



Fast, Reproducible Biopharmaceutical Peptide Mapping Profiling

Global BioPharma Summit

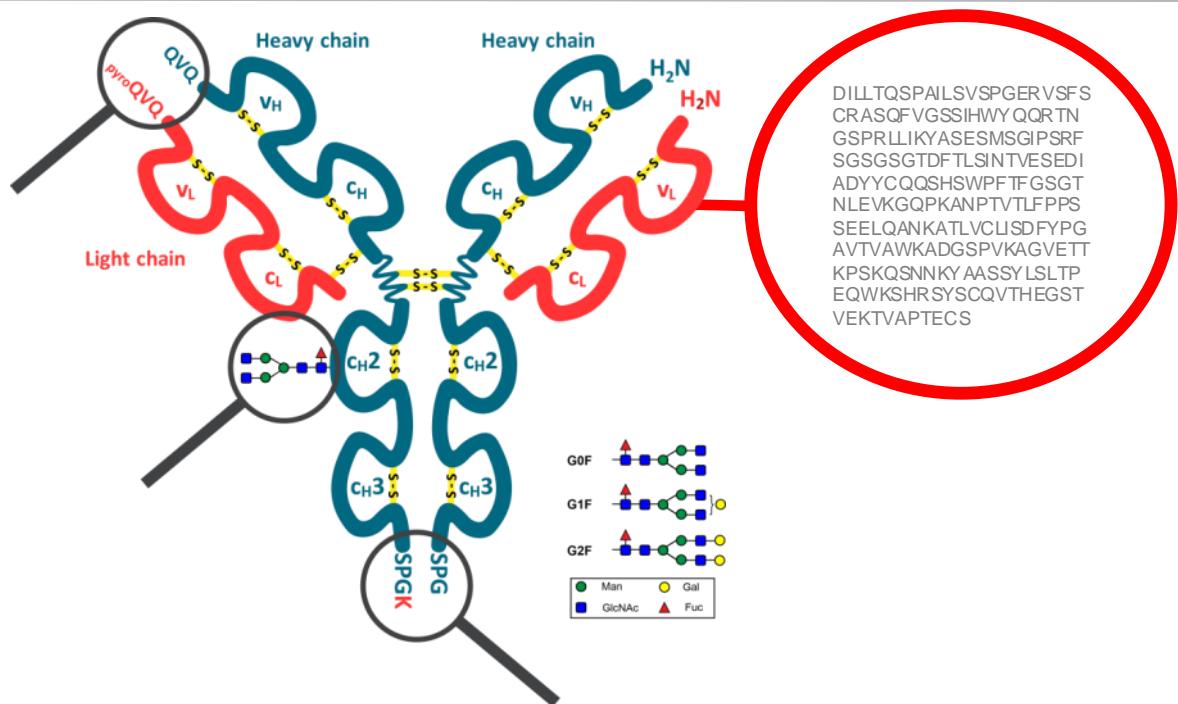
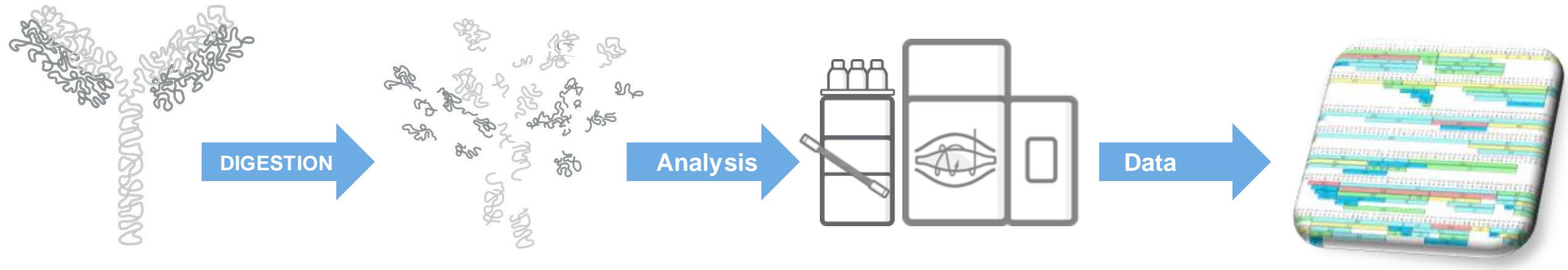
The world leader in serving science

In this presentation you will learn:

1. How to digest biotherapeutic proteins more efficiently and reproducibly
2. How to separate peptides reliably with less variability
3. How a new MS platform allows you to comprehensively map a biologic
4. How new software enables easy correlation of both intact protein and peptide mapping data

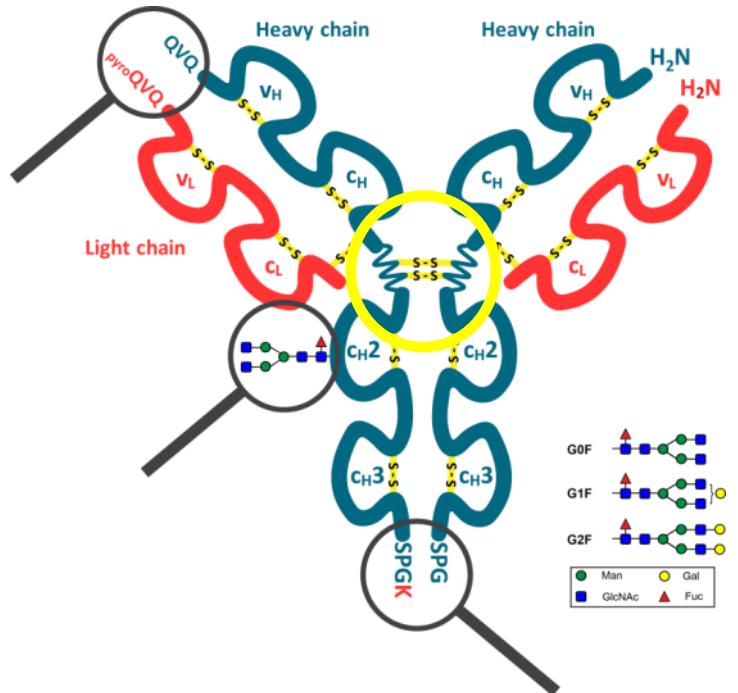
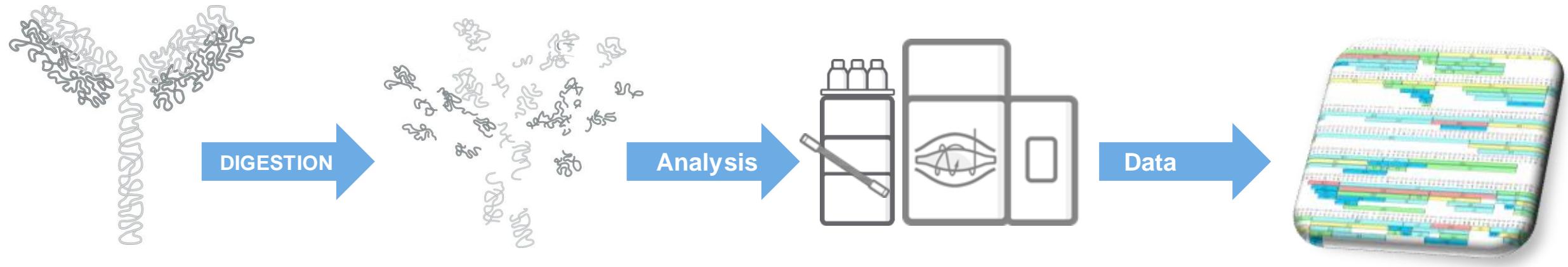


Why Peptide Mapping?



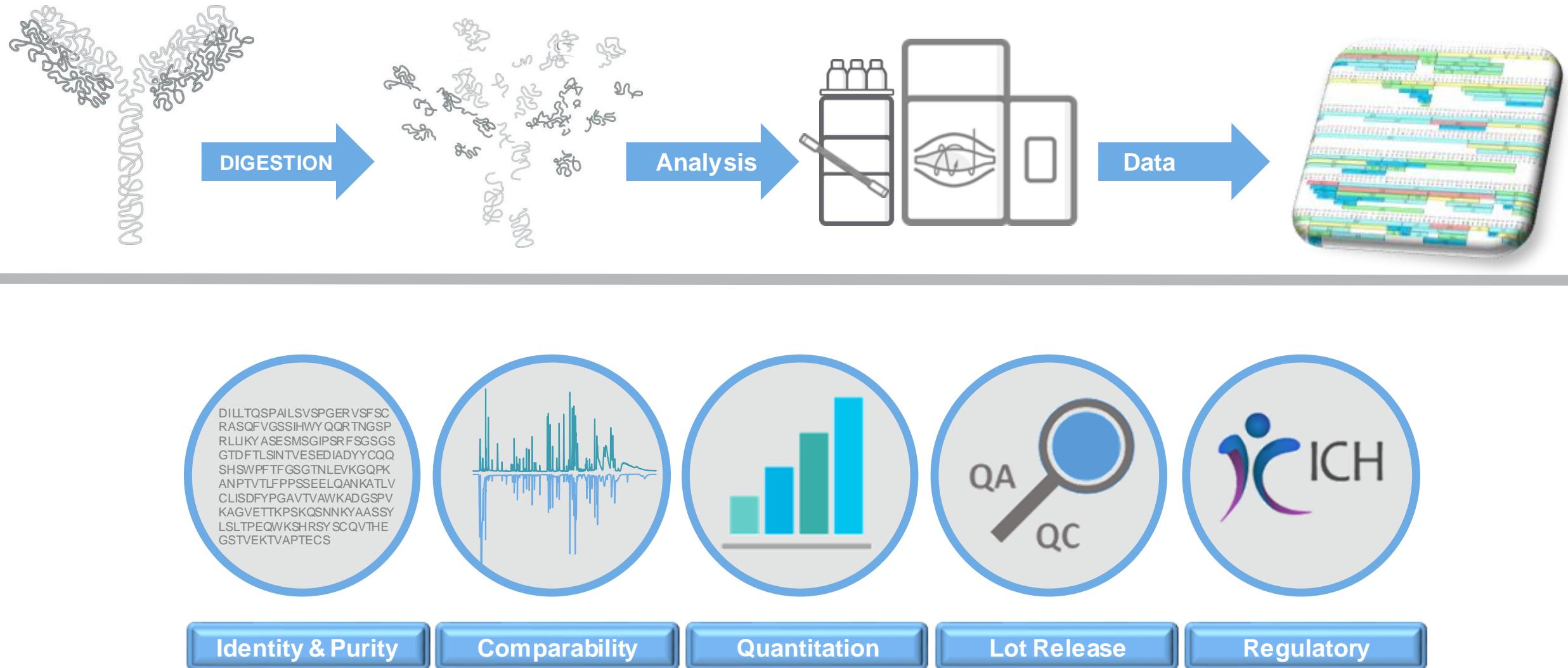
Regulatory

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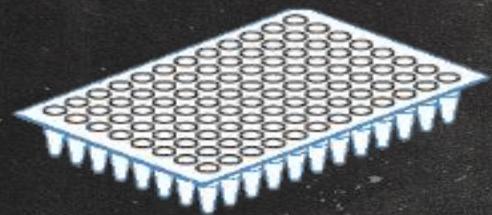


Regulatory

Why Peptide Mapping?



Upgrade Your Maps: Our Workflow Solution



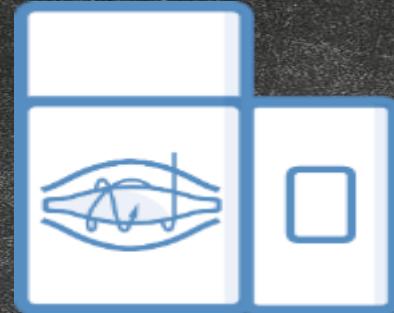
Thermo
Scientific™
SMART Digest™
offer extremely
reproducible and
rapid protein
digestion



Thermo Scientific™
Vanquish™ Flex
UHPLC
is engineered for
high resolution,
reproducible
peptide separations



Thermo Scientific™
Acclaim™ 120 C18
column
is the perfect
column choice to
ensure sharp peaks
during peptide
mapping

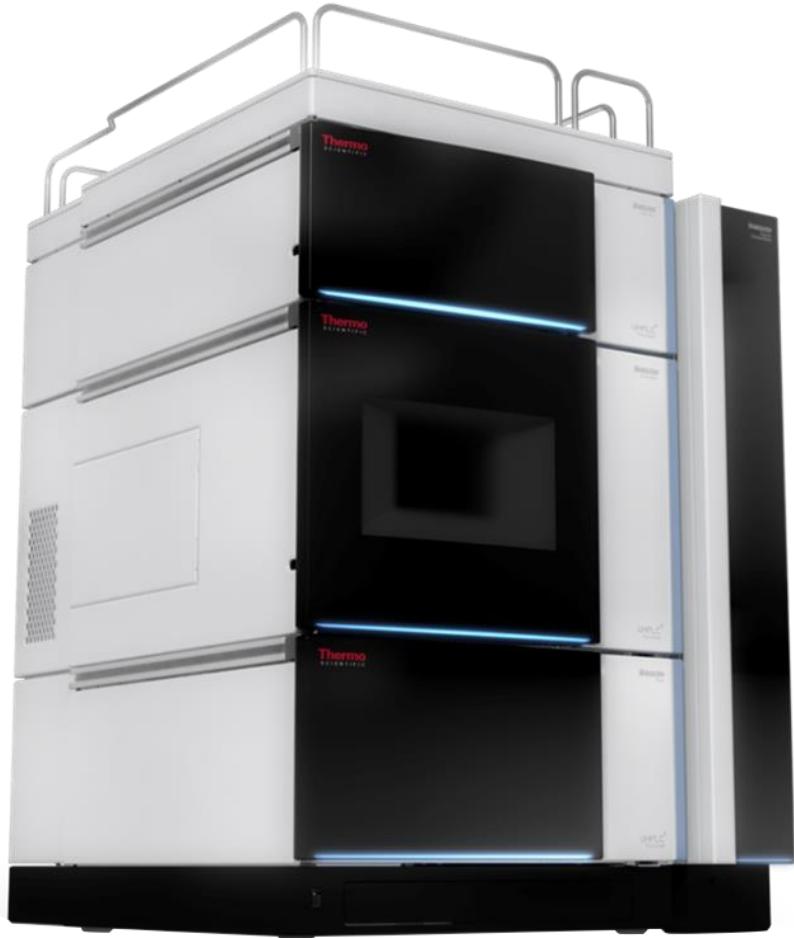


Thermo Scientific™
Q Exactive™ Hybrid
Quadrupole-
Orbitrap™ mass
spectrometers are
the gold standard
for accurate mass
measurement



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

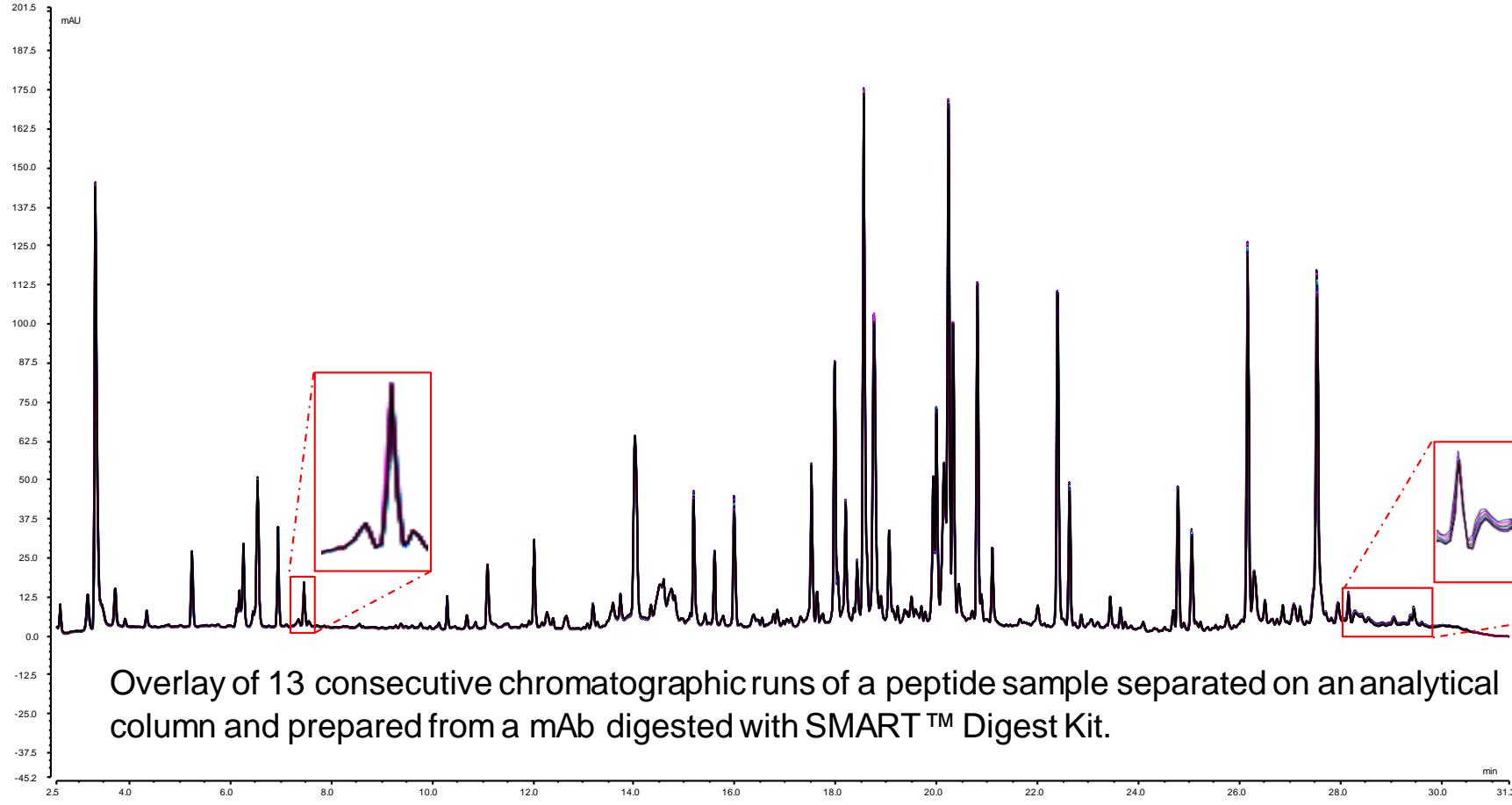
Vanquish Flex UHPLC System



- Developed specifically for biopharma
- Fully biocompatible flow path
- Range of powerful detectors including patented Thermo Scientific™ LightPipe™ Flow Cells
- Class leading retention time reproducibility
- Sample pre-compression for maximum peak retention time reproducibility and column lifetime.
- Ceramic valve technology
- New column thermostatting technology with two modes of temperature control
- **Support for latest innovations in column technology**

Vanquish Flex UHPLC System: Retention Time Reproducibility

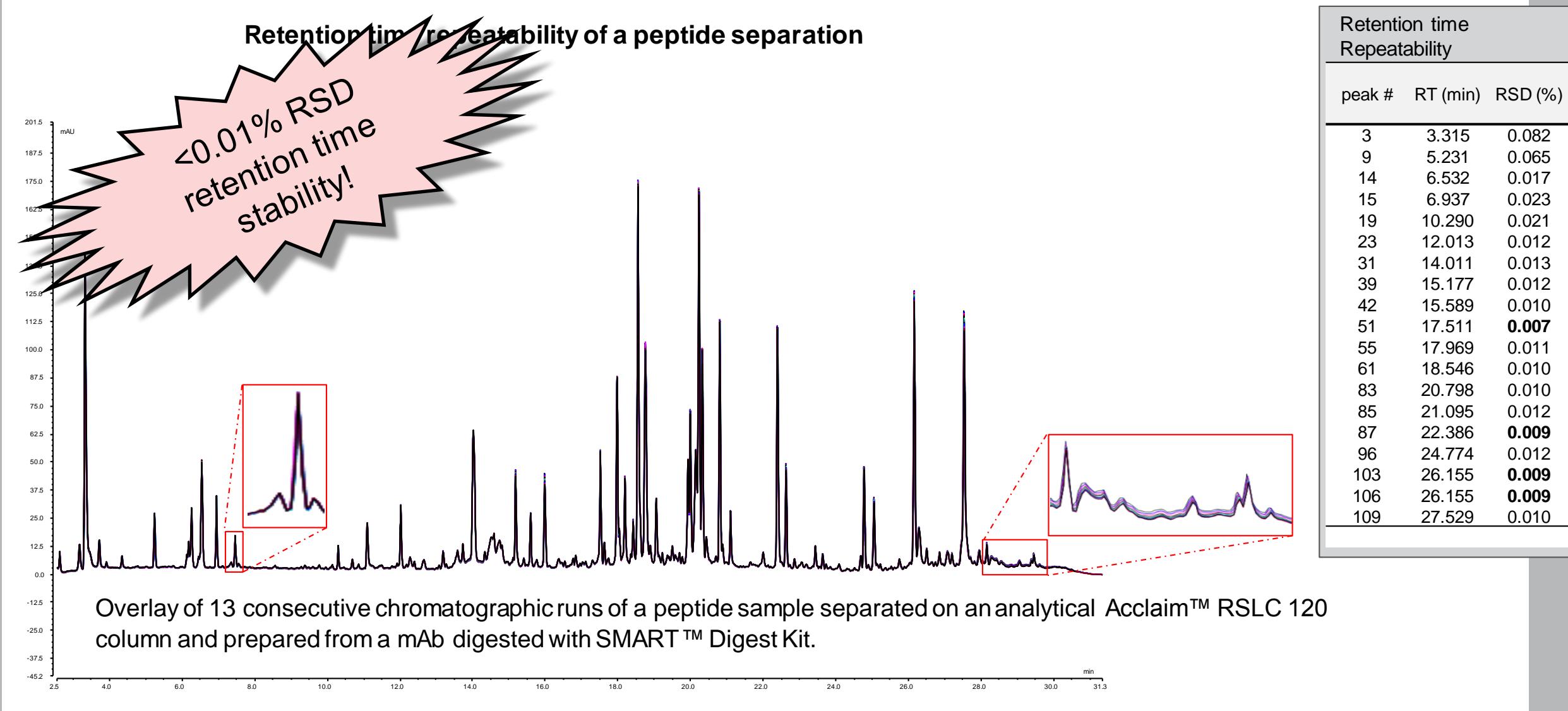
Retention time repeatability of a peptide separation



