



**Thermo Scientific**  
Peptide Mapping Workflows

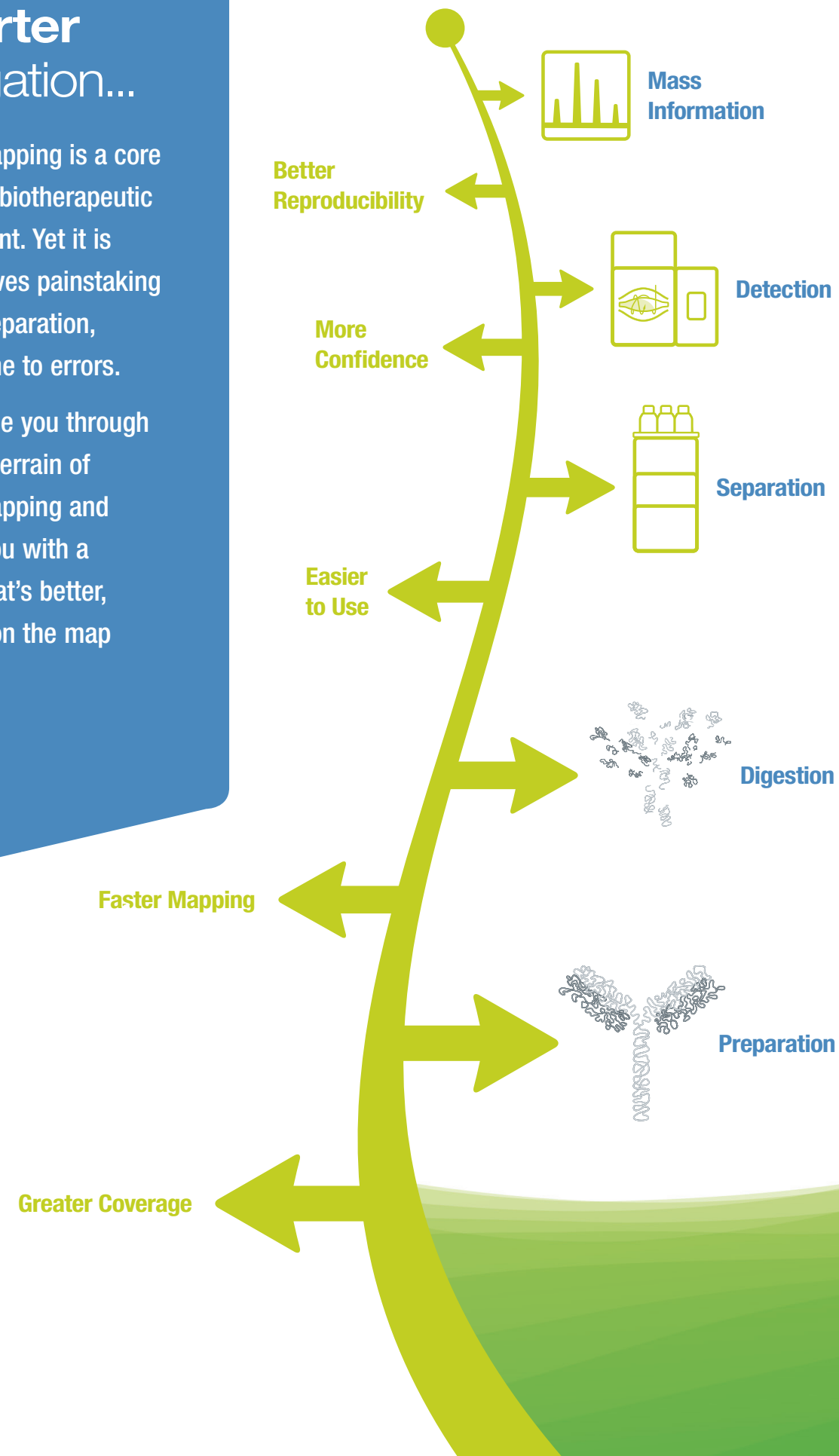
# Upgrade Your Maps

Fast, confident and more reliable peptide mapping.

## Smarter Navigation...

Peptide mapping is a core analytic in biopharmaceutical development. Yet it is slow, involves painstaking sample preparation, and is prone to errors.

