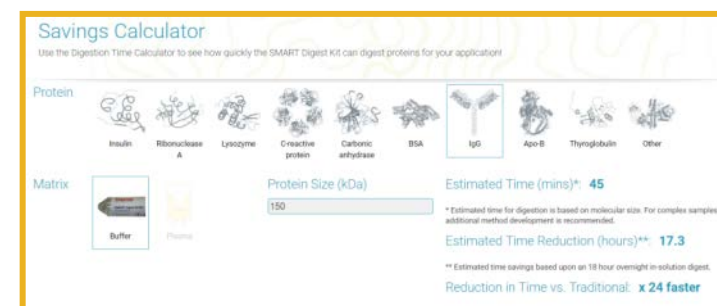
* 200 µL protein solution (100 µg/mL);

IgG in plasma: 17.5 µg/mL

Temperature: 70°C

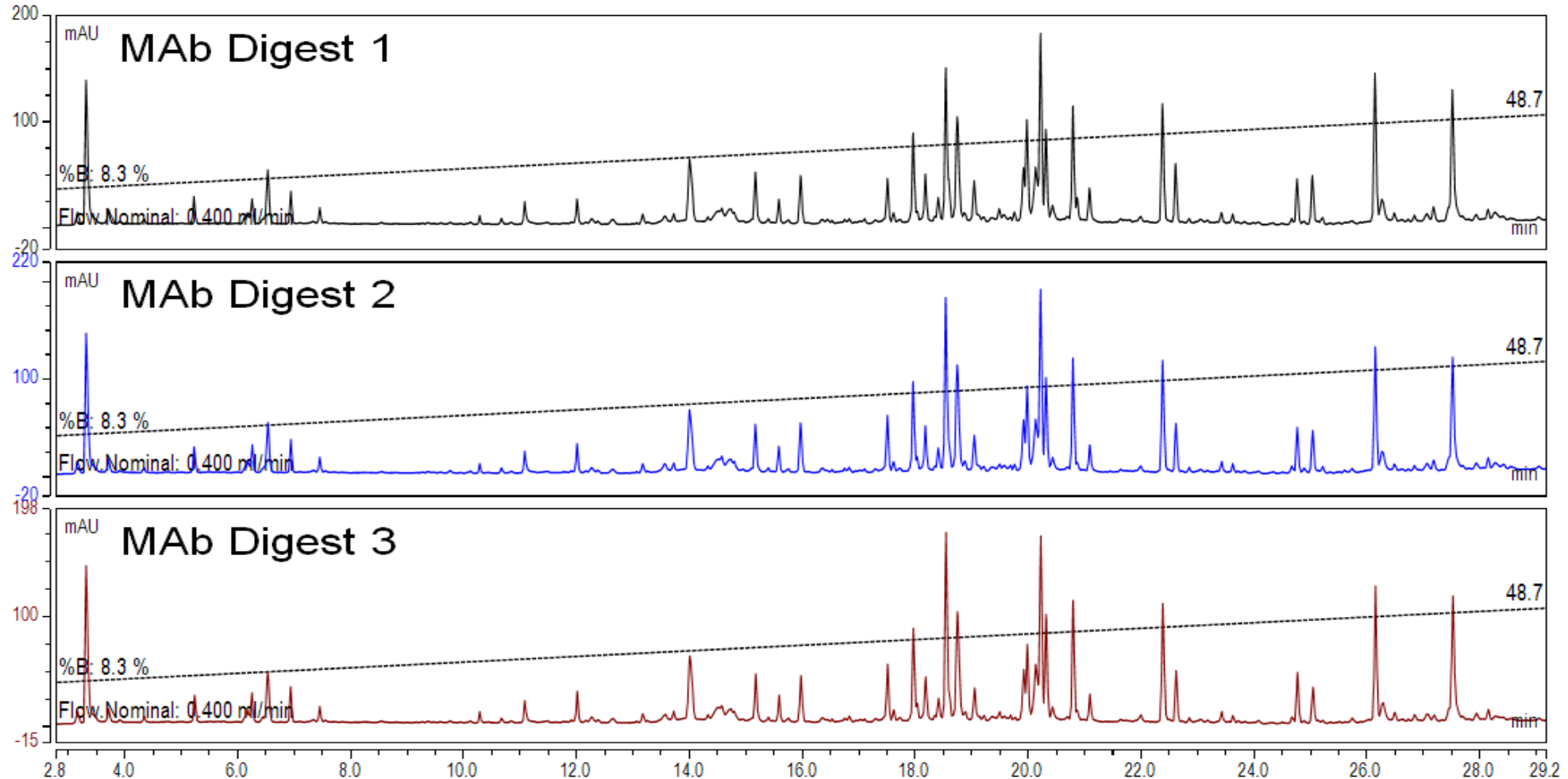


Discover the Savings Calculator:
www.thermofisher.com/upgradeyourmaps



SMART Digest Kits: Outstanding Digestion Reproducibility

Repeatability of a monoclonal antibody SMART Digest Kits digestion

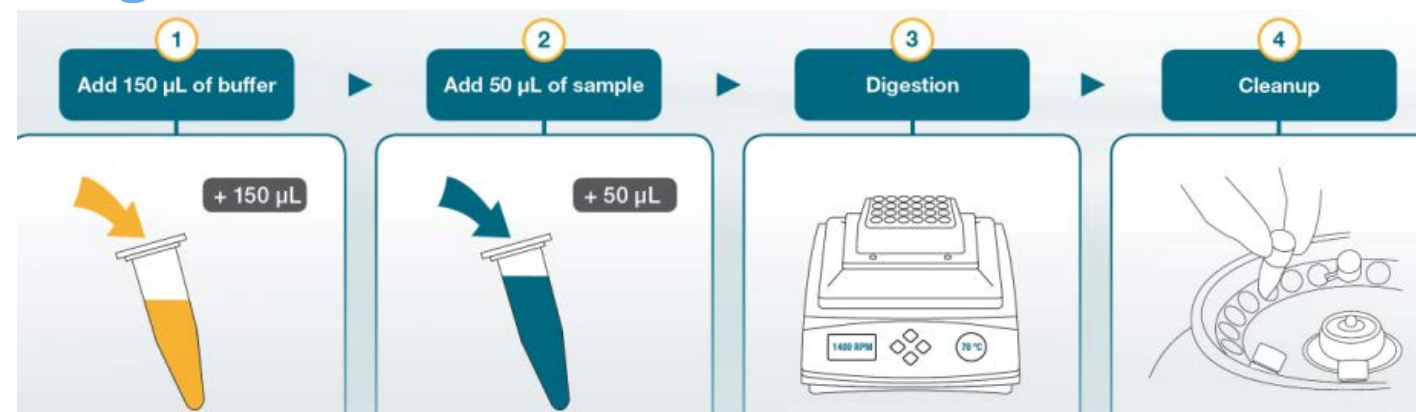


- I want 100 % sequence coverage - **maybe**
- I am quantifying my protein via surrogate peptide monitoring - **no**
- I am worried I may see scrambled disulphides/ my protein has free cysteines – **alkylate before**
- I want to know where my disulphides are! – **yes & no**
- My mass spectrometer won't acquire/is not optimized for higher mass peptides - **maybe**

Protein Digestion with SMART Digest Kits: Tips & Tricks

- How much protein to load? **200 pg - 3.5 mg**
- How long to digest my sample? **Start with the online calculator**
- What are the tubes and heater/shaker? **PCR tubes; heater/shaker *must* have a lid**
- I bet it won't be that simple. **It really is!**
- Will a quick spin will be enough to clean up? **Often, yes! Gel loader tips useful; filter/SPE for complex matrix; divert salts to waste**
- My protein is really difficult to denature- I always add detergents. **OGS has proved not to inhibit digest & led to improved digestion rate**

<http://www.separatedbyexperience.com/smartdigest/>



Impact on Tryptic Digestion when Detergents and Chaotropes are Essential: A Case Study with Ribonuclease A

Valeria Barastri, Thermo Fisher Scientific, Runcorn, UK

Recommended digestion starting conditions for known proteins*	
Protein	Digest Time (min)
Insulin	4
BSA	< 5
Carbonic anhydrase	< 5
Lysozyme	< 5
Apo-B	30
IgG	45
IgG in 50 µL plasma	75
Ribonuclease A	150
Thyroglobulin	240
C-reactive protein	240

