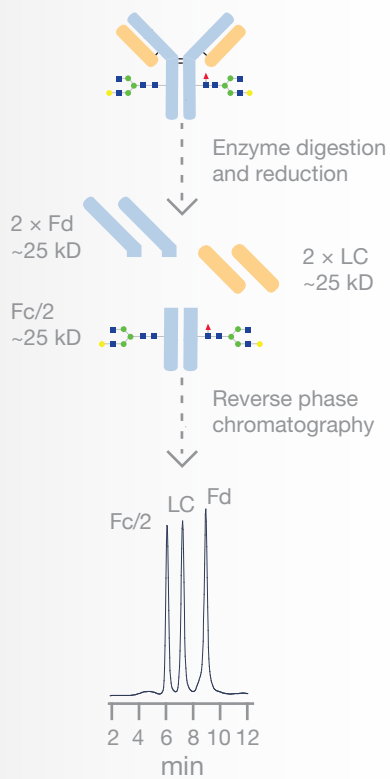


Designed to Fragment the Most Challenging Compounds

New Opportunities for the Characterization of Intact Monoclonal Antibodies

Top-down and middle-down mass spectrometry offer important advantages for the characterization of biotherapeutics with complete molecular specificity. Attaining comprehensive sequence coverage required to confidently verify primary sequence or map post-translational modifications can be challenging. The Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer with UVPD allows for improved characterization of antibodies by generating backbone fragments that complement those obtained with electron and collisional-mediated ion activations.

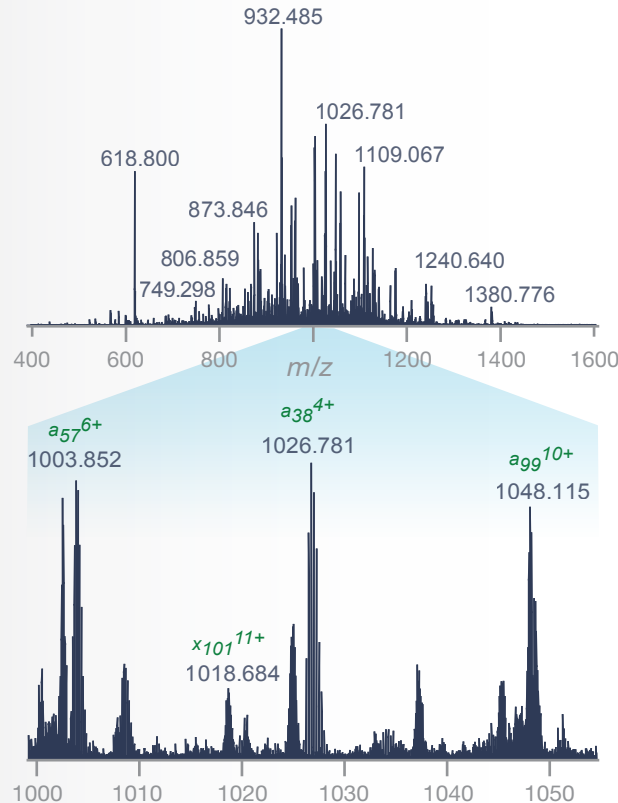
ANTIBODY DIGESTION AND SEPARATION



Every mAb domain is chromatographically separated before UVPD activation.

Fornelli et al., TP 087, ASMS 2017.

HIGH RESOLUTION MS² SPECTRUM OF LIGHT CHAIN (LC)



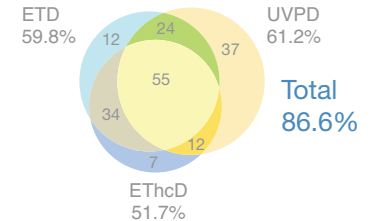
Light Chain UVPD MS² scan was measured at 120,000 FWHM resolution after a UVPD activation time of 8 ms.