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

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

Vanquish Flex UHPLC System: Retention Time Reproducibility



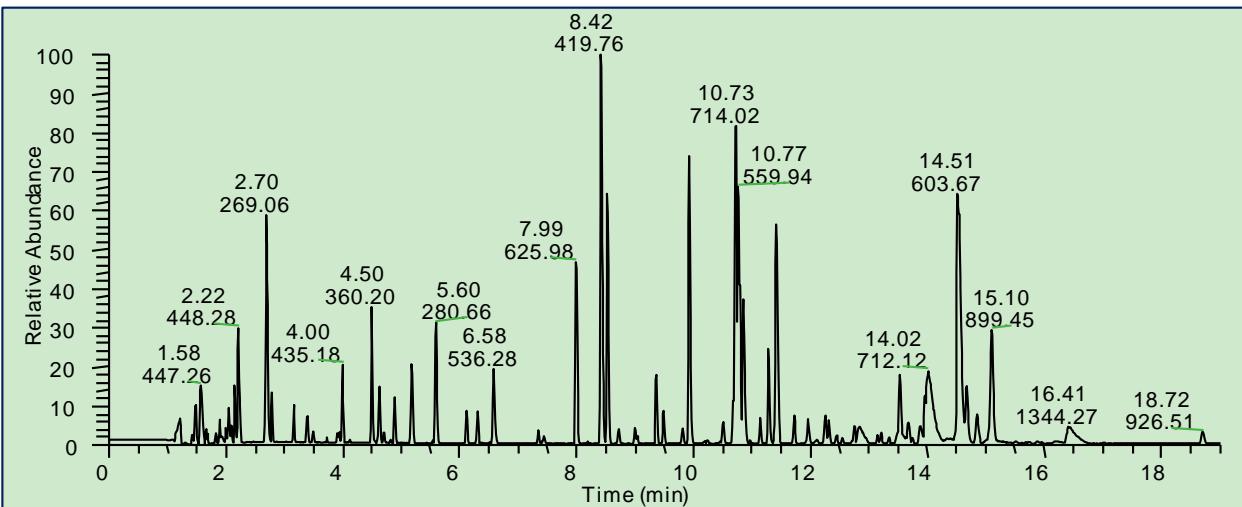
Sample Analysis By Mass Spectrometry

Q Exactive Plus

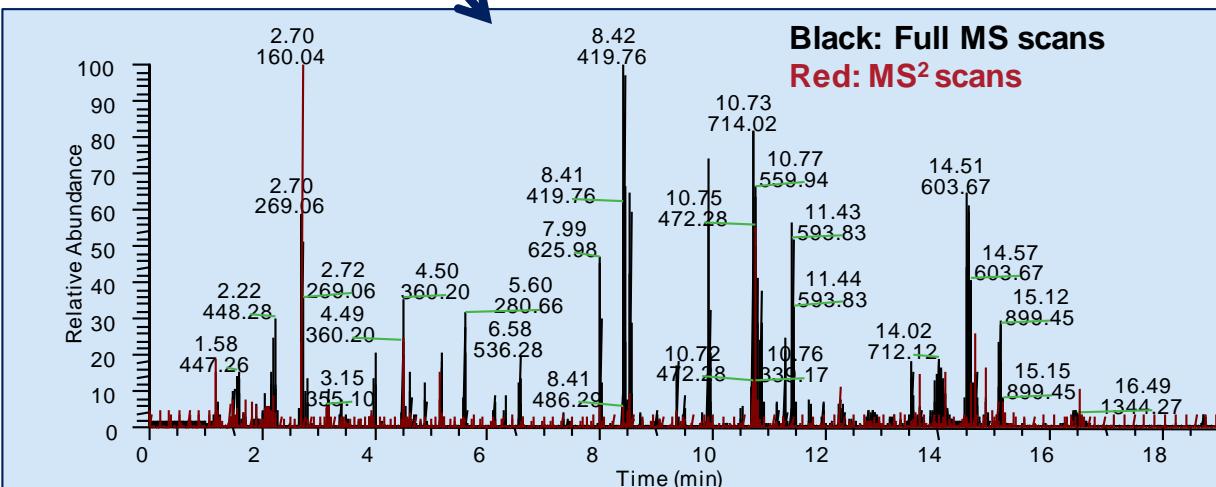


Full MS scan
method
 m/z 200-2000

ddTopN
Method:
Full MS with
dd MS² scans



Base Peak Chromatograms



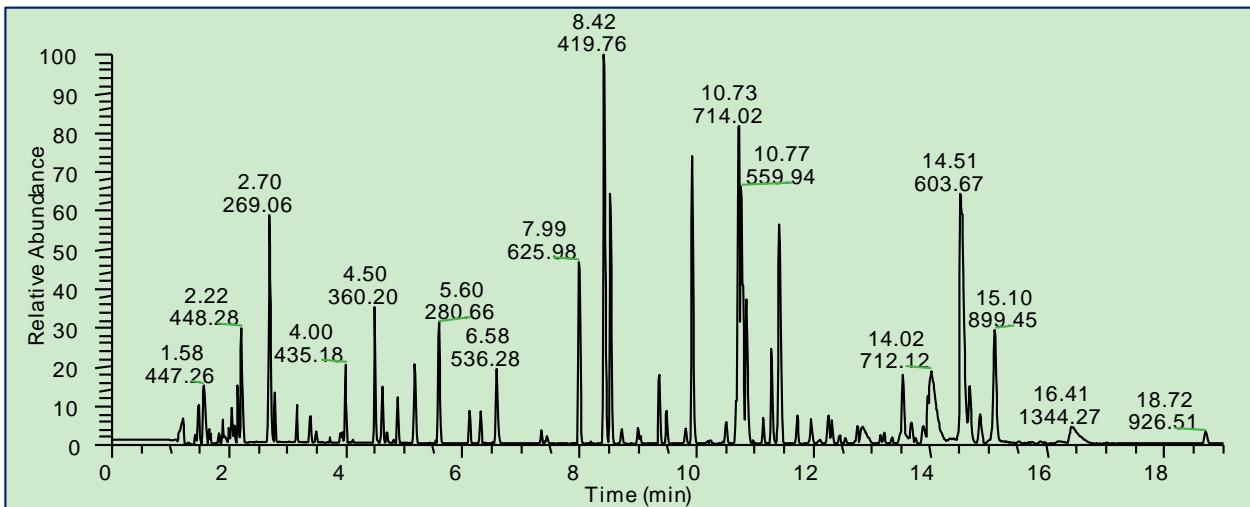
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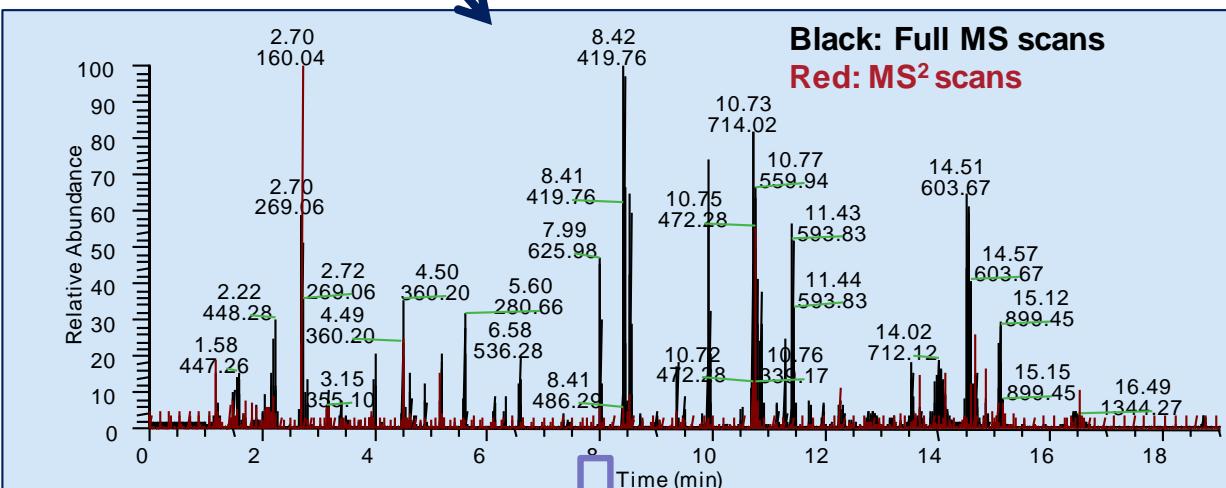


Full MS scan
method
 m/z 200-2000

ddTopN
Method:
Full MS with
dd MS² scans



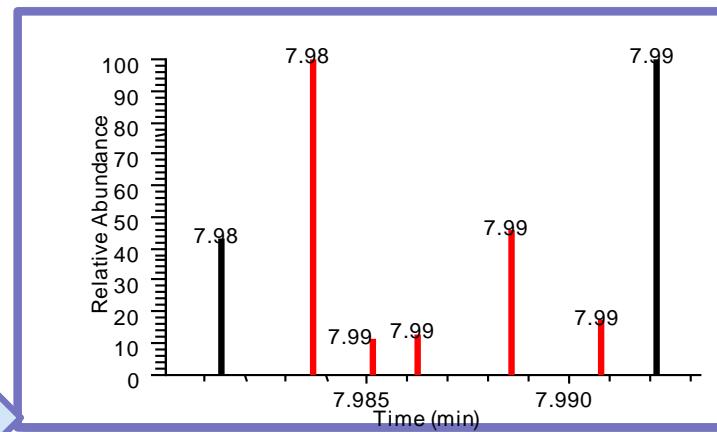
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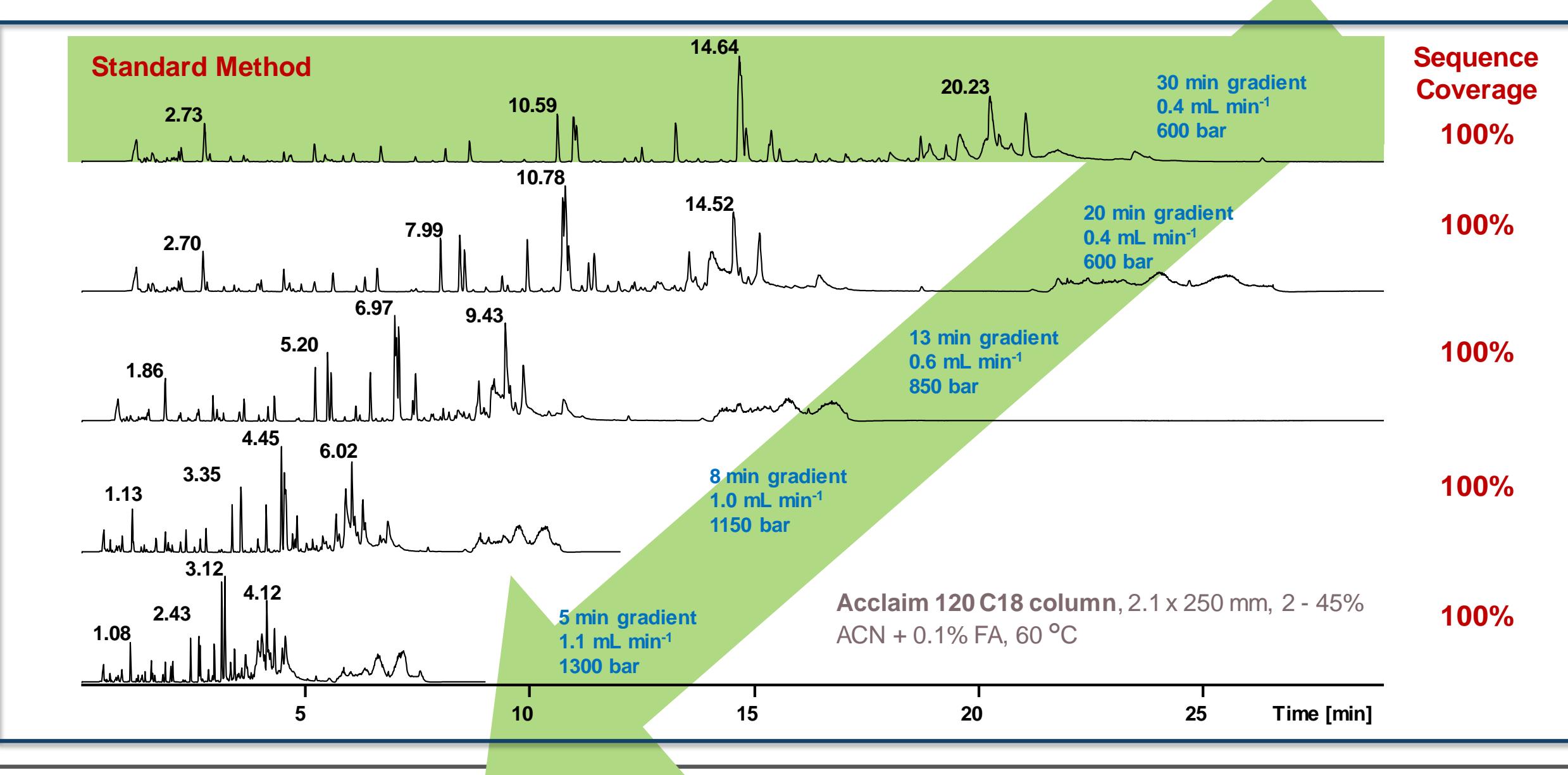
zoom

ddTopN Method with N=5 here:

MS/MS data-dependent analysis of 5 most abundant peptide ions (red) based on precursors obtained in a full scan (black)



Rituximab Analysis With Reducing Gradient



BioPharma Finder

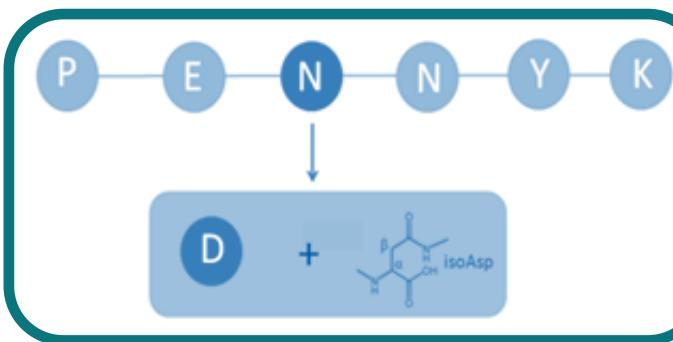
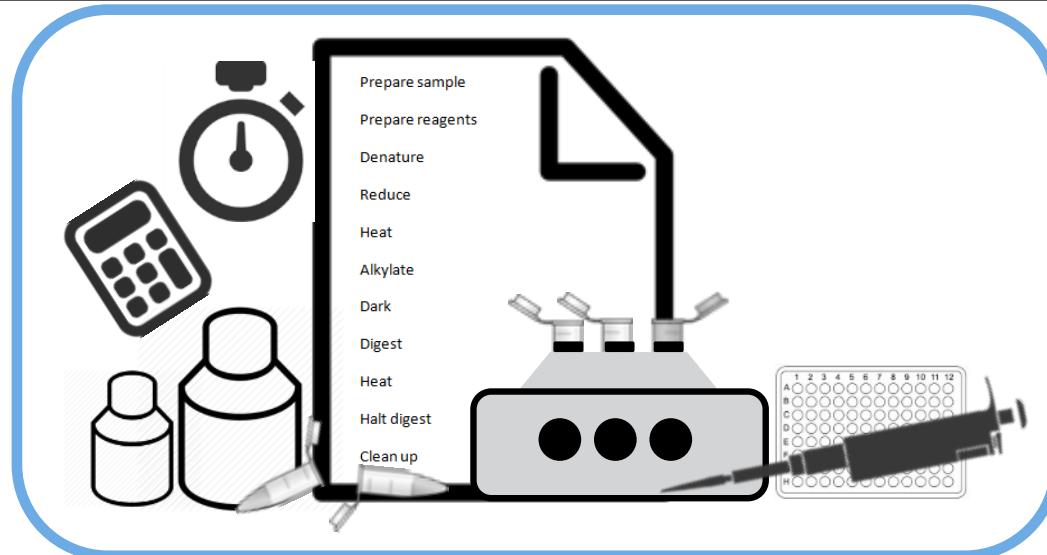
- Single software package for:
 - Intact and Native Mass Analysis
 - Sub-Unit Mass Analysis
 - Peptide Mapping
- Protein Deconvolution of isotopically resolved and unresolved spectra
- Peptide mapping of biotherapeutics & other recombinant proteins
- Automated DAR calculations
- Comparison of biosimilars
- Supports all Orbitrap & ion-trap-based instruments



More confidence, more modifications, less time

What's the Problem with Protein Digestion?

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



The Fundamental Five...or is that Two?



Enzyme



Primary structure



Secondary structure (H bonding)

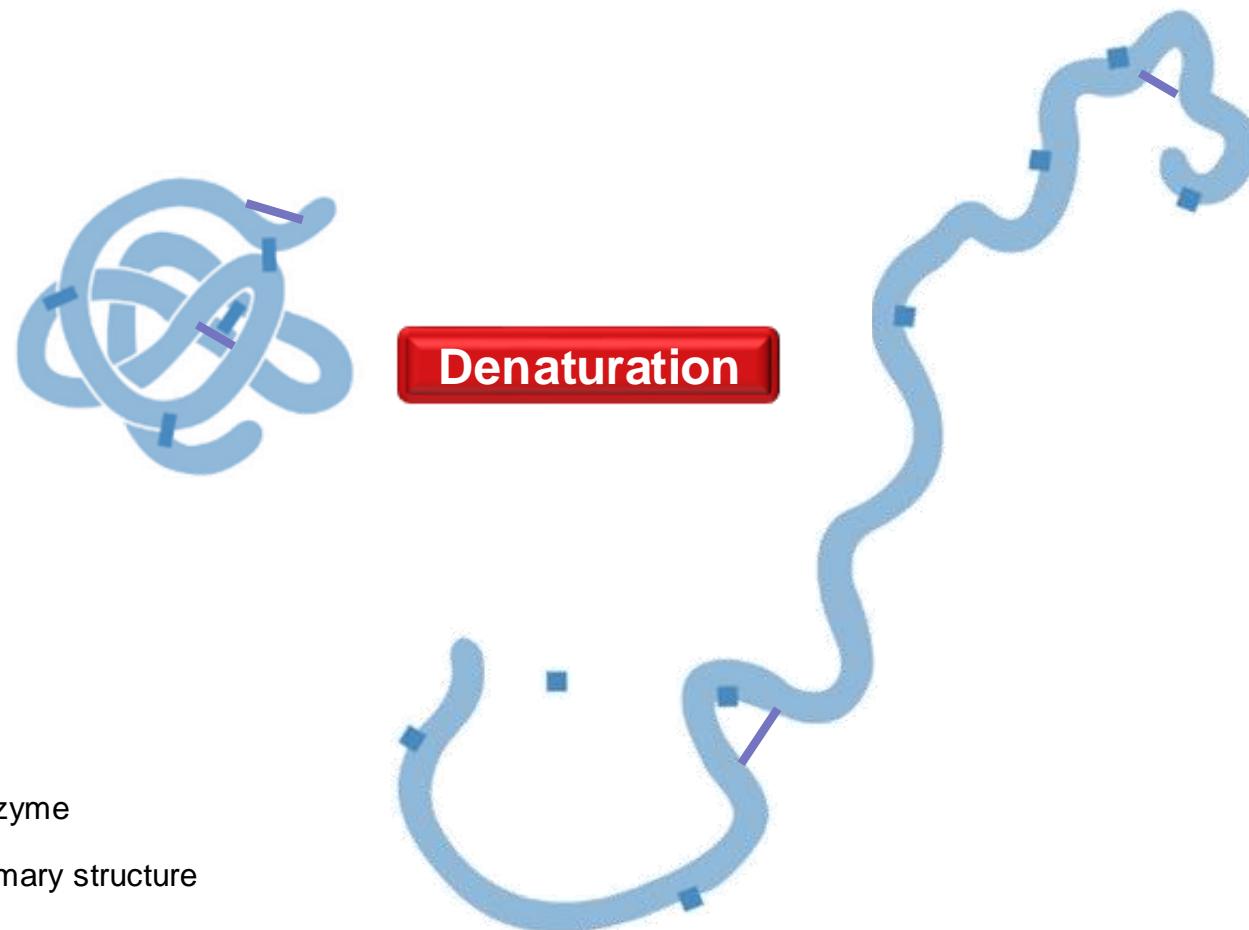


Tertiary structure (disulphide bonding)