Application Note 35531

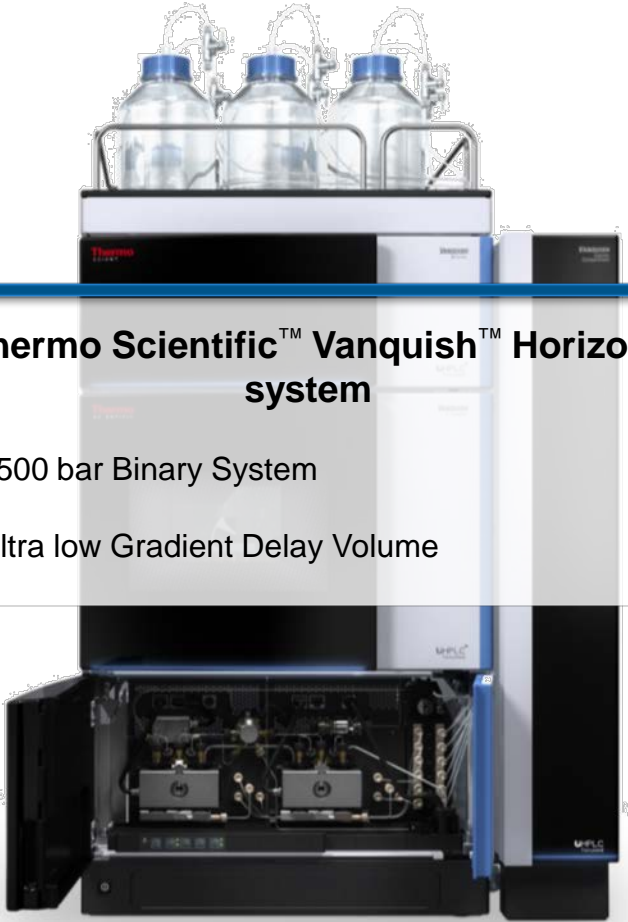


Vanquish UHPLC

Binary & Quaternary
Biocompatible UHPLC
Options

Acclaim Vanquish C18
Column

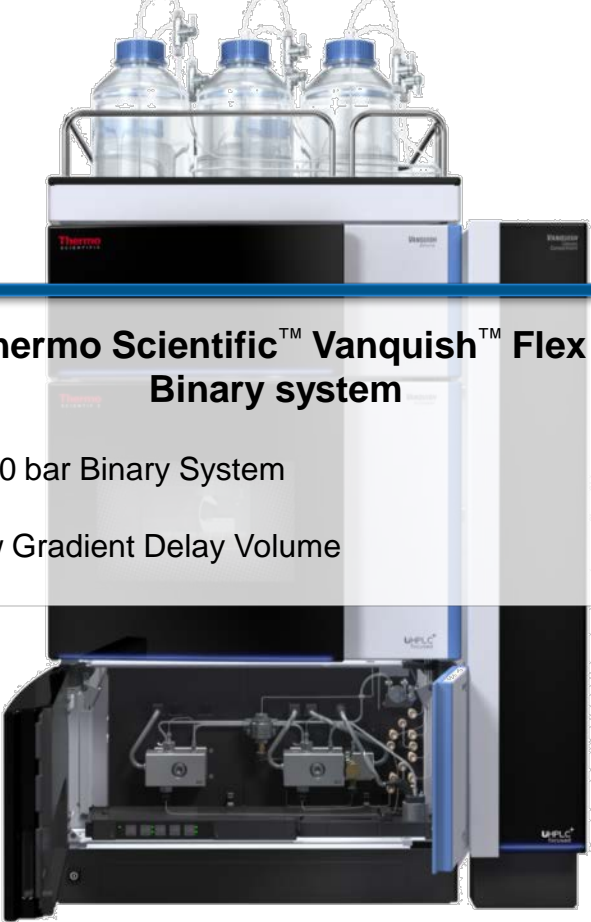
Vanquish Systems – Comparison



Thermo Scientific™ Vanquish™ Horizon system

- 1500 bar Binary System
- Ultra low Gradient Delay Volume

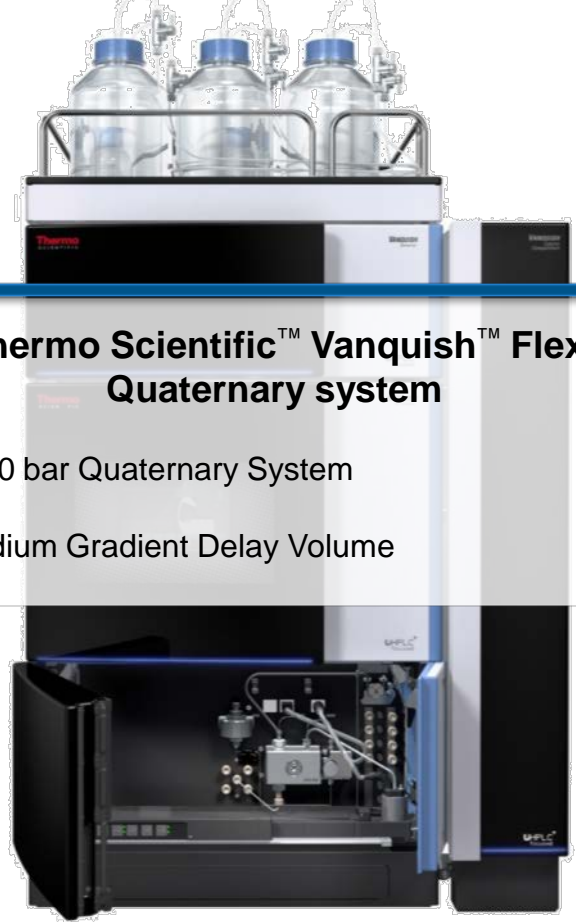
Highest performance for best separation or throughput



Thermo Scientific™ Vanquish™ Flex Binary system

- 1000 bar Binary System
- Low Gradient Delay Volume

Throughput for targeted UHPLC separations



Thermo Scientific™ Vanquish™ Flex Quaternary system

- 1000 bar Quaternary System
- Medium Gradient Delay Volume

UHPLC for resolution or method development

Vanquish Systems – Comparison

Vanquish Platform

- Improved retention time precision
- Biocompatible by default
- Increased sample capacity
- SmartInject sample pre-compression
- Improved sample cooling
- Multiple heating modes

- Active and passive pre-heating
- 4 detection options
- Platform-inherent robustness & maintenance features

Thermo Scientific™ Vanquish™ Horizon system

- 1500 bar Binary System
- Ultra low Gradient Delay Volume



Highest performance for best separation or throughput

Thermo Scientific™ Vanquish™ Flex Binary system

- 1000 bar Binary System
- Low Gradient Delay Volume



Throughput for targeted UHPLC separations

Thermo Scientific™ Vanquish™ Flex Quaternary system

- 1000 bar Quaternary System
- Medium Gradient Delay Volume



UHPLC for resolution or method development

Acclaim VANQUISH C18 Column, 2.1 x 250 mm

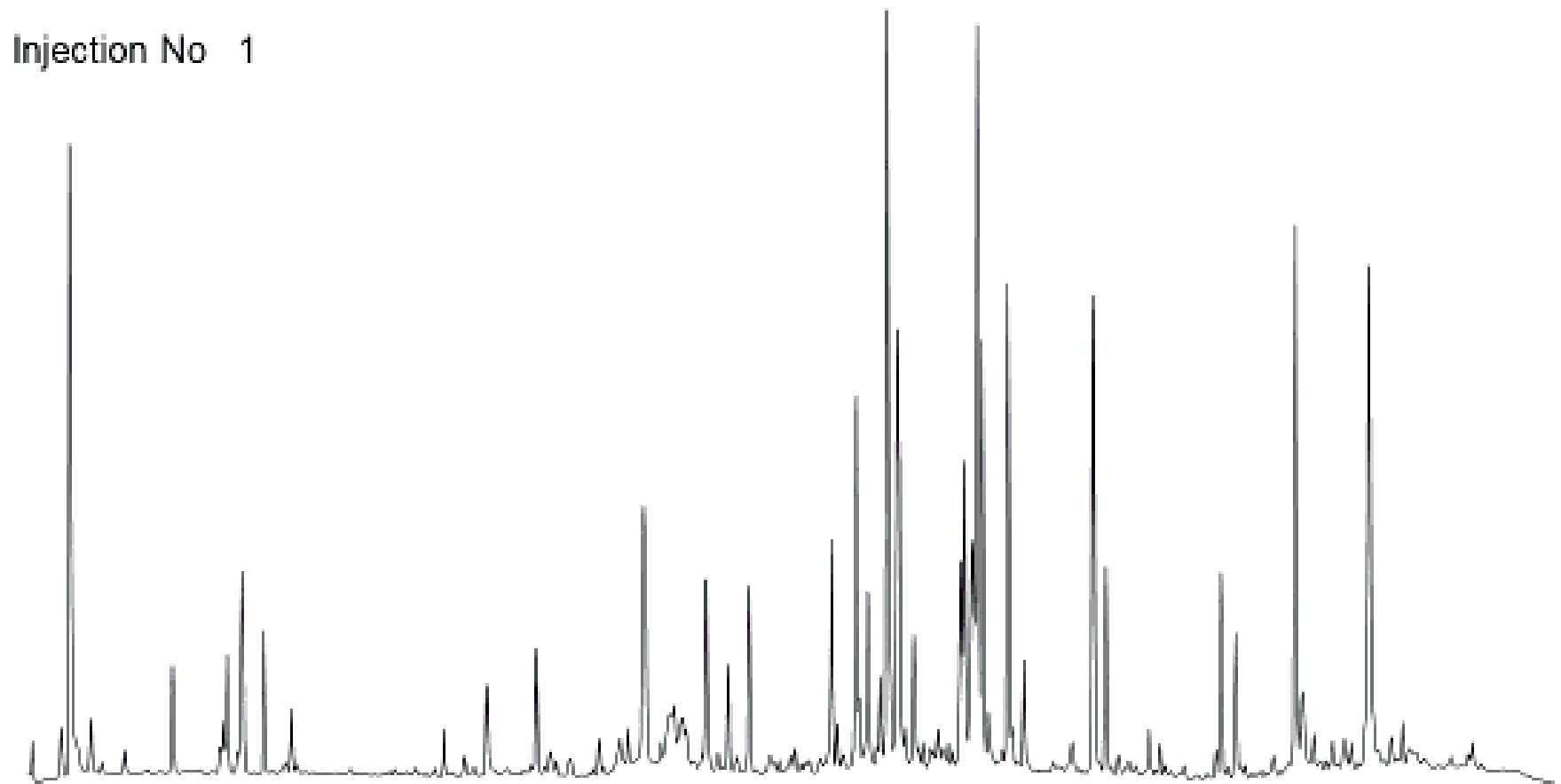


- High resolution peptide separations for increased protein identification
- High loadability for high sensitivity LC/MS
- Designed for TFA-free LC-MS, minimizing ion-suppression effects
- High column-to-column reproducibility
- 1500 bar Vanquish-compatible
- Robust design easy installation using Thermo Scientific™ Viper™ fingertight fittings



Viper™ fingertight fitting

Retention time reproducibility of a peptide separation



Retention time Repeatability

peak #	RT (min)	RSD (%)
3	3.315	0.082
9	5.231	0.065
14	6.532	0.017
15	6.937	0.023
19	10.290	0.021
23	12.013	0.012
31	14.011	0.013
39	15.177	0.012
42	15.589	0.010
51	17.511	0.007
55	17.969	0.011
61	18.546	0.010
83	20.798	0.010
85	21.095	0.012
87	22.386	0.009
96	24.774	0.012
103	26.155	0.009
106	26.155	0.009
109	27.529	0.010

Overlay of 13 consecutive chromatographic runs of a peptide sample separated on an analytical Acclaim™ 120 C18 column and prepared from a mAb digested with the SMART™ Digest Kit.

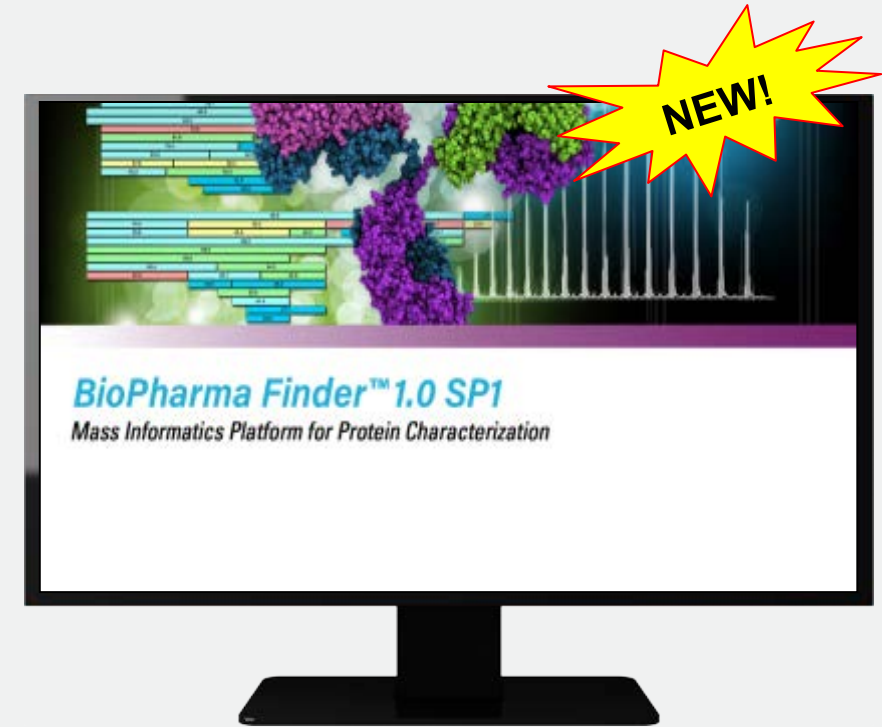


BioPharma Finder

- A single software platform for
- Top-Down & Bottom-Up analysis
 - Dedicated to biotherapeutics

BioPharma Finder

- Intact protein analysis and peptide mapping in one package
- Peptide mapping of biotherapeutics and other recombinant proteins
- Supports all Orbitrap™ & ion-trap-based instruments



**More confidence, more
modifications, less time**

Data Analysis With BioPharma Finder – Peptide Mapping



Thermo BioPharma Finder 1.0

Thermo SCIENTIFIC BioPharma Finder Help

☒ Home

Select an experiment type.

Experiment Types

- Protein Sequence Manager >
- Peptide Mapping Analysis >
- Intact Protein Analysis >

A powerful integrated software solution for in-depth characterization of proteins. Thermo Fisher's BioPharma Finder™ software automates the analysis of LC-MS data for intact mass analysis and LC-MS/MS data for peptide mapping which includes identification and relative quantitation of Disulfide bonds, post-translational Modifications and Sequence Variants. Using the industry-leading, high-resolution, accurate-mass Thermo Scientific™ Orbitrap™ technology with BioPharma Finder software, the most accurate and confident PTM profiles and identifications can be achieved more quickly than ever before.