Let us guide you through the rough terrain of peptide mapping and upgrade you with a solution that's better, wherever on the map you are.



We're ready to upgrade your peptide mapping capabilities. Thermo Scientific™ SMART Digest™ kits give you more confident and reproducible digestion. Thermo Scientific™ Vanquish™ Flex UHPLC speeds up your mapping, whilst giving you better retention time stability. Our range of sub-2µm and solid core particle LC columns including the Thermo Scientific™ Acclaim™ and Accucore™ ranges ensure fast, reliable separations. Thermo Scientific™ BioPharma Finder™ software is easier to use, and ensures you get the greater coverage you demand. Powerful, innovative new solutions for simply better peptide mapping.

- Do you need greater **reproducibility**?
- Do you want **faster** peptide navigation?
- How about greater **sequence coverage**?
- A simple, **easy to use** solution?
- More **confidence** in your peptide assignment?

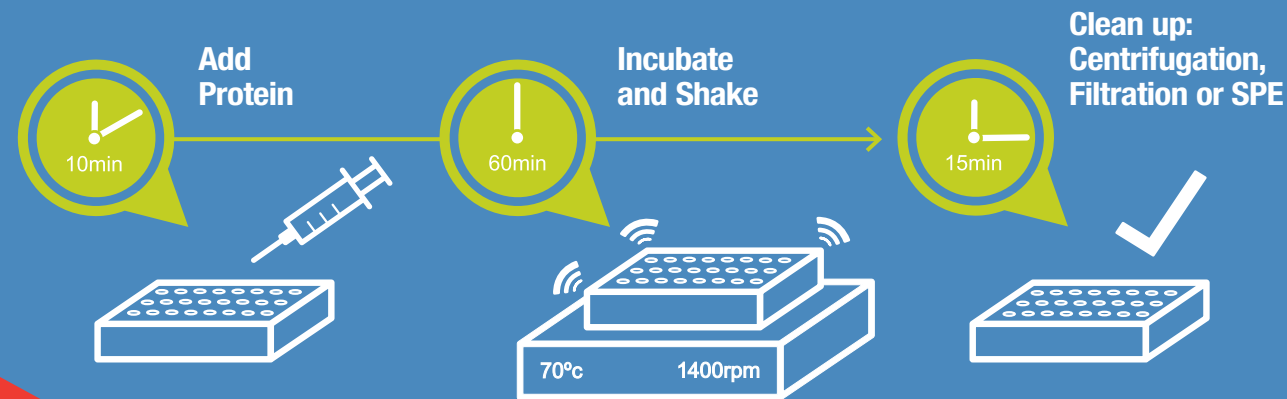
...then let us be your guide to simply better mapping.



# Digestion

Sample preparation for peptide mapping, such as by tryptic digestion, is laborious, difficult, and prone to error. Not only that, it's not always consistent – different

analysts can produce different results. You need confidence in your digestion, that's where SMART Digest Kits come in.



In Solution Digestion, Typically 24 hours

SMART Digestion, Typically 1 hour

More Confidence

Easier to Use

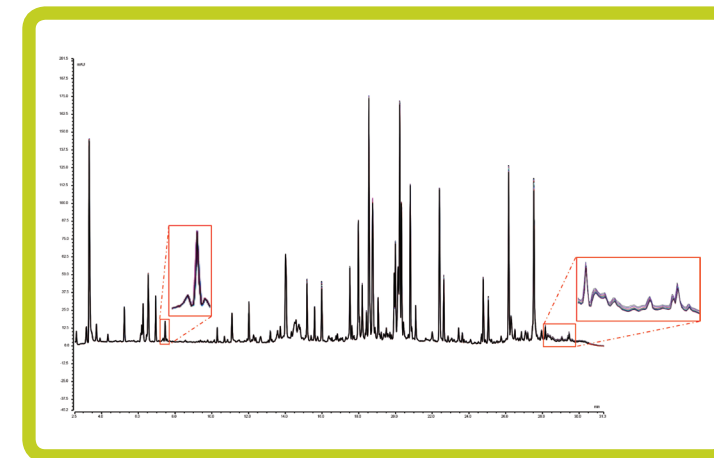
**SMART Digest Kits** are an innovative way of rapidly and reproducibly digesting proteins into their constituent peptides, ready for characterization. The process is simplicity itself: Firstly add the protein and a buffer to the SMART Digest tube. Then incubate and shake, typically for about an hour. You can then optionally centrifuge or filter, and you're done!

