Mass Spectrometry Enables More Definitive Process & Product Development Towards Well-Characterized Biotherapeutics: 

* A Personal Account

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Biotherapeutics Pharmaceutical Sciences
Pfizer, Inc., Andover, MA

September 7, 2023

_CASSS Mass Spec 2023: Symposium on the Practical Applications of Mass Spectrometry in the Biotechnology Industry, Chicago, IL_
Modern Mass Spectrometry (MS) Performance (with Research Grade Instruments)

- At least 40000 FWHM resolution w/ fast acquisition rates
- <2-5 ppm mass accuracy: both MS and MS/MS modes
- Low attomole sensitivity (6 million 50-kDa protein molecules)
- Five orders of magnitude dynamic range
- 50-20,000 m/z mass range (and higher)
- Multiple modes of ion fragmentation (CID, HCD, ETD, EThcD)
The Rise of Mass Spectrometry in Biotech

1990s
- First commercial MALDI & ESI mass spectrometers
- Huge leap in sensitivity & characterization capabilities

2000s
- Commercialization of high-resolution mass spectrometers
- Accurate mass determinations w/ high sensitivity
- Modern characterization & comparability w/ MS begins
- Modern characterization & comparability begins

2010s
- Era of ultrahigh-resolution MS for product characterization & comparability
- Unprecedented increases in sensitivity, speed, resolution & mass accuracy
- Bioprocess support begins for sequence variant and HCP analyses

2020s
- Automated sample preparation & data analysis; in-silico prediction tools
- Improving ease-of-use factor for MS hardware & software
- Implementing more MS-based multi-attribute method (MAM) approaches w/ high resolution mass detectors

MALDI MS of rFVIII 43kDa Domain

ESI-QTOF MS of mAb L Chain

UHR ESI-QTOF MS of mAb H Chain

MALDI MS of rFVIII 43kDa Domain

Pfizer

Biotherapeutics Pharmaceutical Sciences – Analytical Research & Development

MAM 2.0

LEGO® version of Exploris MX Mass Detector
**Enduring MS-based Methods for Heightened Product Characterization**

### Intact Protein Analysis
- Molecular mass
  - Product isoforms
  - Conjugate forms
- Multi-chain architecture

### Subunit Analysis
- Confirm primary structure
  - 100% sequence coverage
- Chain-specific isoforms
  - Conjugate forms

### Peptide Mapping
- Confirm primary structure
  - ≤100% sequence coverage
  - Elucidate disulfide bonds
- Sites of posttranslational & chemical modifications

### N-glycan Profiling
- N-glycan structures (MS)
- N-glycan quantitation (FLR)

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**Intact Protein Analysis**
- **SE- or RP-HPLC-UV/MS** (+/- PNGaseF)
- **SDS-PAGE**

**Subunit Analysis**
- **C4 RP-HPLC-UV/MS** (IdeS digestion \(\rightarrow\) reduction)
- **SDS-PAGE**

**Peptide Mapping**
- **C18 RP-HPLC-UV-MS/MS** (Denaturation/reduction/alkylation or alkylation/denaturation \(\rightarrow\) proteolysis)
- **Edman degradation**
- **Amino acid analysis**

**N-glycan Profiling**
- **HILIC-FLR/MS** (PNGaseF digestion \(\rightarrow\) 2-AB fluorescent labeling \(\rightarrow\) cleanup)
- **HPAEC-PED, AEX-HPLC, GC/MS & NMR**

(Shang et al. J. Pharm. Sci. 2014, 103, 1967)
Contemporary MS-based Methods for Heightened Process Characterization

**Trisulfide Analysis**
- Common mAb modification
- Root cause related to cell culture duration/feeding/pH
- Screen by intact mass & confirm by nr-peptide map

**Sequence Variant (SV) Analysis**
- NGS is frontline SV method to identify genetic mutations
- Final PQ tested w/LC-MS/MS
- Confirm primary structure
  - 100% seq. integrity ≥ 0.1-0.5%