Peptide Mapping Analysis

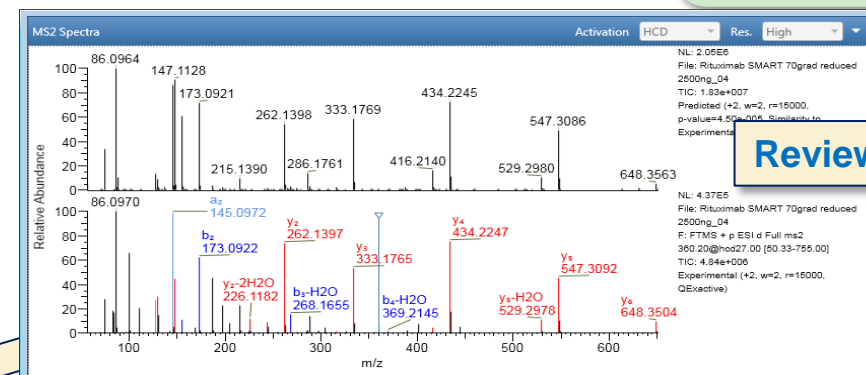
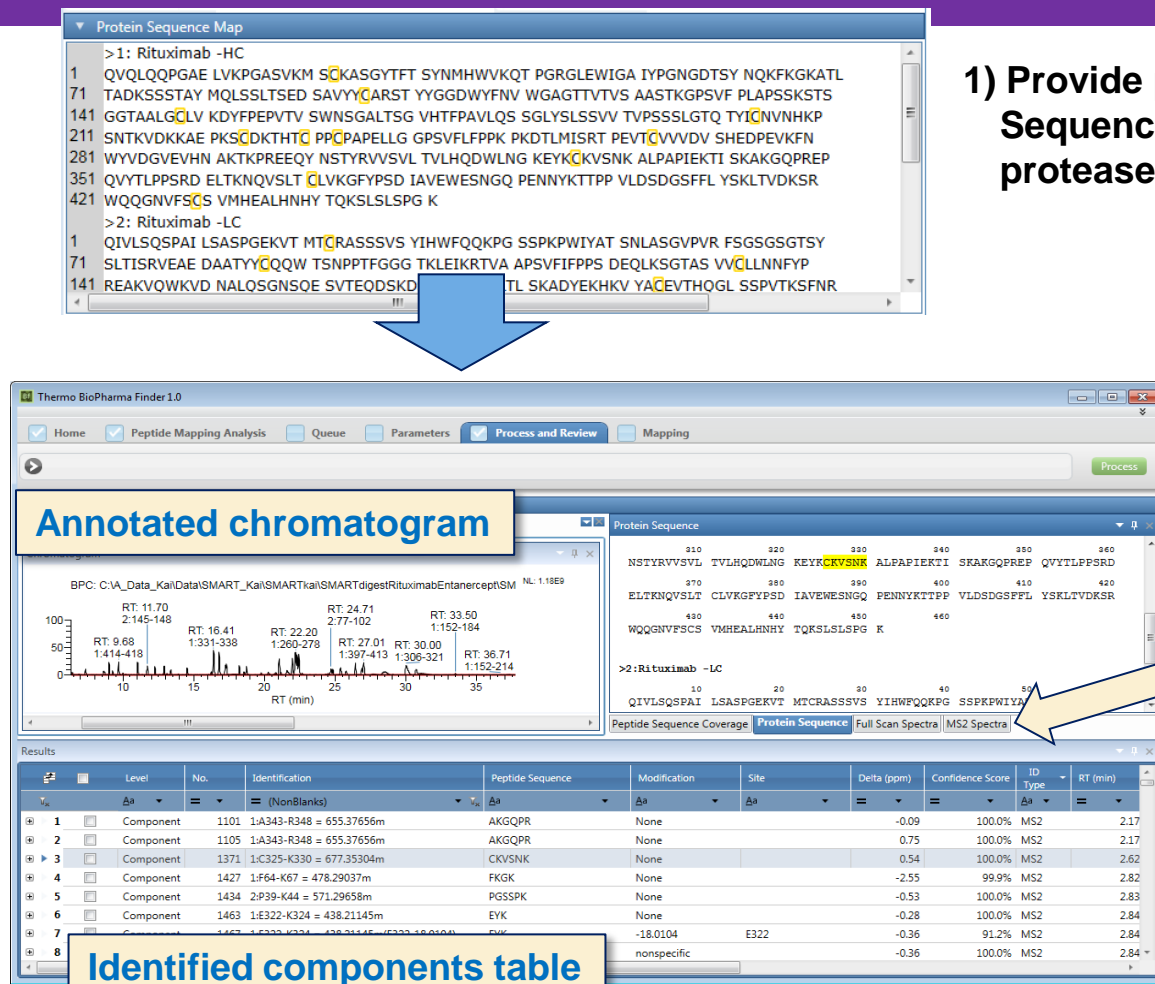
Intact Protein Analysis

Data Analysis With BioPharma Finder – Peptide Mapping



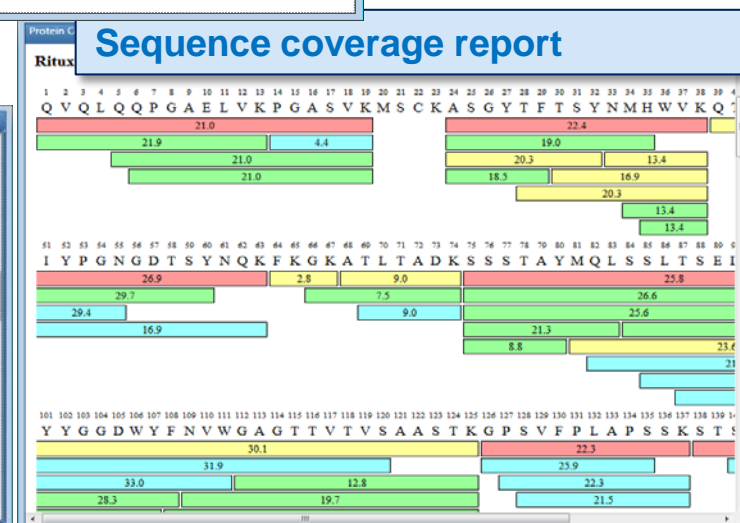
1) Provide protein sequence(s) in the Protein Sequence Manager, define modifications, protease & all search parameters

2) Data Analysis in 3 steps:
a) Component Detection
b) Peptide Identification
c) Quantification



Modification summary

Created	Protein	Residue #	Modification	Category
51	Rituximab -HC	289	-H289+135.9642	Unknown Modification
52	Rituximab -HC	289	-H289-17.0251	Unknown Modification
53	Rituximab -HC	290	-N290+0.9840	Unknown Modification
54	Rituximab -HC	292	-K292-18.0096	Unknown Modification
55	Rituximab -HC	301	N301-A1G0	Glycoform
56	Rituximab -HC	301	N301-A1G0F	Glycoform
57	Rituximab -HC	301	N301-A1G1F	Glycoform
58	Rituximab -HC	301	N301-A2G0	Glycoform
59	Rituximab -HC	301	N301-A2G0F	Glycoform
60	Rituximab -HC	301	N301-A2G1	Glycoform
61	Rituximab -HC	301	N301-A2G1F	Glycoform
62	Rituximab -HC	301	N301-A2G2F	Glycoform
63	Rituximab -HC	301	N301-A2S1G1F	Glycoform
64	Rituximab -HC	301	N301-A2S2F	Glycoform
65	Rituximab -HC	301	N301-M4	Glycoform
66	Rituximab -HC	301	N301-M5	Glycoform
67	Rituximab -HC	311	N301-Unglycosylate	Glycoform
68	Rituximab -HC	311	-T311-18.0106	Artifact



Reference: Zhongqi Zhang. Large-Scale Identification and Quantification of Covalent Modifications in Therapeutic Proteins. *Anal. Chem.* 2009, 81, 8354-8364

BioPharma Finder Software: Protein Sequence Information

Protein Sequence Editor

Variable modifications selection. Import Protein Sequence Default Modifications Save Save As New Cancel

Protein Sequence Information

Target Protein

Name: Rituximab

Category: mAb (chimeric)

Monoisotopic Mass: 144419.259

Average Mass: 144508.74

Chain

Chain: 1

Monoisotopic Mass: 49183.41

Average Mass: 49214.03

Protein Sequence Map

>1: HCA

1 QVQLQQPGAE LVKPGASVKM S KASGYTFT SYNMHWVKQT PGRGLEWIGA IYPGNGDTSY NQKFKGKATL

71 TADKSSSTAY MQLSSLTSED SAVYY ARST YYGGDWYFNV WGAGTTVTVS AASTKGPSVF PLAPSSKSTS

141 GGTAAALGLV KDYFPEPTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ TYINNVNHKP

211 SNTKVDKKA E PKSDKHTQ PPAPPELLG GPSVFLFPK PKDTLMISRT PEVTVVVDV SHEDPEVKFN

281 WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAIEKTI SKAKGQPREP

351 QVYTLPPSRD ELTKNQVSLT CLVKGFPSPD IAVEWESNGQ PENNYKTTTP VLDSDGSFFL YSKLTVDKSR

421 WQQGNVFS S VMHEALHNHY TQKSLSLSPG K

>2: LCA

1 QIVLSQSPAI LSASPGEKVT MTCRASSSVS YIHWFQQKPG SSPKPWIYAT SNLASGVPVR FSGSGSGTYS

71 SLTISRVEAE DAATYYQQW TS NPPTFGGG TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVGLLNNFYP

141 REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLT SKADYEKKHV YACEVTHQGL SSPVTKSFNR

211 GE

>3: HCB

1 QVQLQQPGAE LVKPGASVKM S KASGYTFT SYNMHWVKQT PGRGLEWIGA IYPGNGDTSY NQKFKGKATL

71 TADKSSSTAY MQLSSLTSED SAVYY ARST YYGGDWYFNV WGAGTTVTVS AASTKGPSVF PLAPSSKSTS

141 GGTAAALGLV KDYFPEPTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ TYINNVNHKP

211 SNTKVDKKA E PKSDKHTQ PPAPPELLG GPSVFLFPK PKDTLMISRT PEVTVVVDV SHEDPEVKFN

Disulfide Link Definitions

Chain Number	Amino Acid Site Index	Chain Number	Amino Acid Site Index
1	22	1	96
3	22	3	96
1	148	1	204
3	148	3	204
1	265	1	325
3	265	3	325
1	371	1	429
3	371	3	429
2	23	2	87
2	133	2	193
4	23	4	87
4	133	4	193
1	224	2	213

Manual Input Protein Sequence

Variable Modifications

Modifications

2AA instead of Asn
2AB instead of Asn
Acetylation (N-term)
Arg
Asp
Carbamylation (N-term)
DOTA
DOTA_Mn
DOTA_Cu
DOTA_Zn
Glu
Lys

Modifications Selected for Search

N Terminal

Mono. Mass: 0
Avg. Mass: 0

Add Remove Load Default Mods

C Terminal

Mono. Mass: 0
Avg. Mass: 0

Add Remove Load Default Mods

Side Chain

Mono. Mass: 0
Avg. Mass: 0
Residues:

Add Remove Load Default Mods

Amide (C-term)
Arg
Asp
b ion
Glu
Lys

Acetylation
ADP-ribosylation
Amidation
Carbamylation
Carbamidomethylation
Carboxymethylation
Cysteaminylation
Cysteinylation
Deamidation (N)
Deamidation (Q)
Decarboxylation
Dimethylation

Deamidation (N)
Double Oxidation
Glycation
H2O loss
Hydroxylation
Mannosylation (S)
NH3 loss
Oxidation (MW)
Deamidation (Q)