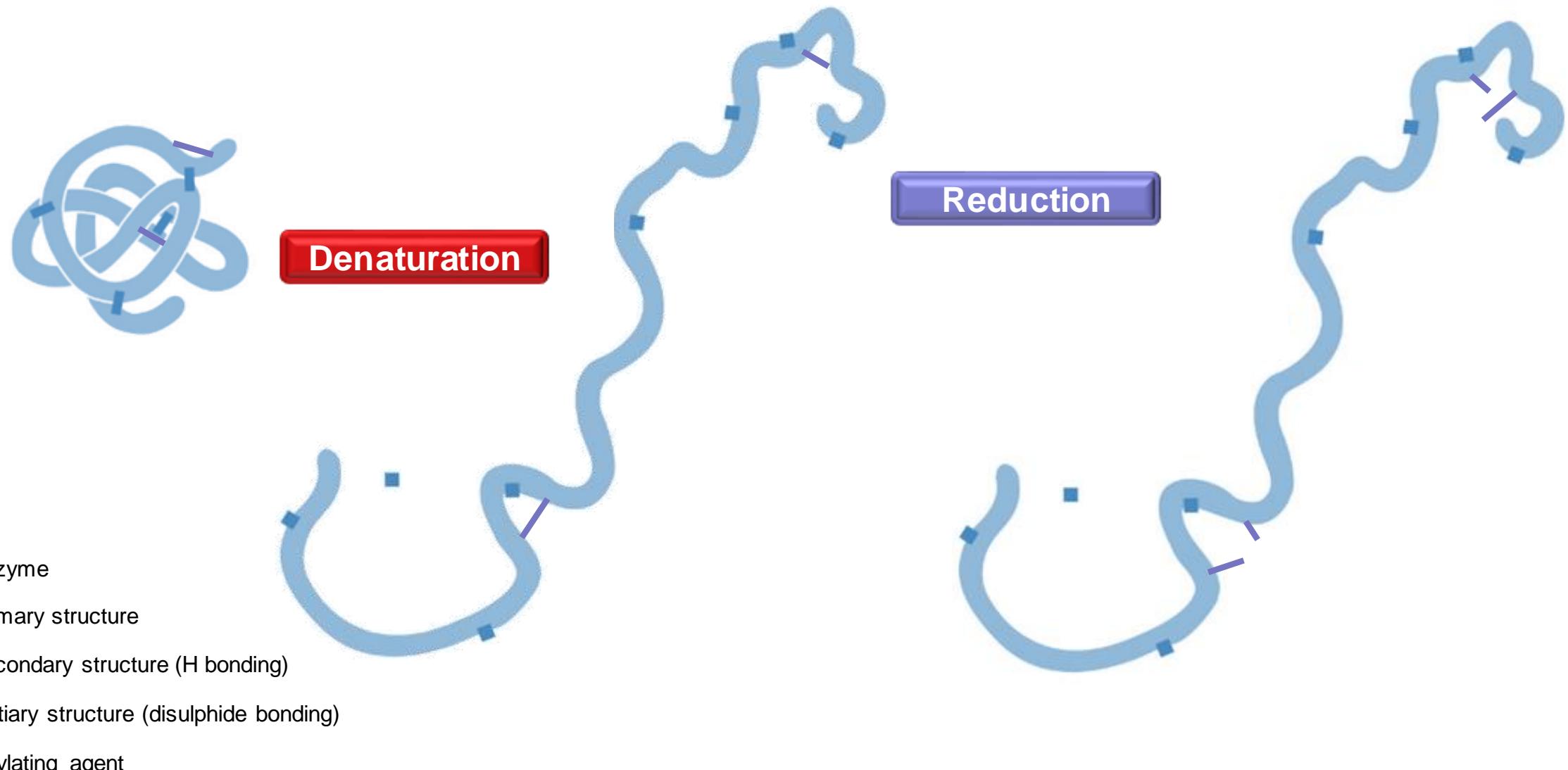


Alkylating agent

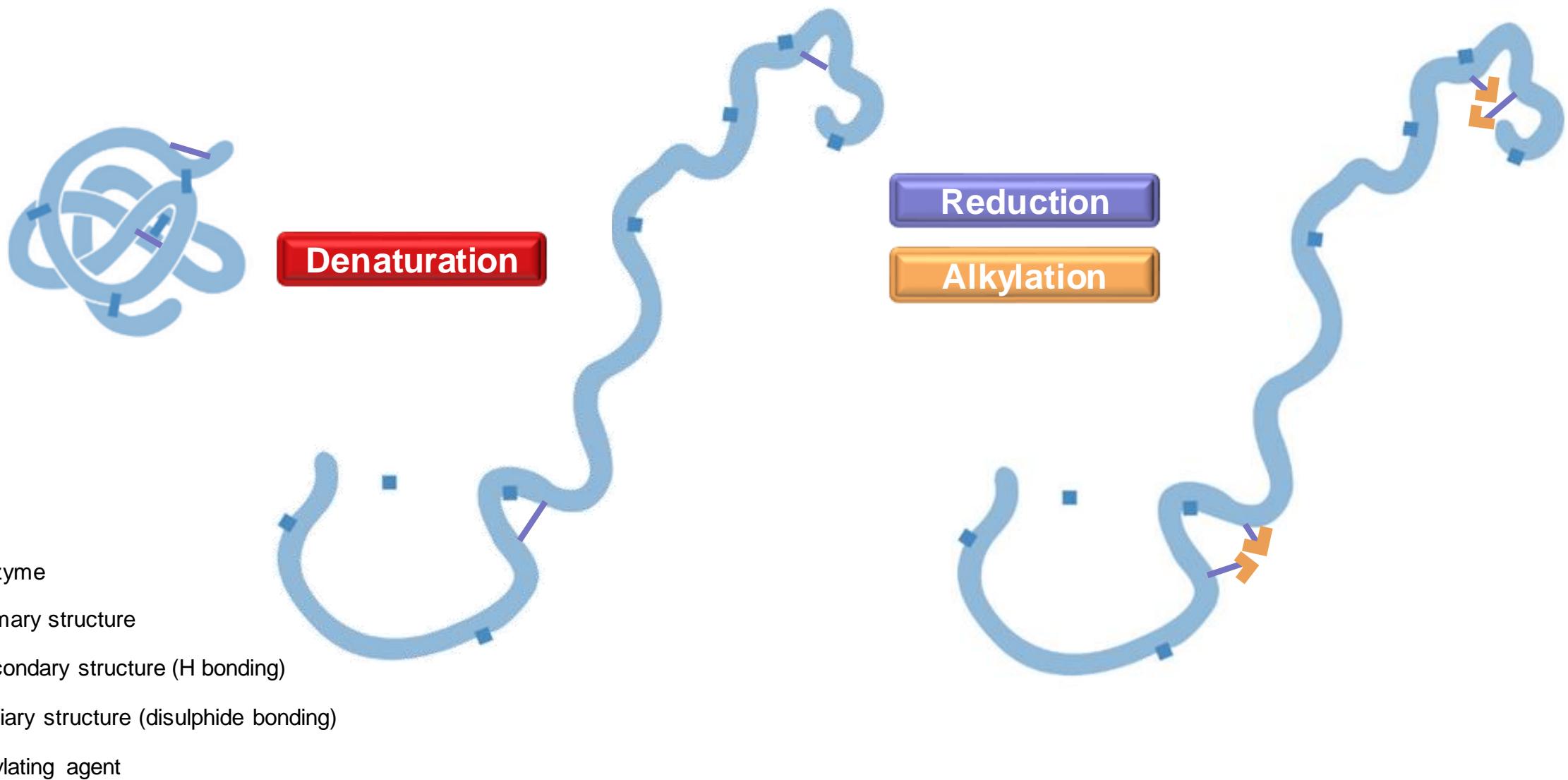
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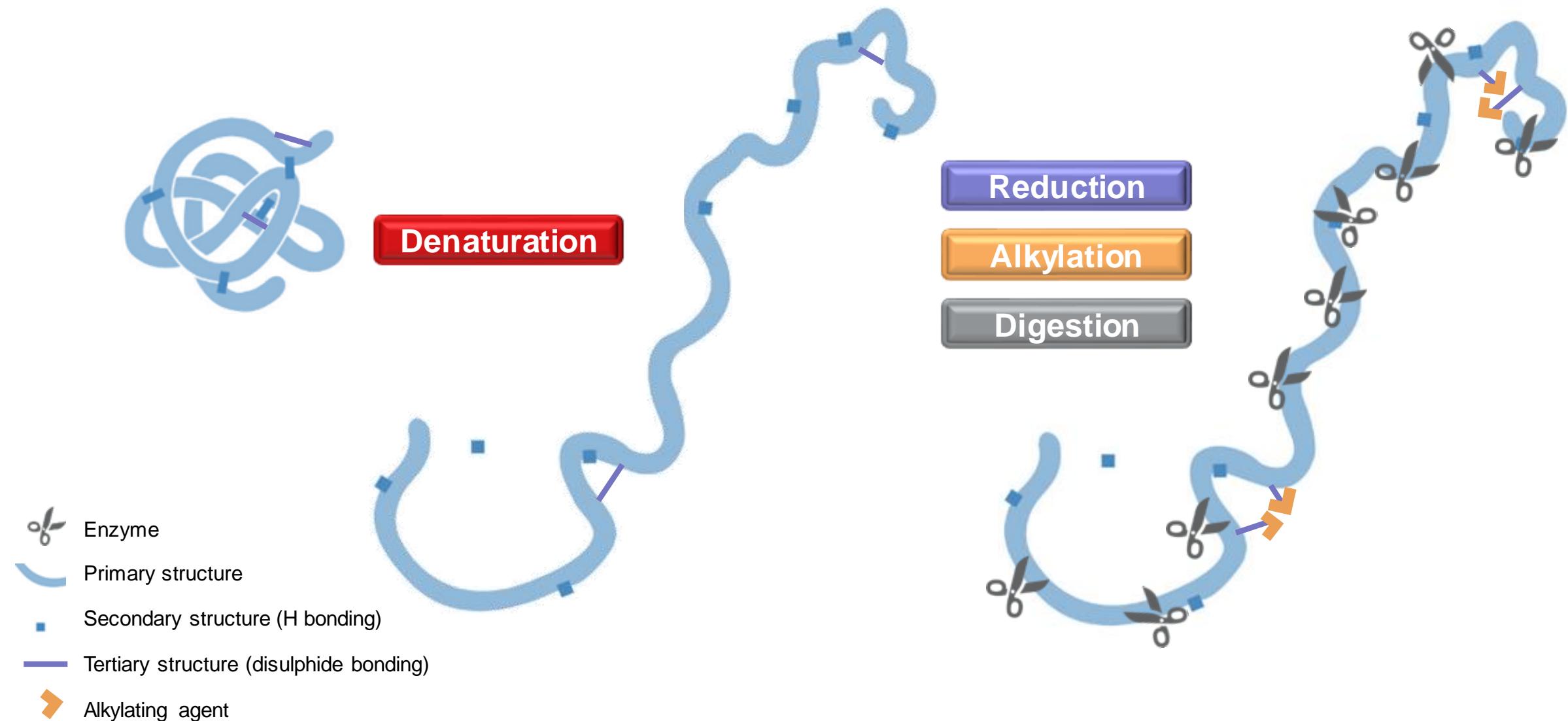
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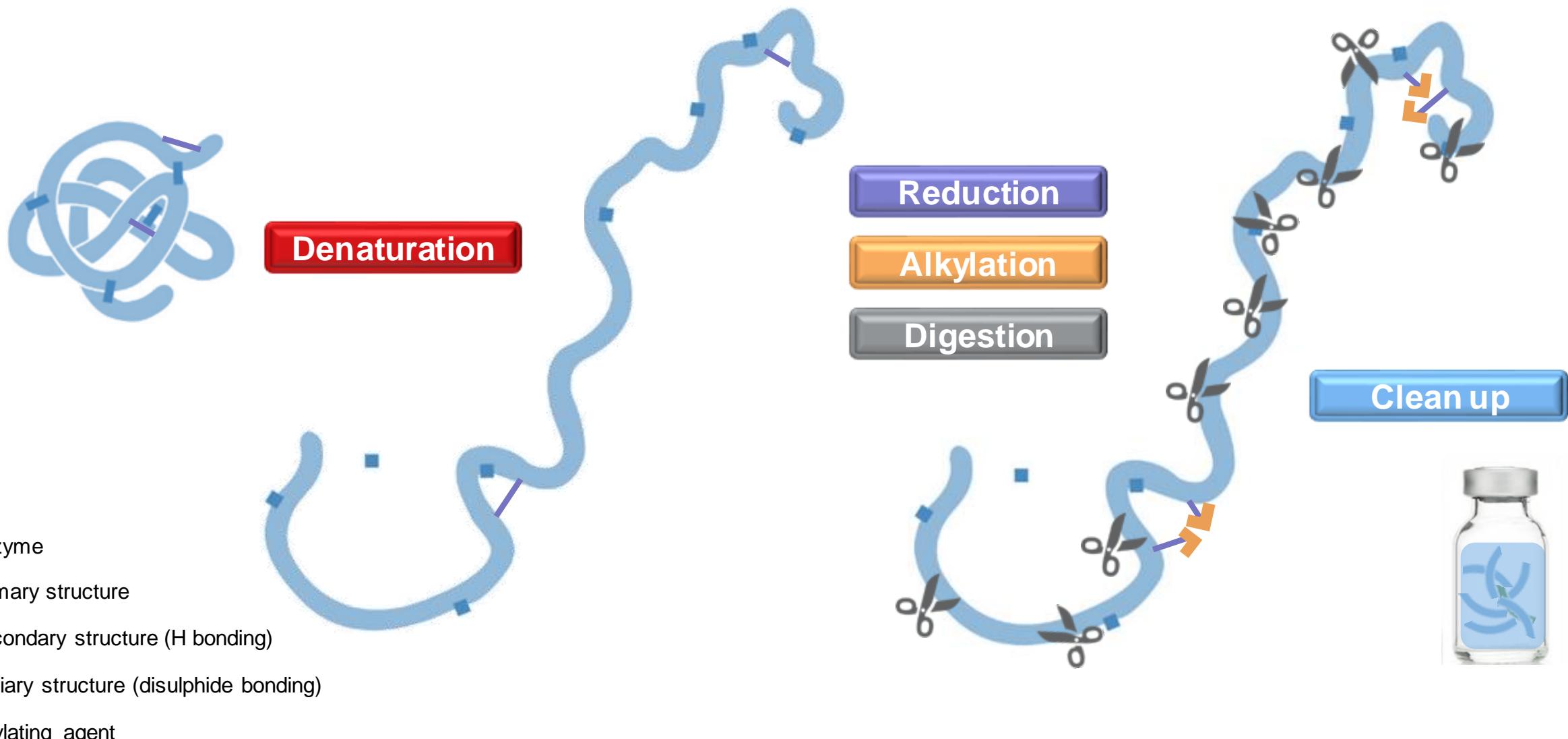
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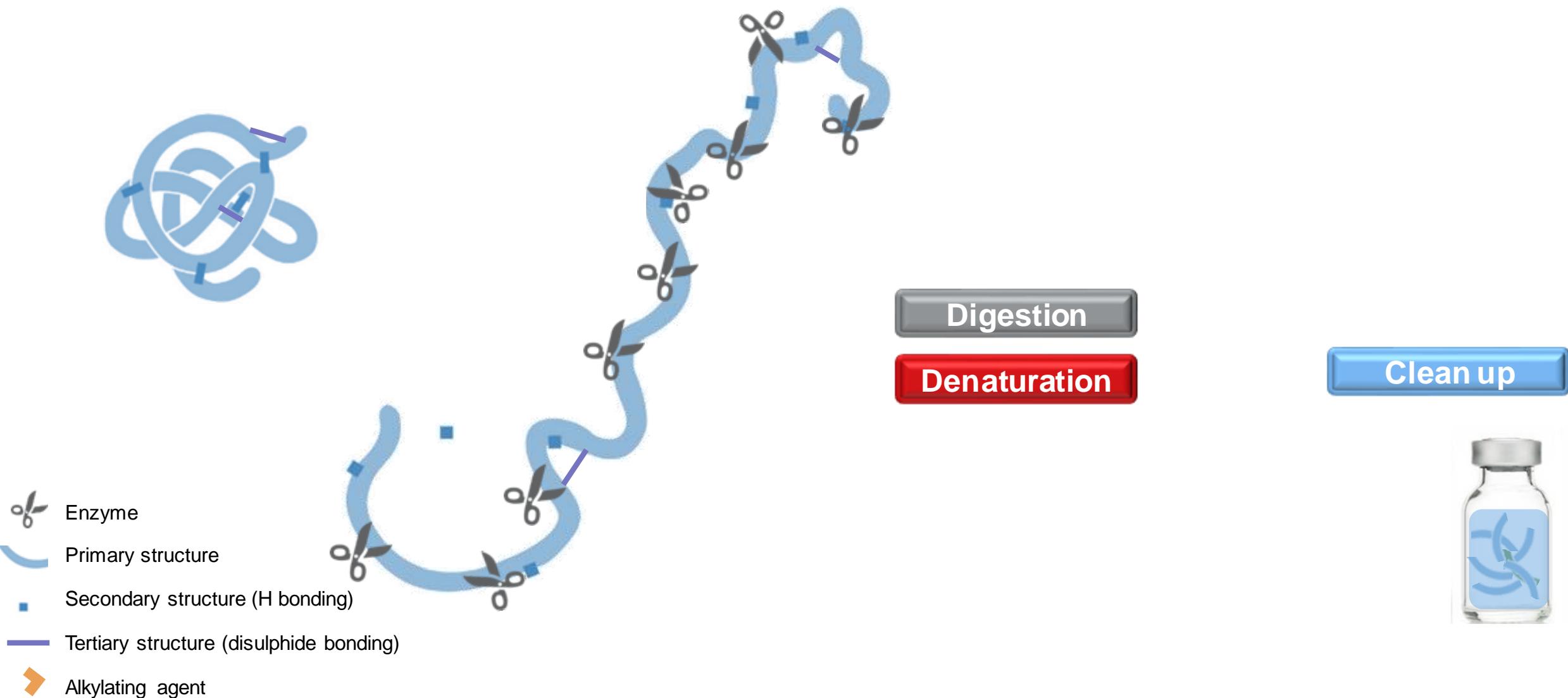
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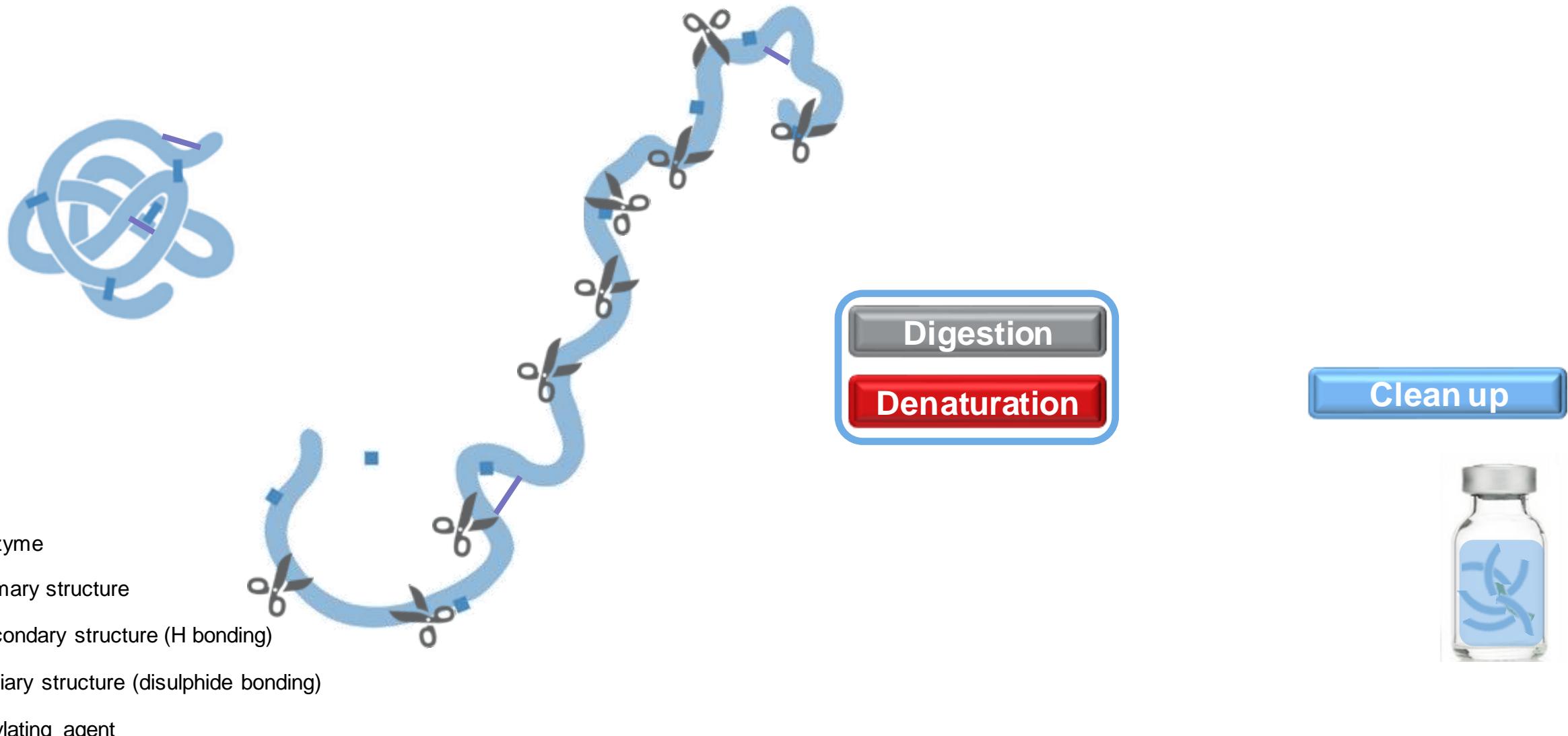
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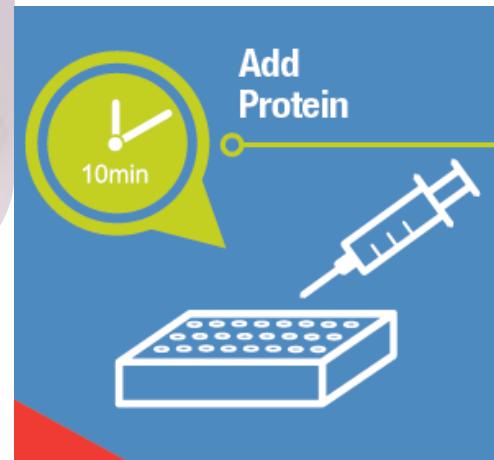
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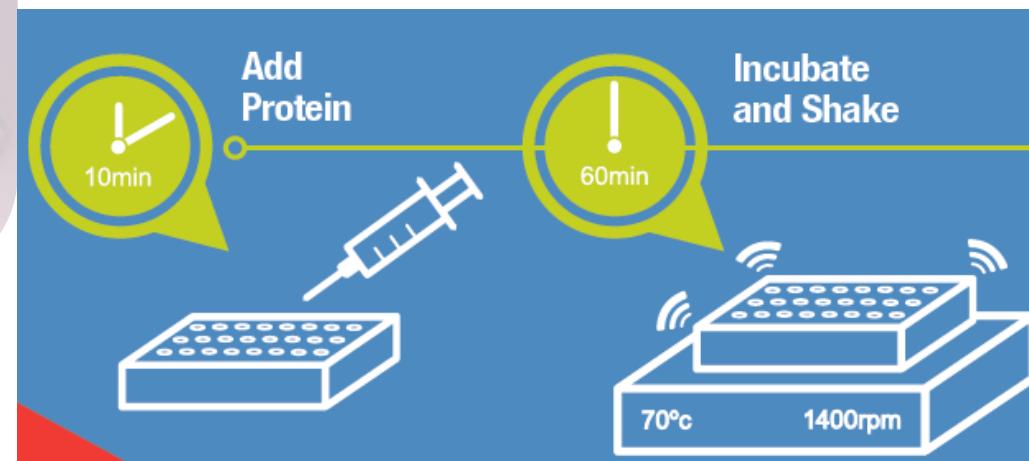
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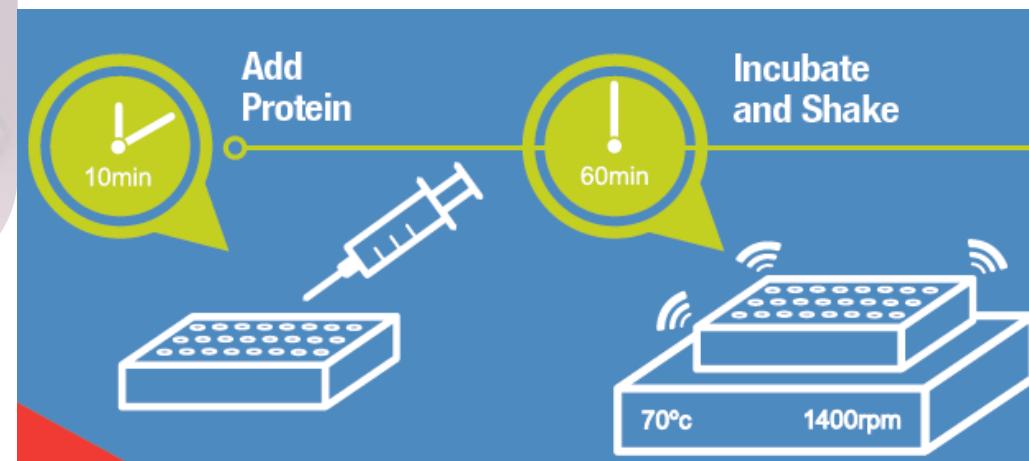
Thermo Scientific SMART Digest Kits



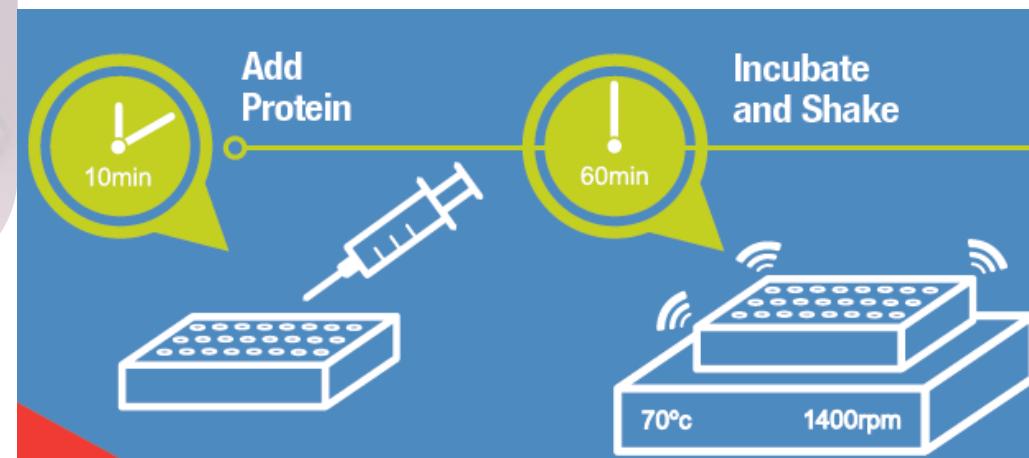
Thermo Scientific SMART Digest Kits



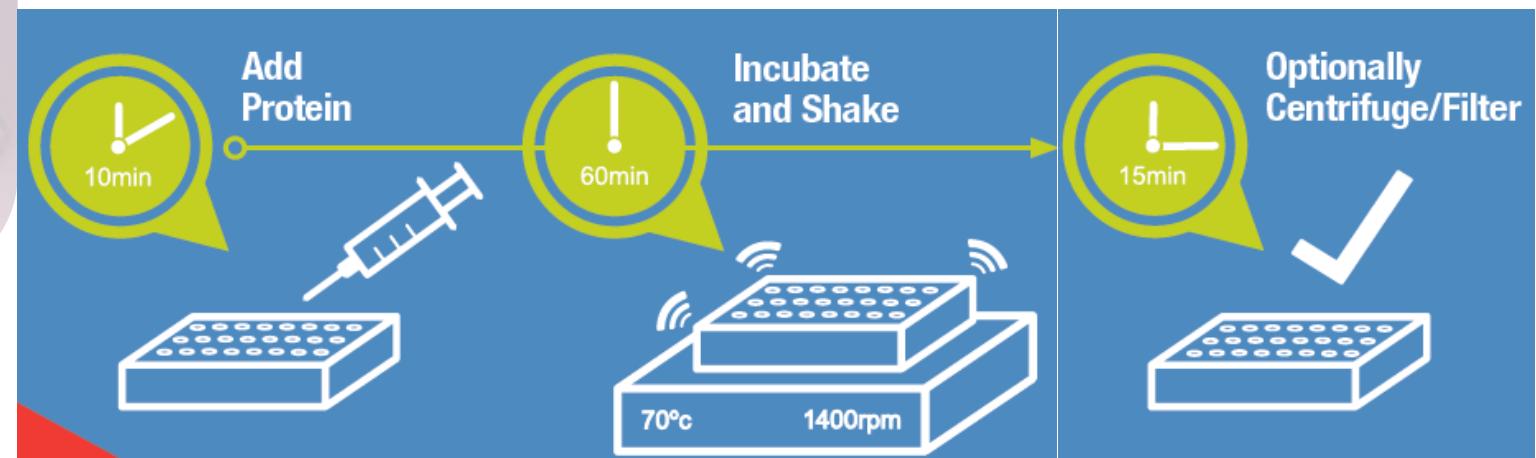
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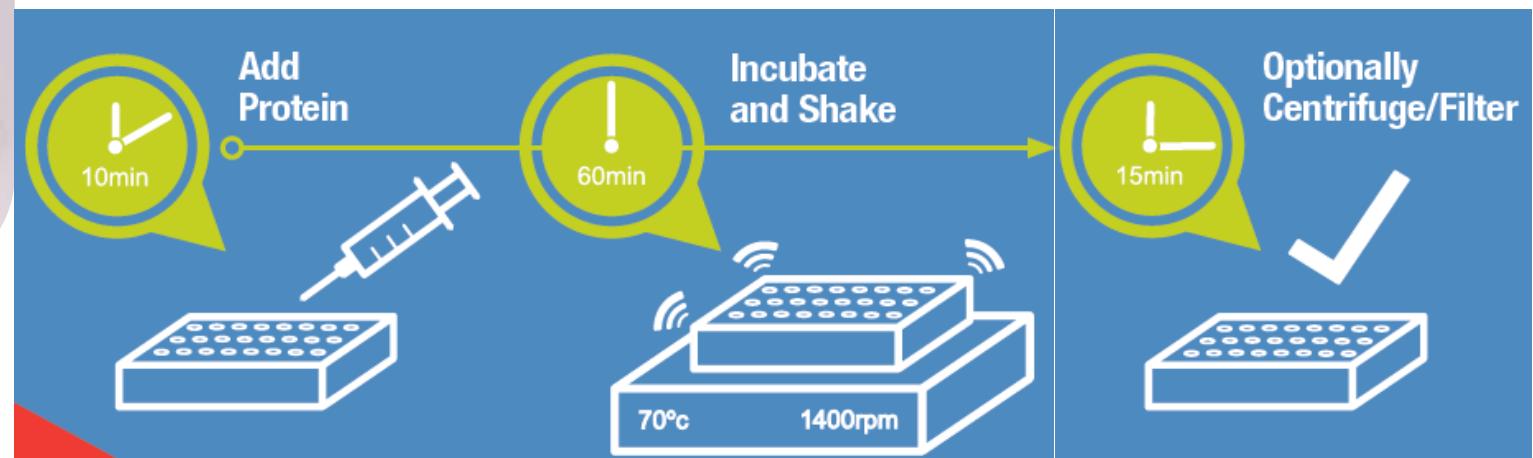
Thermo Scientific SMART Digest Kits