**Misincorporation Analysis**
- AAA is frontline SV method to detect nutrient depletion
- Final PQ tested w/LC-MS/MS
- Confirm primary structure
  - 100% seq. integrity ≥ 0.1-0.5%

**Host Cell Protein (HCP) Analysis**
- Proteomic identification & relative quantitation of individual HCPs
- Augments routine HCP-ELISA
- Ensure no HCPs evade detection above a reportable limit (10 ppm)

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Liquid chromatography-tandem mass spectrometry (LC-MS/MS) | Next-generation sequencing (NGS) | Amino acid analysis (AAA) | Product quality (PQ)
MS Characterization Roadmap Supporting Product & Process Development

**Molecular Design** (Team Supply 0)
- Molecular assessment
  - Calculate molecular properties
  - In silico hotspot prediction
- Hotspot analysis on thermally stressed material by LC-MS/MS
  - Make MAM workbook

**Early Dev. Material** (Team Supplies 1 & 2)
- Product characterization
  - Intact mass, subunit mass, reduced / non-reduced peptide maps, N-glycan profiling
- Process characterization
  - Trisulfide analysis (intact mass)
  - LC-MS/MS – HCP analysis
- MAM: process/stability studies
  - Method dev. for new modalities

**Pilot Manufacturing**
- RM characterization
  - Intact mass, subunit mass, reduced / non-reduced peptide maps, N-glycan profiling
- Process characterization
  - Trisulfide analysis (intact mass)
  - LC-MS/MS – misincorporation analysis
  - LC-MS/MS – HCP analysis

**Phase I Clinical Manufacturing**
- IND authoring
- Off-critical path
  - LC-MS/MS – sequence variant analysis (max cell age)
  - Comprehensive PQA report

**Phase IIa** (Commercial Process Dev.)
- Process characterization
  - Trisulfide analysis (intact mass)
  - LC-MS/MS – misincorporation
  - LC-MS/MS – HCP analysis
- Comparability & MAM

**Phase IIb** (Process Nomination)
- Characterization method qualification as needed

**Phase III** (Process Validation)
- Primary RM characterization
- Forced degradation
- Comparability
- Process characterization
  - LC-MS/MS – HCP analysis
  - LC-MS/MS – sequence variant analysis (max cell age)

**BLA Submission**
- BLA authoring

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**Reference material (RM) | Investigational New Drug (IND) | Product quality attribute (PQA) | Biologics License Application (BLA)**

**Multi-attribute method (MAM) | Liquid chromatography-tandem mass spectrometry (LC-MS/MS) | Host-cell protein (HCP)**
Beginnings of Protein Mass Spectrometry

*Michigan State University 1988-1993: peptide sequencing*

The triple quadrupole turns 40...


**Novel Fragmentation Process of Peptides by Collision-Induced Decomposition in a Tandem Mass Spectrometer: Differentiation of Leucine and Isoleucine**

Richard S. Johnson, Stephen A. Martin, and Klaus Biemann

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA

John T. Stults and J. Throck Watson

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

**Cytochrome C**

Core MALDI Development 1985-1996

Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Biopolymers

F. Hillenkamp, M. Karas, R.C. Beavis and B.T. Chait

ANAL. CHEM., VOL. 63, NO. 24, DEC. 15, 1991, 1193 A

Core ESI Development 1984-1996

Electrospray Ionization for Mass Spectrometry of Large Biomolecules


SCIENCE, VOL. 246, p. 64
Genetics Institute circa 1993

- Structural Biochemistry - MS subgroup
  - Hubie Scoble, Director (Sanofi, consultant)
  - Steve Martin, Manager (Waters, retired)
  - James Vath (Cure Ventures)
  - Wen Yu (AstraZeneca)
  - Mike Huberty