Proteins are heat-denatured for digestion, so denaturing agents and reduction/alkylation steps are not needed. It's a high throughput technique that can be readily automated. The kit comes as 12 x 8 stripe digest tubes in PCR format or in bulk format for use with 96 well plates. Optionally a Thermo Scientific™ SOLAp™ Solid Phase Extraction (SPE) Plate can be included for use with more complex plasma-based sample sets. Reproducibility is typically significantly better than that of traditional tryptic protein digestion and often with increased sensitivity.

# SMART Digest Kits

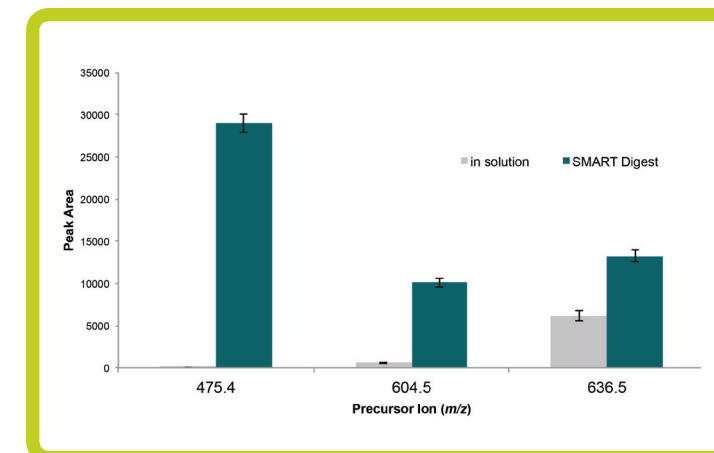


“Amazingly stable”  
David Goodsell,  
Protein Data Bank



## Better Reproducibility

Overlay of 13 consecutive chromatographic runs of a peptide sample separated on an analytical Thermo Scientific™ Acclaim™ 120 C18 column (RP18, 2.2 μm, 2.1 x 250 mm) and prepared from a mAb, digested with the SMART Digest kit.



## Better Sensitivity

Measurement of serum Thyroglobulin after tryptic digestion of serum samples.

- SMART Digest: 25% plasma, 3.5 h digestion
- In-solution digest: 20% plasma, R/A, 4 + 16 h digestion

For in-solution protocol, see for example: Clarke et al. (2012), J. Investigative Medicine, 60(8)

# Separation

The characterization of biotherapeutics places rigorous demands on liquid chromatography systems. Biocompatibility, reproducibility and high performance are necessary. However, it's also important for an LC system to offer flexibility and ease of use. Vanquish Flex UHPLC has all of this and more.

More Confidence

Faster Mapping

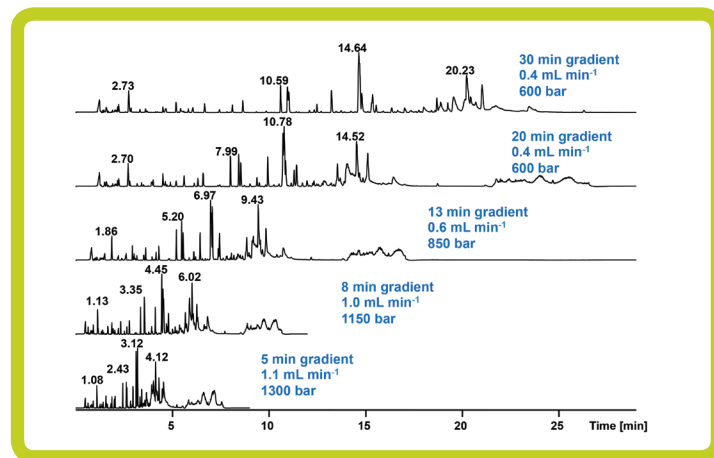
## What about the Choice of Column?

For perfect peptide mapping, the LC column needs to produce excellent resolution and reliable reproducibility. The Acclaim RSLC 120 C18 column is ideal for the job, and is our recommended LC column of choice for peptide mapping applications.



## Faster Mapping

Total ion chromatograms obtained from peptide mapping experiments of rituximab applying gradient lengths from 30 to 5 minutes.

