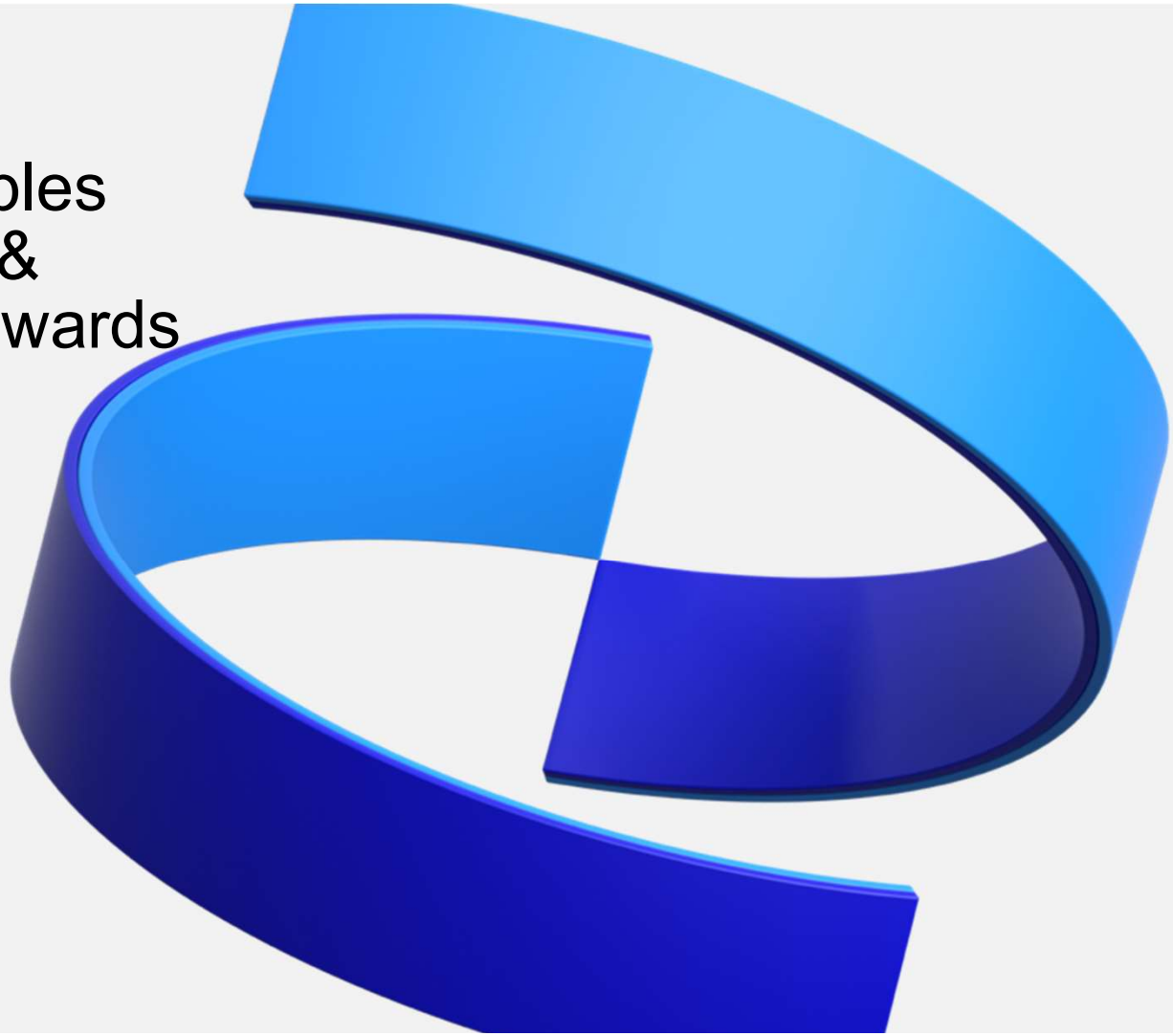
Mass Spectrometry Enables More Definitive Process & Product Development Towards Well-Characterized Biotherapeutics:

A Personal Account

Jason C. Rouse
Analytical Research & Development
Biotherapeutics Pharmaceutical Sciences
Pfizer, Inc., Andover, MA

January 25, 2022

2022 CASSS WCBP Conference - 25th Anniversary
Washington D.C.

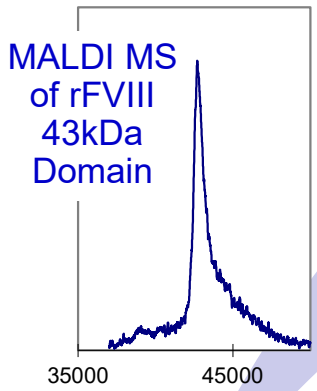


Modern Mass Spectrometry (MS) Performance *(with Research Grade Instruments)*

- 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)

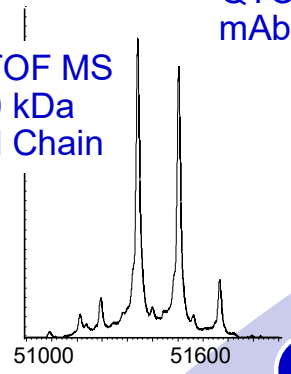
Timp and Timp, Sci. Adv. 2020, 6, 1.

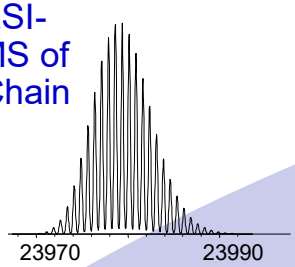
The Rise of Mass Spectrometry in Biotech



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

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
- Improving ease-of-use factor for MS hardware & software
- Implementing more MS-based multi-attribute method (MAM) approaches w/ high resolution mass detectors

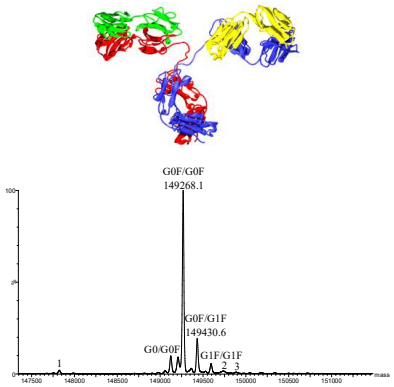
MAM 2.0

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**

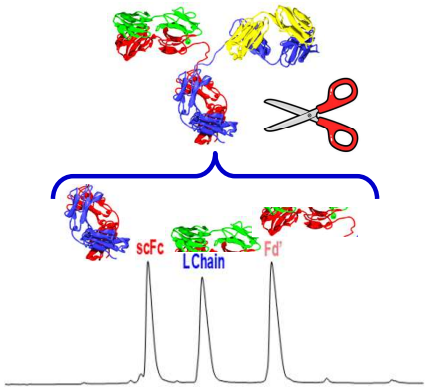


SE- or RP-HPLC-UV/MS
(+/- PNGaseF)

SDS-PAGE

Subunit Analysis

- **Confirm primary structure**
  - **100% sequence coverage**
- **Chain-specific isoforms**
  - **Conjugate forms**

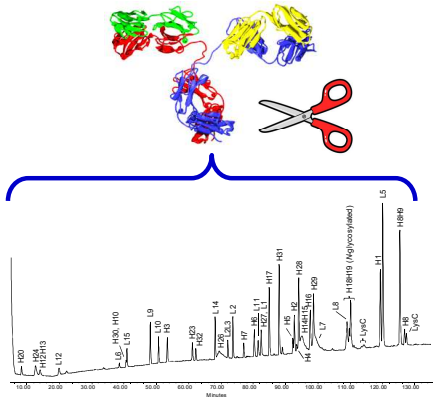


C4 RP-HPLC-UV/MS
(IdeS digestion → reduction)

SDS-PAGE

Peptide Mapping

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

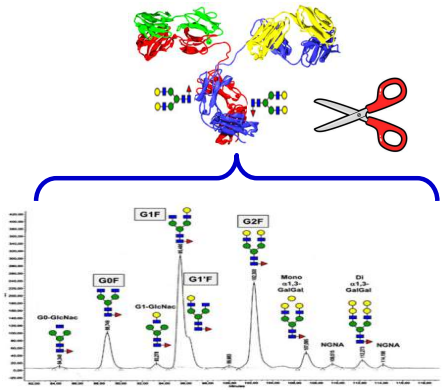


C18 RP-HPLC-UV-MS/MS
(Denaturation/reduction/alkylation or alkylation/denaturation → proteolysis)

Edman degradation
Amino acid analysis

N-glycan Profiling

- **N-glycan structures (MS)**
- **N-glycan quantitation (FLR)**



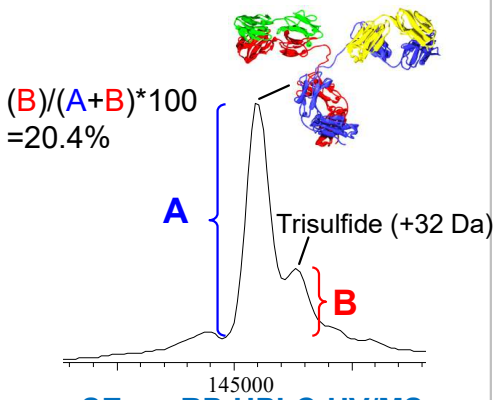
HILIC-FLR/MS
(PNGaseF digestion → 2-AB fluorescent labeling → cleanup)
(Shang et al. J. Pharm. Sci. 2014, 103, 1967)