JEOL HX-110/ HX-110 4-sector mass spectrometer
(equipped w/ fast-atom bombardment [FAB])

Bruker Reflex I mass spectrometer
(MALDI-TOF MS)

My Postdoc Research Projects
- Optimized continuous-flow FAB on JEOL HX-110/
  HX-110 4-sector mass spectrometer for peptides
- Benchmarked peptide ion fragmentation by MALDI-
  PSD to high and low energy CAD on JEOL HX-
  110/HX-110 4-sector mass spectrometer
- Developed MALDI cleanup methods for sensitive
  analysis of released N-linked glycans
- Elucidated N-linked glycan structures and isomers by
  MALDI, PSD and glycosidases
Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

1. Deposit purified protein sample (1-5 pmol) & let dry

2. Deposit 1 μL of SA matrix on dried sample

Bruker Reflex I TOF-MS

KE = 1/2mv^2 & v = D/t \rightarrow t = d\sqrt{m/2KE} \approx \sqrt{m/z}

SA matrix

3,5-methoxy-4-hydroxycinnamic acid
(6 mg in 500 μL 33% ACN/0.1% TFA)

Deposit purified protein sample (1-5 pmol) & let dry

Deposit 1 μL of SA matrix on dried sample

337 nm UV Laser

PHYSICAL REVIEW LETTERS 1999, VOL 83:2, p444

3,5-methoxy-4-hydroxycinnamic acid
(6 mg in 500 μL 33% ACN/0.1% TFA)
sPSGL-1 – P-selectin Glycoprotein Ligand-1 (1994)

- Site-specific analysis of a bioactive O-glycan in sPSGL-1
- MALDI-PSD-TOF MS
- Small-scale beta-elimination with NaOH (i.e., de-O-glycosylation)


Monitoring sPSGL-1 “Glyco-Engineering” by MALDI-TOF MS (1994)


McEver and Cummings. J Clin Invest. 1997; 100(3); 485-491

Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2)

High-pH anion exchange chromatography with pulsed electrochemical detection:
- Dionex CarboPac PA-100 column
- MPA & MPB: 0.1M NaOH
- MPB: 0-500 mM NaAcetate
- 1 mL/min
MALDI-TOF MS Analysis of rFVIII HPAEC Fractions (released EndoH N-glycans)
Sequential Glycosidase Digestion of Unknown Fraction 12

Dr. Terry D. Butters,
Oxford Glycobiology Institute

α1-3 Glucosidase II
30 min Drop Dialysis

α1-2 Mannosidase
30 min Drop Dialysis

\[ \text{Hex} \text{HexNAc} \]

\[ [\text{M+Na}]^{+} 1541.0 \]

\[ \Delta 162 = -1 \text{Glc} \]

\[ \Delta 162 \times 2 = -2 \text{Man} \]

\[ \equiv \text{Man}5 \ (1054.9) \]

Rouse and Strang, ASMS Conference 1998
Modern N-Glycan Profiling by LC-FLR/MS: Recombinant Factor IX (rFIX)

- DOE-optimized sample prep with 2-AB fluorescent dye
- Mixed-mode NP-/AEX-HPLC with MS-compatible mobile phases & fluorescence detect.
- New NH₂-polymer columns
- Positive/Negative ion ESI MS

Method adapted from Anumula & Dhume Glycobiology 1998; 8(7), 685.