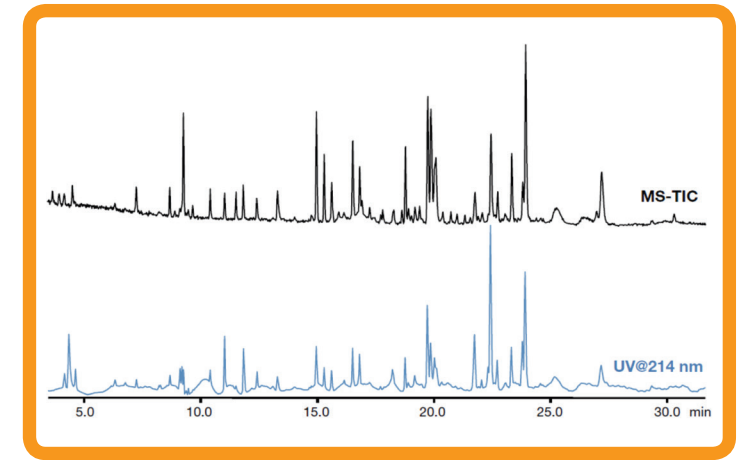


# Vanquish Flex UHPLC

## Easy Transfer from LC-MS to LC-UV

Peptide mapping is typically done using mass spectrometric detection. However, for certain batch testing and QA/QC applications, analysts are increasingly turning to LC-UV, a simpler and faster technique. This relies on LC performance that is high enough to provide data of sufficient quality.

In the data shown right, mass spectrometric data is compared to LC-UV data. The Vanquish Flex has a highly stable flow delivery as well as sample pre-compression, and this results in excellent retention time precision and increased confidence in the peak assignment. The data comparison clearly shows good confirmation between the LC-MS data (upper trace) and the LC-UV data (lower trace). Vanquish Flex is therefore extremely well suited to being used in a routine LC-UV peptide mapping in a quality control environment.



Overlaid chromatograms of the total ion current (TIC, upper trace), measured using LC-MS and the UV trace at 214 nm (measured using Vanquish Flex UHPLC, lower trace) of a SMART Digest Kit digested rituximab sample.



- Sample pre-compression for excellent retention time stability and column robustness
- Extremely high pressure flow path to ensure high peak capacity and peak area precision
- Easy integration with MS
- Multiple detector options

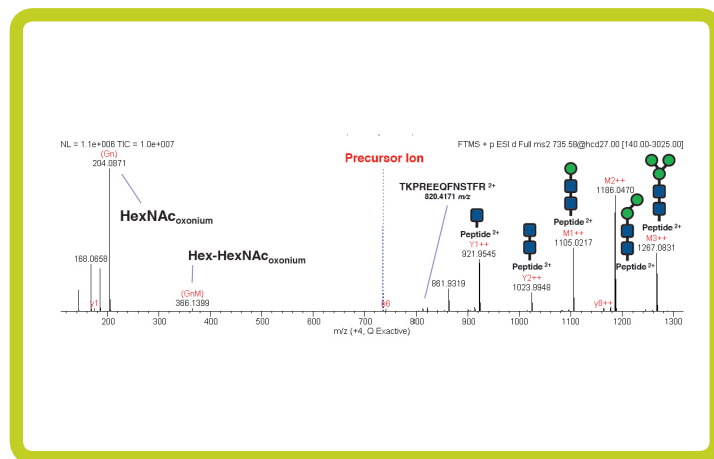


# Mass Informatics

The collation, interpretation and deconvolution of peptide mapping mass spectrometry data is as important as the analysis. You need a software package that allows the identification of all peptides, gives confirmation of the amino acid sequence, identifies the site and type of any modifications and maps the disulfide bonds. Welcome to BioPharma Finder.

## What about Mass Spectrometry?

Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometers are ideal for peptide mapping applications where maximum characterization is required and full sequence information needed. It is extremely accurate, typically identifying component compounds to within plus minus 3ppm. Furthermore, it is linear across four and a half orders of magnitude, proving ideal for complex and varied peptide mixtures.

