New, rapid & reproducible peptide mapping workflows

Pharma & Biopharma Tours | 2016
Why Peptide Mapping?

- Essential step in biotherapeutic characterization
- Used from discovery to QC
- Routine… but slow and painstaking
What Are The Pain Points?

- **Time and effort**
  
  *labour intensive, little standardization, slow digestion*

- **Digestion variability**
  
  *different protocols and operators = different results*

- **Lack of reproducibility** – and a lack of data confidence

- **Difficult** – Requires skill from digestion to data handling
We Have Called Our Solution: Upgrade Your Maps!

- Full workflow solution
- Robust and reliable
- Fast and reproducible
- Easy and convenient
- Gives you:
  - Sequence verification
  - Modifications analysis
  - Sequence variants
  - Relative abundance quantification
<table>
<thead>
<tr>
<th>What’s The Goal of Upgrade Your Maps?</th>
<th>Sample preparation, separation, MS detection &amp; data analysis of proteins.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Confirm amino acid sequence with 100% coverage, identify and quantify modifications, identify variants.</td>
</tr>
<tr>
<td>Most Important:</td>
<td>Make it simpler! little training, results within a few hours.</td>
</tr>
</tbody>
</table>
Upgrade Your Maps: Our Workflow Solution

Thermo Scientific™ SMART Digest™ kits 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.
Wide Range of Bioanalytical Characterization Methods

- Protein A capture of IgG
- SEC aggregation screening
- IEC pH-gradient charge variant analysis
- ADC DAR determination
- Intact MAb RP separation
- Digestion and peptide mapping
SMART Digest Kits

From this…

To this…!

- Simple, three step protocol
- Quicker than in-solution digestion
- Reduces operator variability
- Increases reproducibility
- Higher throughput
- Ability to automate
SMART Digest Kits: What Is The Benefit?

What it is?
Highly stable, immobilized trypsin digestion

What is the benefit?
- Proteins are heat-denatured for digestion
- No additional denaturing agents
- No reduction / alkylation required
- No handling of mutagenic substances
- Minimized trypsin autolysis
- Enzyme can be use in excess
SMART Digest Kits: IgG digestion in 45 min

Thermal denaturation of IgG

DSC thermogram of IgG (6 mg/ml; mouse IgG2b) in a 10 mM phosphate buffer pH 8.1; 0.5°C/min

Native IgG Digest Profile
monitoring VVSVLTVLHQDWLNGK

- Heat flow (in μW)
- Temperature (in °C)
- Response (Peak Area)

- 50°C
- 60°C
- 70°C

Digest Time (minutes)
SMART Digest Kits: Outstanding Digestion Reproducibility

Monoclonal antibody SMART digestion by three different analysts

MAb Digest 1

MAb Digest 2

MAb Digest 3
Rituximab Peptides for Disulfide Mapping

Reduced peptides

Reduced

Non- Reduced

Disulfides

WVL: 214 nm
Flow: 400 µl/min
%B: 10.0 %
%C: 0.0 %
mAU
min
Acclaim 120 C18 Column

- High resolution - better protein identification
- 250mm in length
- High loadability - high sensitivity LC/MS
- High column-to-column reproducibility
- 1500 bar Vanquish UHPLC compatible
- Viper™ fittings: robust & easy to install

Viper™ finger tight fitting
Acclaim 120 C18 Column: High Peak Capacity

BSA digest
30min gradient
Acclaim 120 C18 2.2µm 2.1 x 250mm
Peak capacity - 380

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time</th>
<th>RSD (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.486</td>
<td>0.095%</td>
</tr>
<tr>
<td>2</td>
<td>6.852</td>
<td>0.099%</td>
</tr>
<tr>
<td>3</td>
<td>8.078</td>
<td>0.058%</td>
</tr>
<tr>
<td>4</td>
<td>9.691</td>
<td>0.045%</td>
</tr>
<tr>
<td>5</td>
<td>12.099</td>
<td>0.037%</td>
</tr>
<tr>
<td>6</td>
<td>12.715</td>
<td>0.036%</td>
</tr>
<tr>
<td>7</td>
<td>13.481</td>
<td>0.029%</td>
</tr>
<tr>
<td>8</td>
<td>16.336</td>
<td>0.030%</td>
</tr>
<tr>
<td>9</td>
<td>18.065</td>
<td>0.030%</td>
</tr>
<tr>
<td>10</td>
<td>18.996</td>
<td>0.027%</td>
</tr>
<tr>
<td>11</td>
<td>19.251</td>
<td>0.021%</td>
</tr>
<tr>
<td>12</td>
<td>19.968</td>
<td>0.028%</td>
</tr>
<tr>
<td>13</td>
<td>22.288</td>
<td>0.026%</td>
</tr>
<tr>
<td>14</td>
<td>22.815</td>
<td>0.026%</td>
</tr>
<tr>
<td>15</td>
<td>24.917</td>
<td>0.022%</td>
</tr>
</tbody>
</table>

19 repeats overlaid
Vanquish Flex UHPLC System

- Developed for biopharma
- Fully biocompatible flow path
- Powerful detectors – all workflows
- Sample pre-compression for maximum peak retention time reproducibility and column lifetime
- Ceramic valve technology
- Column thermostatting technology with two temperature control modes
- Supports latest column technology
Retention time reproducibility of a peptide separation

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 SMART™ Digest Kit.