Himakshi Patel and Matt Thompson, Pfizer, 2008-2009
In 2000, the ESI-Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometer Arrives

**Q-Tof is well suited for characterizing proteins...**
- High resolving power (~11,000 in V-mode)
- High mass accuracy: ≤30 ppm for peptides / N-glycans
  (NaI ext. cal. & DXC) ≤50 ppm for intact proteins
- Wide usable “mass” range (m/z 50-4000)
- Efficient ion transmission
- Powerful MaxEnt-1 & MaxEnt-3 deconvolution software

**Parts-per-million mass accuracy:**
\[
\frac{[M_r (O)bs.) - M_r (Theor.)]}{M_r (Theor.)} \times 10^6
\]

High resolving power (~11,000 in V-mode)

**Sample isolation / cleanup**
- Accurate, intact protein mass analysis by QTOF MS (if possible)

**Reduce complexity:**
- Subunit or domain mapping with on-line RP-HPLC/QTOF MS
- RP-, AEX-, CEX-, SEC-HPLC with off-line nanoESI-QTOF MS

**Specific structural details:**
- Peptide map (LC/MS)
- N-glycan profile (MALDI-TOF MS)

**Data analysis:**
- Correlate intact mass data with peptide map and N-glycan profile data to define primary structure

Adapted from schematic of Q-Tof-2 (Micromass MS Technologies, Waters Corp.)
MALDI-TOF MS of Intact rhBMP-2 (Isolated from Phenyl RP-HPLC-UV)

rhBMP-2 N-terminal Heterogeneity

~28.6 kDa
<Q283
Q283
~30.6 kDa
T266
Q283
~32.6 kDa
T266
T266

Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2)

<Q/<Q, <Q/Q & Q/Q Forms
28845.3
28862.3
28879.4

<Q/T & Q/T Forms
30862.0

Covalent Dimer Fraction

Monomer Fraction

<Q & Q Forms

<Q/Man6
Q/Man6
14384.9

T Forms

s-s-Cys

Man6
Man7 D1
Man8 D1/D3

Rouse, Abbatangelo, Haq, Marzilli, Nemeth-Cawley, Patel, Rathore, Jankowski, Porter, & Scoble, ACS NERM 2001, University of NH
Comparison of ESI-QTOF & MALDI-TOF MS for Covalent rhBMP-2 Dimer

On-line Phenyl RP-HPLC/QTOF MS (MaxEnt-1)
T/T, Man6/Man6 (32518.5)

<Q/<Q, Man6/Man6 (28520.0)
<Q/T, Man6/Man6 (30519.3)

<Q/<Q, <Q/Q & Q/Q Isoforms
<Q/T & Q/T Isoforms
T/T Isoforms

MALDI-TOF MS

Pfizer
Biotherapeutics Pharmaceutical Sciences – Analytical Research & Development
Rouse, Abbatiello, Haq, Marzilli, Nemeth-Cawley, Patel, Rathore, Jankowski, Porter, & Scoble, ACS NERM 2001, University of NH
rhBMP-2 Comparability Study: Phenyl RP-HPLC-UV / ESI QTOF MS

**Process 1**
- AU (214 nm)
- T266
- Monomer
- Dimer
- <Q & Q Forms

**Process 2**
- AU (214 nm)
- Monomer
- Dimer

**Process 3**
- AU (214 nm)
- Monomer
- Dimer

Rouse, Abbatiello, Haq, Marzilli, Nemeth-Cawley, Patel, Rathore, Jankowski, Porter, & Scoble, ACS NERM 2001, University of NH
The mass spectra of intact rhBMP-2 indicated the 3 processes produced comparable DS (according to predetermined acceptance criteria):

- Mass differences between the same isoforms were < 1.3 Da
- All isoform masses were < 1.6 Da (50 ppm) from theoretical values
- Similar isoform distributions were observed (slight redistribution in process 2)
- No new isoforms were detected

All routine testing and characterization studies together supported structural and functional comparability of rhBMP-2 DS.