HPAEC-PED, AEX-HPLC, GC/MS & NMR

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

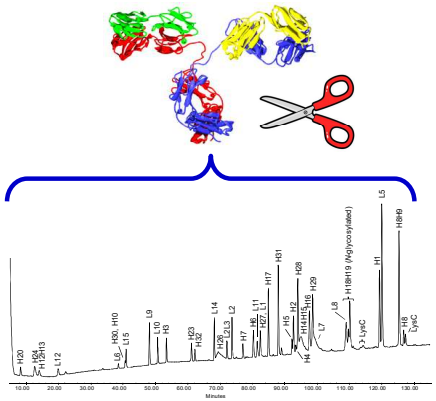


SE- or RP-HPLC-UV/MS (+/- PNGaseF)

(Gu et al. Anal. Biochem. 2010, 410, 89)

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%

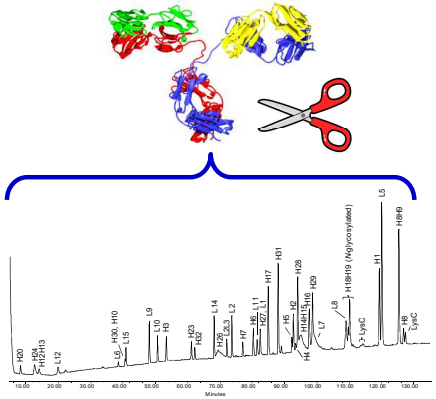


LC-MS/MS – peptide mapping (Denaturation/reduction/alkylation → desalt → proteolysis → bioinformatics)

(Lin et al. mAbs 2019, 11, 1; Wong et al. Biotechnol. Adv. 2018, 36, 168)

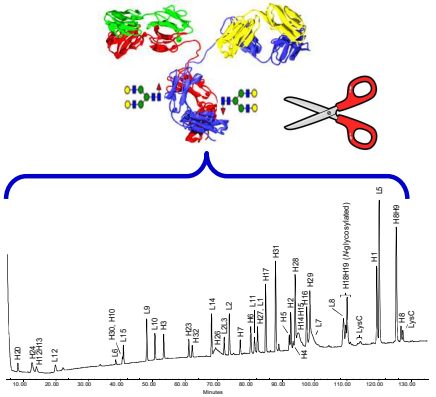
Misincorporation Analysis

- 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)



LC-MS/MS – peptide mapping (Denaturation/reduction/alkylation → desalt → proteolysis → database search)

(Zhang et al. LC/GC Supp. 2021. 39. 25)

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 on stability sample sets**
- **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

Multi-attribute method (MAM) | Liquid chromatography-tandem mass spectrometry (LC-MS/MS) | Host-cell protein (HCP)

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)

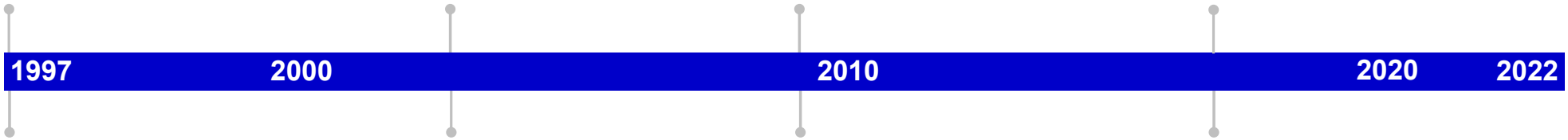
BLA Submission

- **BLA authoring**

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

Timeline of CASSS Symposia and MS Topics

CASSS is at the forefront for integrating the MS community with the larger biotherapeutics development & regulation communities to discuss innovative approaches and share experiences & best practices



1st CASSS WCBP Symposium
(January 1997)

Agenda (characterization)
MS - Carbohydrate analysis
MS - Posttranslational modifications
MS - Sequencing proteins/glycans

1st CASSS Mass Spec Symposium
(September 2004)

Agenda
Intact protein analysis
Antibody characterization
MS in QC

ASMS
1st Biotherapeutics Session
(June 2009)

Agenda
IdeS Enzyme & LC/MS
New LC/UHR ESI-QTOF MS
Antibody characterization
Peptide sequencing
Peptide mapping

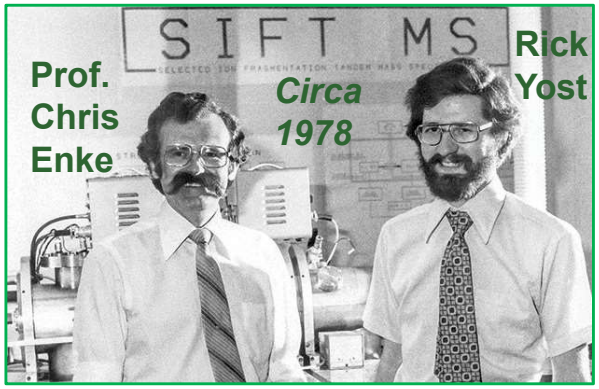
21st CASSS WCBP Symposium
(January 2017)

Agenda (characterization)
Multi-attribute Method (LC/MS)
Sequence variants
Biosimilars
High-throughput LC/MS for PQAs

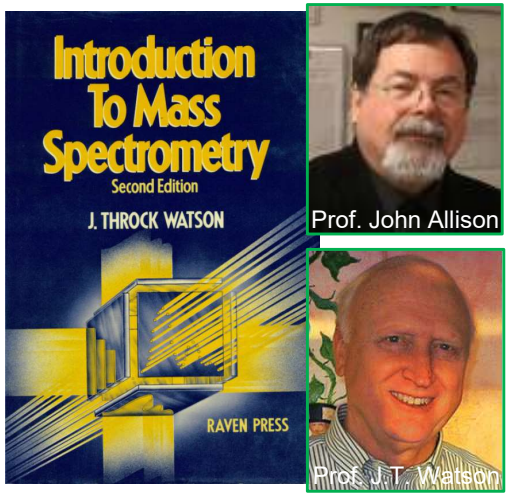
CASSS Well-Characterized Biotechnology Products (WCBP) Symposium: Overview – Past, Present & Future; Brian Nunnally, May 2015

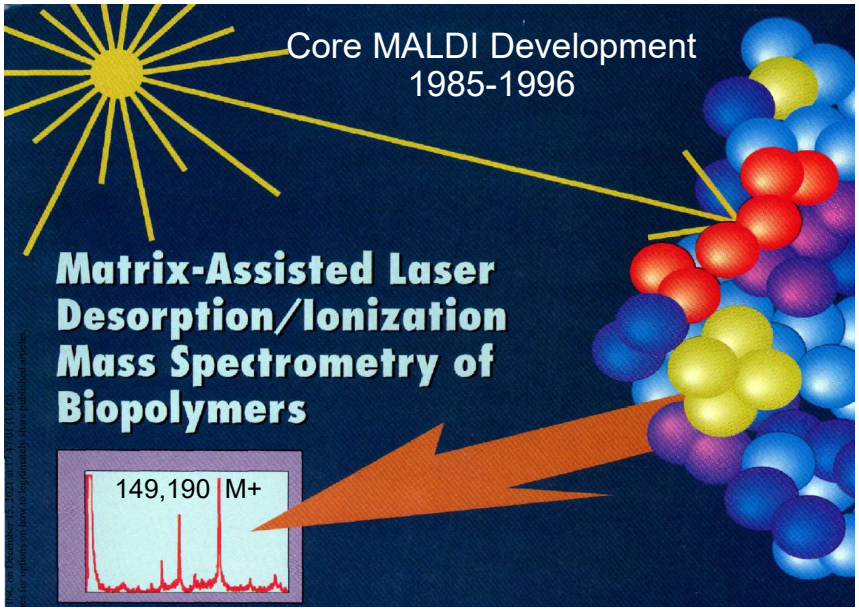
Beginnings of Protein Mass Spectrometry

Michigan State University 1988-1993: peptide sequencing



The triple quadrupole turns 40...
C&E News: Mar. 5, 2018, Vol 96:10 | 15-18



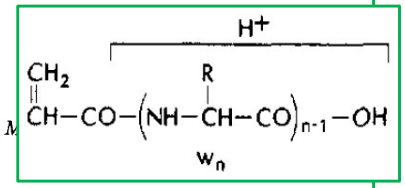


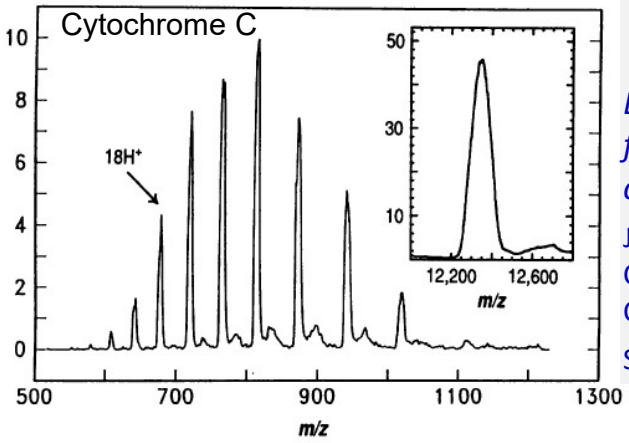
F. Hillenkamp, M. Karas, R.C. Beavis and B.T. Chait
ANAL. CHEM., VOL. 63, NO. 24, DEC. 15, 1991, 1193 A