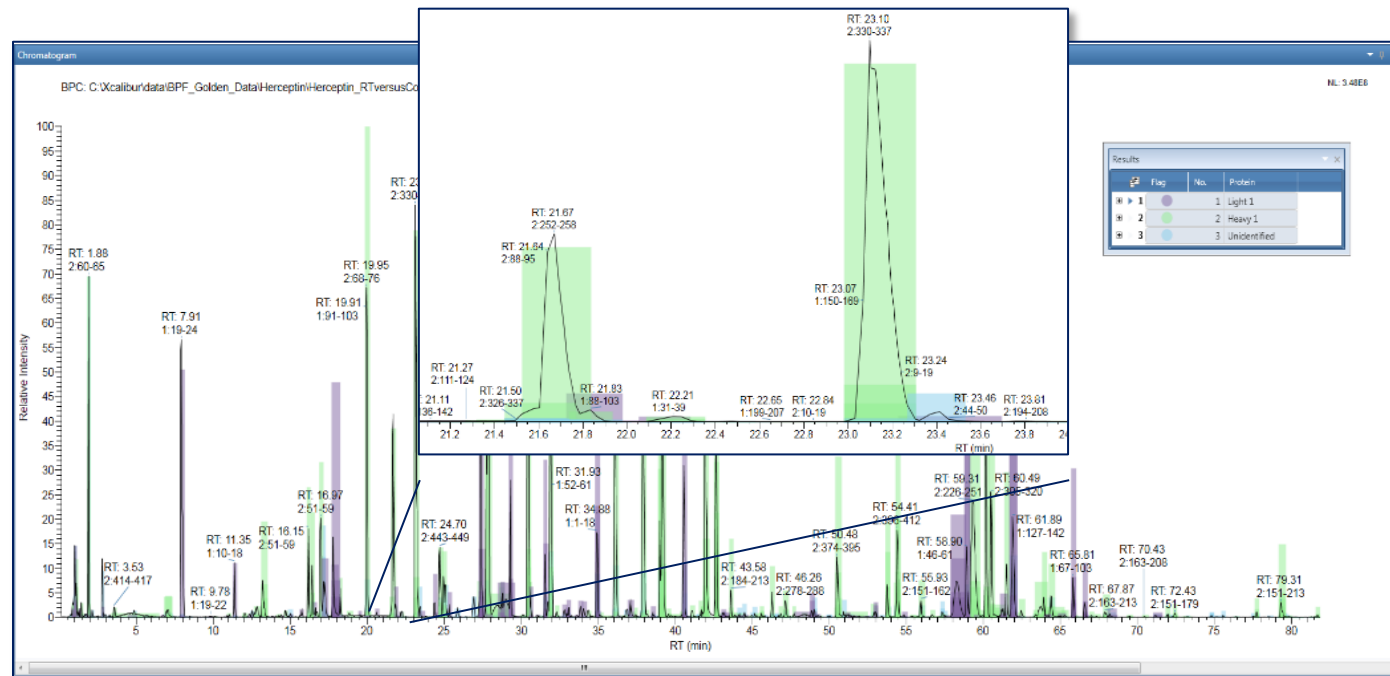
Modification Editor

Max # Modifications: 3 N Gly: CHO

Specification of custom modifications

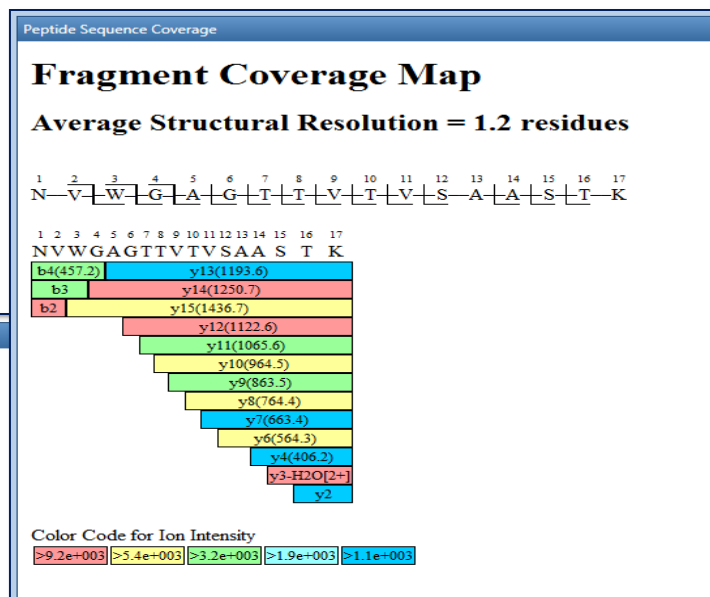
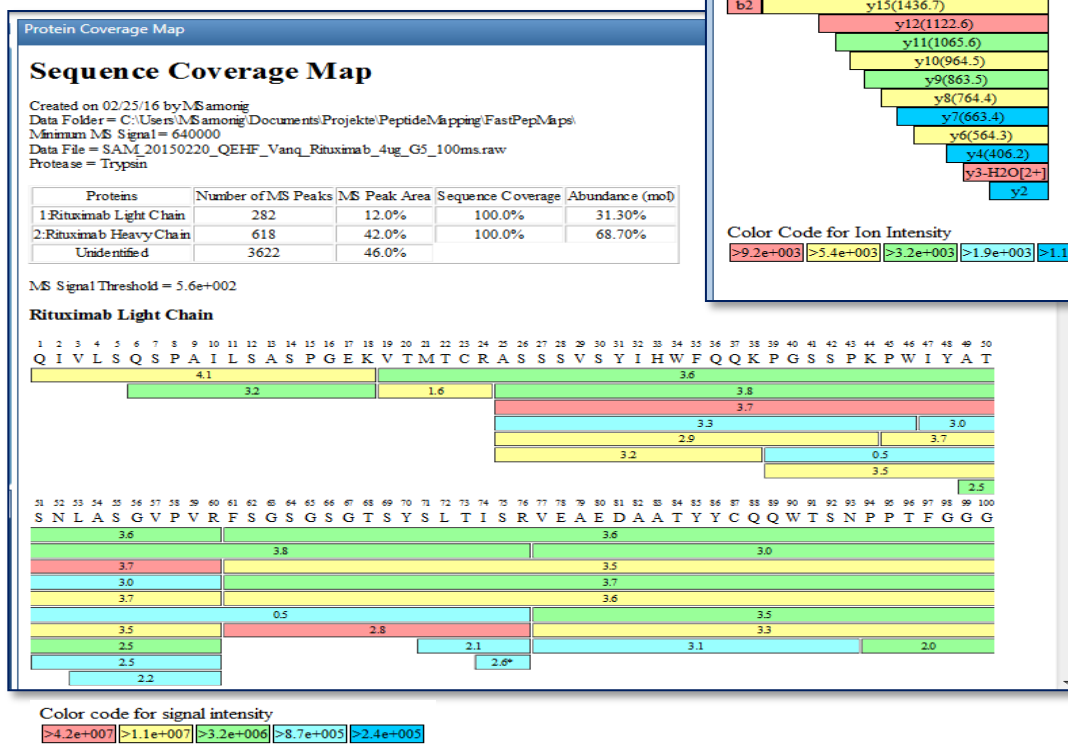
pre-defined set of glycosylation profiles:
human
CHO

Data Analysis With BioPharma Finder: Chromatographic Peak Shading



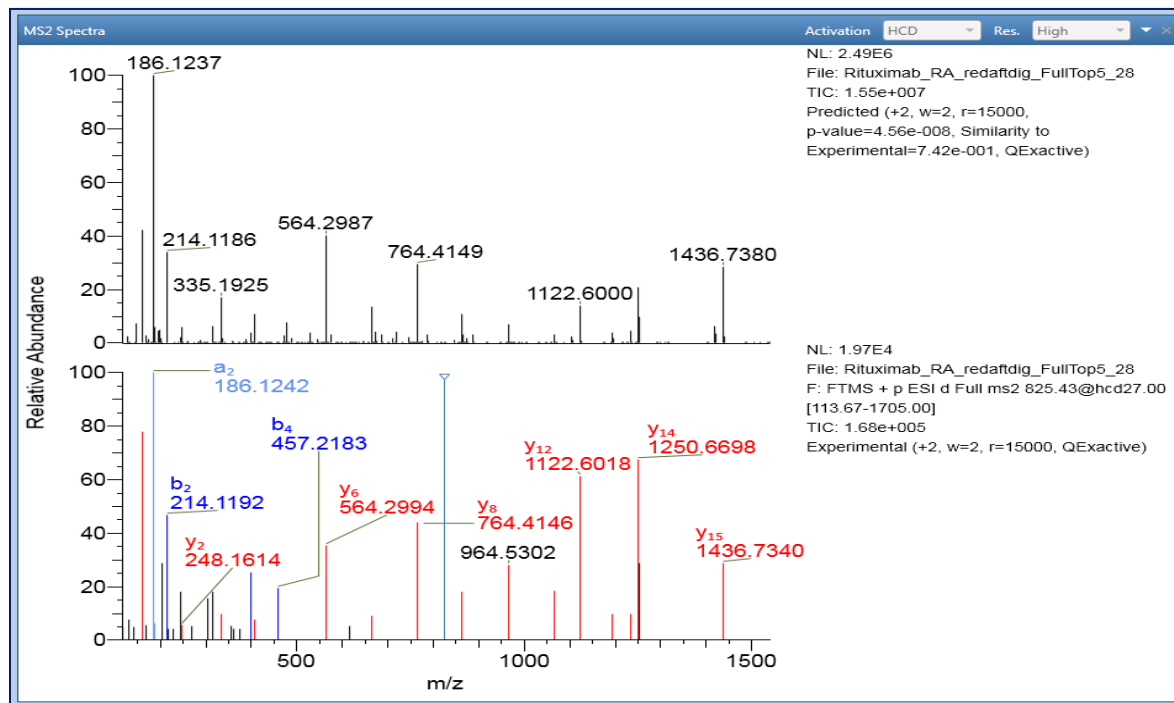
- Protein sequence manager stores sequence information for quick use
- Maximum throughput through simple method editor, allowing batch analyses
- Interactive results display allows you to review data how you want
 - Sequence/fragment coverage maps
 - Chromatographic shading – never miss a thing
 - Compare real and predicted spectra
 - Powerful modification and results summary

Data Analysis With BioPharma Finder: Coverage Mapping



- Protein sequence manager stores sequence information for quick use
- Maximum throughput through simple method editor, allowing batch analyses
- Interactive results display allows you to review data how you want
 - Sequence/fragment coverage maps
 - Chromatographic shading – never miss a thing
 - Compare real and predicted spectra
 - Powerful modification and results summary

Data Analysis With BioPharma Finder: Spectral Confidence



Theoretically predicted fragment ion and their relative intensities are calculated for all peptide candidates and compared to the measured MS/MS spectrum

- Protein sequence manager stores sequence information for quick use
- Maximum throughput through simple method editor, allowing batch analyses
- Interactive results display allows you to review data how you want
 - Sequence/fragment coverage maps
 - Chromatographic shading – never miss a thing
 - Compare real and predicted spectra
 - Powerful modification and results summary