Emerging Ultrahigh Mass Measurement Techniques (2022)

Charge Detection MS (CDMS)  Adeno-associated Virus (AAV)

CDMS is orthogonal to AUC for quantitation of empty / partial / full AAV capsids
- Well-suited for analysis of low conc. samples; requires minimal volumes
- Sufficient resolution of partially packaged AAV capsids (~2% rel. abundance)

Viral capsids up to 150 MDa also were successfully analyzed by CDMS

Only AUC & CDMS show true linearity


Wörner et al. Molecular Therapy: Methods & Clinical Development 2022, 24, 40.
Characterization of Biotherapeutics by Mass Photometry (MP) (2023)

- Sensitive mass measurement of single molecules in solution, in their native state
- Light scattered by a molecule that has landed on measurement surface interferes with light reflected by that surface. The interference signal scales linearly with mass.
- MP enables quick quality checks of glycoproteins & mRNA to support projects

MP analysis of recombinant influenza B Phuket HA (Protomer mass: 73.3 kDa)

- Observed masses within 2% of theoretical masses
- Dimer under investigation; also present in CGE & CDMS

mRNA Case study:
Two mRNA samples with different poly(A)-tail lengths

AAAAAAA

595 kDa (mRNA w/ 0A; Theor. Mass: 608 kDa)

Poly-lysine coated glass required for mRNA analysis

619 kDa (mRNA w/ 75A; Theor. Mass: 632 kDa)

5'-Cap

Dimer 1175 kDa

Dimer 1221 kDa

AAAAAA
Important Mass Spectrometer Characteristics & New Directions

- Research-grade mass spectrometers are defined by ultimate performance such as sensitivity, resolution, and mass accuracy.

- New “Smart” UHPLC mass detectors are being developed with improved “ease-of-use” for hardware/software operation.
  - Opens-up LC/MS access to more colleagues (w/ more manageable training) for supporting routine MS workflows!
Modern View of Mass Spectrometry in Process & Product Dev. Labs

- Reference material
- Drug substance
- Drug product
- In-process samples
- Stability samples

- LC/MS – intact protein analysis
  - Characterization
  - Trisulfide analysis

- LC-MS/(MS) – peptide mapping
  - Characterization
  - MAM
  - HCP analysis
  - Seq. variant analysis
  - Misincorporation analysis
- LC/MS – subunit analysis
- N-glycan profiling

- LC ‒ MS/MS
- UHPLC

Fit-for-purpose LC/MS setup
(high resolution / accurate mass)
Built-in reliability-serviceability
(service requested by system)
Simple operation / maintenance
(auto tuning & calibration)

Research Grade Mass Spectrometer
(State-of-the-art, $$$

Advanced software
(standalone)
3rd party informatics
(annotated datasets)
Database searching

Transfer key PQA characterization
knowledge via attribute workbook

Transfer key PQA characterization
knowledge via attribute workbook

Easy-to-use/intuitive software
(standalone or enterprise)
Attribute focused data stream
(automated analysis/annotation;
pre-defined peak integrations,
calculations & reports)

Workstation

UHPLC Mass detector

Complex analysis

Automated sample prep

Direct analysis

In-depth Characterization

Routine PQA monitoring (MAM)

Transfer key PQA characterization
knowledge via attribute workbook

Sitasuwans et al. mAbs 2021, 13, 1.: hands-on time; precision

贺滕丸等. mAbs 2021, 13, 1.: 手工时间; 精度

Deploying the multi-attribute method (MAM) across sites at Pfizer, Thermo Scientific Case Study (cs73683)
Chemical Modifications in Complementarity-Determining Regions (CDRs)

Potential CDR Modifications
- Asn deamidation
- Asp isomerization
- Met/Trp oxidation
- Asp-Pro cleavages

...which can occur during manufacturing, storage, administration, and *in vivo* circulation,
...and possibly affect mAb target binding

- Haberger et al. mAbs 2014, 6, 327.
- Lu et al. mAbs 2019, 11, 45.
Elucidated & Cataloged CDR Sequence Instabilities across 95 mAbs
...provided enhanced S-F & molecular design knowledge, and laid groundwork for in silico hotspot prediction

Used the latest LC-MS/MS technologies...
Reduce (DTT)  Alkylate (IAA)  Desalt (BioSpin6)  Trypsin/Lys-C (pH 8.2, 37C)