Anal. Chem. **1987**, *59*, 2621–2625

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,[1] and Klaus Biemann*
Department of Chemistry, Massachusetts Institute of Technology, Cambridge, M
John T. Stults and J. Throck Watson
Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824





Core ESI Development
1984-1996

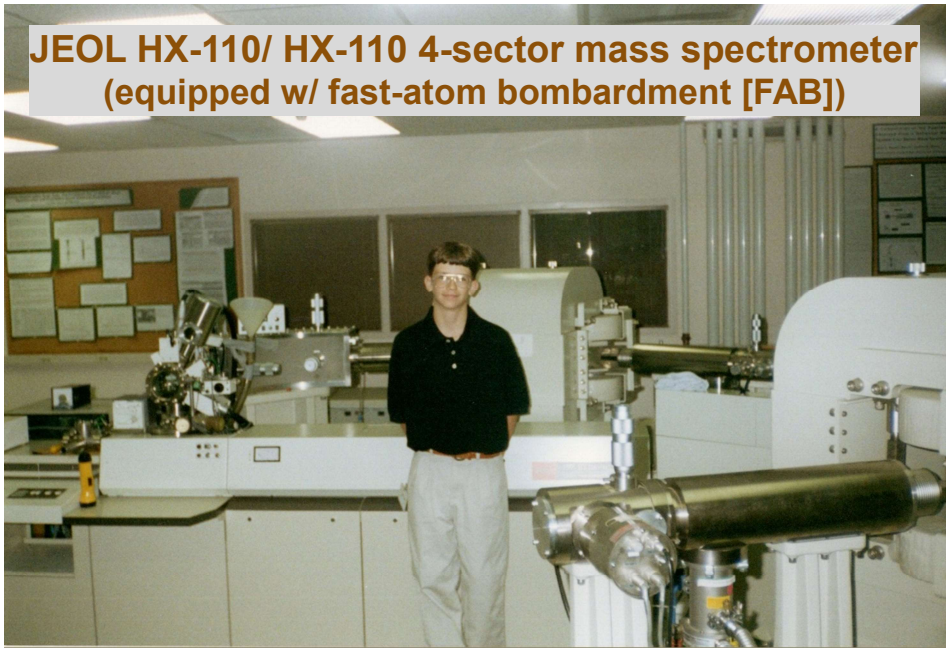
Electrospray Ionization for Mass Spectrometry of Large Biomolecules

J.B. FENN, M. MANN, C.K. MENG, S.F. WONG, C.M. WHITEHOUSE (1989)

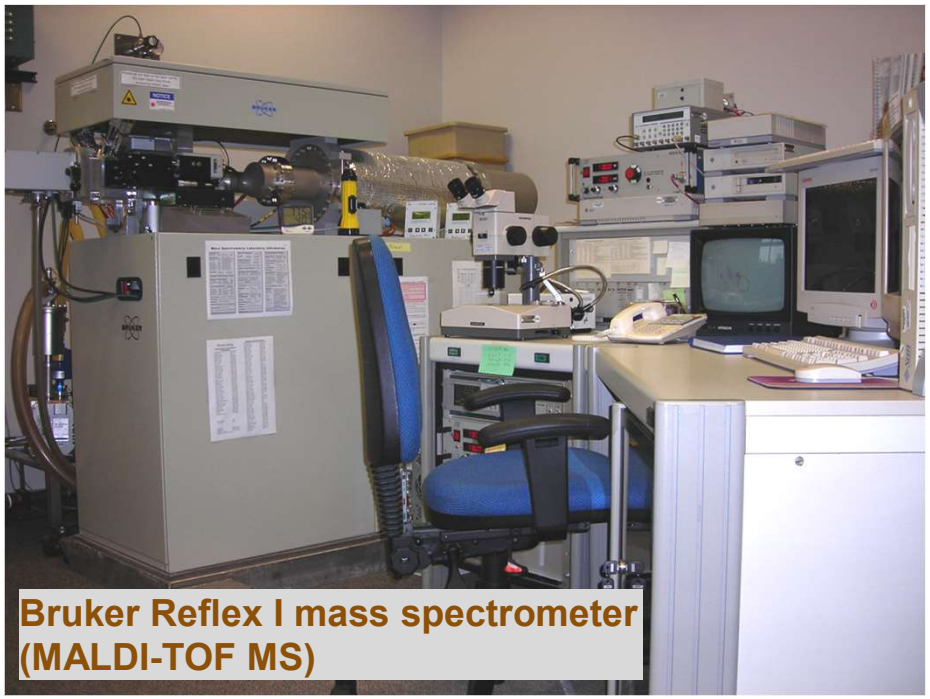
SCIENCE, VOL. 246, p. 64

Genetics Institute circa 1993

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



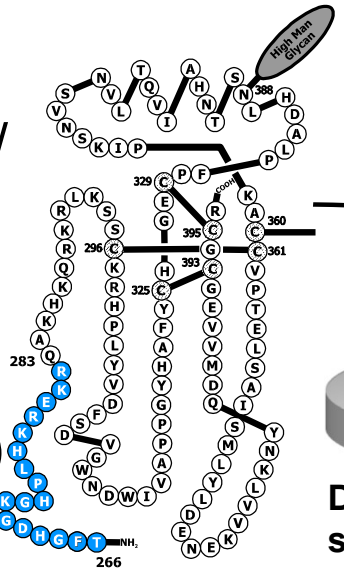
JEOL HX-110/ HX-110 4-sector mass spectrometer (equipped w/ fast-atom bombardment [FAB])



Bruker Reflex I mass spectrometer (MALDI-TOF MS)

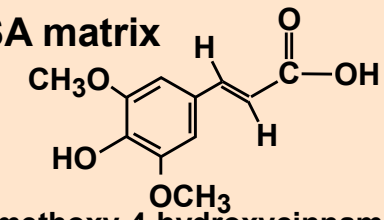
- My Postdoc Research Projects
  - Optimized continuous-flow FAB on JEOL HX-110/ HX-110 4-sector mass spectrometer for peptides
  - Benchmarked peptide ion fragmentation by MALDI-PSD to high and low energy CAD on JEOL HX-110/HX-110 4-sector mass spectrometer
  - Developed MALDI cleanup methods for analysis of released N-linked glycans
  - Elucidated N-linked glycan isomers by MALDI, PSD and glycosidases

Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)



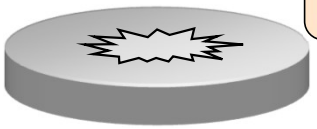
1

SA matrix

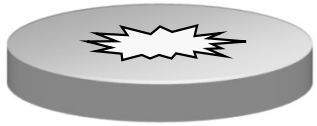


3,5-methoxy-4-hydroxycinnamic acid
(6 mg in 500 µL 33% ACN/0.1% TFA)

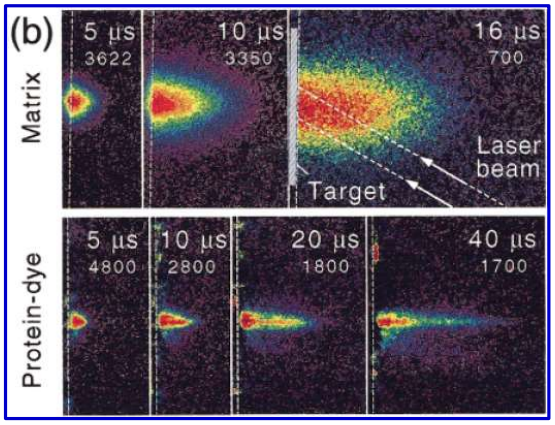
2



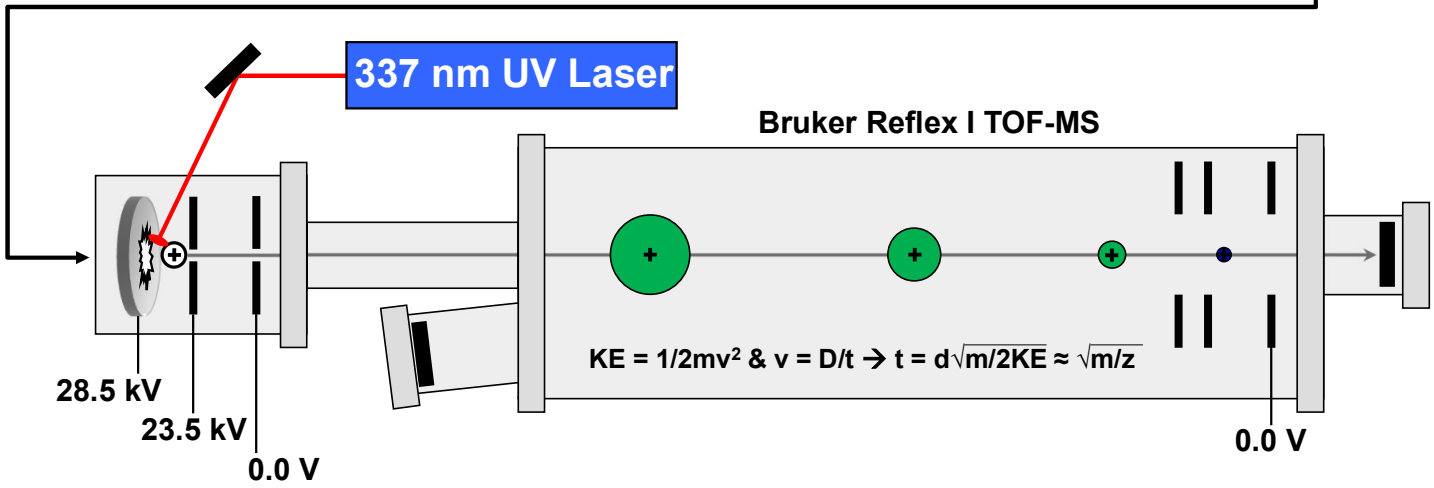
Deposit purified protein sample (1-5 pmol) & let dry