Data Analysis With BioPharma Finder: Modification Quantification

Thermo BioPharma Finder 1.0

Thermo SCIENTIFIC BioPharma Finder

Home Peptide Mapping Analysis Parameters Queue Process and Review Mapping

Real Time Optimization

Results Chromatogram

parameter optimization with real time reprocessing

quantification comparison

Peptide Sequence	Modification	Delta (ppm)	Confidence Score	M/Z	Avg MS Area: cond_1	%CV: cond_1	Avg MS Area: cond_2	%CV: cond_2	Avg MS Area: cond_3	%CV: cond_3
1 HGLDNYR	Isomerization	-1.26	100.0%	437.712	2.60E+05	13	2.24E+05	24	4.43E+05	23
2 HGLDNYR	Deamidation	-4.10	100.0%	438.206	1.91E+05	5	8.18E+04	21	2.87E+05	5
3 HGLDNYR	H2O loss	-1.28	100.0%	428.707	2.07E+05	4	7.94E+04	9	3.86E+04	9
4 HGLDNYR	Deamidation	-1.02	100.0%	438.204	1.33E+05	2	5.44E+04	16	2.08E+05	15
5 FESNFNTQATNR	GasPhase ~T58+21.9	1.18	94.2%	725.819	1.88E+06	5	1.16E+06	25	1.63E+06	23
6 FESNFNTQATNR	Deamidation	-2.72	100.0%	715.323	2.11E+06	4	1.19E+06	8	2.69E+06	12
7 FESNFNTQATNR	H2O loss	-1.43	99.9%	705.824	4.67E+05	6	1.89E+05	6	4.93E+05	2
8 GYSLGNWV	nonspecific,H2O loss	0.32	99.9%	439.214	0.00E+00	0	0.00E+00	0	3.93E+05	8
9 NTDGSTDYGLQINSR	Deamidation	-2.66	100.0%	877.916	2.50E+06	5	1.59E+06	4	3.40E+06	6
10 FESNFNTQATNRNTDGTSDY	GasPhase ~T58+21.9	-1.03	100.0%	1063.156	4.07E+04	3	1.29E+06	16	4.60E+04	14
11 IVSDGNGMNAWVAWR	Lys	-2.06	100.0%	601.971	5.99E+05	4	2.12E+07	8	4.31E+05	3
12 IVSDGNGMNAWVAWR	Lys	-1.65	100.0%	902.453	9.51E+04	4	3.18E+06	4	0.00E+00	0
13 IVSDGNGMNAWVAWR	Oxidation	-1.80	100.0%	846.403	1.22E+06	5	5.65E+05	14	1.41E+06	5
14 KIVSDGNGMNAWVAWR	Glycation	-0.97	100.0%	656.322	1.04E+06	5	2.97E+05	173	0.00E+00	0
15 KIVSDGNGMNAWVAWR	Glycation	-0.60	100.0%	656.321	5.37E+04	50	7.72E+05	83	1.37E+06	14

Level	No.	Raw File Name	Condition	MS Area	Delta (ppm)	Confidence Score	ID Type	RT (min)	M/Z	Charge State	Mono Mass Exp.	Avg Mass Exp.	Mono Mass Theo.
1	Raw File	1	Lysozym_LA_cond_1	4.11E+04	-0.60	100.0%	Full	19.81	656.321	3	1964.9426	1965.68	1964.9414
2	Raw File	2	Lysozym_LA_cond_1	8.48E+04	-0.23	100.0%	MS2	19.80	656.320	3	1964.9419	1965.47	1964.9414
3	Raw File	3	Lysozym_LA_cond_1	3.52E+04	-0.48	100.0%	MS2	19.80	656.321	3	1964.9424	1965.81	1964.9414
4	Raw File	4	Lysozym_LP_cond_2	2.78E+04	-0.54	100.0%	Full	19.77	656.322	3	1964.9425	1965.74	1964.9414
5	Raw File	5	Lysozym_LP_cond_2	1.15E+06	0.14	100.0%	MS2	19.76	656.322	3	1964.9412	1966.04	1964.9414
6	Raw File	6	Lysozym_LP_cond_2	1.14E+06	0.21	100.0%	MS2	19.76	656.322	3	1964.9410	1966.00	1964.9414
7	Raw File	7	Lysozym_LW_cond_3	1.16E+06	-2.40	100.0%	Full	19.62	656.323	3	1964.9462	1966.02	1964.9414
8	Raw File	8	Lysozym_LW_cond_3	1.49E+06	-1.28	100.0%	MS2	19.87	656.323	3	1964.9440	1966.02	1964.9414
9	Raw File	9	Lysozym_LW_cond_3	1.46E+06	-0.97	100.0%	MS2	19.88	656.323	3	1964.9434	1966.02	1964.9414

- Protein sequence manager stores sequence information for quick use
- Maximum throughput through simple method editor, allowing batch analyses
- Interactive results display allows you to review data how you want
 - Sequence/fragment coverage maps
 - Chromatographic shading – never miss a thing
 - Compare real and predicted spectra
 - Powerful modification and results summary

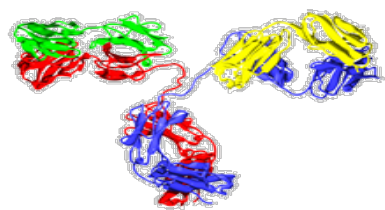


Application examples

Transfer your Maps:
Sensitive LC-MS to Routine LC-UV

Speed up your Maps:
UHPLC peptide mapping

Transfer your Maps: Sensitive LC-MS to Routine LC-UV



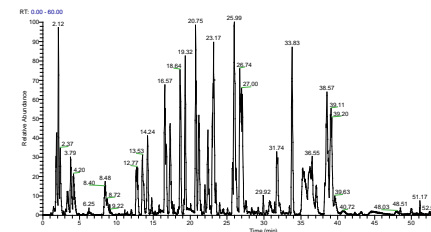
Intact Ab

SMART
Digest
followed
by optional reduction

Peptides



Bottom up



- Peptide Map
- PTMs
- Impurities

```
EVQLVESGGGLVQPGGSLRLSCAASGFFNIKDTYIHW  
VRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISA  
DTSKNTAYLQINSGLTQTYICNVNHKPSNTKVDKKVE  
PPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTL  
MISRTPEVTCVVDVSHEDNKALPAPIEKTIKAKGQ  
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV  
EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDK  
RWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
```

Sequence/PTMs unknown or
need to be confirmed



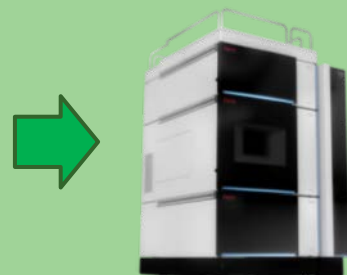
Peptide identification by MS
and MS/MS

Fast analysis

Method Transfer to LC-UV

Result Transfer

- Sequence and PTMs known.
- No further information required
- Stability studies, QA/QC
- Batch release



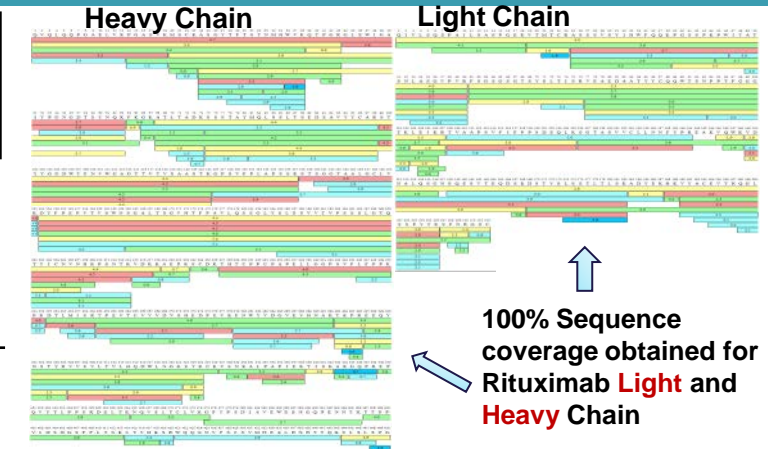
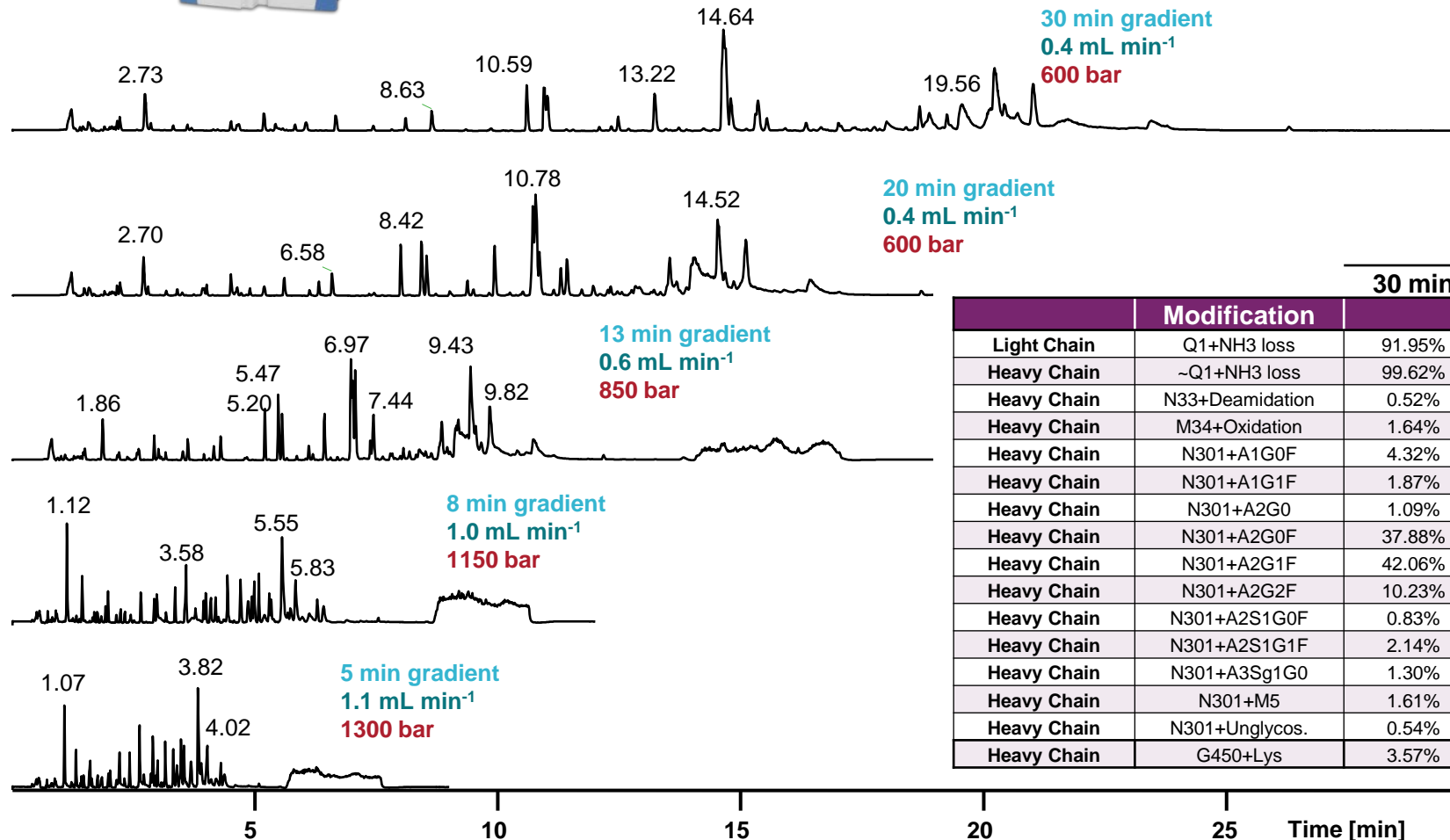
Peptide identification by
unknown and reference
sample chromatogram
comparison (retention time
comparison)

High degree of
confidence on retention
time determination is
required!