Orbitrap Lumos (HCD & EThcD)  High selectivity  Low-artifact digests

Compiled a hotspot database→shared across orgs

mAb1 L Chain CDR Hotspot Database

<table>
<thead>
<tr>
<th>CDR</th>
<th>Rel. Abund.</th>
<th>mab1 L Chain CDR Hotspot Database</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIVMTQTPLSLSVTGPQPASISCRSSQSLV 30</td>
<td>High (&gt;5%)</td>
<td></td>
</tr>
<tr>
<td>HSNGNTFLYWLQKPGQSPQLIYRVSNRF 60</td>
<td>Low (1-5%)</td>
<td></td>
</tr>
<tr>
<td>SGVPDRFGSFGSGTDFTLKIISVEAEVGV90</td>
<td>Potential</td>
<td></td>
</tr>
<tr>
<td>YCFQATHVPTFGGTLKVEIK 91</td>
<td>Framework</td>
<td></td>
</tr>
</tbody>
</table>

Pfizer Structural & Computational Biology Team
Biotherapeutics Pharmaceutical Sciences – Analytical Research & Development

S. Philip & E. Stephens

Orbitrap Lumos
(HCD & EThcD)  High selectivity  Low-artifact digests

Used the latest LC-MS/MS technologies...
Reduce (DTT)  Alkylate (IAA)  Desalt (BioSpin6)  Trypsin/Lys-C (pH 8.2, 37C)


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<td>Low (1-5%)</td>
<td></td>
</tr>
<tr>
<td>SGVPDRFGSFGSGTDFTLKIISVEAEVGV90</td>
<td>Potential</td>
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</tr>
<tr>
<td>YCFQATHVPTFGGTLKVEIK 91</td>
<td>Framework</td>
<td></td>
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</table>

Pfizer Structural & Computational Biology Team
Biotherapeutics Pharmaceutical Sciences – Analytical Research & Development


...and developed a structure-based hotspot prediction algorithm for deamidation, isomerization & oxidation
Trastuzumab Light Chain CDR-1

Antigen Binding Fragment (Fab) Region

<table>
<thead>
<tr>
<th>Trastuzumab Material</th>
<th>D^{28}V (L-CDR1)</th>
<th>N^{30}T (L-CDR1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T=0 control</td>
<td>ND</td>
<td>88.8 / 9.7 / 0.8 / 0.7</td>
</tr>
<tr>
<td>4w, 40C, Tris pH 7.5</td>
<td>ND</td>
<td>28.2 / 69.8 / 0.6 / 1.4</td>
</tr>
<tr>
<td>4w, 40C, His pH 5.8</td>
<td>ND</td>
<td>75.7 / 12.2 / 2.9 / 9.2</td>
</tr>
<tr>
<td>4w, 40C, Glu pH 4.5</td>
<td>ND</td>
<td>89.2 / 2.7 / 2.3 / 5.8</td>
</tr>
<tr>
<td>Harris et al. 2001 (%)</td>
<td>---</td>
<td>~15% (CEX-HPLC)</td>
</tr>
<tr>
<td>Sydow et al. 2014 (%)</td>
<td>---</td>
<td>11% → 24% (His, pH 6)</td>
</tr>
</tbody>
</table>
**New Structure-based mAb CDR “Hotspot Prediction” Algorithm**

**mAb CDR Hotspot Database (95 mAbs)**
- Unstressed material (T=0)
- Stressed material
  - Tris buffer† (pH 7.5, 40°C, 4wks)
  - His buffer (pH 5.8, 40°C, 4wks)
  - Glu buffer (pH 4.5, 40°C, 4wks)
- LC-MS/MS – peptide maps
  - Trypsin digestion at pH 8.2, 30min
  - Trypsin digestion at pH 6.0, overnight