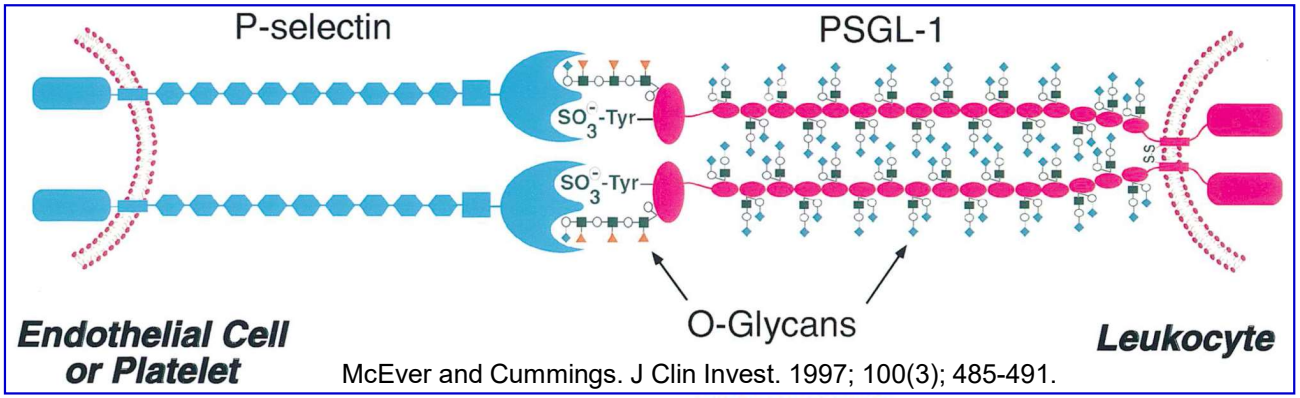
Deposit 1 µL of SA matrix on dried sample



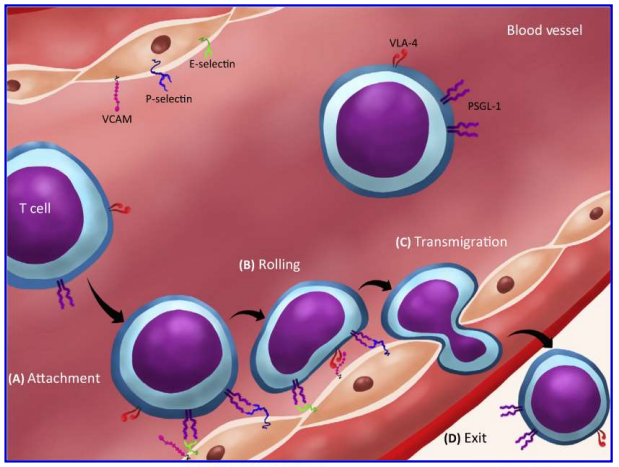
PHYSICAL REVIEW LETTERS 1999, VOL 83:2, p444



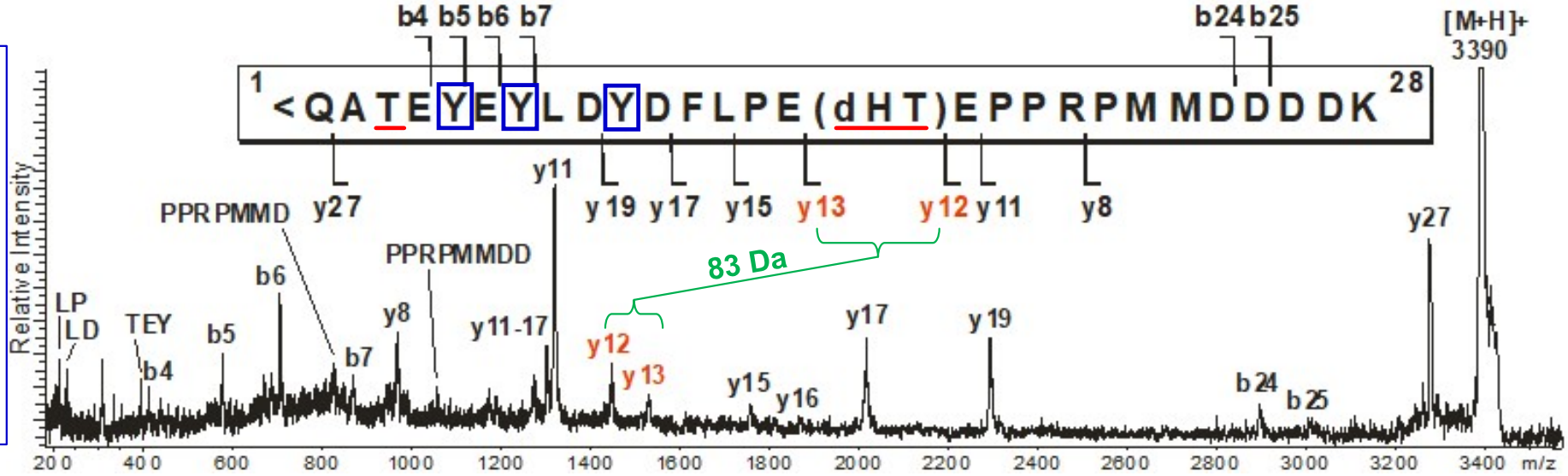
sPSGL-1 – P-selectin Glycoprotein Ligand-1 (1994)