Speed up your Maps: Short and Long UHPLC Methods for Peptide Mapping



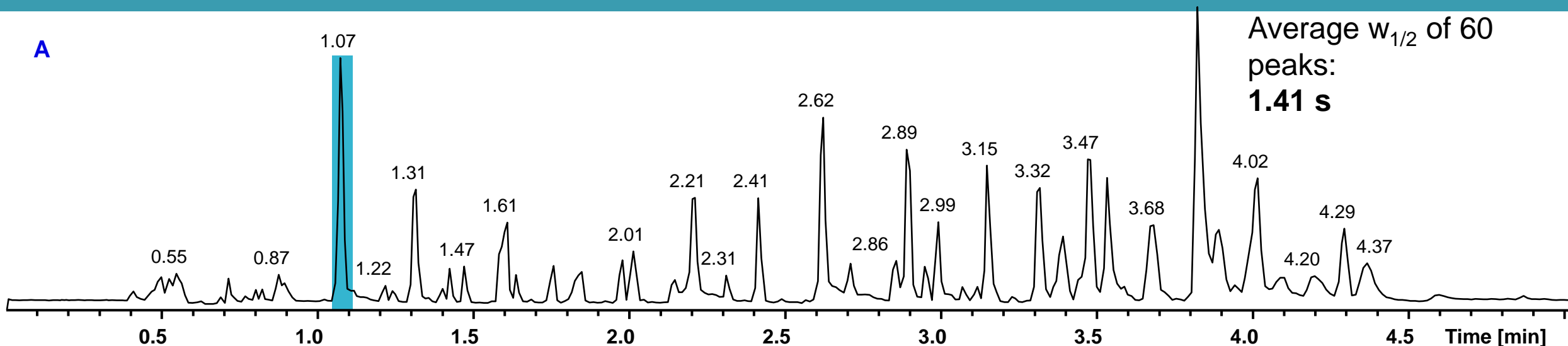
Thermo Scientific™ Vanquish™
UHPLC coupled to a Thermo
Scientific™ Q Exactive™ HF MS



	Modification	Gradient Time					σ
		30 min	20 min	13 min	8 min	5 min	
Light Chain	Q1+NH3 loss	91.95%	91.17%	89.69%	90.93%	26.57%	28.80%
Heavy Chain	~Q1+NH3 loss	99.62%	99.67%	99.61%	99.68%	99.69%	0.04%
Heavy Chain	N33+Deamidation	0.52%	0.51%	0.58%	-	0.51%	0.03%
Heavy Chain	M34+Oxidation	1.64%	1.54%	1.73%	1.42%	1.45%	0.13%
Heavy Chain	N301+A1G0F	4.32%	4.42%	3.83%	3.52%	3.38%	0.46%
Heavy Chain	N301+A1G1F	1.87%	1.91%	1.72%	3.32%	1.46%	0.73%
Heavy Chain	N301+A2G0	1.09%	1.02%	1.02%	-	0.98%	0.05%
Heavy Chain	N301+A2G0F	37.88%	37.11%	38.59%	40.48%	43.12%	2.41%
Heavy Chain	N301+A2G1F	42.06%	41.89%	43.42%	43.20%	43.35%	0.75%
Heavy Chain	N301+A2G2F	10.23%	10.17%	9.81%	10.36%	10.05%	0.21%
Heavy Chain	N301+A2S1G0F	0.83%	0.86%	-	-	-	0.02%
Heavy Chain	N301+A2S1G1F	2.14%	-	-	-	-	-
Heavy Chain	N301+A3Sg1G0	1.30%	-	-	-	-	-
Heavy Chain	N301+M5	1.61%	1.59%	1.66%	1.87%	1.86%	0.14%
Heavy Chain	N301+Unglycos.	0.54%	0.90%	0.76%	0.83%	0.97%	0.16%
Heavy Chain	G450+Lys	3.57%	3.56%	3.92%	3.40%	3.15%	0.28%
median							0.19%

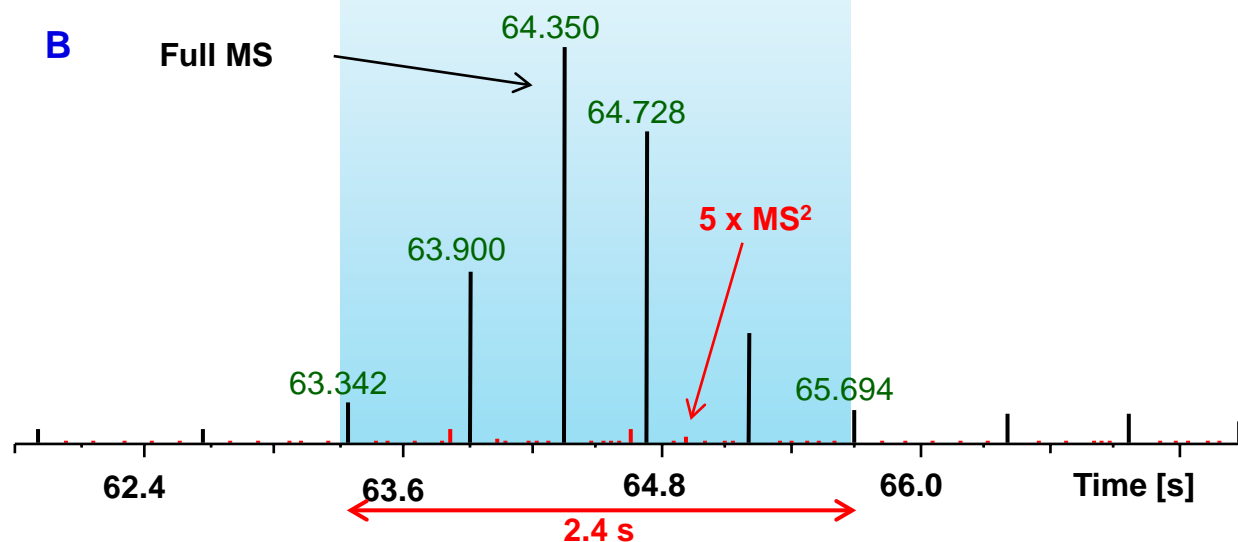
Speed up your Maps: Fast UHPLC Peptide Mapping

A



B

Full MS



6 Full MS scans

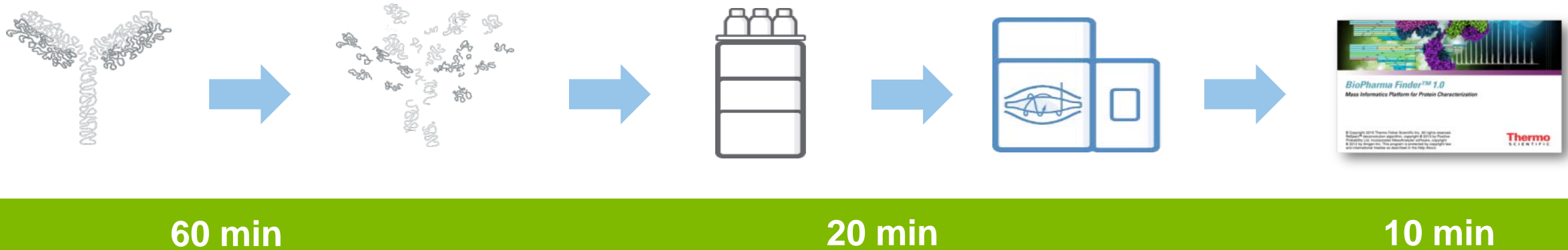
5 x Top5 MS² scans

$\Sigma=31$ scans over a chromatographic peak
of 2.4 s width ($w_{1/2} = 0.9$ s)

A) Total ion chromatogram of a five minute gradient separation of Denosumab.

B) Data point distribution for a Full MS / ddMS² Top5 method during a representative chromatographic peak.

Peptide Mapping Workflow: Time Saving



- Complete workflow can be completed in less than 90 minutes
- MS analysis is rapid, stand-alone LC-UV even quicker
- Even inexperienced analysts can obtain highly reproducible results

Selected Application Notes on Peptide Mapping

LC-UV-MS Peptide Mapping Development for Easy Transfer to LC-UV QA/QC

Martin Samonig, Remco Swart
Thermo Fisher Scientific, Germering, Germany

Application Note 1138

Key Words
Monoclonal Antibody, Spectrometer, Bio Characterization,

Goal
Prove the suitability of LC-UV-MS setup.

High-Throughput Peptide Mapping with the Vanquish UHPLC System and the Q Exactive HF Mass Spectrometer

Martin Samonig¹, Kai Scheffer², Remco Swart³, and Jonathan Joseph⁴
¹Thermo Fisher Scientific, Germering, Germany
²Thermo Fisher Scientific, Dusseldorf, Germany
³Thermo Fisher Scientific, San Jose, CA, USA

Application Note 1135

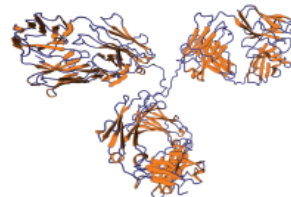
Key Words
Monoclonal Antibodies, Acclaim C18 RSLC Column, Q Exactive HF Mass Spectrometer, Biocompatible UHPLC, Biotherapeutics Characterization, Biopharma

Goal
Report on the benefits of a fast analytical platform employing highly efficient chromatography in combination with fast and high-resolution quadrupole Thermo Scientific™ Orbitrap™ mass spectrometry technology as a tool for fast identification and quantification of sequence truncations, glycosylation and post-translational or artificial modification of recombinant monoclonal antibodies.

Introduction

Monoclonal antibodies, or mAbs, (Figures 1 and 2) are the major element in the fastest growing sector of biopharmaceuticals within the pharma industry. By 2016, eight of the top ten drugs will be therapeutic proteins. Their manufacture is accomplished in bacterial or eukaryotic expression systems, requiring extensive purification of the target product. During drug development and production, the quality of biopharmaceuticals needs to be closely monitored.

Various analytical methods have been used to study quality attributes such as structural integrity, aggregation, glycosylation pattern or amino acid degradation. Because of their high information content and versatility, characterization methods based on high-performance liquid chromatography and mass spectrometry are among the most powerful protein characterization techniques. Proteins can be enzymatically digested to obtain peptides enabling their analysis by means of peptide mapping experiments.



Here, we report a fast and sensitive approach that combines enzymatic digestion, fast chromatographic separation, high-resolution mass spectrometry, and rapid data processing to handle the large amount of samples in diverse biopharma workflows. In this study we have analyzed two commercially available drug products: rituximab (trade names MabThera and Rituxan®) and denosumab (trade names Prolia® and XGEVA®).

Experimental

The two drug products rituximab and denosumab were denatured for 30 min in 7 M urea and 50 mM Tris HCl at pH 8.0. The samples were reduced with 5 mM DTT for 30 min at 37 °C; alkylation was performed with 10 mM IAA for 30 min at room temperature, and the reaction was quenched by addition of 10 mM DTT. Thermo Scientific™ Pierce™ Trypsin Protease (MS Grade) was added and digestion allowed to proceed overnight at 37 °C. Digests were stopped by addition of TFA to approximately pH 3.0.

Abbreviations	
ACN: Acetonitrile	mAb: Monoclonal antibody
DTT: Dithiothreitol	PTM: Post-translational modifications
FA: Formic acid	TFA: Trifluoroacetic acid
IAA: Iodoacetamide	

Thermo
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thermoscientific

APPLICATION NOTE

No. 1159

SMART Digest compared to classic in-solution digestion of rituximab for in-depth peptide mapping characterization

Authors: Martin Samonig¹, Alexander Schwahn², Ken Cook³, Mike Oliver⁴, and Remco Swart¹

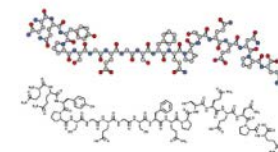
¹Thermo Fisher Scientific, Germering, Germany; ²Thermo Fisher Scientific, Basel, Switzerland; ³Thermo Fisher Scientific, Hemel Hempstead, United Kingdom; ⁴Thermo Fisher Scientific, Runcorn, United Kingdom

Key words

SMART Digest, tryptic digestion, in-solution protein digestion, monoclonal antibody, mAb, Vanquish, reversed phase, mass spectrometry, Q Exactive, Orbitrap, biopharmaceutical, biomolecules, peptide mapping

Goal

To compare the results achieved by using the newly developed Thermo Scientific™ SMART Digest™ kit to those obtained from classic in-solution protein digestion methods, focusing on protein sequence coverage and identified post-translational modifications (PTMs), including deamidation, oxidation, and glycosylation. A Thermo Scientific™ Acclaim™ VANQUISH™ C18 column with conventional water/acetonitrile-based gradients and



the Thermo Scientific™ Vanquish™ Flex UHPLC system were used for separation in combination with the Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer.