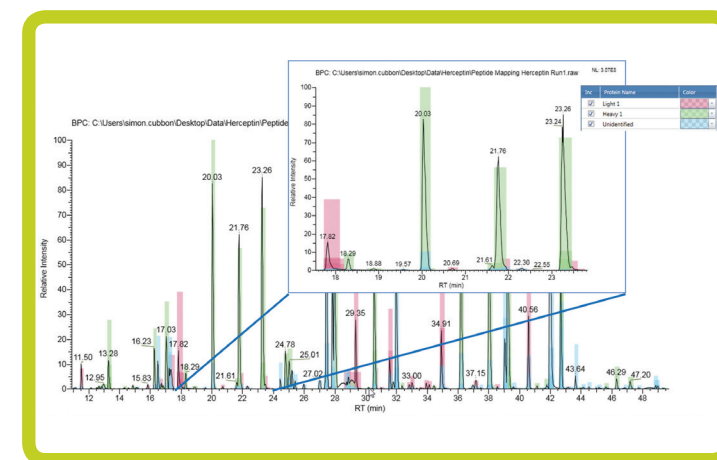
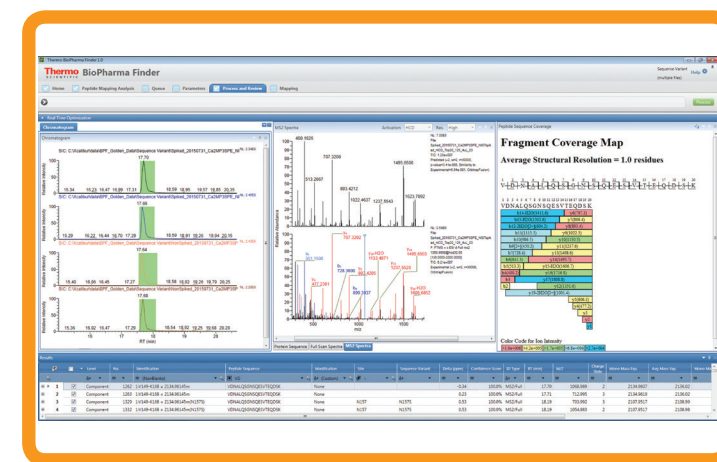


MS/MS spectra of the glyco-peptide aa 290-302 (TKPREEQFN\*STFR, \*=AG20), as measured using Vanquish Flex UHPLC - Q Exactive HF MS and detected with BioPharma Finder.

# BioPharma Finder Software

100% coverage peptide mapping and intact protein characterization in one package

The ability to perform Sequence Variant Searching, disulfide link analysis, de novo sequencing with quantitation of post-translational modifications assures you won't miss anything. Multiple interactive plot display options mean you can see what you want, when you need it.



“...it's **everything we need** from our biopharma software”

BioPharma Finder is also an excellent tool for batch-to-batch comparability, essential in routine peptide mapping applications. Interactive visualization tools such as chromatographic shading afford increased efficiency in data mining and interpretation.

- Integrated workflows for intact protein to peptide mapping analysis
- Interactive visualization tools
- Superior deconvolution and peptide identification algorithms

## Smarter Navigation

It's time for an upgrade. The peptide mapping workflow is ready for new technologies, new solutions, and better answers. Let us guide you to a world of better, more confident, and more reproducible peptide mapping.

“ This workflow has made my **mapping much easier**, and frees my lab staff for more valuable tasks

*Leading US Biopharma*

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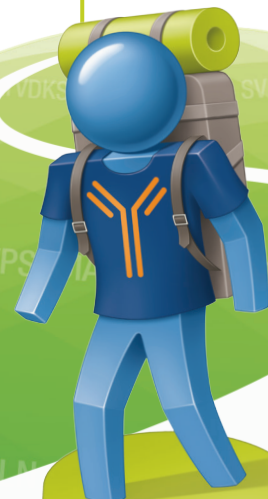
Greater Coverage

Faster Mapping

Easier to Use

More Confidence

Better Reproducibility



## Ease of Use

SMART Digest Kits

## Reproducibility

Acclaim RSLC 120 C18

## Speed

Vanquish Flex UHPLC

## Confidence

Q Exactive Plus Mass Spectrometer

## Coverage

BioPharma Finder Software

“

Being able to sort for sequence variants gives me the **confidence** that I'm not overlooking any

*Shannon Williams, Coherus Biosciences*

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