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

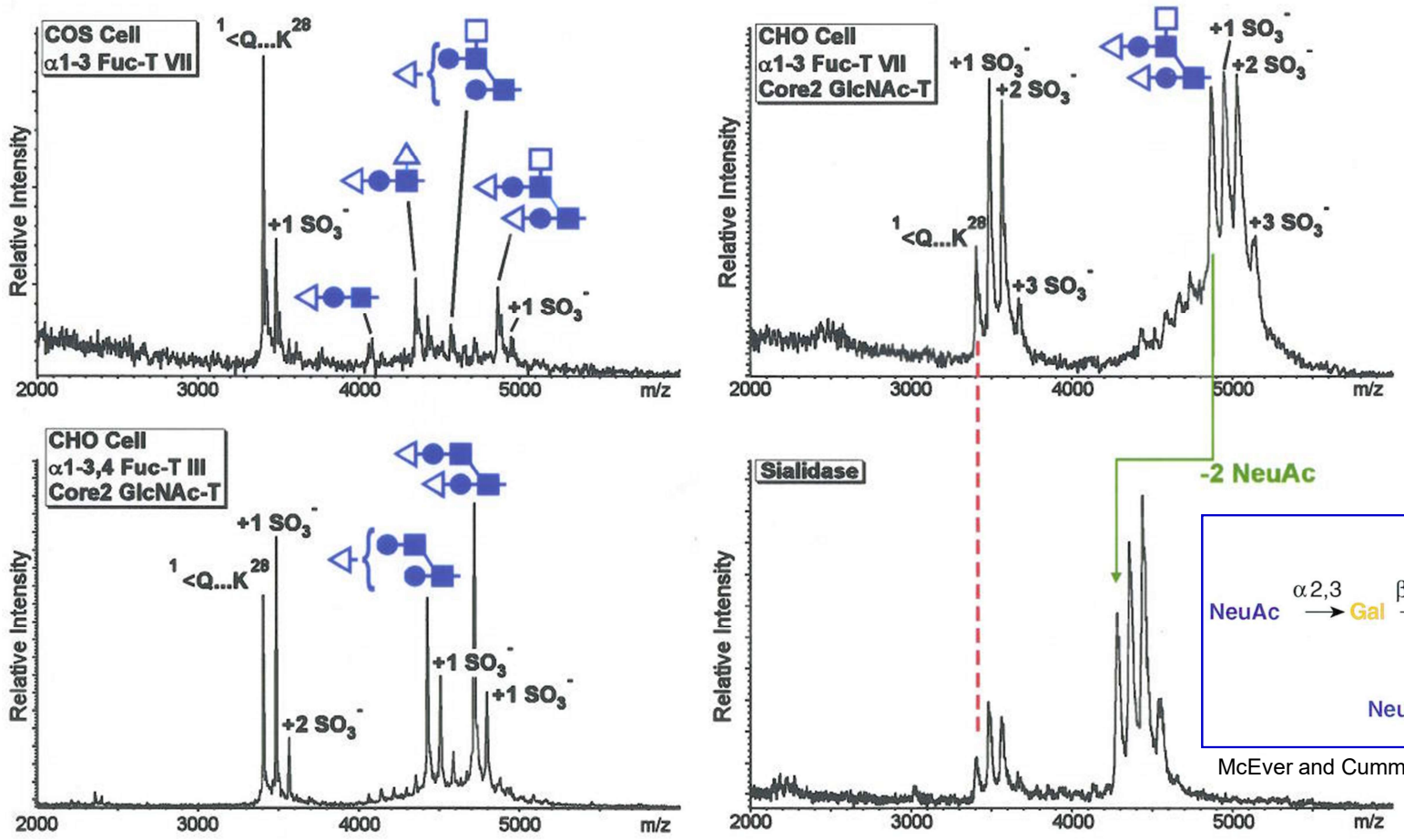


- ✓ **Site-specific analysis of a bioactive O-glycan in sPSGL-1**
- ✓ **MALDI-PSD-TOF MS**
- ✓ **Small-scale beta-elimination with NaOH (i.e., de-O-glycosylation)**

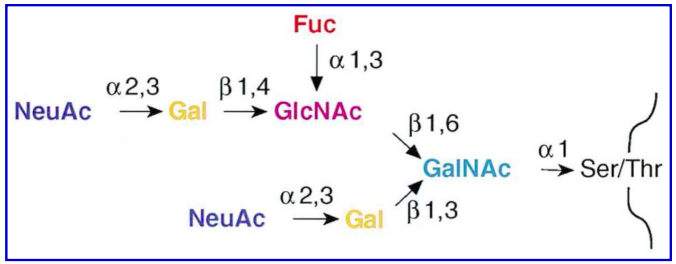


Rouse, Camphausen, Cornell, Kitchen, Yu, Hardy, Harris, and Scoble, ASMS Conference 1998.

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



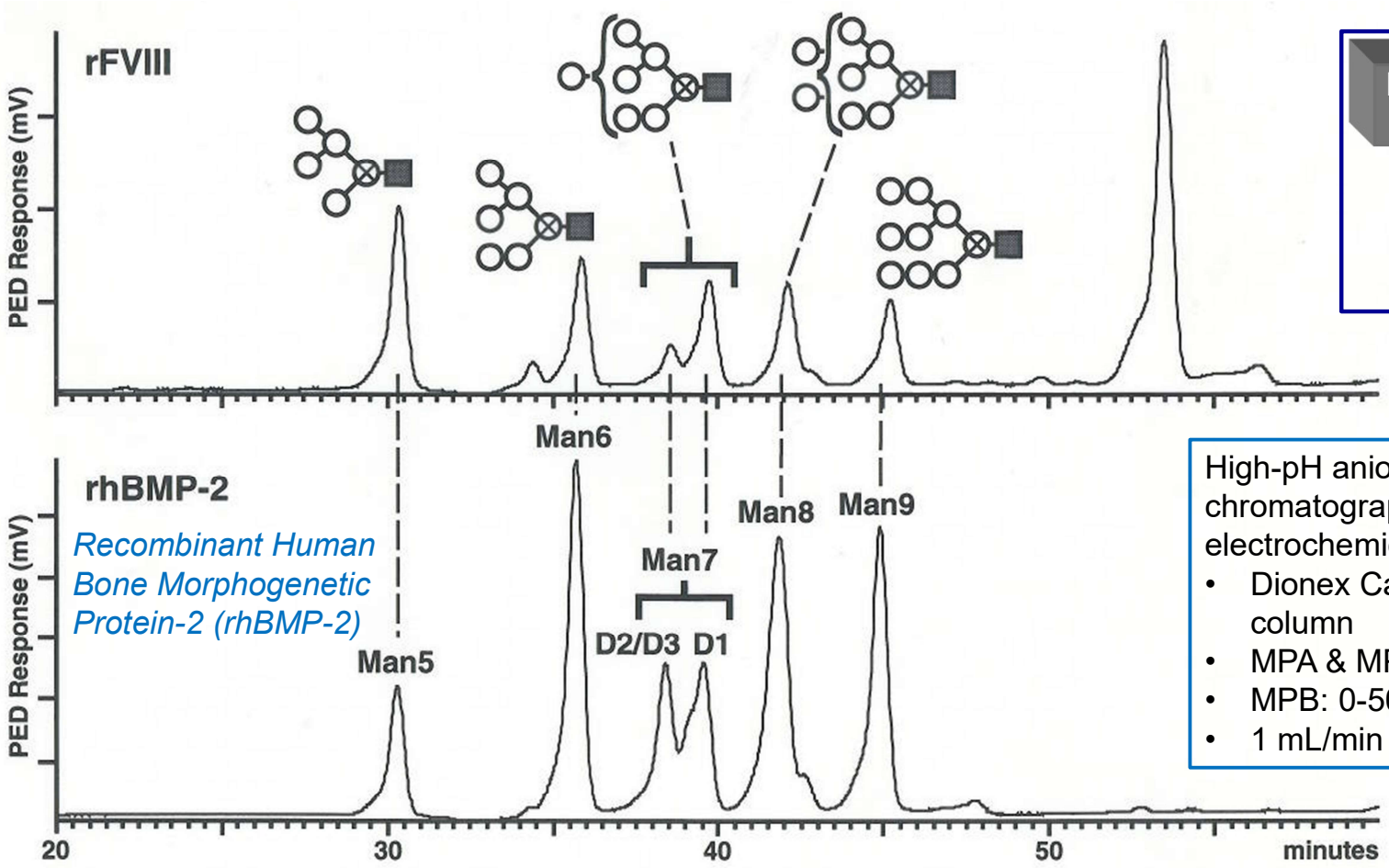
Target bioactive O-glycan

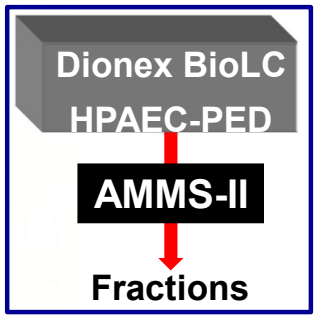


McEver and Cummings. J Clin Invest. 1997; 100(3); 485-491

Rouse, Camphausen, Cornell, Kitchen, Yu, Hardy, Harris, and Scoble, ASMS Conference 1998.

Profiling EndoH-released N-glycans by HPAEC-PED (1995)