SEQUENCE COVERAGE FOR THREE mAb DOMAINS

Fc/2

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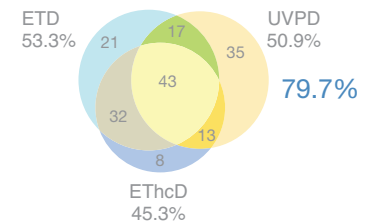
25 N G P S V F L F P P K P K I D I T L M I I S R I T P I E V I T C
26 V I V I D V I S E I D I E V I K I F I N W I Y V D I G V E I V H N
51 A K T K I P R E E Q I Y N I S T Y R I V I V S V L T V L H Q
76 D I W I N I G I K E Y I K I K I V I S I N I K A I L P A I P I E I K I T I I
101 S K I A I G I Q I P R I E I P Q V I Y T I L P I P I S R I D I E L I T K I N
125 Q V S L I T C I L V I K G F I Y I P I S D I I A I V E I W I E I S N G Q I
150 P I E N N Y K I T T I P I P V I L D I S D I G S I F I F L Y S K I L T
175 V D K S I R W I Q I Q I G N I V I F I S I C I S V M H I E A L I H N H Y
200 T Q K I S L S L S P G C
    
```



LC

```

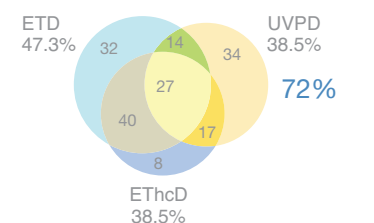
25 N Q I V L S Q S P A I L S I A I S I P G E K V T M T C R I A
26 S I S S I V I S Y I H I W I Q I Q I K I P G S S I P I K I W I I Y A T
51 S I N L I A I S G I V I P V R F I S G S G S G T I S Y S L T I I S
76 R V E A I E D A A T Y I C Q Q I W I T S N I P I P I F I G I G I
101 I T K L E I K I R T I V A I A I P I S V I F I I P I P I S D E I Q I L I K I
125 S I G T I A S I V I V C L L I N H F Y P R E A K V Q W K V D
150 N I A I L I Q I S G N S Q I E S V I T E I Q D S I K I D I S I T I Y I S I L S
175 S I T I L T L S K I A I D I Y E I K I H K V I Y A I C E I V I T H Q G I L
200 S I S I P I V I T I K I S F I N R G E C C
    
```



Fd

```

25 N Q V Q L Q I Q I P I G I A E I L V K I P G A S V K I M S I C K I A S
26 G Y I T I F I T S Y I N M H W I V I K Q T I P G R I G I L E I W I I G A
51 I I Y I P I G I N G D I T M S Y I N Q K I F I K G K A I T I L T A D K S
76 S S I T A Y I M I Q L I S I S L I T S E D S A V Y Y C A R I S T
101 Y Y G G D W I Y F I N V W G I A I G T T V T V S A A I S T K
125 G I P I S V F I P I L A I P S S I K S T I S G I G T A A L G I C L V
150 K D I Y F I P I P I V T V S W N S G A L T S G V H T I F I P
175 A V L I Q S S G L Y I S I L S I S I V I T I V I P I S S I S L G I T Q
200 T I Y I C I N V N H K I P I S N T I K V I D I K K A I E I P I K S C D
225 K T H I T I C I P I P I C I P A I P E L L G C
    
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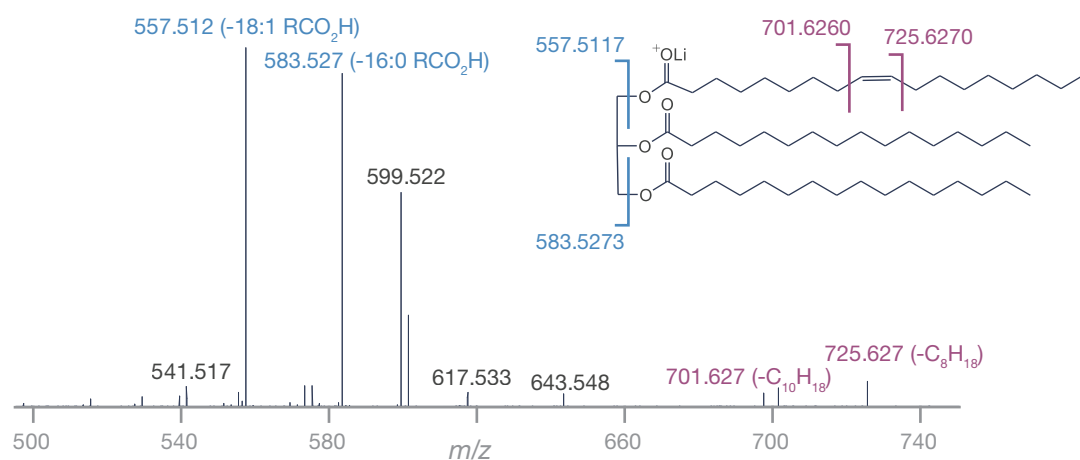


LEFT: UVPD provided 61.2% sequence coverage for Fc/2, 50.9% for the LC and 38.5% for Fd. The a and x ions are indicated in green, b and y ions in blue, and c and z ions in red. **RIGHT:** UVPD provided 20.4% unique coverage for Fc/2 when compared to ETD and ETHcD, 20.7% for LC and 19.8% for Fd. When combining the LC/MS results from ETD, ETHcD and UVPD, the bond coverage reached 72%, 79.7% and 86.6% respectively for Fd, LC and Fc/2.