Denaturation

Digestion

Thermo Scientific SMART Digest Kits

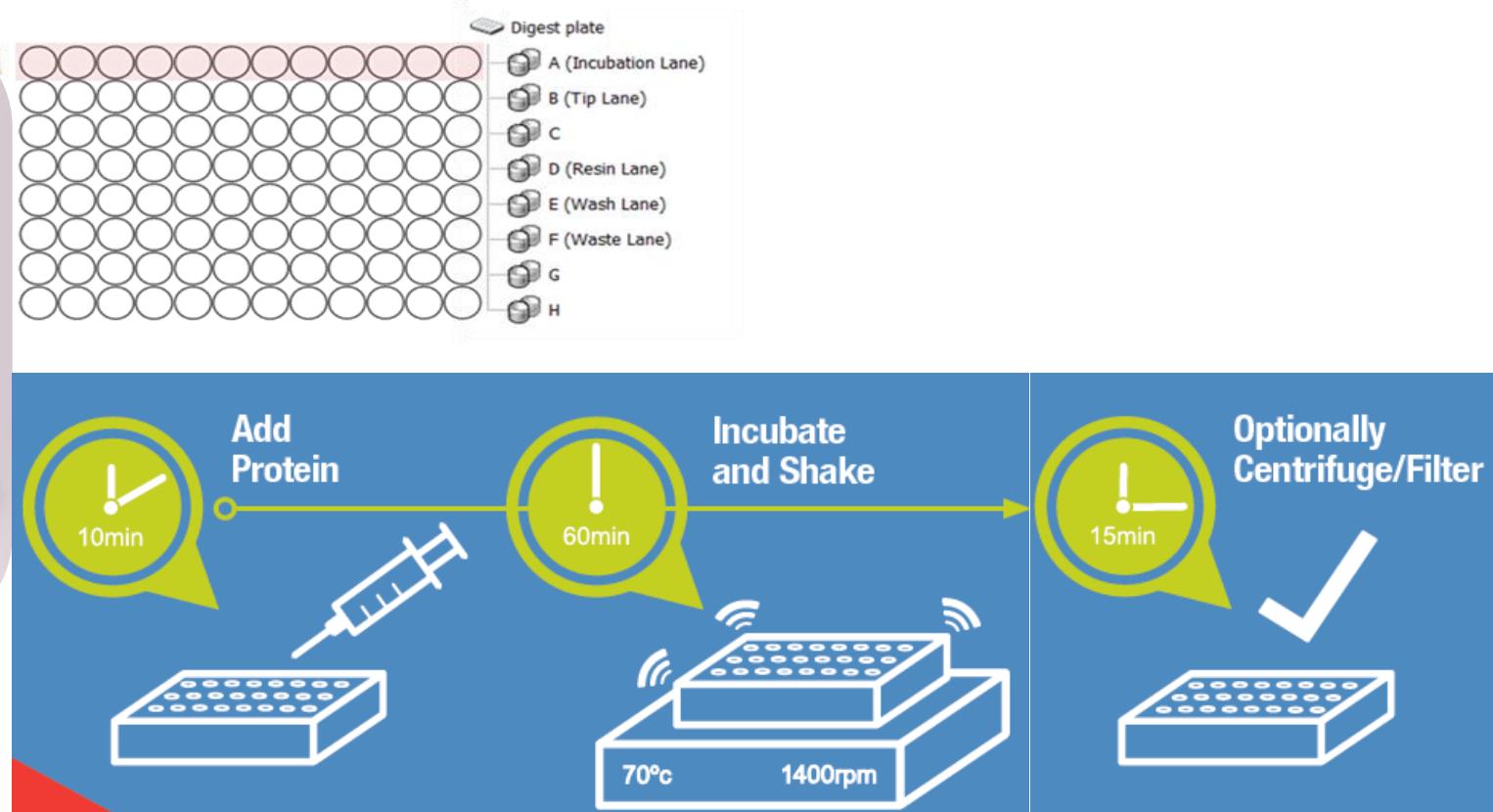


Denaturation

Digestion

Clean up

Thermo Scientific SMART Digest Kits

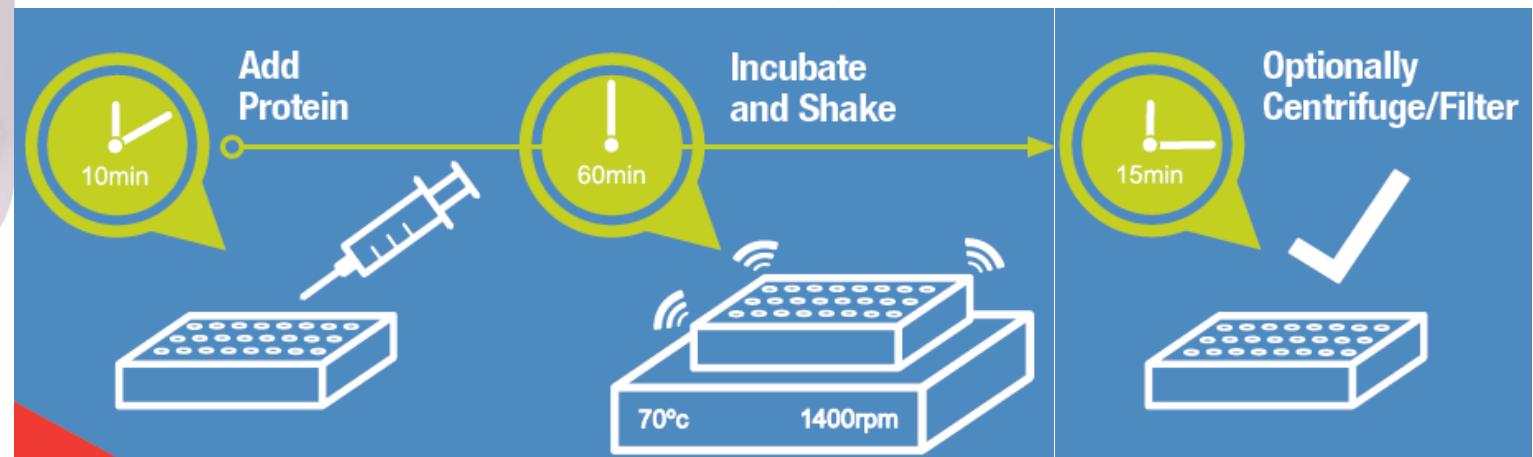


Denaturation

Digestion

Clean up

Thermo Scientific SMART Digest Kits

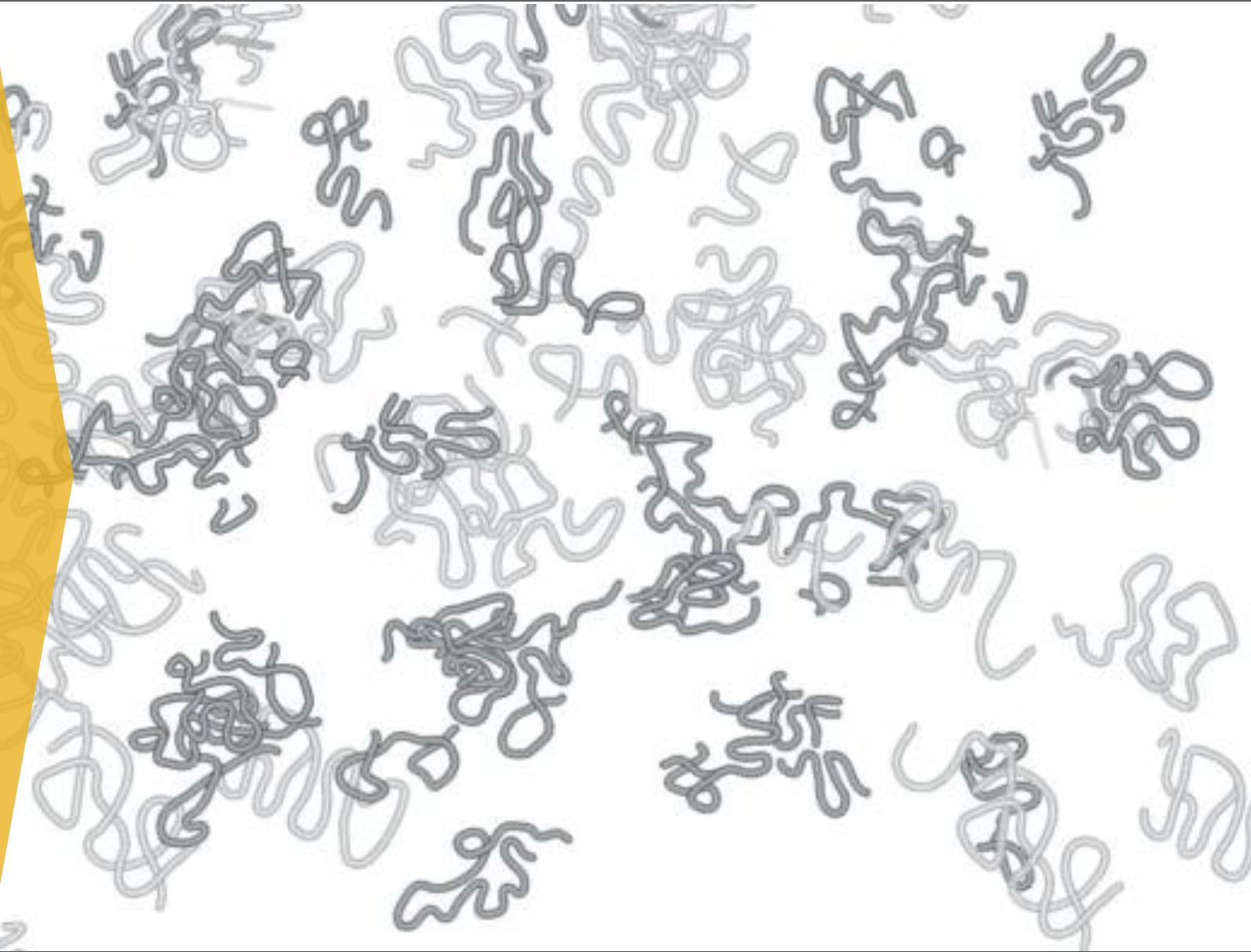


Denaturation

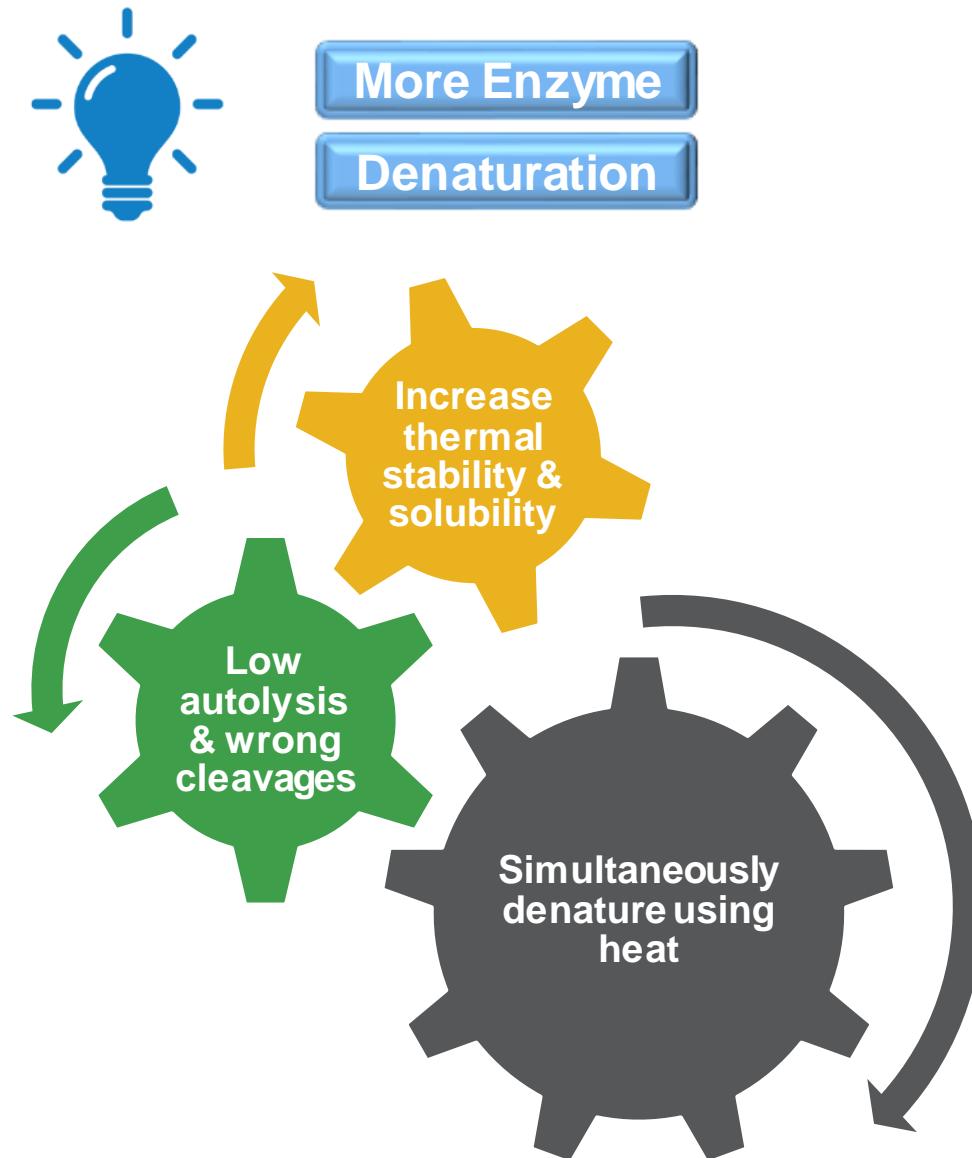
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Clean up

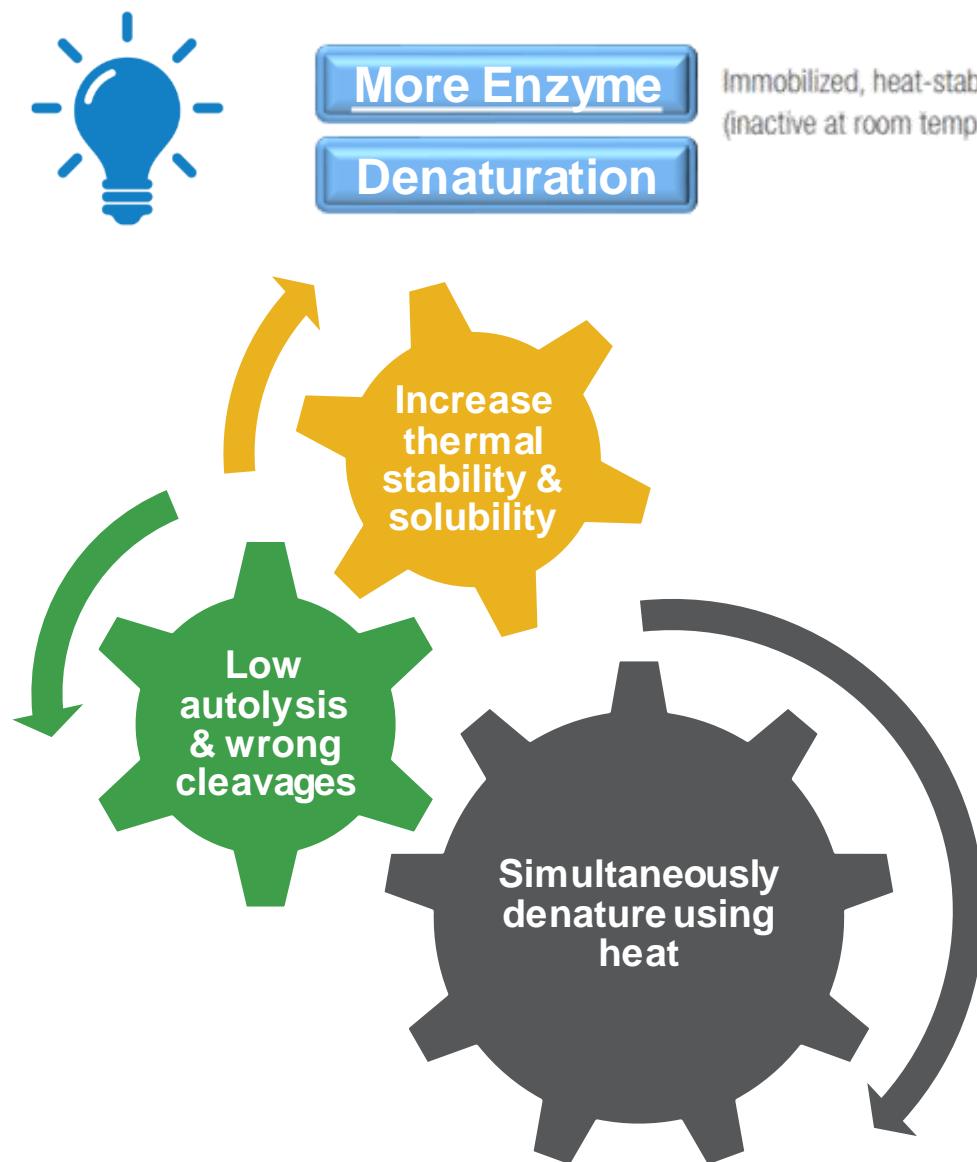
The Science of SMART



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



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



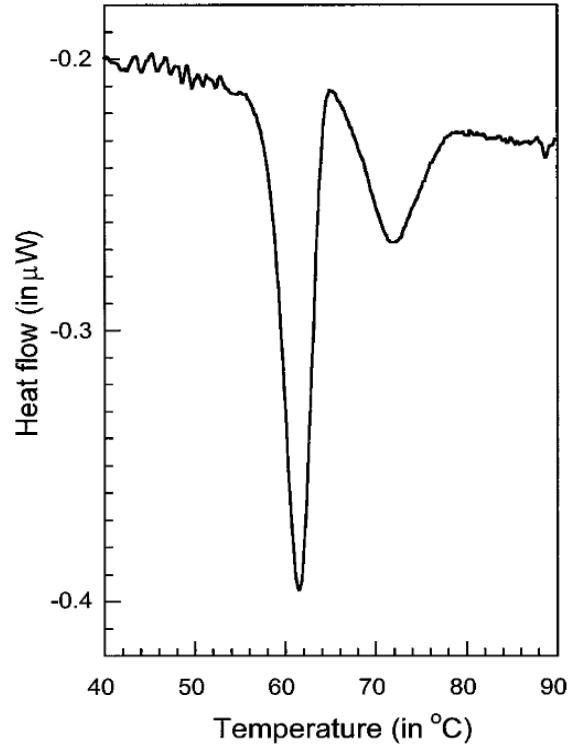
- **Save time**
 - Reagent prep/denaturation, reduction/alkylation
- **Simplify**
 - Only 2 steps
- **Increase robustness**
 - Stable, reproducible tryptic activity
 - Untrained users
 - Easy method transfer

Optimize the protein for Heat Denaturation!

Protein

Denaturation

Thermal denaturation of IgG



394

Biophysical Journal Volume 78 January 2000 394–404

Anal. Chem. 2000, 72, 2667–2670

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

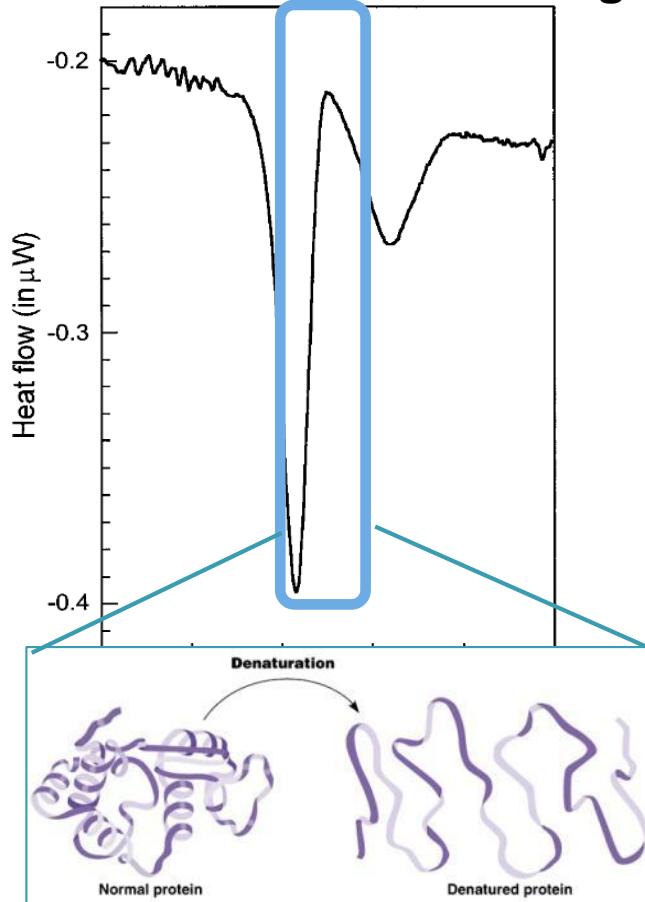
Thermal Denaturation: A Useful Technique in Peptide Mass Mapping

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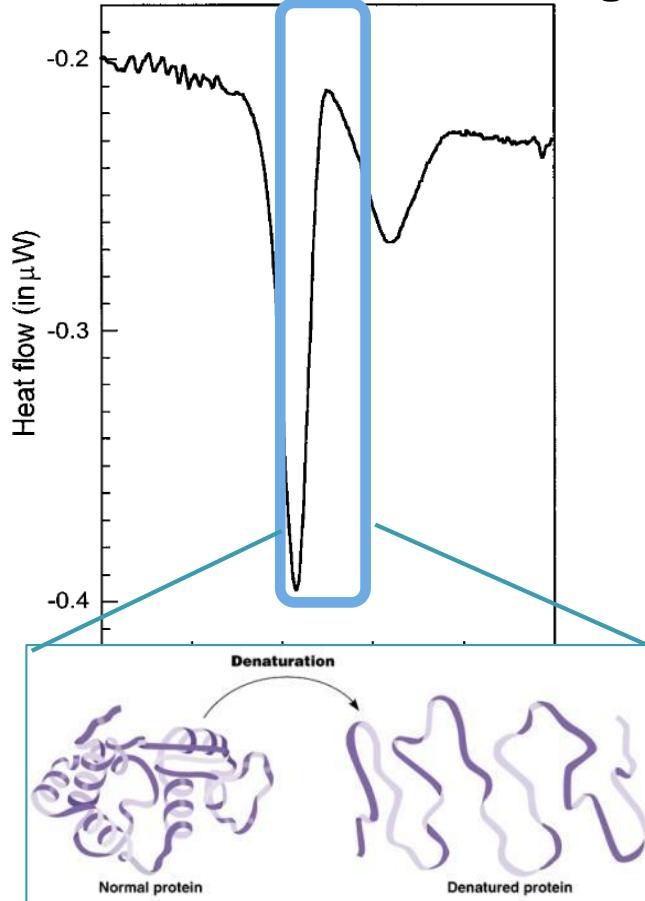
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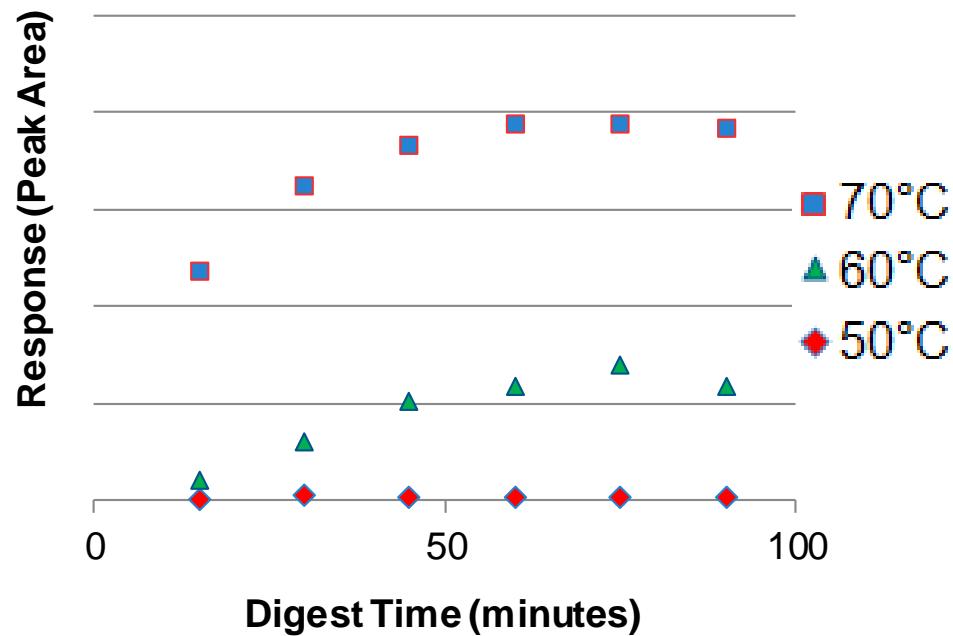
Protein