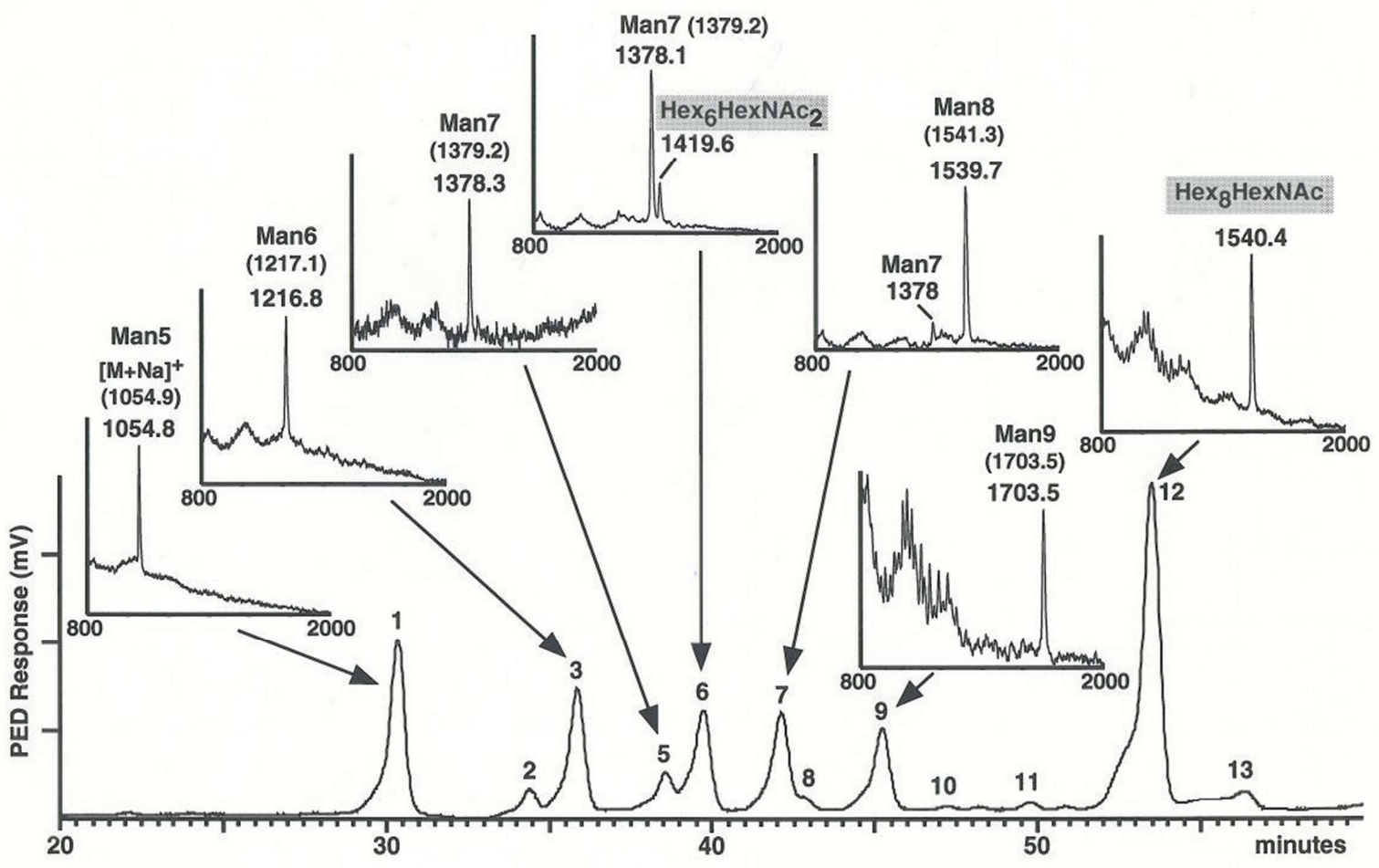


High-pH anion exchange chromatography with pulsed electrochemical detection:

- Dionex CarboPac PA-100 column
- MPA & MPB: 0.1M NaOH
- MPB: 0-500 mM NaAcetate
- 1 mL/min

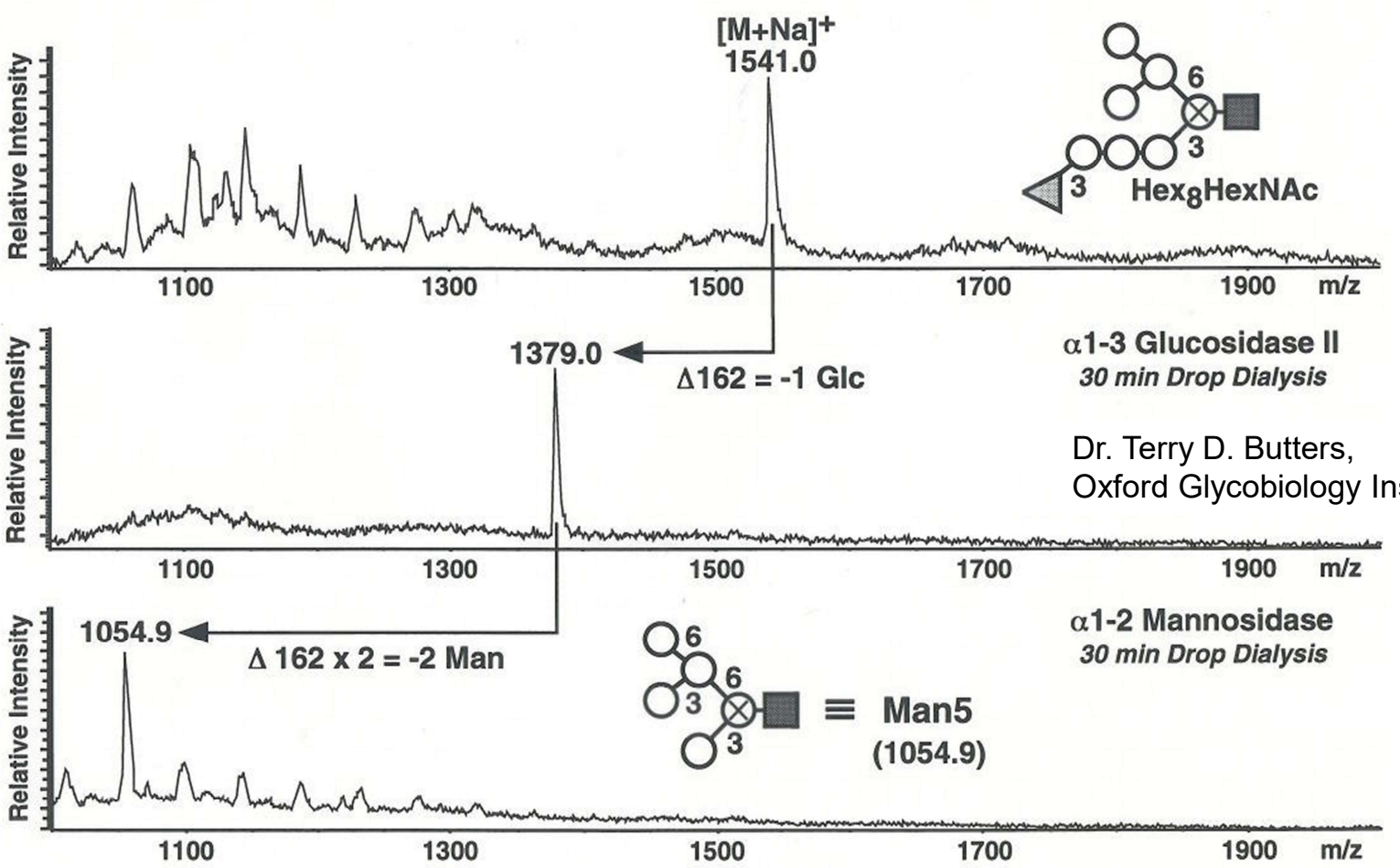
Rouse and Strang, ASMS Conference 1998

MALDI-TOF MS Analysis of rFVIII HPAEC Fractions (released EndoH N-glycans)



Rouse and Strang, ASMS Conference 1998

Sequential Glycosidase Digestion of Unknown Fraction 12



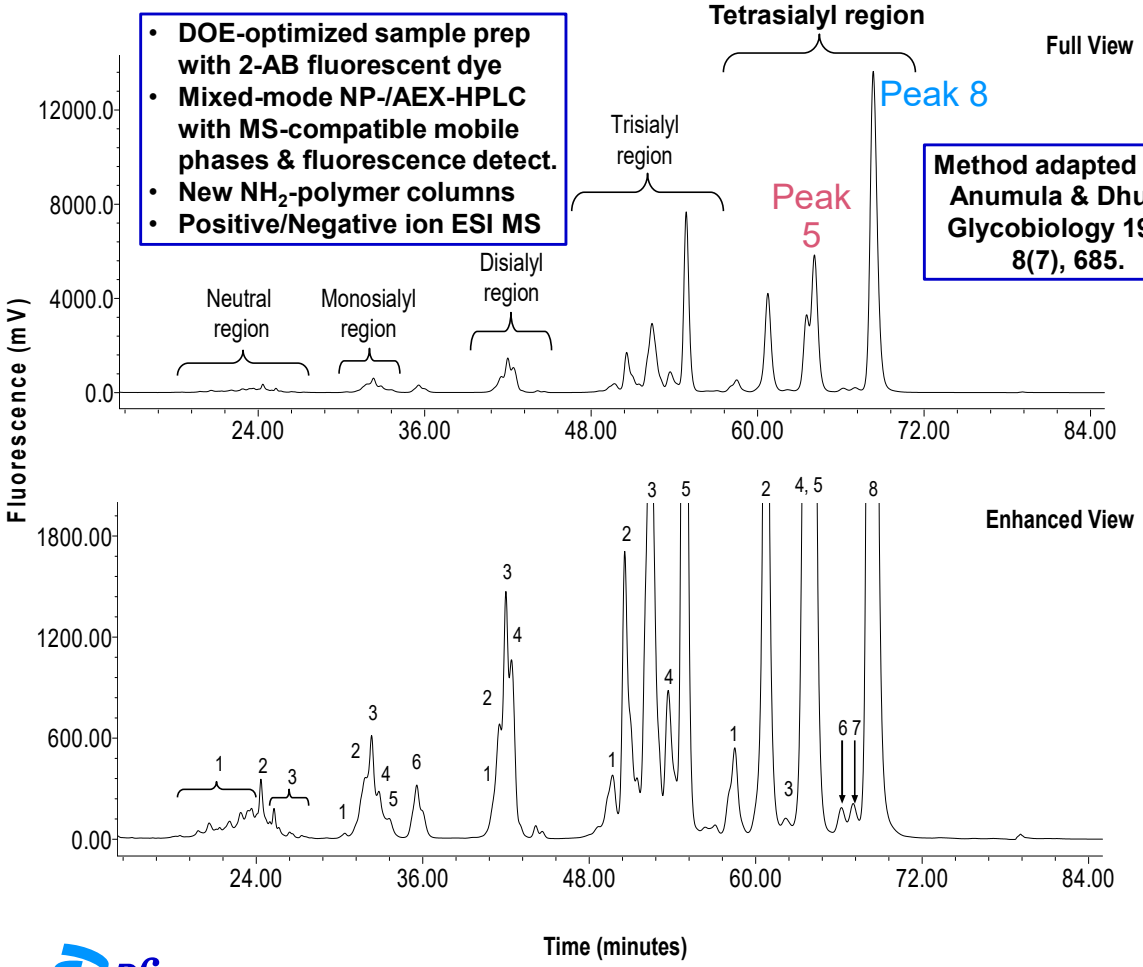
Dr. Terry D. Butters,
Oxford Glycobiology Institute