Revolutionizing Lipid Structural Elucidation

Locating a double bond in the acyl chain of lipid molecules used to be impossible with soft fragmentation techniques such as CID or HCD. The Orbitrap Fusion Lumos MS with UVPD delivers similar fragmentation behavior to that of CID or HCD, but also provides diagnostic ions indicative of double bond locations, as well as other unique structurally diagnostic information that could not be previously obtained. UVPD is a unique fragmentation mode for unambiguous characterization of various lipid classes and represents a major breakthrough for lipid research.

HRAM UVPD MS² SPECTRUM OF [M+Li]⁺ PRECURSOR IONS OF TG 16:0/16:0/18:1

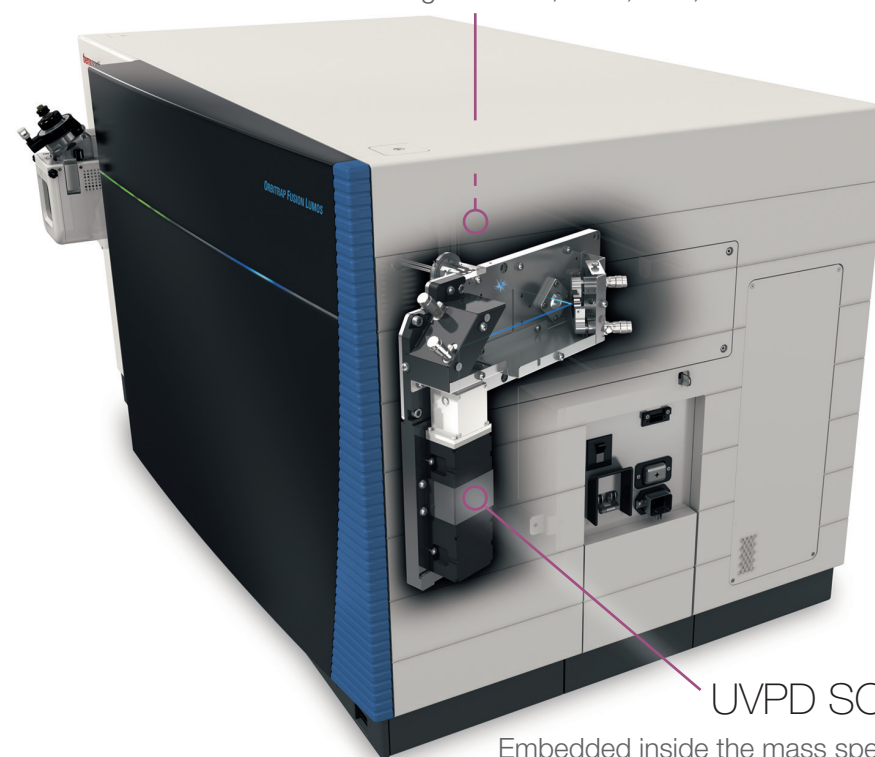


“The implementation of UVPD-MS/MS and -MSⁿ on the Orbitrap Fusion Lumos promises an unprecedented level of structural information for the improved identification and characterization of lipids, that is not possible to obtain with any other commercial mass spectrometry system. This includes localization of their site(s) of unsaturation, assignment of complex lipid backbone and headgroup identities, and the differentiation of isomeric lipids within complex mixtures.”

— Gavin Reid, Professor, University of Melbourne

DUAL-PRESSURE LINEAR ION TRAP

MSⁿ and sensitive mass analysis of fragments resulting from CID, HCD, ETD, ETHcD and UVPD



UVPD SOURCE

Embedded inside the mass spectrometer for optimal performance and reliability; the UVPD MSⁿ fragments are generated in the linear ion trap and can be detected by either ion trap or Orbitrap

Specifications: Class 1 213 nm CryLaS laser system with 2.5 kHz repetition rate delivering >1.2 μJ per pulse.

Find out more at thermofisher.com/lumos