**Database Trends:**
Prevalent Hotspot Motifs
Hotspot positions in CDRs

**Input:** Crystal Structure

**Calculate:** Accessible Surface Areas (%ASAs)

**Motif-based prediction (91% false discovery rate)**

**Trastuzumab Heavy Chain**
1EVQLVESGGGLVQPGGLRLSCAASGFNIKDTYIHWRQRKPEKRLESGVHHVYPTNYGTYR 60
61ADSVQGRFTISADTKNTAYLQSLRAEDTAVYYCQRWGGDFYAMDYWGQGTVTVSS 120

**Trastuzumab Light Chain**
1DIQMTQSPSSLSASVQDRVTITCRASQDDNVNTAVAWYQQKPGKAPKLLIYSASFLYGVP 60
61RFSGSRSGTDFLTISSLQPEDFATYYCQHYTTPPGTGQYIKRTIVALAPSVFIFPP 107

**Structure-based prediction (90% accuracy rate; 58% false discovery rate; MCC=56%)**

<table>
<thead>
<tr>
<th>mAb</th>
<th>CDR</th>
<th>Site</th>
<th>Motif</th>
<th>%ASA (x)</th>
<th>%ASA (x+1)</th>
<th>B-turn Type</th>
<th>B-turn Position</th>
<th>Sec. Structure</th>
<th>Predicted Hotspot &gt;5%</th>
<th>Exp. Hotspot Level (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab L-CDR1 (4hkz)</td>
<td>28</td>
<td>DV</td>
<td>66.8</td>
<td>0</td>
<td>--</td>
<td>--</td>
<td>Loop</td>
<td>No</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>H-CDR1</td>
<td>30</td>
<td>NT</td>
<td>70.6</td>
<td>48.5</td>
<td>II'</td>
<td>2&amp;3</td>
<td>Loop</td>
<td>Investigate</td>
<td>71.8</td>
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<tr>
<td>H-CDR2</td>
<td>55</td>
<td>NG</td>
<td>54.8</td>
<td>68.6</td>
<td>I</td>
<td>4&amp;--</td>
<td>Loop</td>
<td>Investigate</td>
<td>7.3</td>
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<tr>
<td>H-CDR3</td>
<td>99</td>
<td>W</td>
<td>18.8</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Sheet</td>
<td>No</td>
<td>0.6</td>
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<td>102</td>
<td>DG</td>
<td>83.0</td>
<td>84.0</td>
<td>I'</td>
<td>2&amp;3</td>
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<td>Investigate</td>
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<tr>
<td></td>
<td>107</td>
<td>M</td>
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<td>--</td>
<td>Loop</td>
<td>No</td>
<td>0.6</td>
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<tr>
<td></td>
<td>108</td>
<td>DY</td>
<td>0.2</td>
<td>34.5</td>
<td>--</td>
<td>--</td>
<td>Loop</td>
<td>No</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

**Provides hotspot access to more colleagues ● Speeds-up hotspot analysis ● Create MAM workbooks at risk ● Cross-check MS assignments**
Summary

- MS has evolved significantly over 25+ years, providing more in-depth, high-quality information faster
  - MS is the analytical characterization workhorse for definitive elucidation of primary structure & modifications
  - MS is a decisive characterization tool during molecular assessment and early process development
    - If needed, minor improvements to the platform process can occur in “real-time” without affecting timelines
  - MS is an essential element of commercial process dev. and comparability (similarity) exercises
    - Rapidly assess effect of manufacturing improvements on product quality attributes & batch consistency
    - Directly visualize the intact protein isoforms that constitute pre-change & post-change comparability batches
- The pace and breadth of biotherapeutics process & product development are increasing every year!
  - Demand is shifting to smaller, more reliable, easier-to-use instruments with automatic calibration & tuning
    - Automated sample preparation/data analysis, and in silico prediction tools, will improve access & productivity
    - Continued quantum leaps in capability, performance & ease-of-use from our vendor partners are essential!
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