Denaturation

Thermal denaturation of IgG



Native IgG Digest Profile monitoring VVSVLTVLHQDWLNGK



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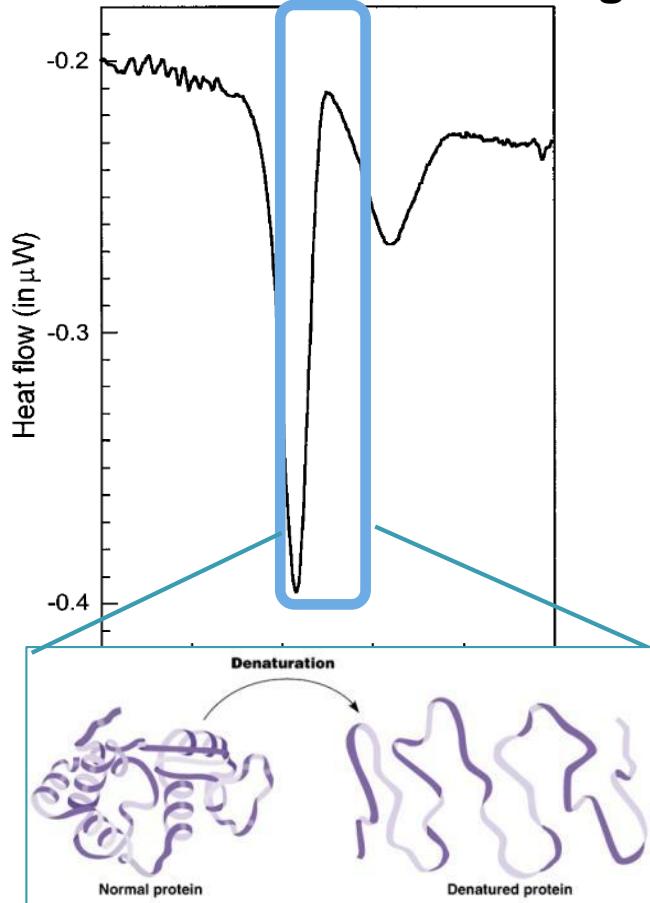
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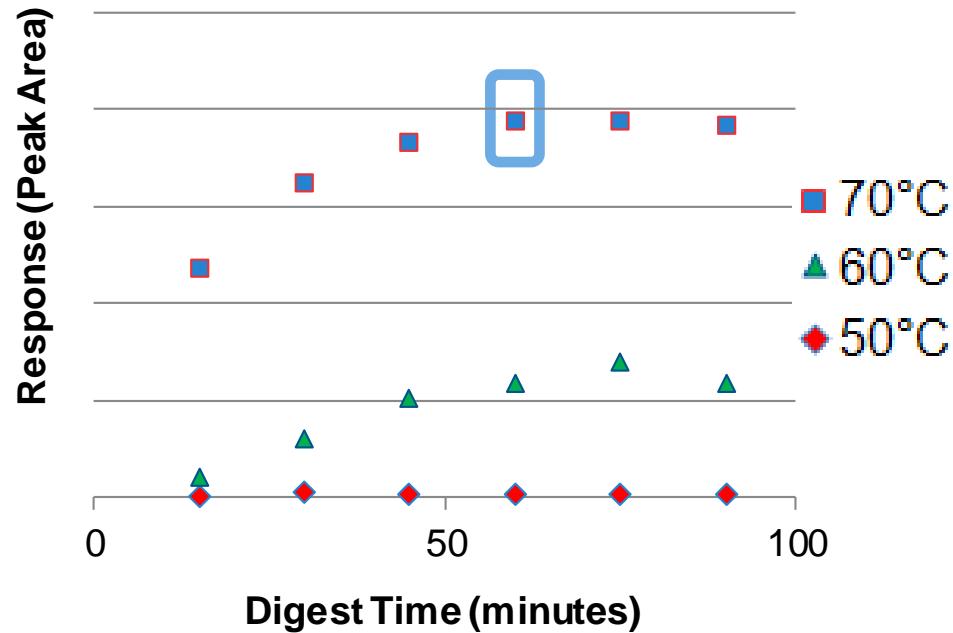
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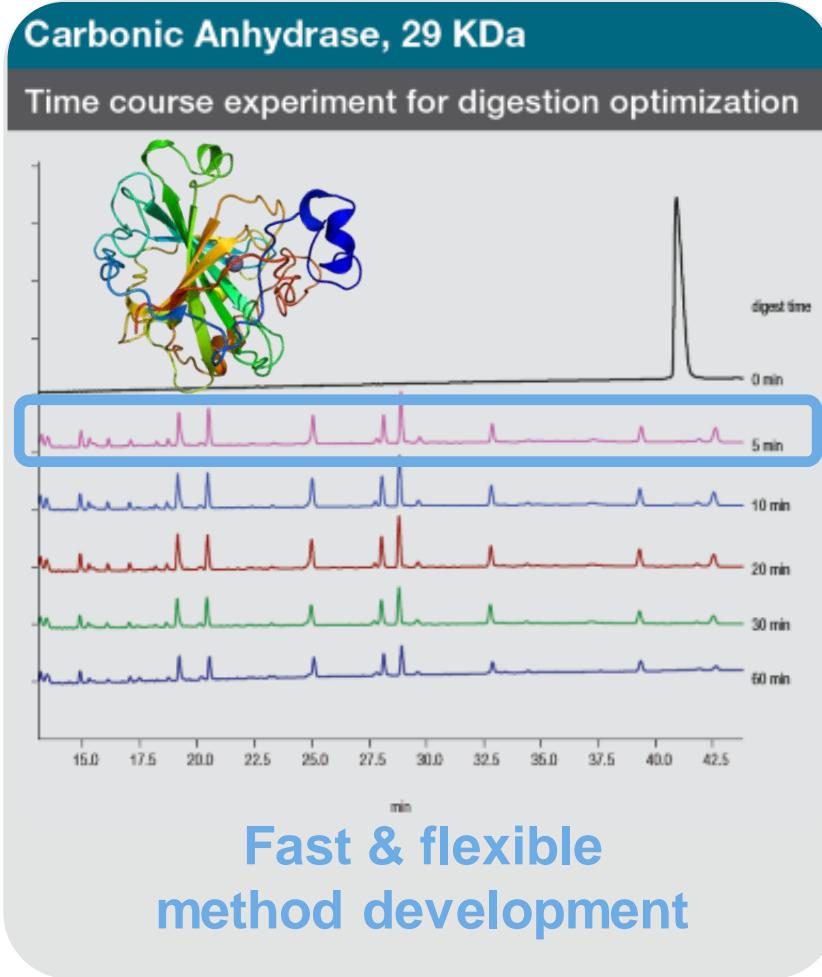
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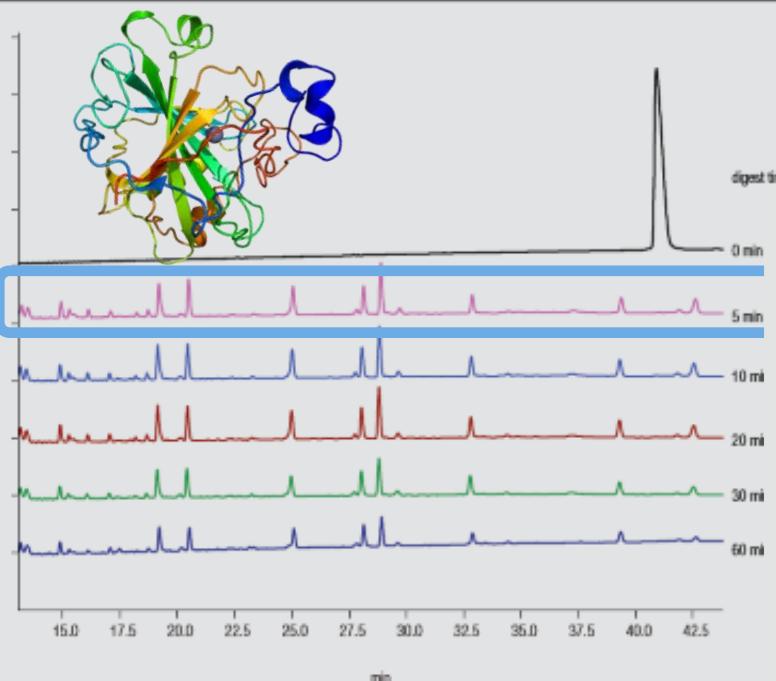
3 Reasons to change to SMART digestion



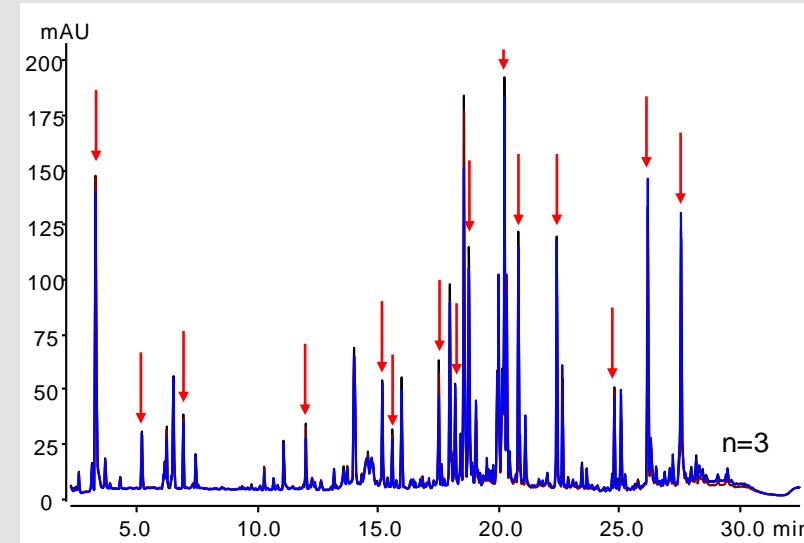
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Carbonic Anhydrase, 29 KDa

Time course experiment for digestion optimization



Fast & flexible
method development



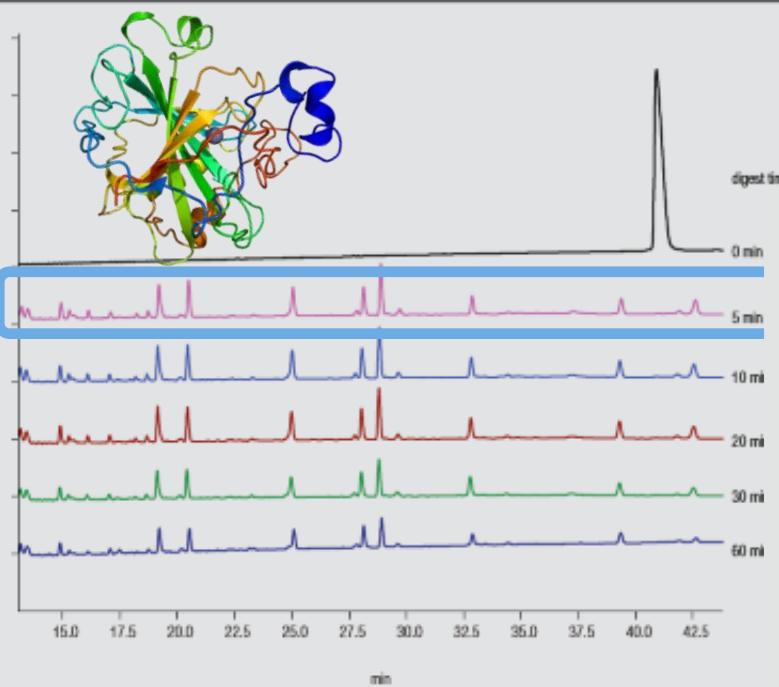
RSDs: RT 0.024 %; Peak Area 2.82 %
(n=5 users, based on 15 peaks)

Standardized reproducibility

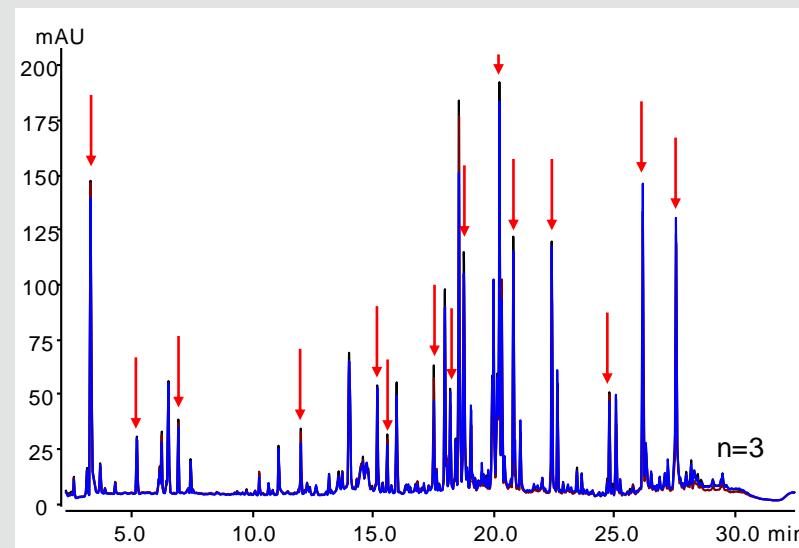
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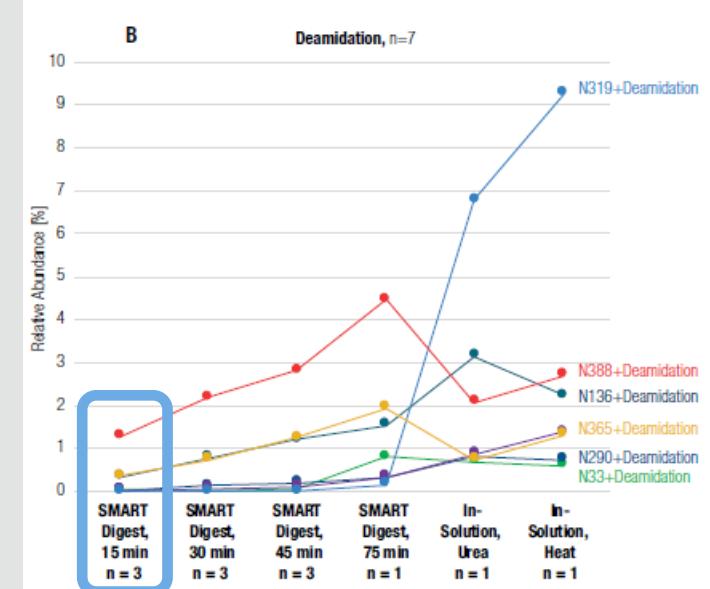


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Reduced complexity

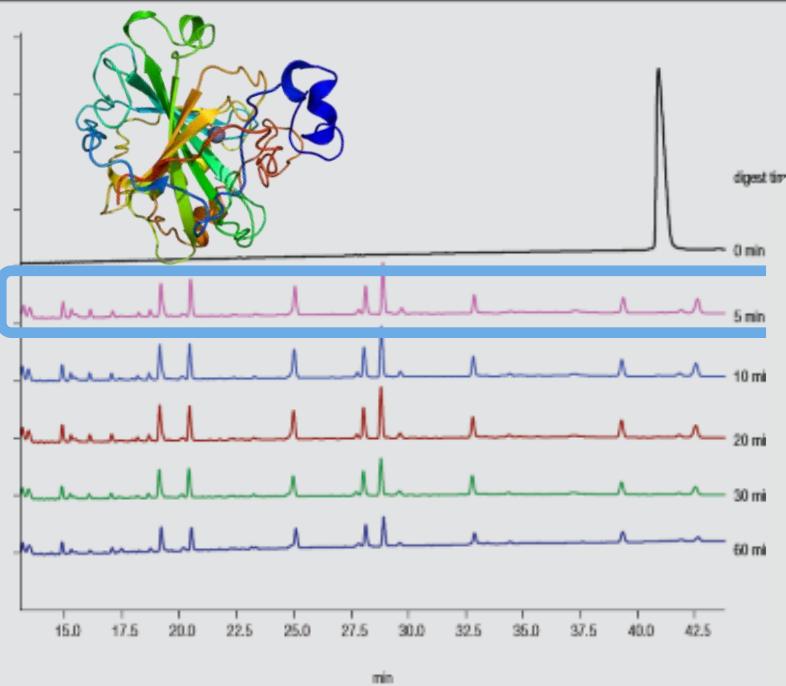
APPLICATION NOTE

SMART Digest Compared to Classic In-Solution Digestion of Rituximab for In-Depth Peptide Mapping Characterization

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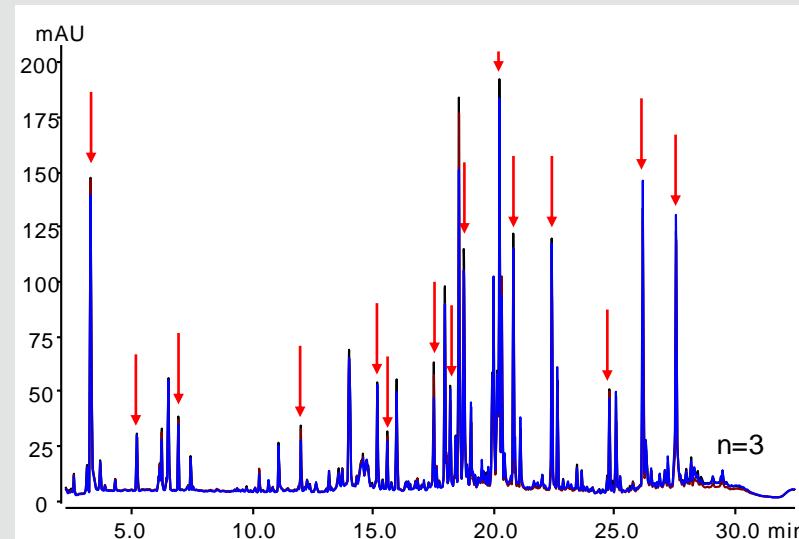
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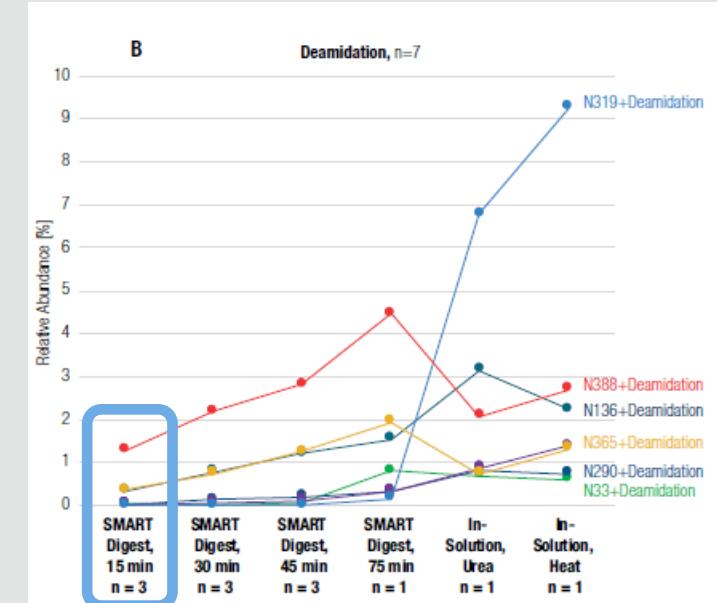
Fast & flexible
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Automation!



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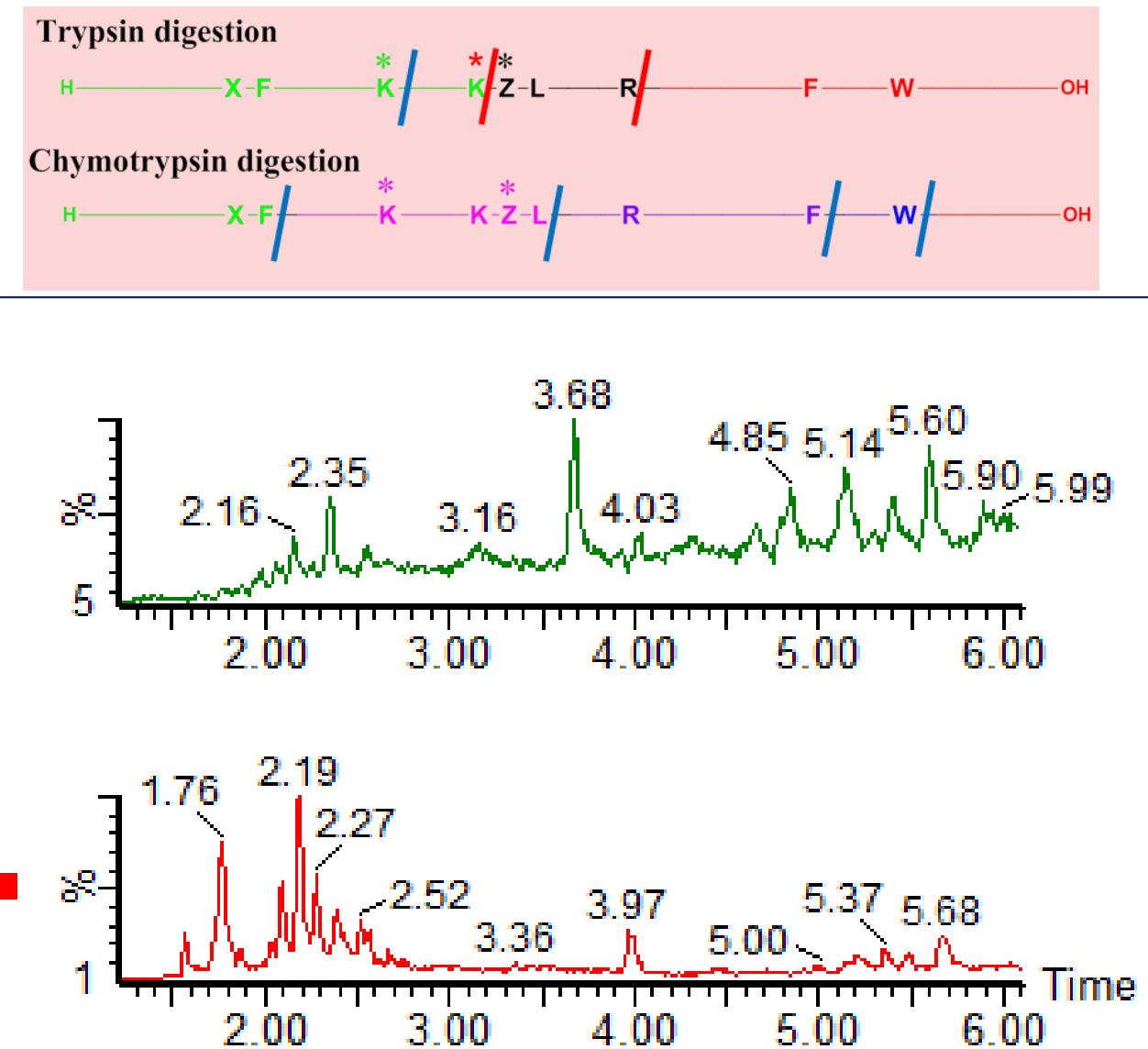