<table>
<thead>
<tr>
<th>peak #</th>
<th>RT (min)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.315</td>
<td>0.082</td>
</tr>
<tr>
<td>9</td>
<td>5.231</td>
<td>0.065</td>
</tr>
<tr>
<td>14</td>
<td>6.532</td>
<td>0.017</td>
</tr>
<tr>
<td>15</td>
<td>6.937</td>
<td>0.023</td>
</tr>
<tr>
<td>19</td>
<td>10.290</td>
<td>0.021</td>
</tr>
<tr>
<td>23</td>
<td>12.013</td>
<td>0.012</td>
</tr>
<tr>
<td>31</td>
<td>14.011</td>
<td>0.013</td>
</tr>
<tr>
<td>39</td>
<td>15.177</td>
<td>0.012</td>
</tr>
<tr>
<td>42</td>
<td>15.589</td>
<td>0.010</td>
</tr>
<tr>
<td>51</td>
<td>17.511</td>
<td>0.007</td>
</tr>
<tr>
<td>55</td>
<td>17.969</td>
<td>0.011</td>
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<tr>
<td>61</td>
<td>18.546</td>
<td>0.010</td>
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<tr>
<td>83</td>
<td>20.798</td>
<td>0.010</td>
</tr>
<tr>
<td>85</td>
<td>21.095</td>
<td>0.012</td>
</tr>
<tr>
<td>87</td>
<td>22.386</td>
<td>0.009</td>
</tr>
<tr>
<td>96</td>
<td>24.774</td>
<td>0.012</td>
</tr>
<tr>
<td>103</td>
<td>26.155</td>
<td>0.009</td>
</tr>
<tr>
<td>106</td>
<td>26.155</td>
<td>0.009</td>
</tr>
<tr>
<td>109</td>
<td>27.529</td>
<td>0.010</td>
</tr>
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</table>
Vanquish Flex: Rituximab Analysis With Reducing Gradient

**Standard Method**

- 30 min gradient
- 0.4 mL min⁻¹
- 600 bar

**Acclaim 120 C18 column, 2.1 x 250 mm, 2 - 45% ACN + 0.1% FA, 60 °C**
Vanquish Flex: Rituximab Analysis With Reducing Gradient

**Standard Method**

5 min gradient
1.1 mL min\(^{-1}\)
1300 bar

8 min gradient
1.0 mL min\(^{-1}\)
1150 bar

13 min gradient
0.6 mL min\(^{-1}\)
850 bar

20 min gradient
0.4 mL min\(^{-1}\)
600 bar

30 min gradient
0.4 mL min\(^{-1}\)
600 bar

Acclaim 120 C18 column, 2.1 x 250 mm, 2 - 45% ACN + 0.1% FA, 60 °C
Vanquish Flex: Rituximab Analysis With Reducing Gradient

**Standard Method**

- 30 min gradient
  - 0.4 mL min\(^{-1}\)
  - 600 bar
- 20 min gradient
  - 0.4 mL min\(^{-1}\)
  - 600 bar
- 13 min gradient
  - 0.6 mL min\(^{-1}\)
  - 850 bar
- 8 min gradient
  - 1.0 mL min\(^{-1}\)
  - 1150 bar
- 5 min gradient
  - 1.1 mL min\(^{-1}\)
  - 1300 bar

**Acclaim 120 C18 column**, 2.1 x 250 mm, 2 - 45% ACN + 0.1% FA, 60 °C

**Sequence Coverage**

- 100%
- 100%
- 100%
- 100%
- 100%
Q Exactive Plus Mass Spectrometer

- Ideal for intact proteins and peptides
- Very high accuracy
- High dynamic range
- Gold standard Orbitrap™ detector
- Optional intact protein mode.

High Accuracy

95% of the 1067 identified components are in +/- 3 ppm

High Dynamic range

Components are identified over 4.5 orders of magnitude in intensity.

(0.02% TFA in buffer A and B)
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

ddTopN Method with N=5: MS/MS data-dependent analysis of 5 most abundant peptide ions (red) based on precursors obtained in a full scan (black)
BioPharm 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

NEW!
Data Analysis With BioPharma Finder: Peptide Mapping

- Protein sequence manager stores sequence information for quick use
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- Protein sequence manager stores sequence information for quick use
- Maximum throughput through simple method editor, allowing batch analyses
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  - Powerful modification and results summary
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- Interactive results display allows you to review data how you want
  - Sequence/fragment coverage maps
  - Compare real and predicted spectra
  - Powerful modification and results summary
  - Chromatographic shading – never miss a thing
Sequence Coverage Obtained For 5 Minute Gradient

100% Sequence coverage obtained for light and heavy chain of Rituximab
Peptide Mapping: Method Transfer From LC-MS to LC-UV

Sequence/PTMs unknown or needs confirming → Peptide identification by MS and MS/MS → Fast analysis

Method Transfer to LC-UV

Sequence and PTMs known, no further information required
Stability studies, QA/QC, batch release)

Identification by unknown and reference sample chromatogram comparison

High degree of confidence in retention time is required!
Peptide Mapping Workflow: Time Saving

- Complete analysis can be done in less than 90 minutes
- MS analysis is rapid, stand-alone LC-UV even quicker
- Inexperienced analyst can obtain reproducible results from LC-UV
Thank You – Any Questions?