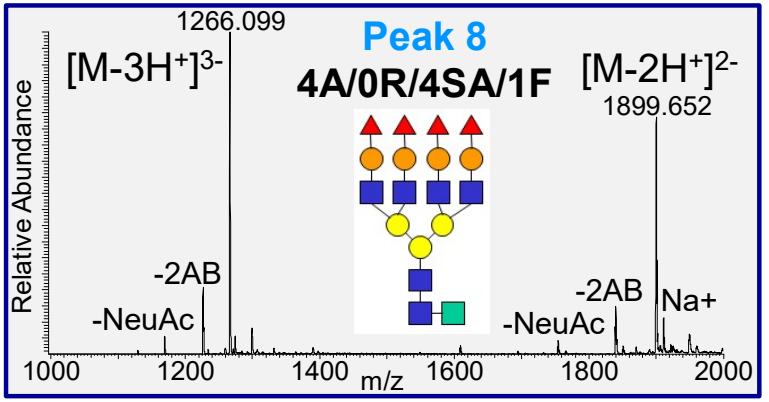
Rouse and Strang, ASMS Conference 1998

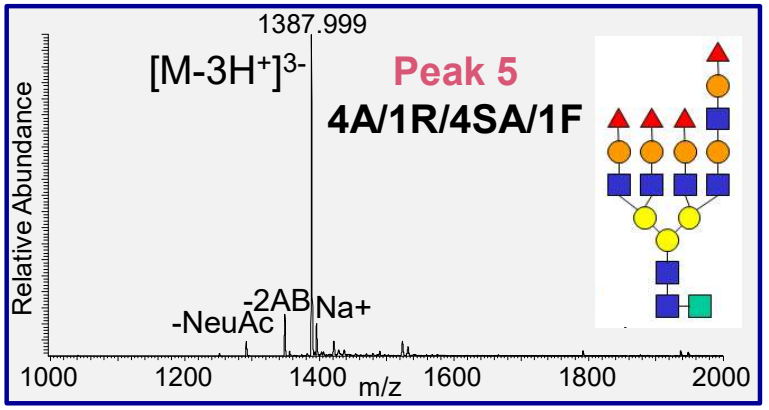
Modern N-Glycan Profiling by LC-FLR/MS: Recombinant Factor IX (rFIX)

- **DOE-optimized sample prep with 2-AB fluorescent dye**
- **Mixed-mode NP-/AEX-HPLC with MS-compatible mobile phases & fluorescence detect.**
- **New NH_2-polymer columns**
- **Positive/Negative ion ESI MS**

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

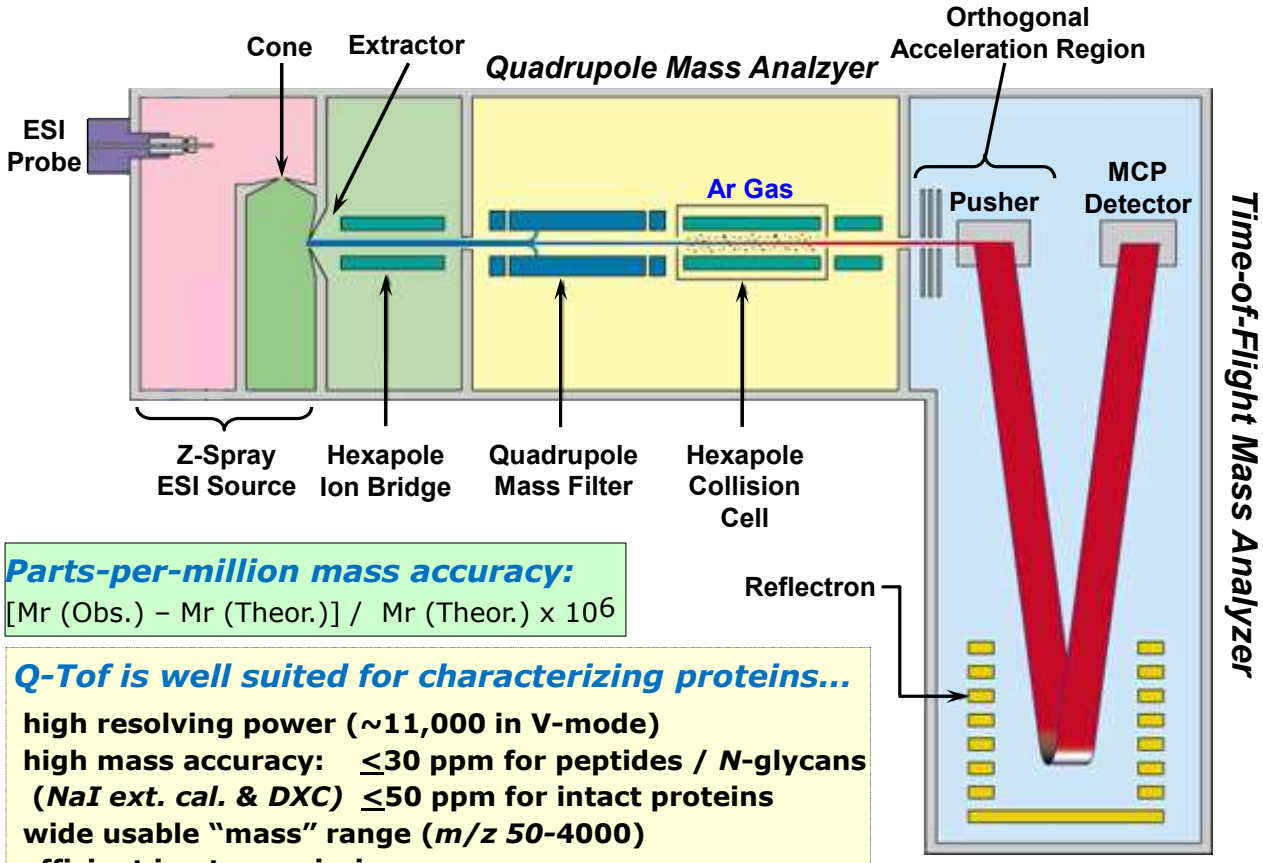






Himakshi Patel and Matt Thompson, Pfizer, 2008-2009

In 2000, the ESI-Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometer Arrives

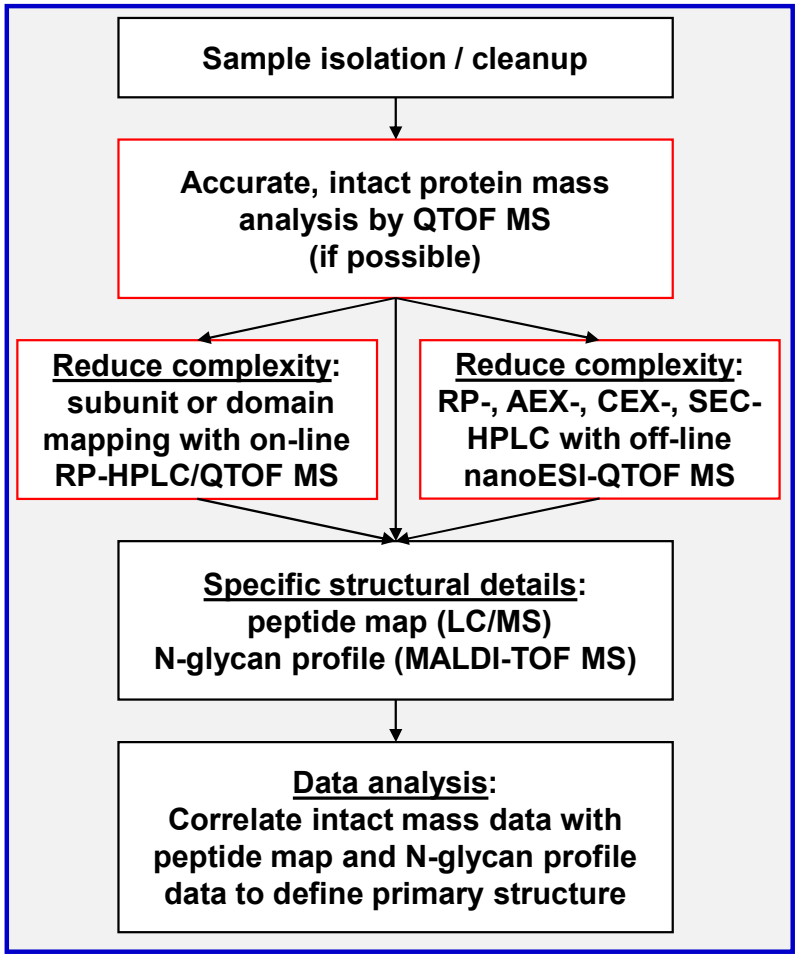


Parts-per-million mass accuracy:

[Mr (Obs.) – Mr (Theor.)] / Mr (Theor.) x 10^6

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



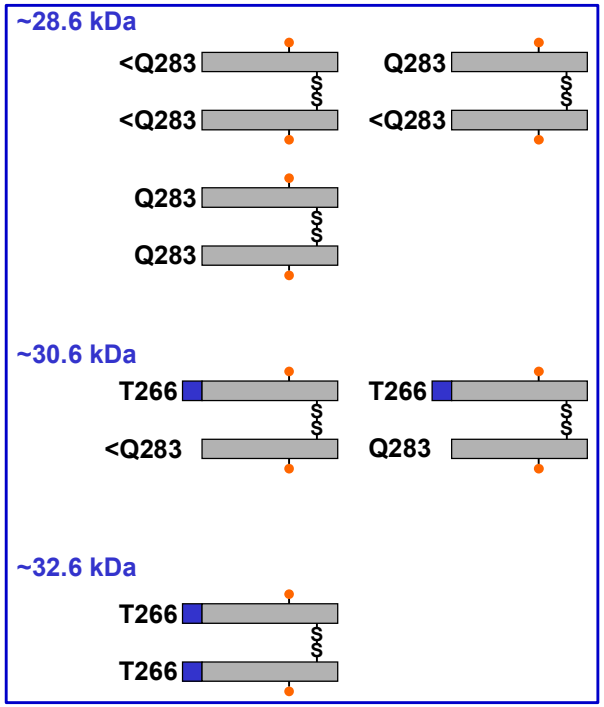
Adapted from schematic of Q-Tof-2 (Micromass MS Technologies, Waters Corp.)