Reduced complexity

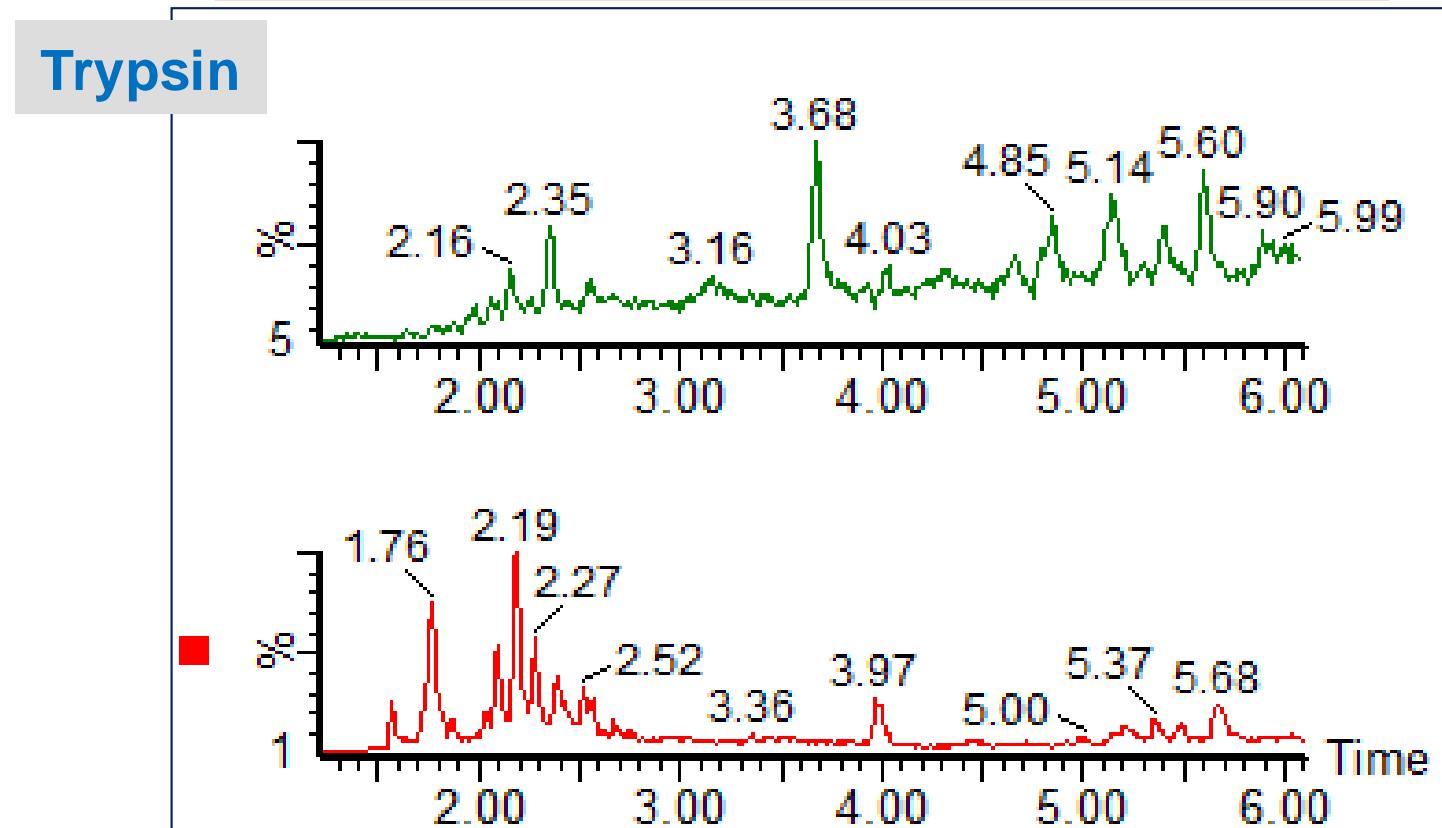
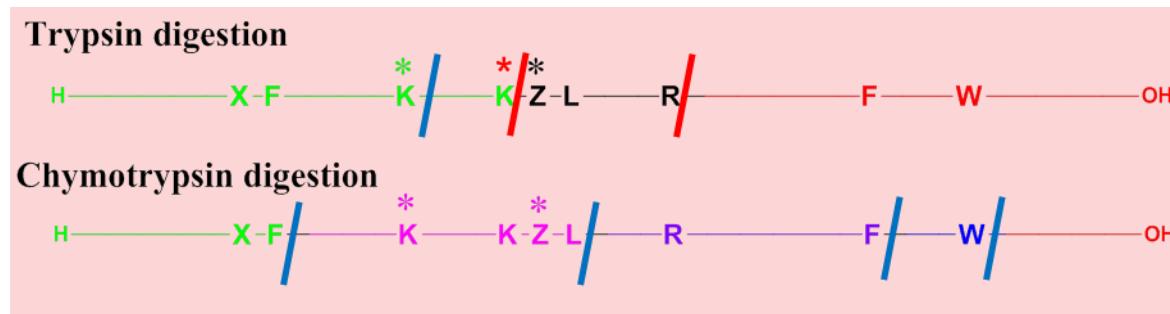
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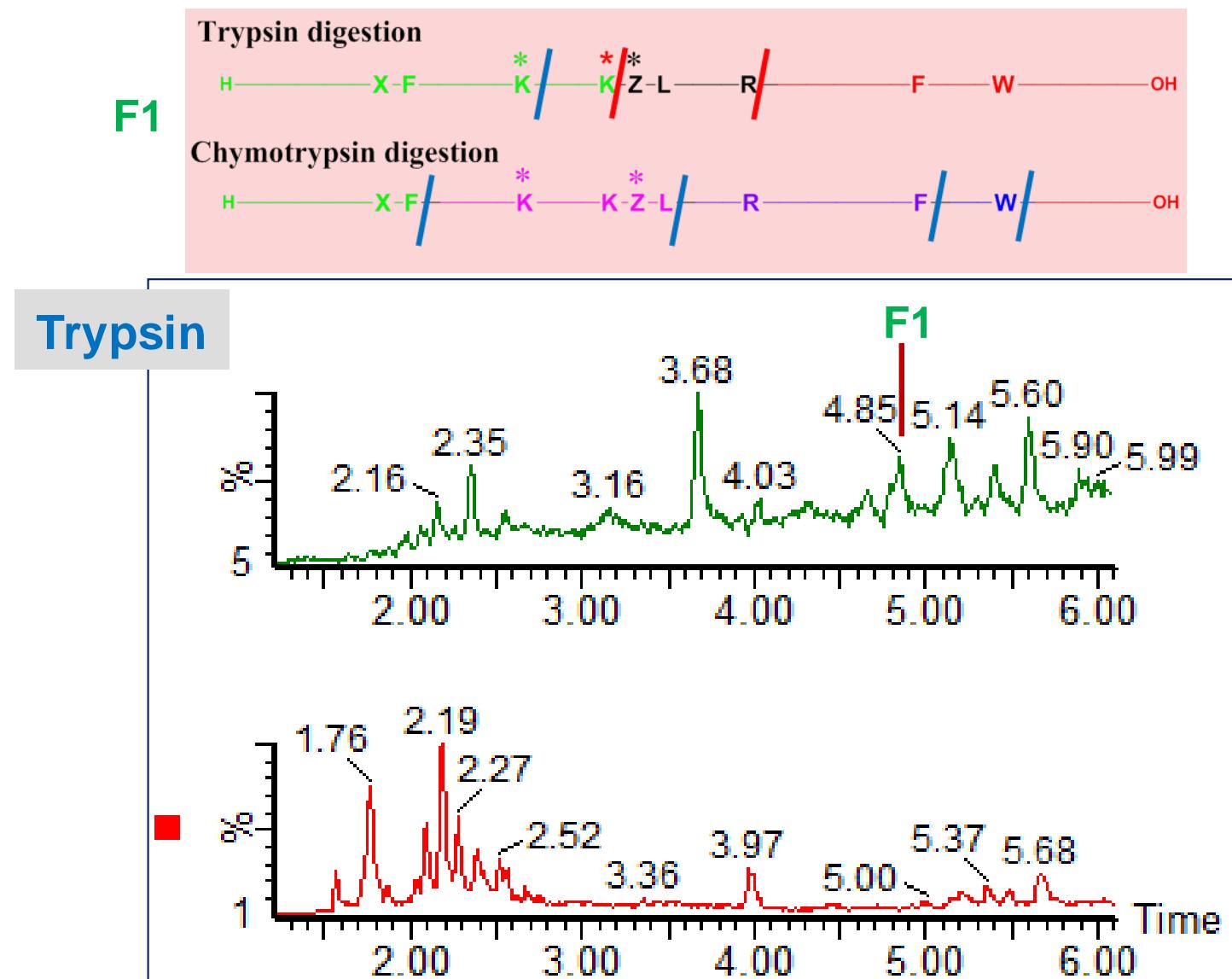
SMART Digestion (Trypsin or Chymotrypsin) and HR-LC/MS/MS of the modified peptide



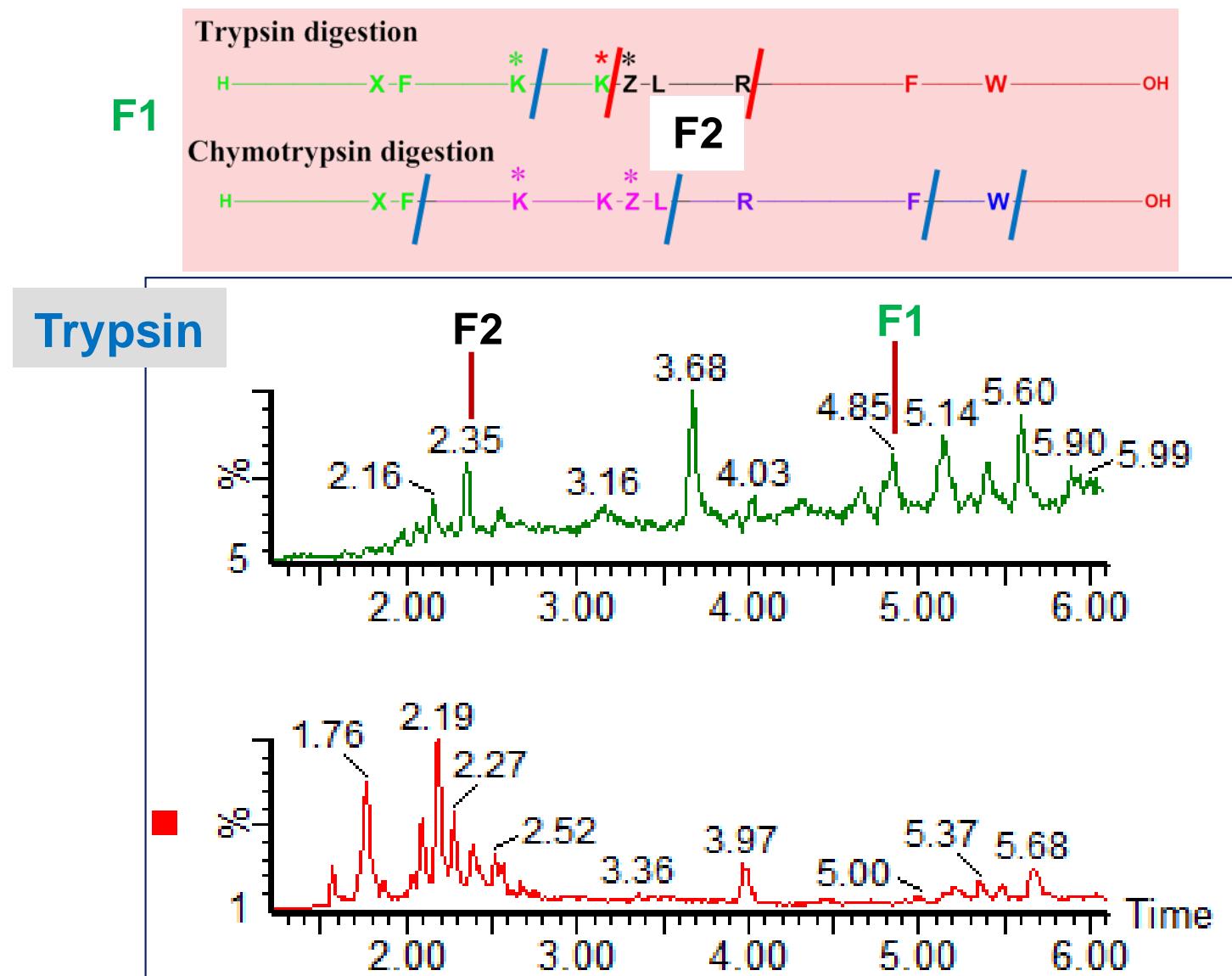
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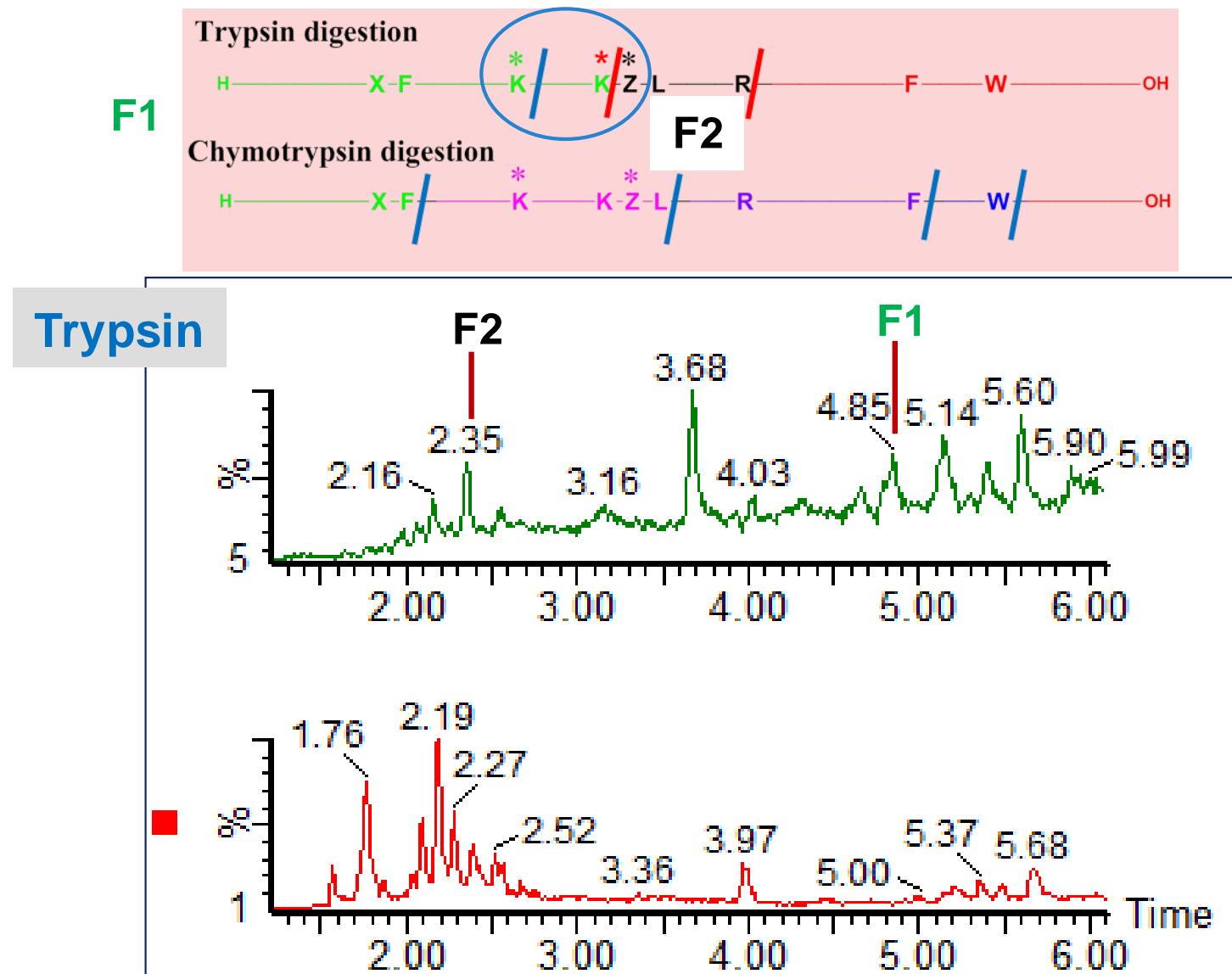
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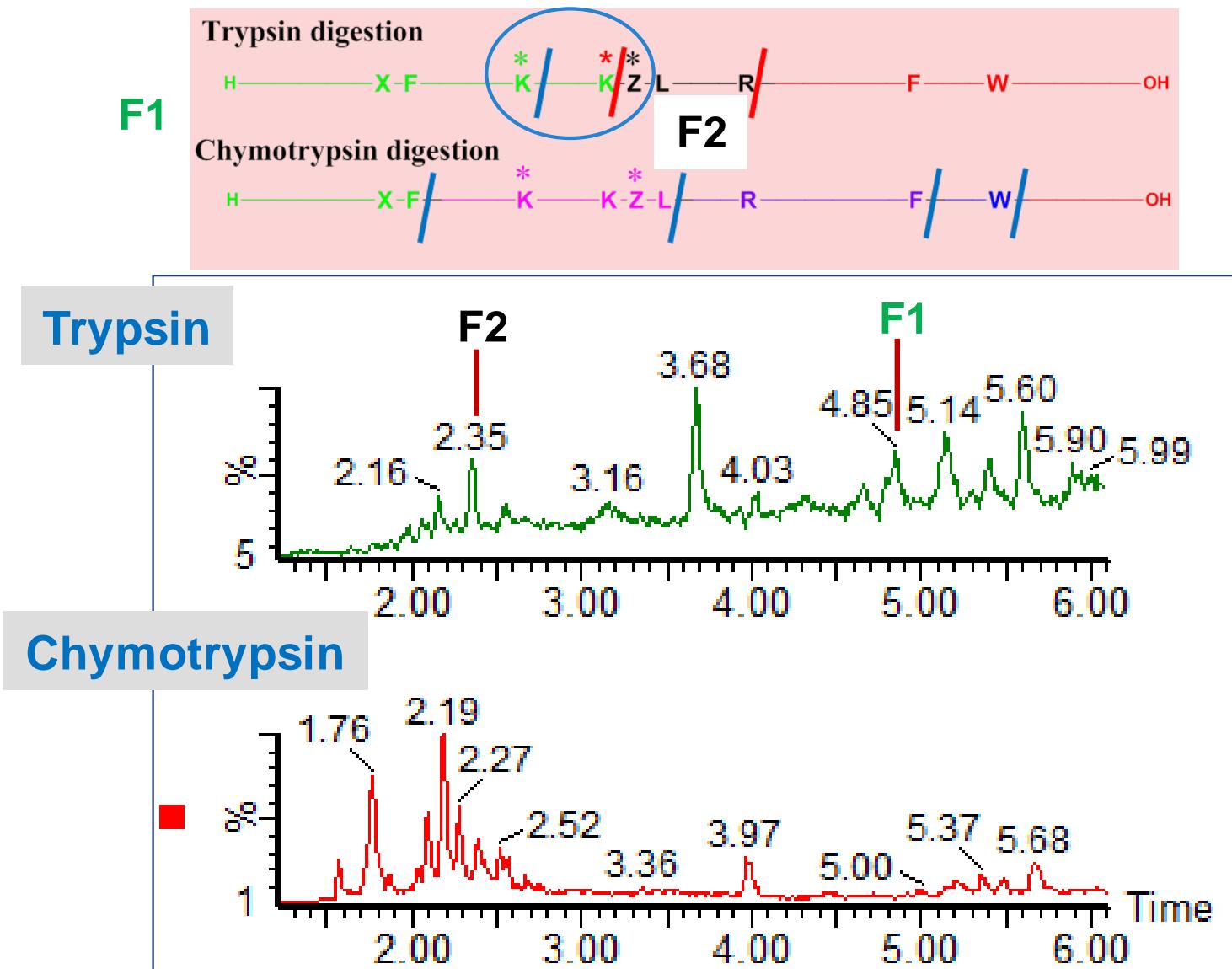
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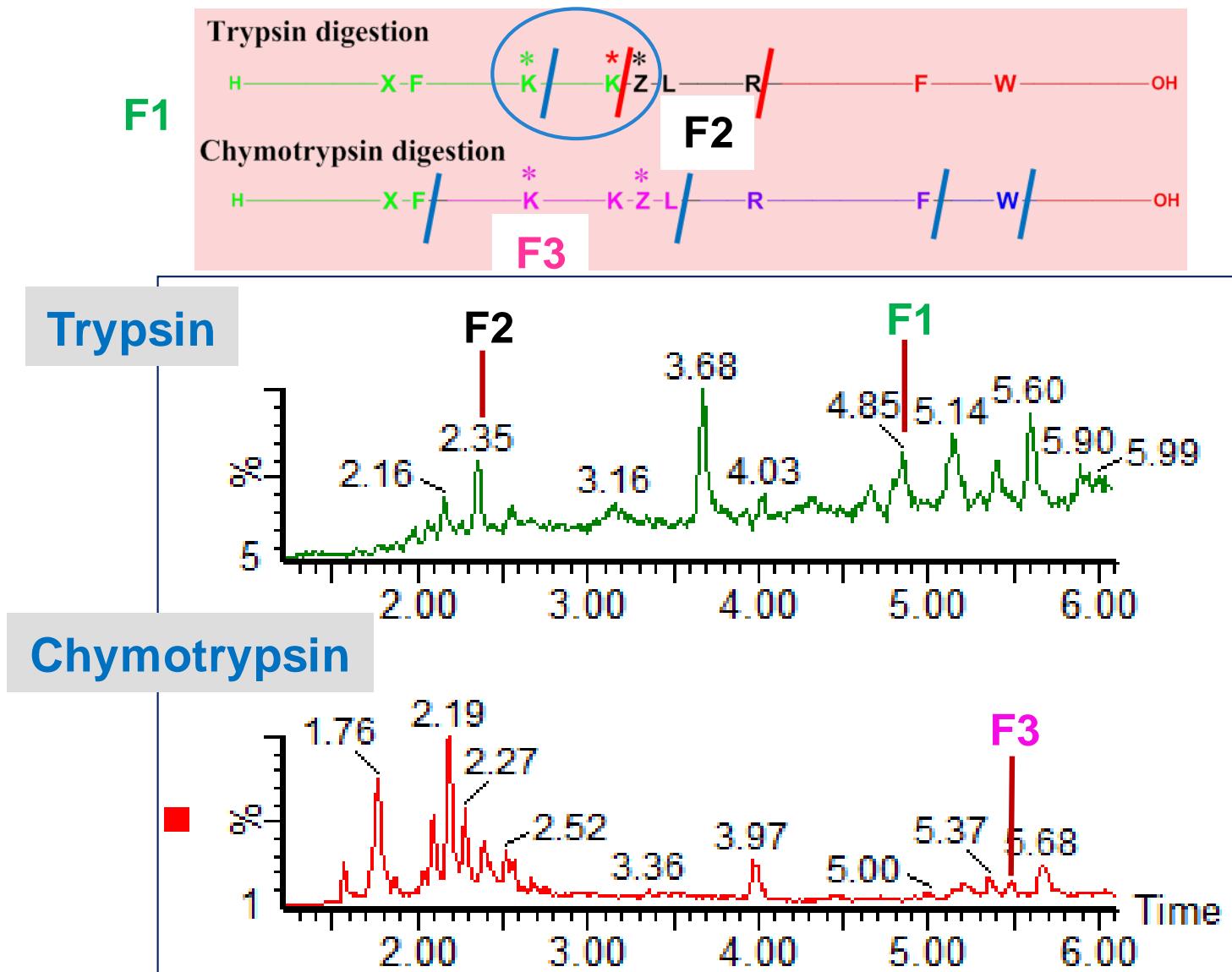
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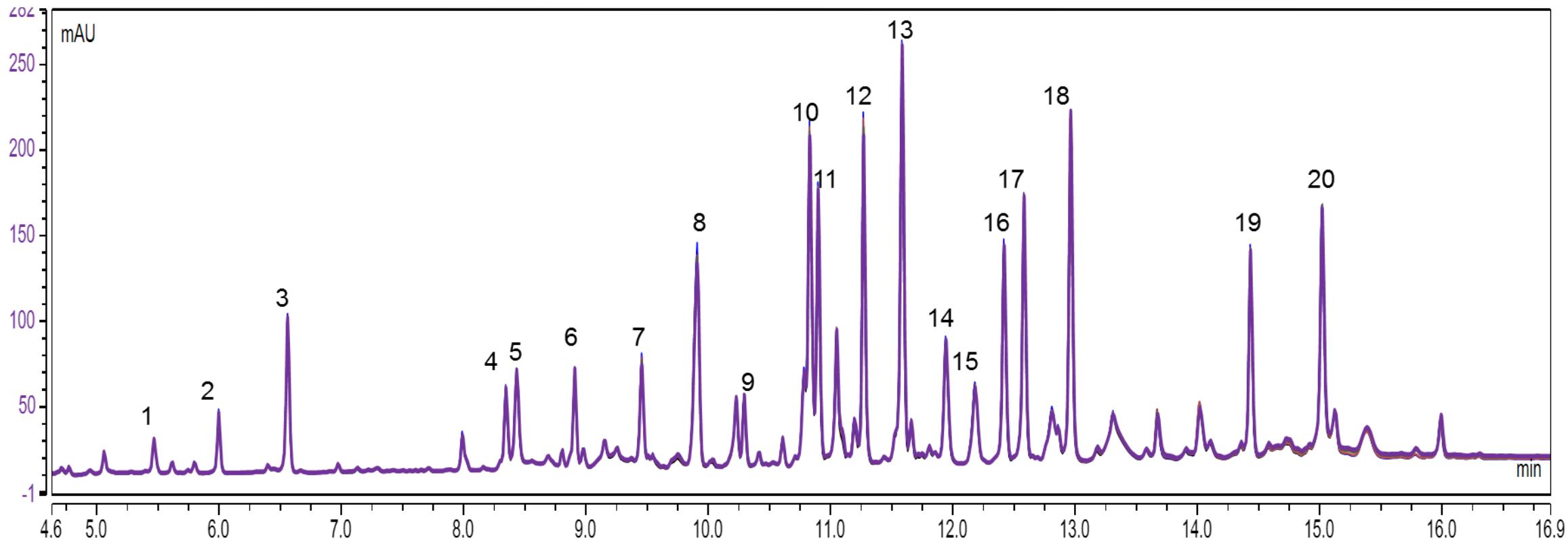
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You have the theory now for the real data!

Reproducibility
and Precision!