Introduction

Peptide mapping is a common technique in the biopharmaceutical industry to characterize monoclonal antibodies (mAbs) for the determination of product identity and stability. Many conventional sample preparation methods are time consuming with digestion times of several hours and can introduce modifications such as deamidation, oxidation, and carbamylation.¹ In this study, two classic in-solution digestion approaches were compared to the recently developed SMART Digest kit method to quantify the extent of post-translational and chemical modifications of a therapeutic recombinant mAb. The critical requirements for each

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Selecting Buffers to Remove Uncertainty in Tryptic Digestion

Valeria Girelli, Philip Humphries, Thermo Fisher Scientific, Runcorn, UK

Application Note 1179

Key Words
Peptides, proteins, digestions, Accuracy, biopharmaceuticals, bi

Goal
To demonstrate how uncertainty associated with protocols, resulting

thermoscientific

Questions Answers

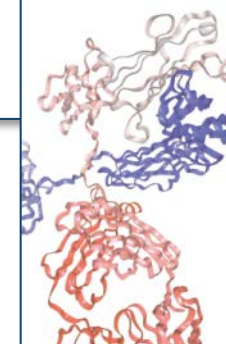
SMART Digest Kit Facilitating perfect digestion

The modern biopharmaceutical and protein research laboratory is tasked with providing high quality analytical results, often in high-throughput, regulated environments. One of the key areas which affects these requirements is sample preparation. Current technologies employed are subject to high levels of irreproducibility, poor sensitivity, and protracted methodologies that often require 24 hours to achieve full digestion.

The Thermo Scientific™ SMART Digest™ kits remove these issues by providing a digestion solution which is:

- Fast
- Simple
- Highly reproducible

Following are some frequently asked questions relating to how the technology works and how it can be implemented.



ThermoFisher
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On-Demand Webinar Series on Peptide Mapping

- ☐ **TAKE THE SMART ROUTE TO PROTEIN DIGESTION - How to increase reproducibility whilst reducing preparation time.**

Wednesday, September 28, 2016, 03:00 PM BST

- ☐ **PEPTIDE SEPARATIONS WITH PINPOINT PRECISION - How to achieve ultimate retention time reproducibility & high resolution separation of peptides.**

Wednesday, October 12, 2016, 03:00 PM BST

- ☐ **DON'T MISS A THING ON YOUR PEPTIDE MAPPING JOURNEY - How to get full coverage peptide maps using high resolution accurate mass spectrometry.**

Wednesday, October 26, 2016, 03:00 PM BST

- ☐ **EASILY NAVIGATE ALL BIOTHERAPEUTIC MODIFICATIONS - How to confidently compare and interpret your peptide maps with powerful, yet intuitive software.**

Wednesday, November 09, 2016, 03:00 PM GMT



bit.ly/UYPepMaps

Questions?

Acknowledgements

- Kai Scheffler
- Alexander Schwahn
- Ken Cook
- Martin Samonig
- Mike Oliver
- Valeria Barattini
- Mauro De Pra
- Jennifer Sutton

bit.ly/BPM_4

Thank you for your attention!

BioPhar Moore

Your guide to the evolving bio/pharma universe.

Backup slides

Let's Optimize the Digestion Buffer!



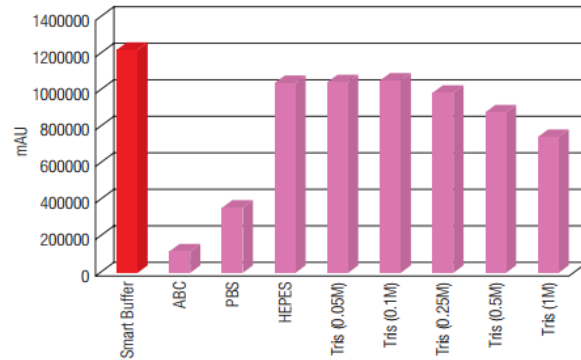
Enzyme

Solubility

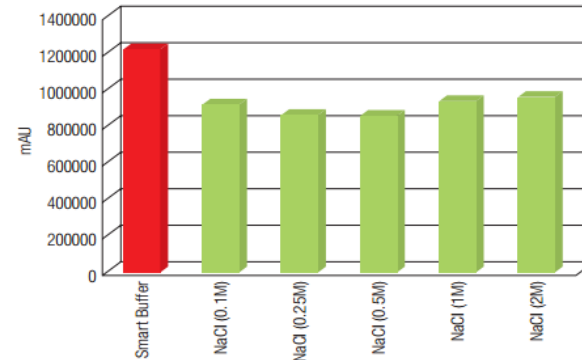
Denaturation

Diffusion

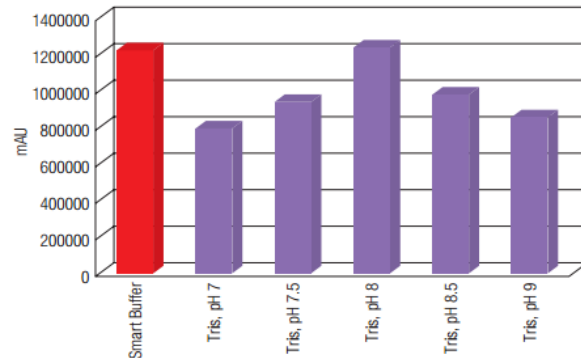
A Effect of buffering ion



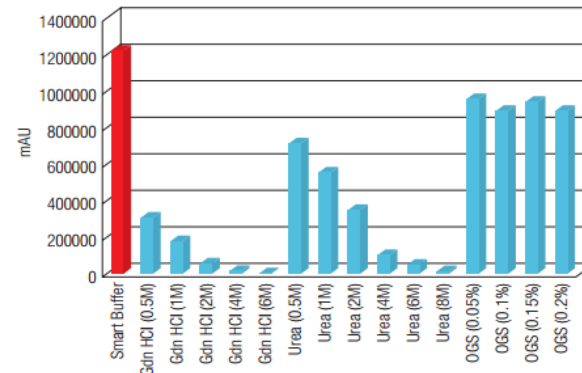
B Effect of salt concentration



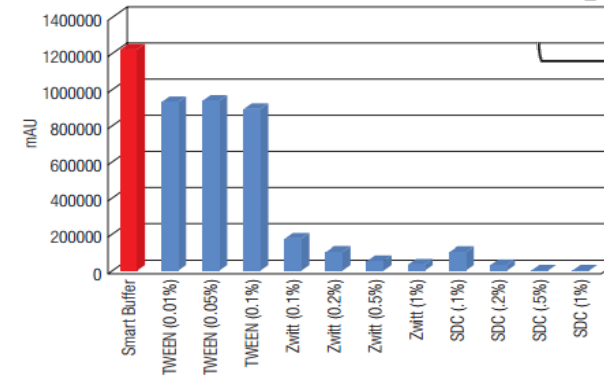
C Effect of pH



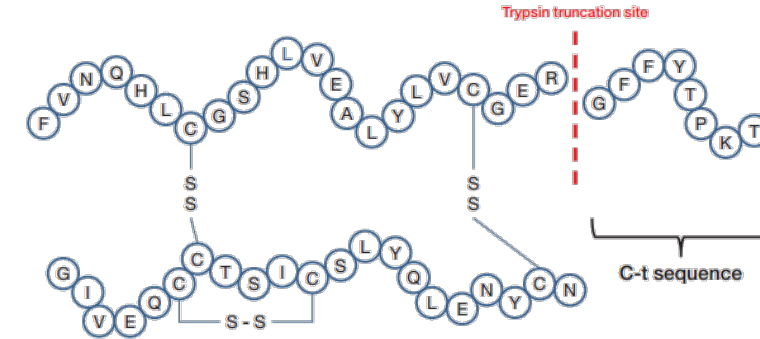
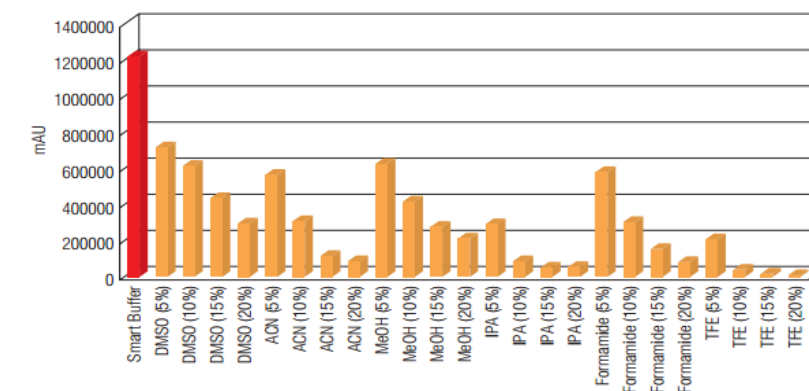
D Effect of chaotropes



E Effect of detergents



F Effect of co-solvents



Insulin amino acid sequence, C-t peptide was monitored

Selecting Buffers to Remove Uncertainty in Tryptic Digestion

Valeria Barattini, Philip Humphries, Thermo Fisher Scientific, Runcorn, UK

Application Note 21179

Let's Optimize the Digestion Buffer!