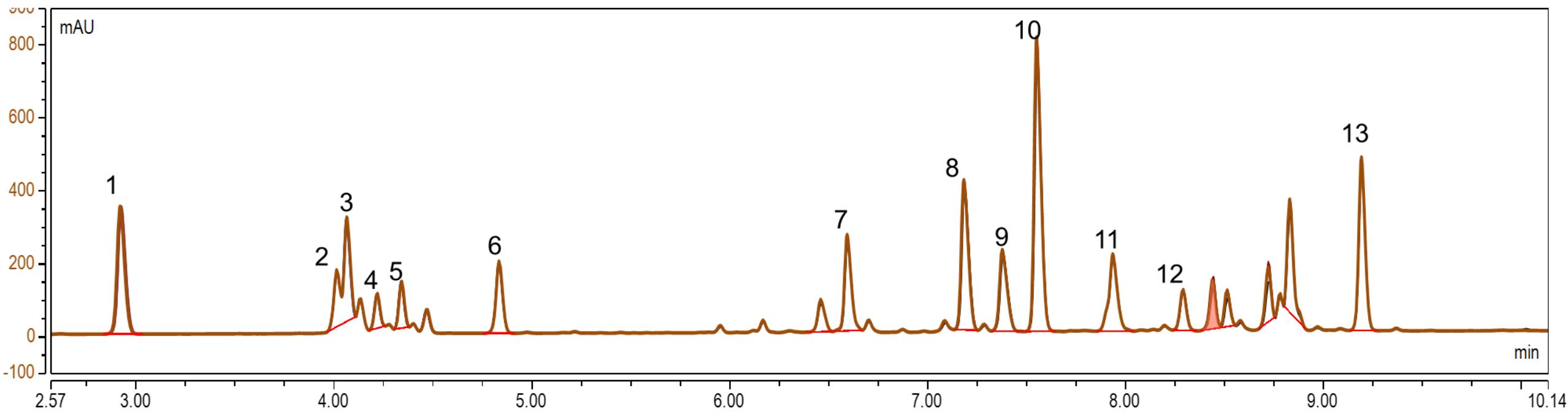
5 different Rituximab Digests by 5 Different Seminar Attendees



Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10
2.54	2.41	1.89	3.39	3.53	2.16	4.41	2.10	2.10	3.65

Peak 11	Peak 12	Peak 13	Peak 14	Peak 15	Peak 16	Peak 17	Peak 18	Peak 19	Peak 20
1.96	3.51	3.72	2.26	2.91	1.97	3.28	2.62	3.16	1.20

5 Cytochrome C digests with Magnetic SMART and the Kingfisher

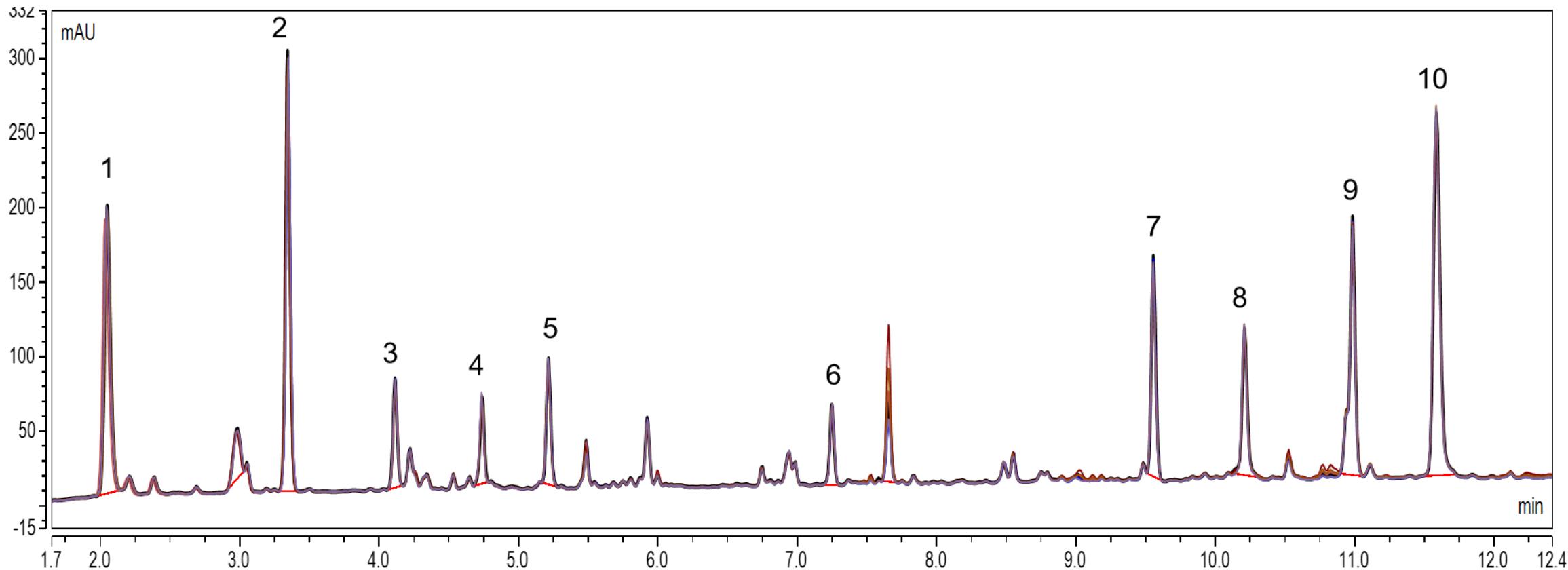


Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10
2.75	1.87	2.45	0.71	1.27	1.9	3.6	2.09	2.35	3.92
0.12	0.03	0.05	0.04	0.03	0.02	0.02	0.03	0.03	0.02

Peak 11	Peak 12	Peak 13
1.11	0.72	2.42
0.01	0.01	0.01

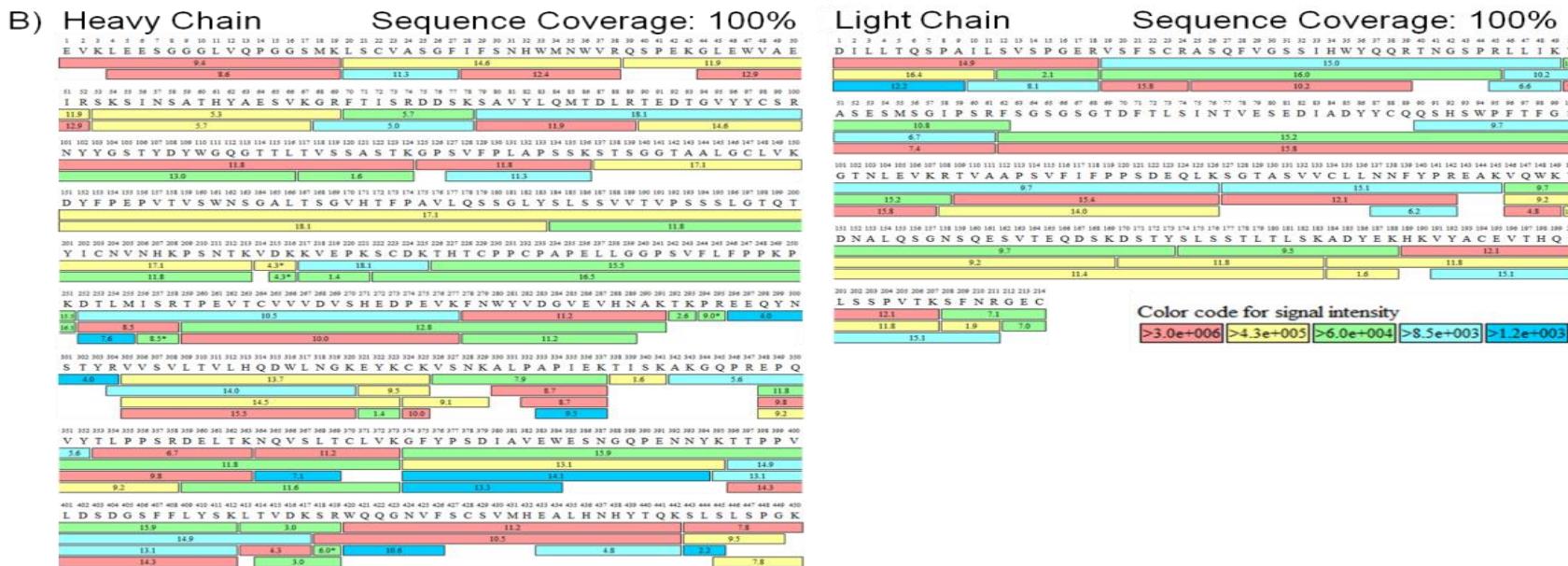
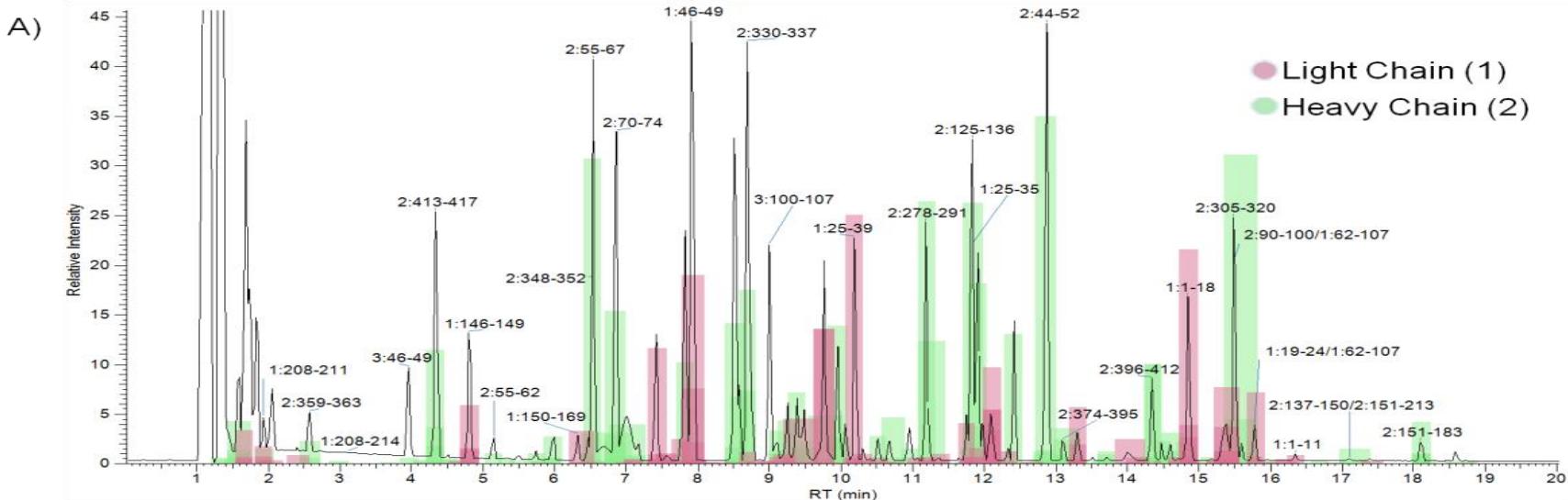
Peak areas and retention times are automated and reproducible with the Magnetic SMART, Kingfisher and Vanquish UHPLC

6 Carbonic Anhydrase Digests – Kingfisher SMART

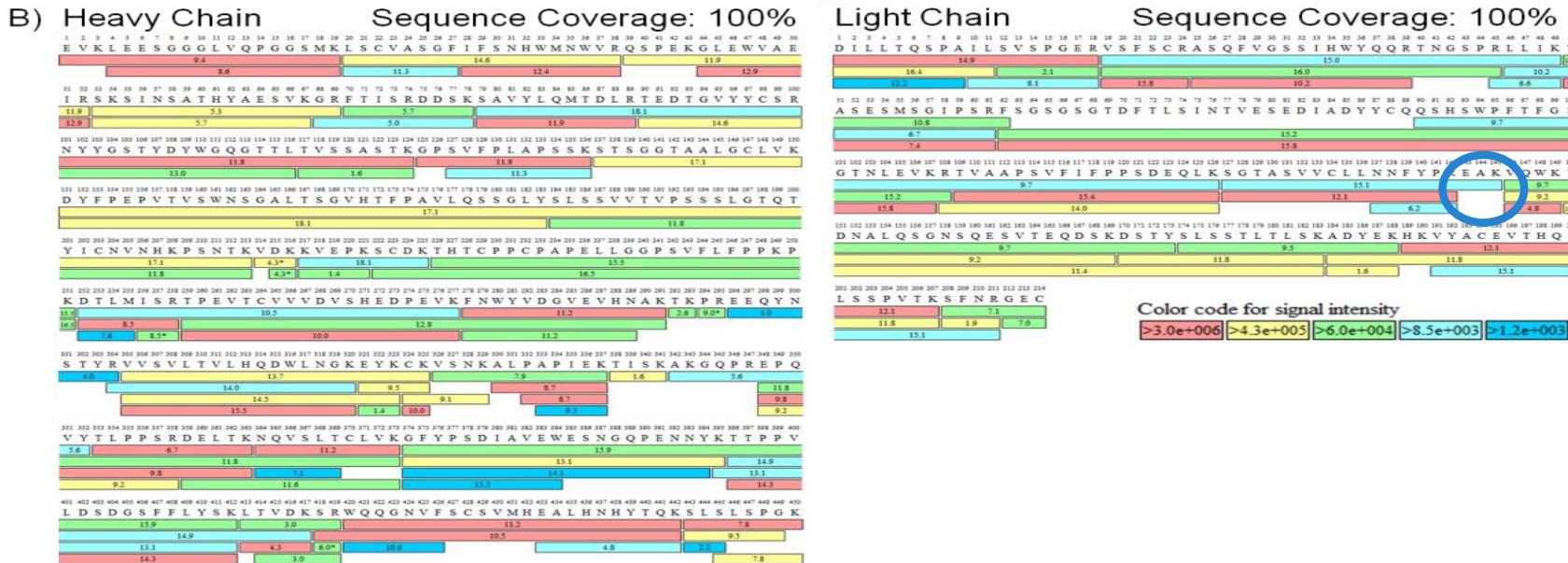
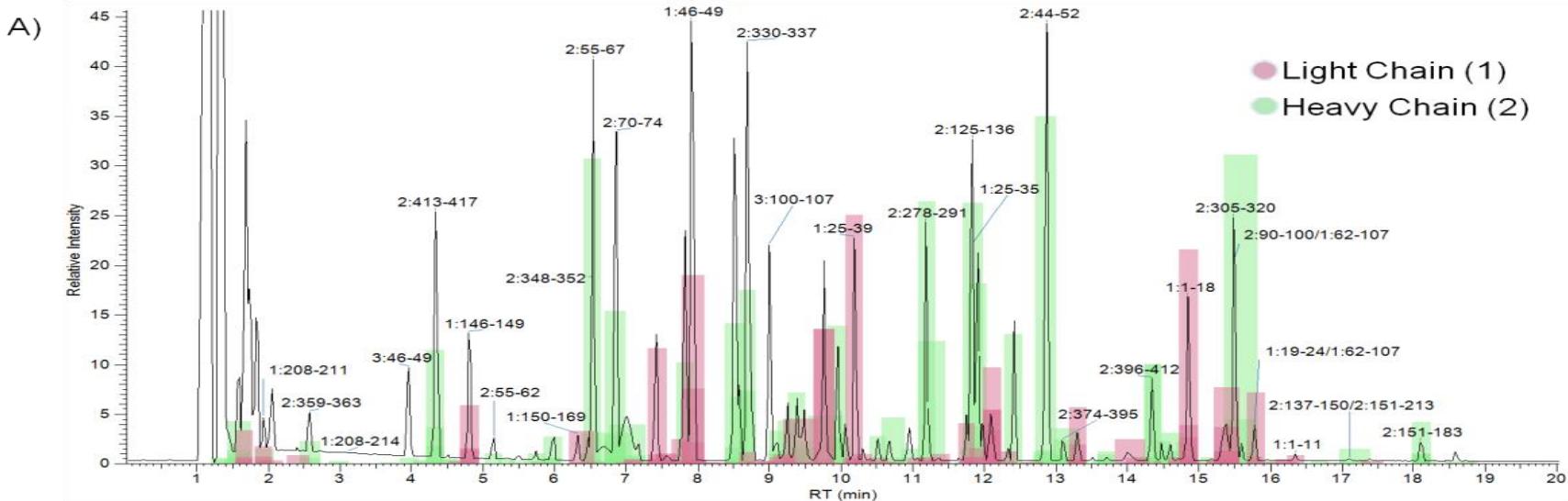


Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10
2.12	1.46	1.53	3.03	1.71	1.26	1.51	2.6	1.14	1.64
0.32	0.1	0.03	0.04	0.02	0.02	0.01	0.01	0.01	0.01

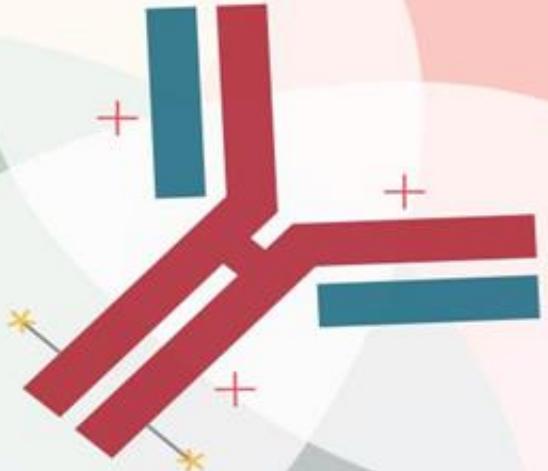
Sequence Coverage Map from Infliximab using the Magnetic SMART Digest



Sequence Coverage Map from Infliximab using the Magnetic SMART Digest



Model Therapeutic protein

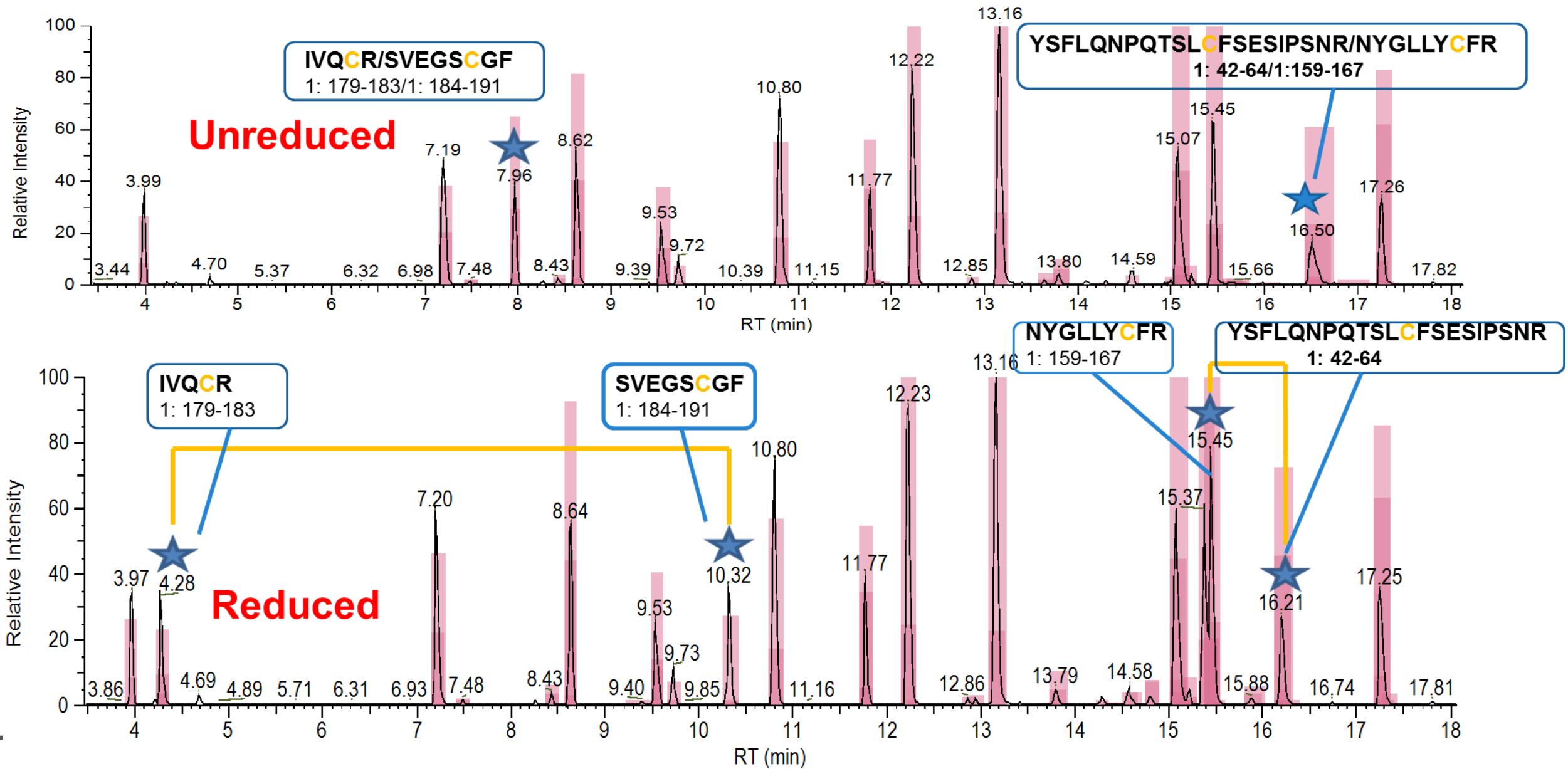


ThermoFisher
SCIENTIFIC

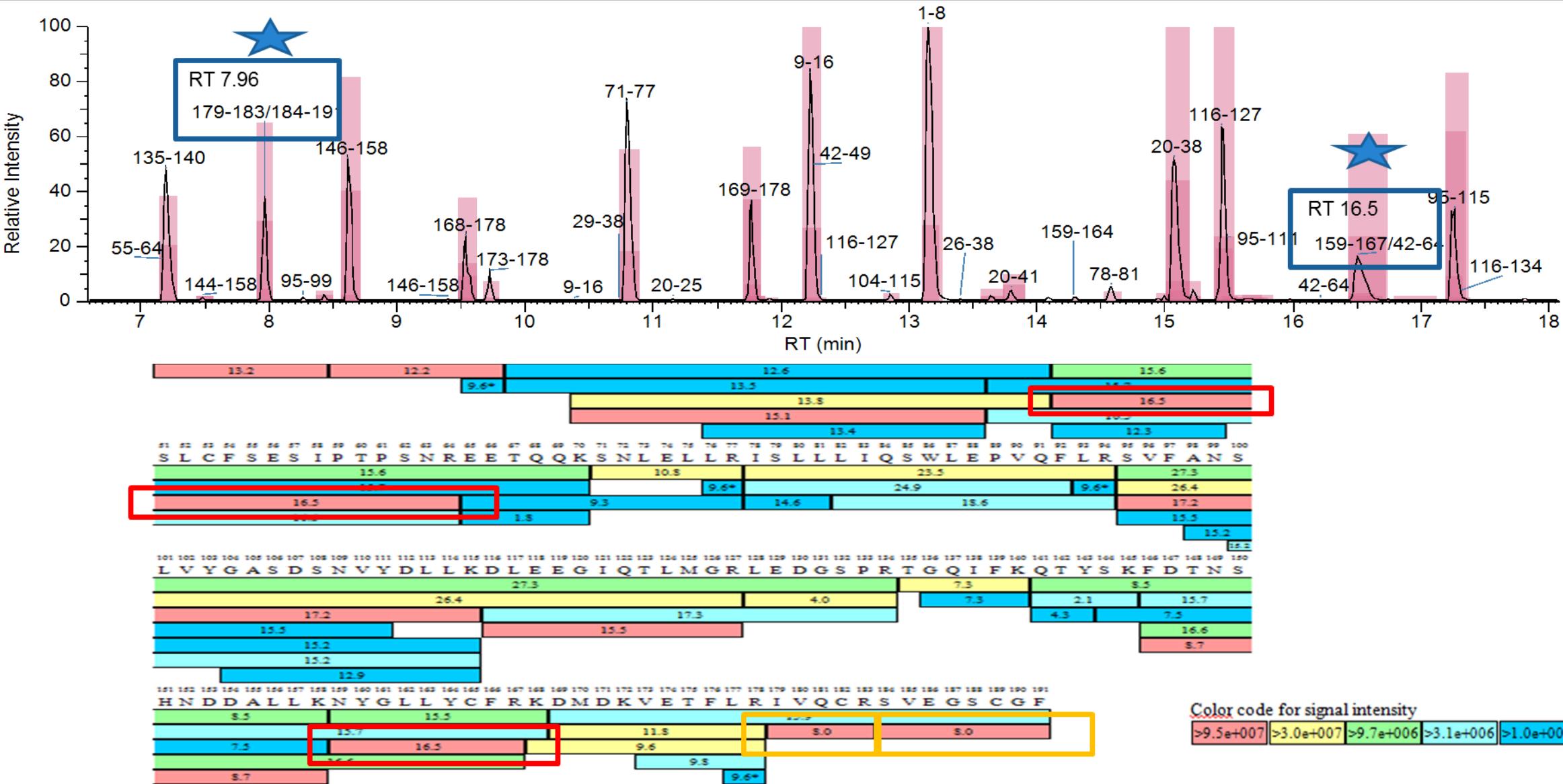
Somatotropin Sequence

FPTIPLSRLF DNAMLRAHRL HQLA^DTYQE FEEAYIP^KEQ KYSFLQNPQT SLC^CFSESIPT PSN^REETQQK
SNLELLRISL LLIQS^WLEPV QFLR^SVFANS LVYGASDSNV YDLL^KDLEEG IQTLMGRLED GS^PRTGQIFK
QTYSKFDTNS HNDDALLKNY GLLYCFRKDM DKVETFLRIV QCR^SVEGSCG F