Enzyme

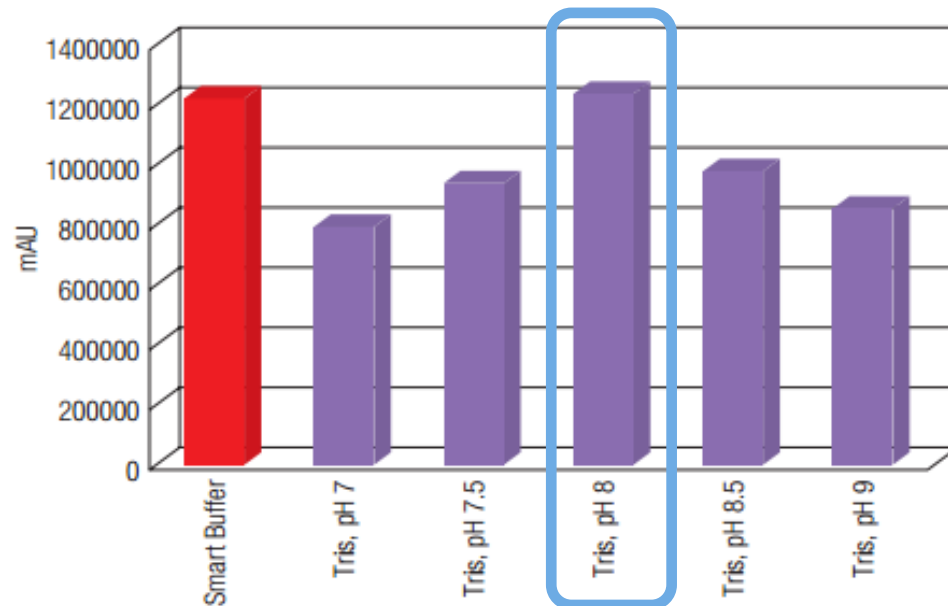
Solubility

Denaturation

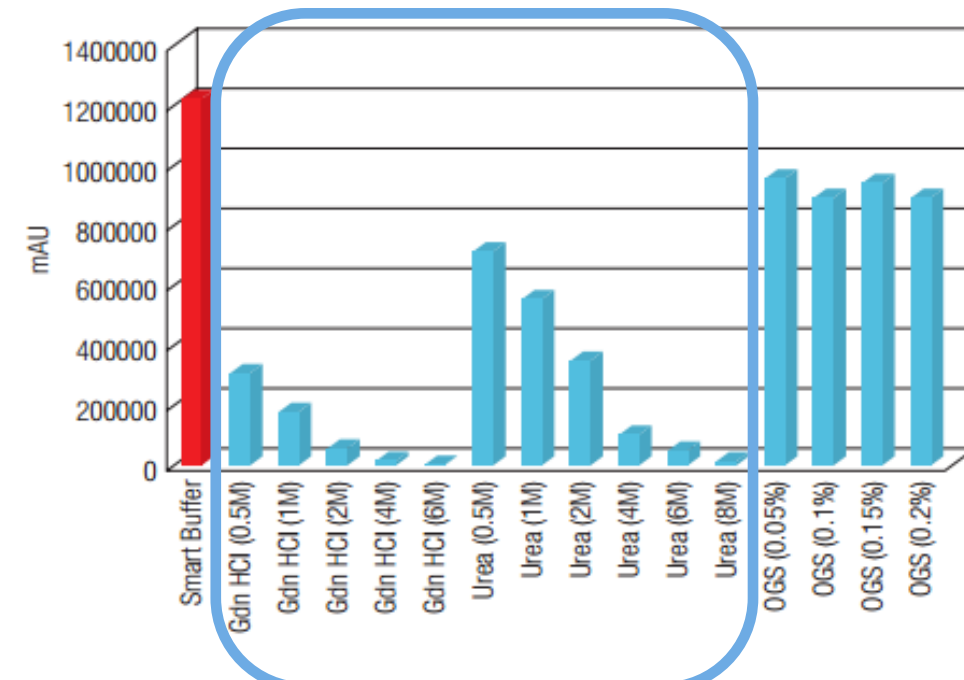
Diffusion

Chemical Name	CAS No.	EINECS No.	Kit Component	Weight %
Water	7732-18-5	231-791-2	2	50-95%
Glycerol	56-81-5	200-289-5	2	< 20%
Tris Base	77-86-1	201-064-4	2	< 10%
Tris-HCl	1185-53-1	214-684-5	2	< 10%
Calcium Chloride	10043-52-4	233-140-8	2	< 10%
Sodium Azide	26628-22-8	247-852-1	2	< 0.1%

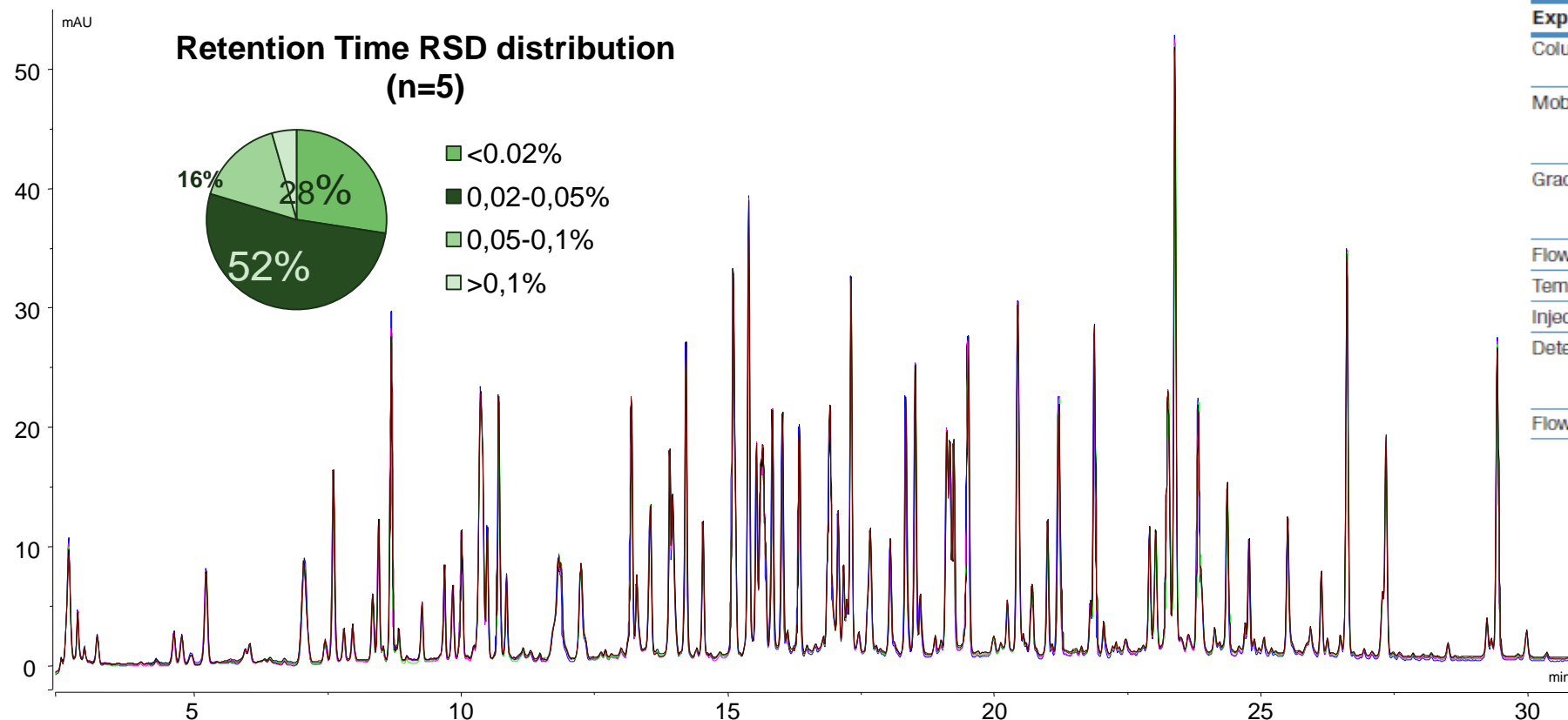
C Effect of pH



D Effect of chaotropes



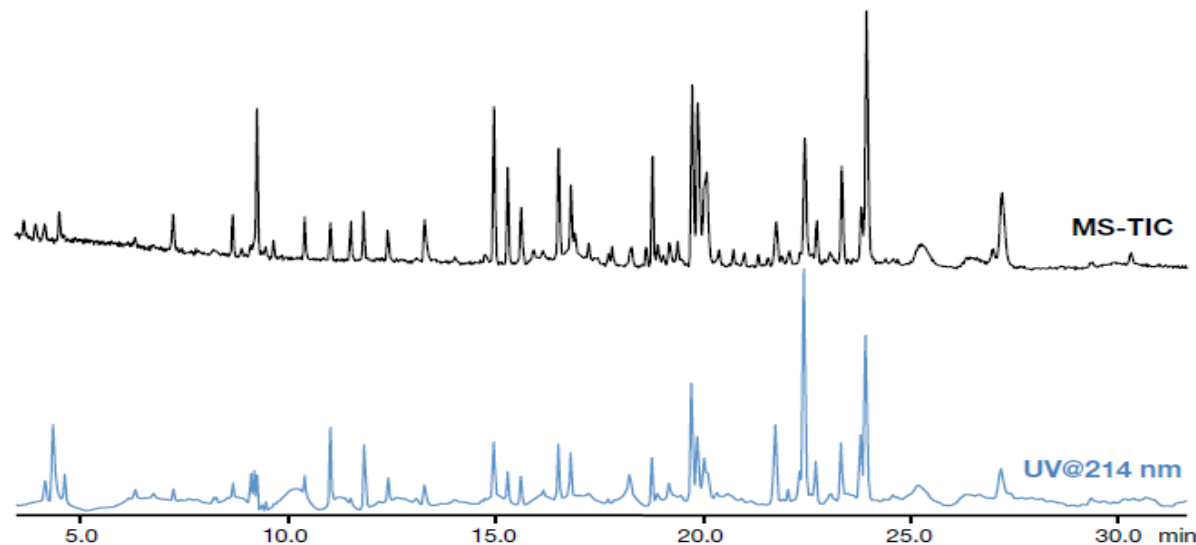
Outstanding retention time reproducibility for confident peptide annotation



Experimental Conditions

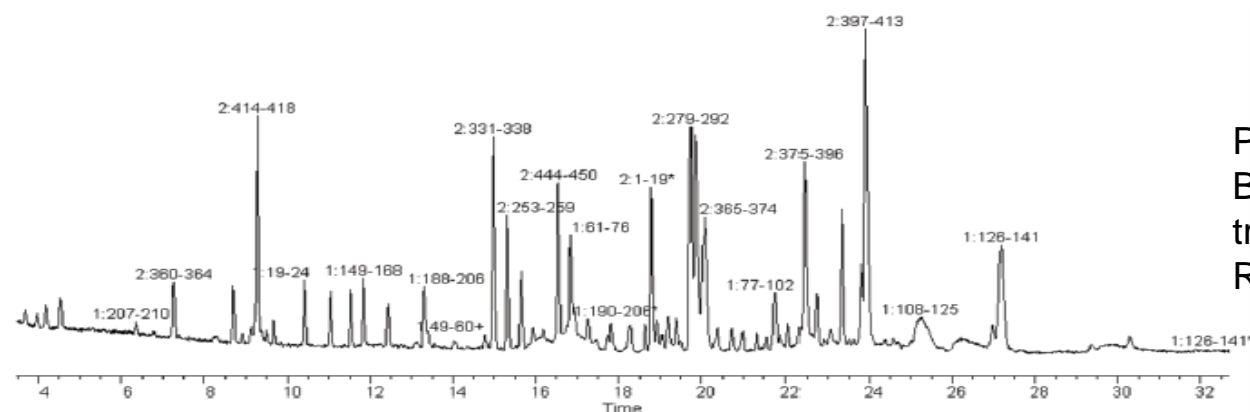
Column:	Acclaim RSLC 120, C18, 2.2 µm Analytical (2.1 x 250 mm, P/N 074812)
Mobile Phase:	A: 0.05% TFA in water (P/N TFA 85183) B: 0.04% TFA in 8/2 acetonitrile/water (v/v), (P/N acetonitrile TS-51101)
Gradient:	0–30 min: 4%–55% B, 30–31 min: 55%–100% B, 31–35 min: 100% B, 35–36 min: 100%–4% B, 36–56 min: 4% B
Flow Rate:	0.3 mL/min
Temperature:	50°C still air
Injection Volume:	2 µL
Detection:	214 nm Data collection rate: 10 Hz Response time: 0.4 s
Flow Cell:	10 mm LightPipe

Transfer your Maps: Sensitive LC-MS to Routine LC-UV

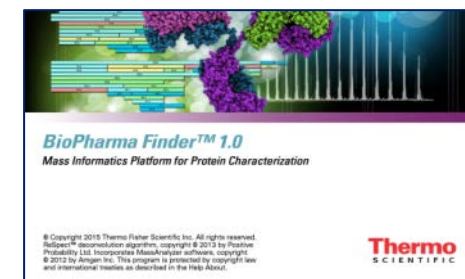


Total Ion Current (TIC) of a SMART digested Rituximab sample, measured by Q Exactive MS

Vanquish Flex: UV detection SMART digested Rituximab sample.



Peak assignment with Biopharma Finder of the tryptic peptides from Rituximab.



Why Peptide Mapping?

- It's an absolute essential step in biotherapeutic characterization
- It's used at every stage, from discovery to QC
- It's routine... but slow and painstaking

➔ **we can make it easier!**

What Are The Peptide Mapping Pain Points?

- **Effort and Time consuming**
 - labour intensive, multi-step sample preparation, with little standardization
 - overnight digestion causes delay of results
- **Variability in digestion**
different protocols and operators produce different results
- **Lack of reproducibility** – leading to a lack of data confidence
- **Difficult** – from digestion to data interpretation multiple skills are required.