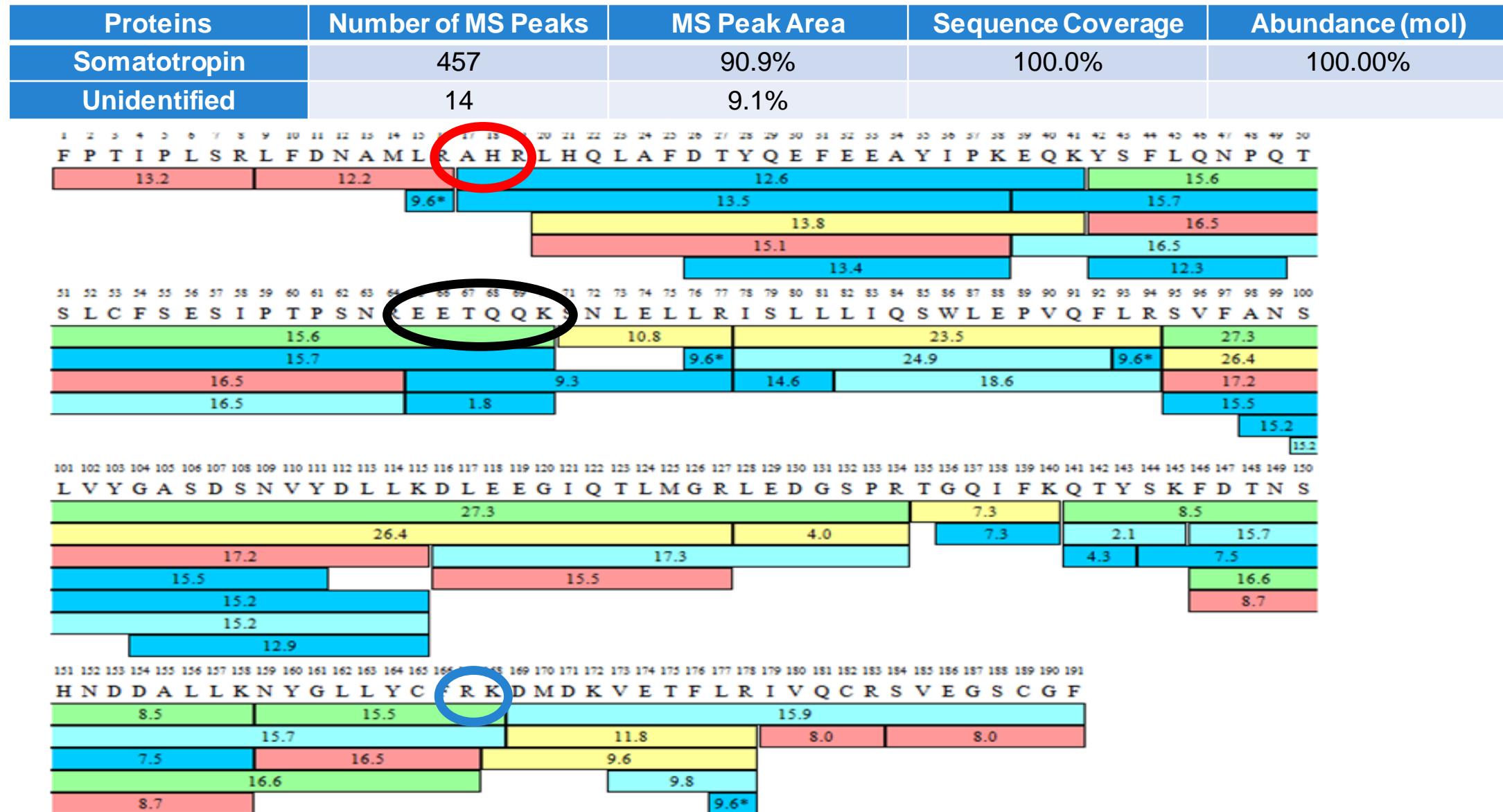
Effect of Reduction with TCEP after the Digestion



Finding the Disulphide Linkages with BioPharma Finder Software

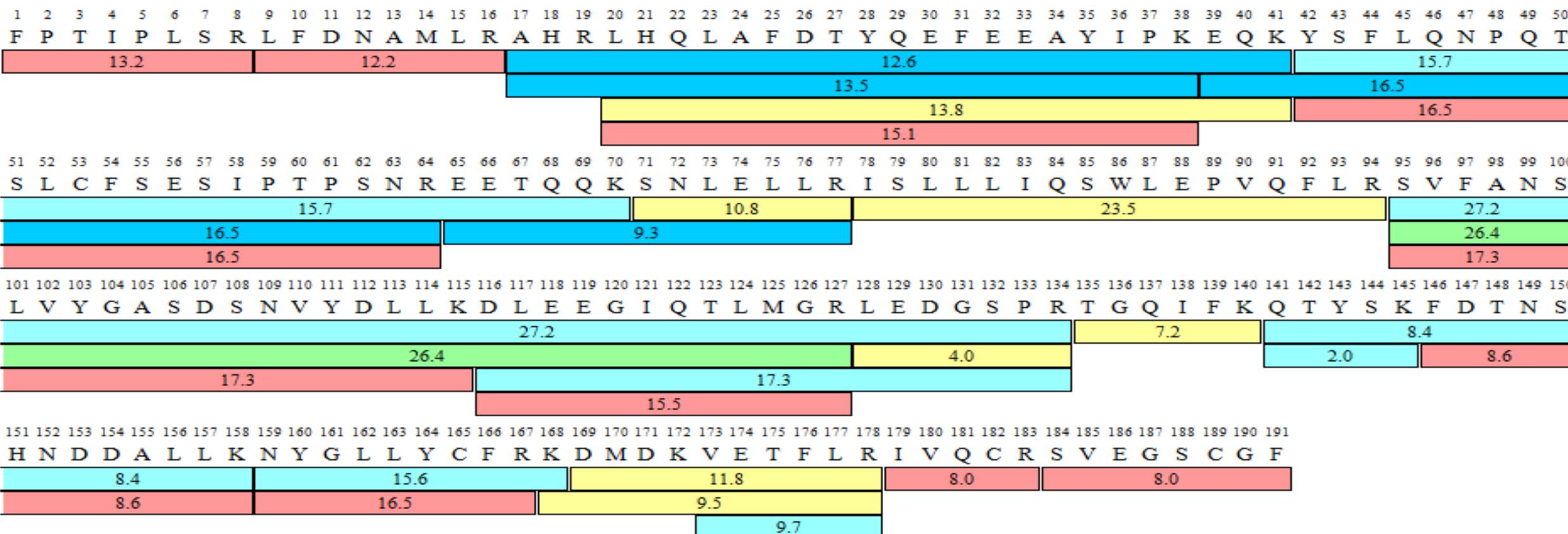


How to Achieve 100% Sequence Coverage



MS Only Data Acquisition

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
Somatotropin	205	89.6%	100.0%	100.00%
Unidentified	4	10.4%		



Chemical Modifications Present in Proteins

Position	Sequence	Retention time [Mod/Native]	Modification	% 5 min	% 15 min
N149	FDTNSHNDALLK	9.43 / 8.68	Deamidation	1.05	1.64
M170	DMDKVETFLR	9.58 / 10.31	Oxidation	0.59	0.60
M14	LFDNAMLR	10.41 / 12.28	Oxidation	1.52	1.34
M125	DLEEGIQTLMGR	12.40 / 15.48	Oxidation	0.18	0.21
D130	LEDGSPR	3.95 / 4.06	Isomerization	0.18	0.55

Chemical Modifications Present in Proteins

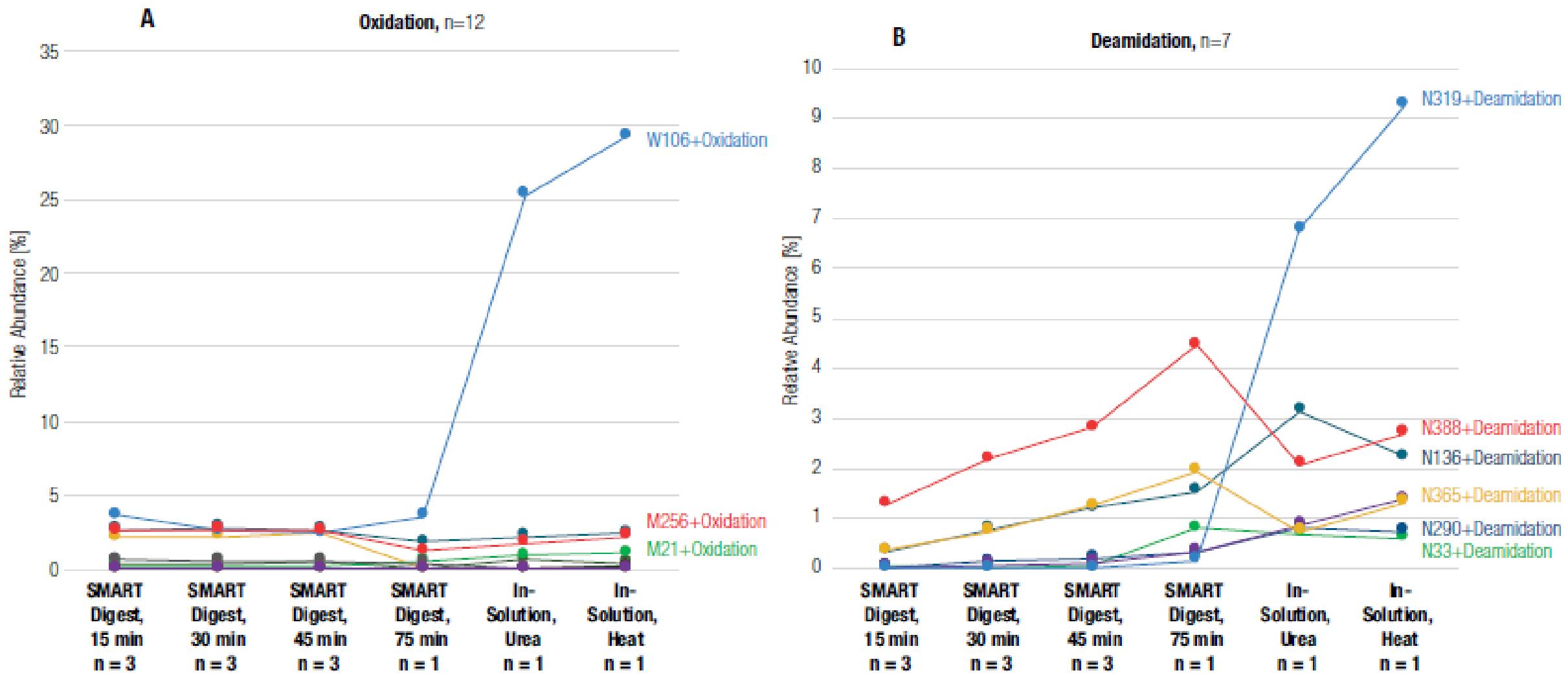


Figure 6. Relative abundance of 12 identified oxidations (A) and 7 deamidations (B) in different runs with various digestion methods.

Chemical Modifications Present in Proteins

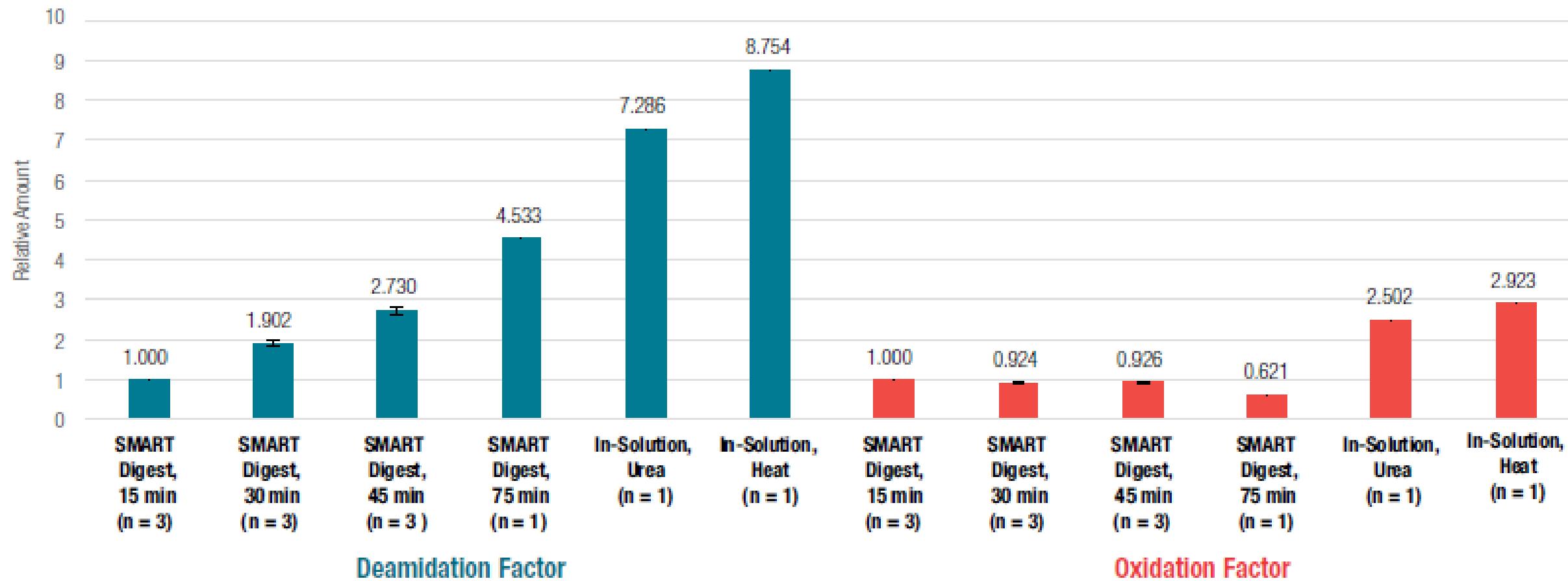
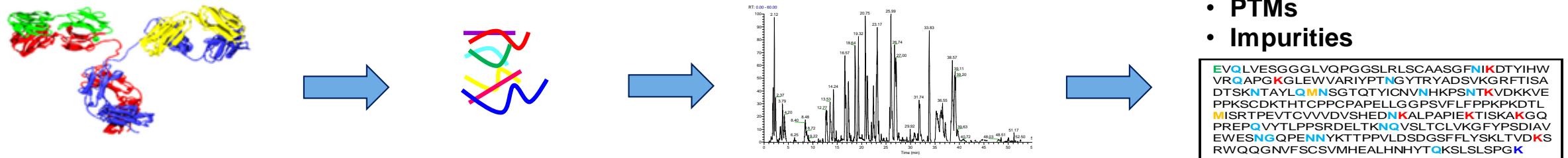


Figure 7. Relative amount of total deamidation and oxidation modifications measured for the six different digest conditions (Normalization to SMART Digest, 15 min).

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

Peptide Mapping of Therapeutic Monoclonal Antibodies



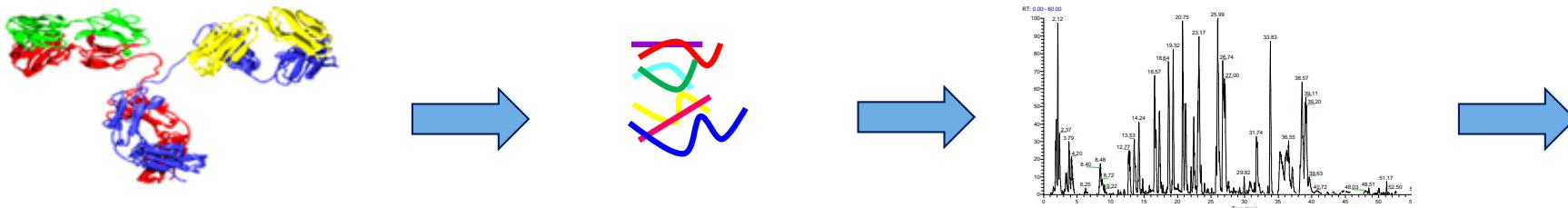
Sequence/PTMs unknown or needs confirming



Peptide identification by MS and MS/MS

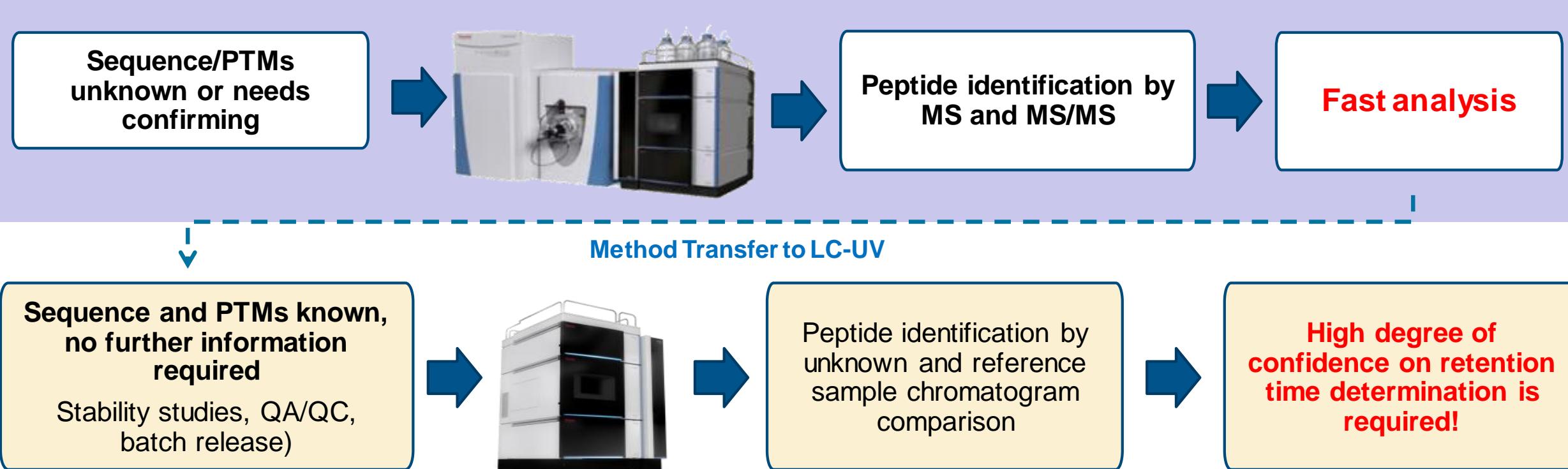
Fast analysis

Peptide Mapping of Therapeutic Monoclonal Antibodies

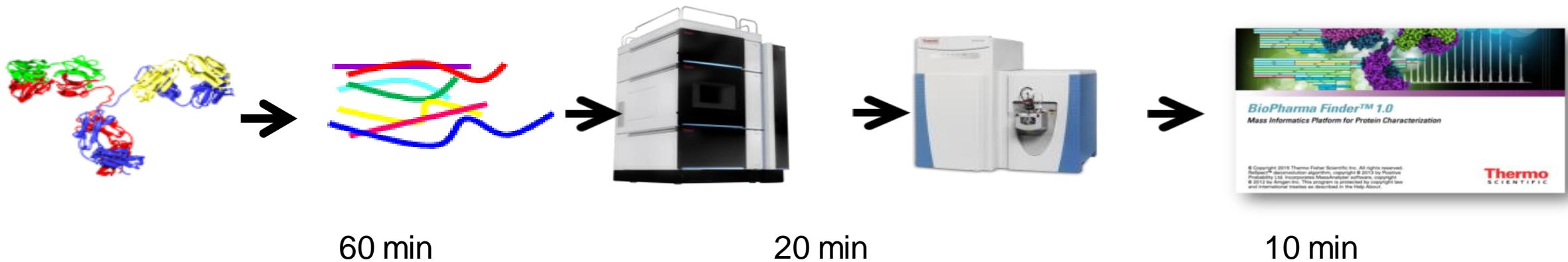


- Peptide Map
 - PTMs
 - Impurities

**EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHW
VRQAPKG**K**LEWVARYPIPT**N**GYTRYADSVKRFTISA
DTSKNTAY**L**QMN**S**GQT**T**YICNVN**H**NKPSNT**V**KDKVKE
PPKSCDKTH**T**HTCPCPAPELLG**G**PSVFLPPKPKD**T**
MISRTPEVTCV**V**DVSHED**N**KLAPAPI**E**KTISAK**G**
PREP**V**QYTLPSSR**D**ELTK**N**QVSLTCLVK**G**FYPSDIAV
EWES**N**QPENNY**K**TPV**P**VLDS**N**QFV**F**FLSKLTVD**K**
RWQQGN**V**FCS**V**MEHALHN**Y**T**Q**KSLS**S**SPG**K****



Overview of the Peptide Mapping Workflow

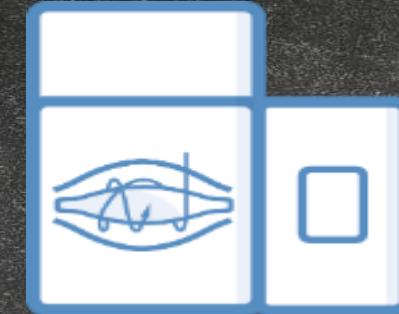
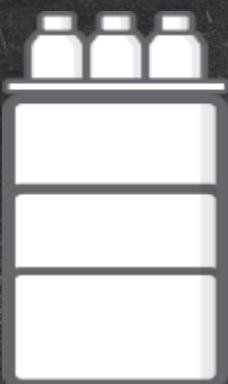
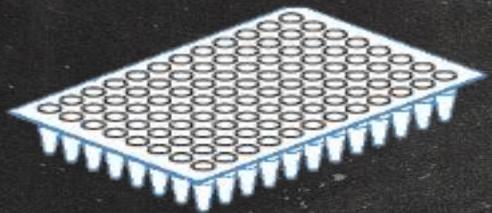


Combining all the components of this Peptide Mapping work flow a complete analysis can be done in less than 90 minutes.

Can be set up and verified by MS very quickly.

A relatively inexperienced analyst can obtain reproducible results from UV with simple sample preparation and walk up UHPLC analysis.

Our Workflow Solution Gives You....



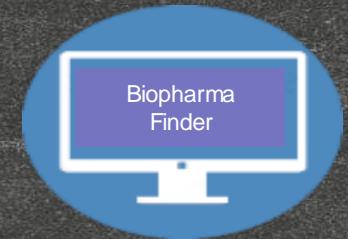
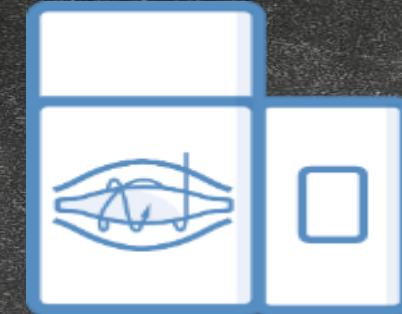
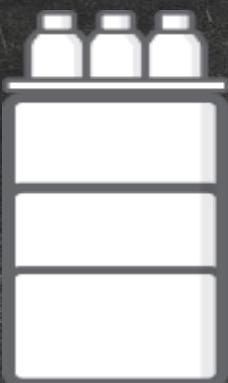
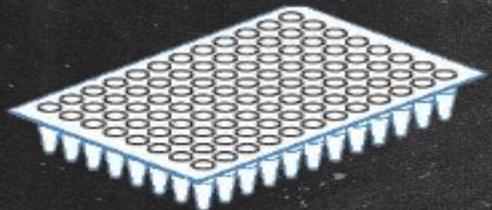
Hugely better reproducibility and robustness!

Massive time and labour savings!

Better sequence coverage!

More integrated software!

Our Workflow Solution Gives You....



Hugely better reproducibility and robustness!

Massive time and labour savings!

Better sequence coverage!

More integrated software!

- * Unique automated Digestion
- * Class leading chromatography and UHPLC
- * Highest resolution, highest sensitivity Orbitrap MS systems
- * Integrated Industry specific Software

Summary

- Game changing automation of protein digestion for ultimate precision and reproducibility
- Robust and highest reproducibility UHPLC system for peptide mapping
- Most sensitive and highest resolution mass spectrometer with the Q Exactive
- Industry specific BioPharma Finder software
- Complete workflow enables ease of use and time/cost savings
- Easy method transfer and reduced need for highly skilled operators