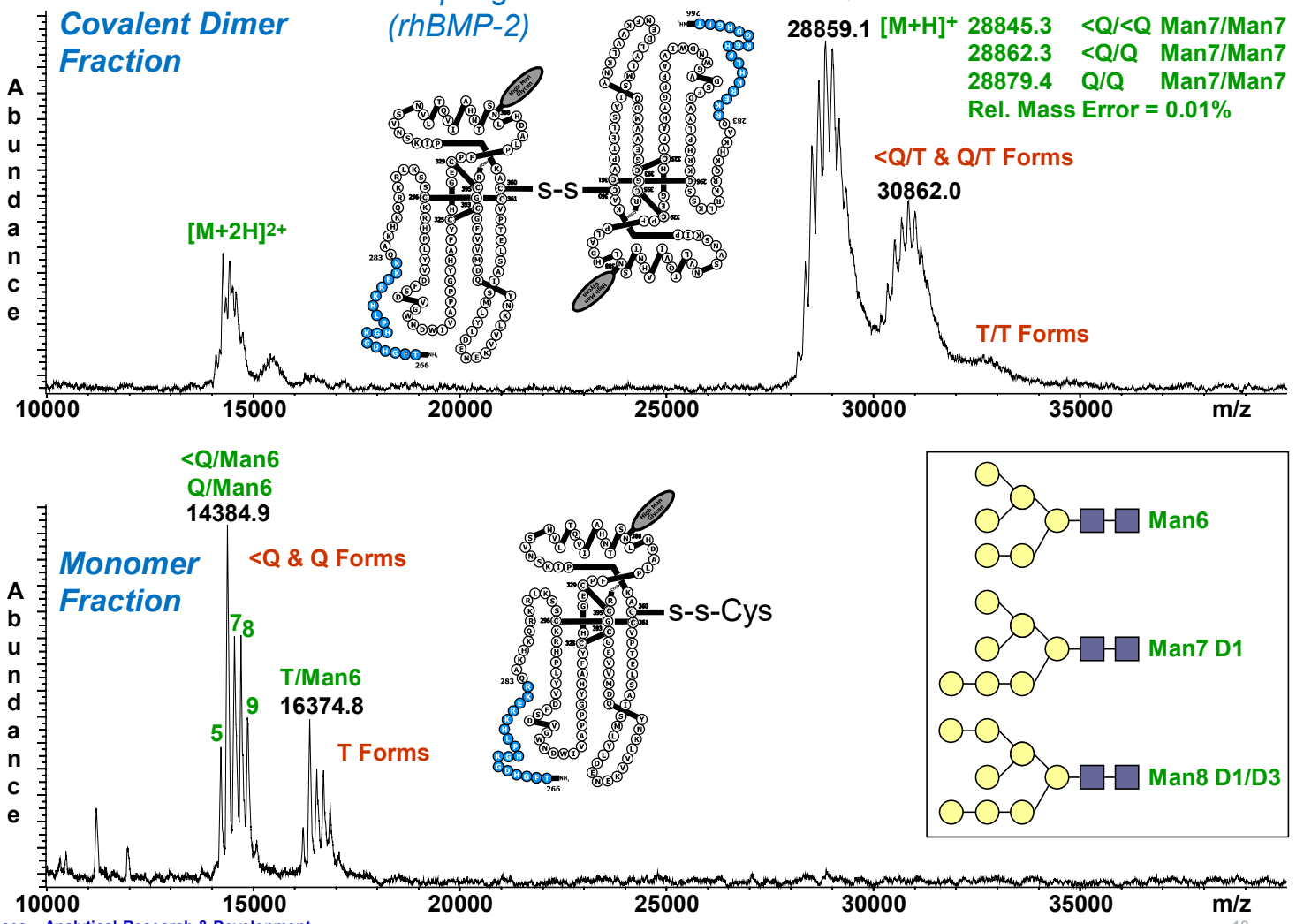
MALDI-TOF MS of Intact rhBMP-2

(*Isolated from Phenyl RP-HPLC-UV*)

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

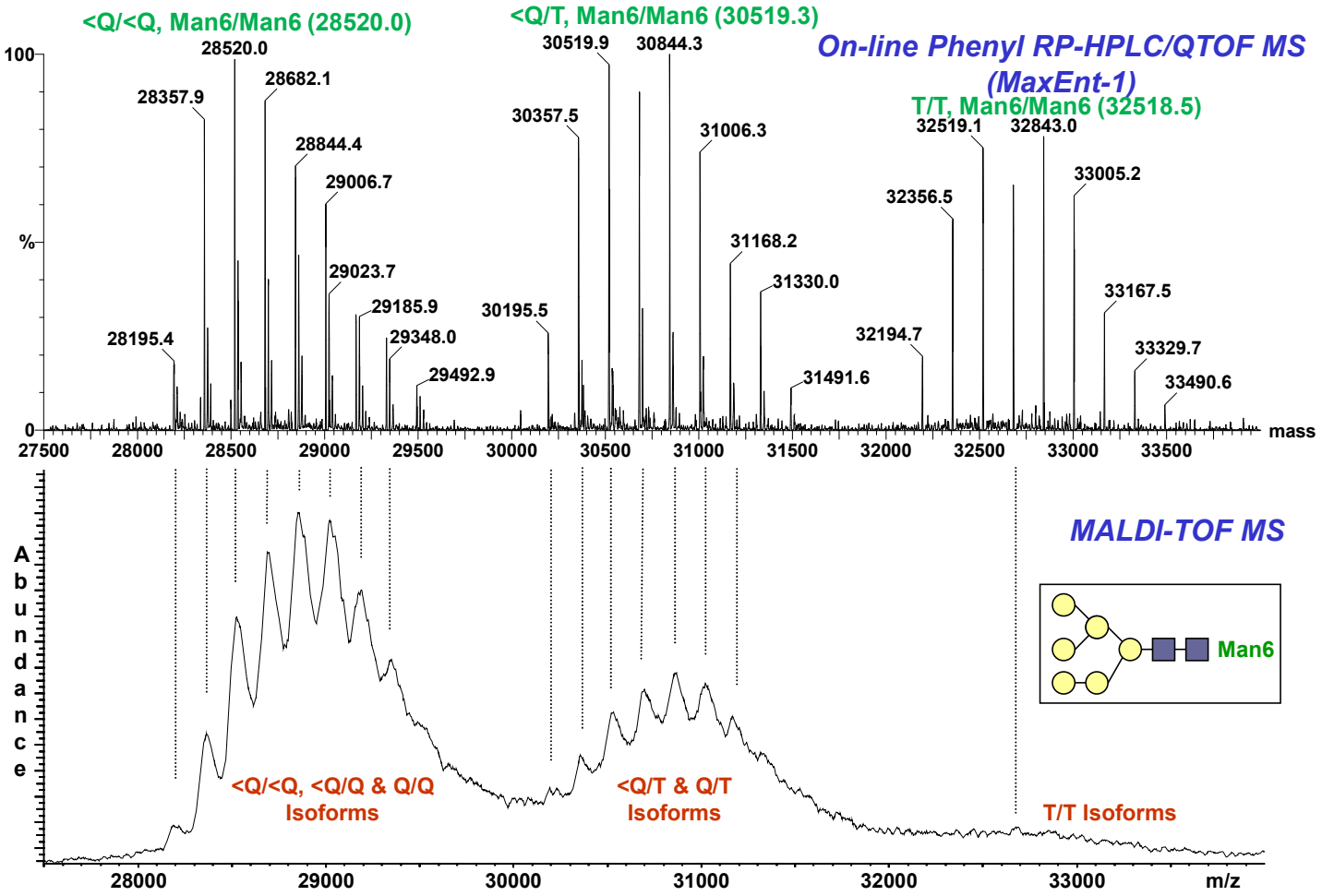
rhBMP-2 N-terminal Heterogeneity





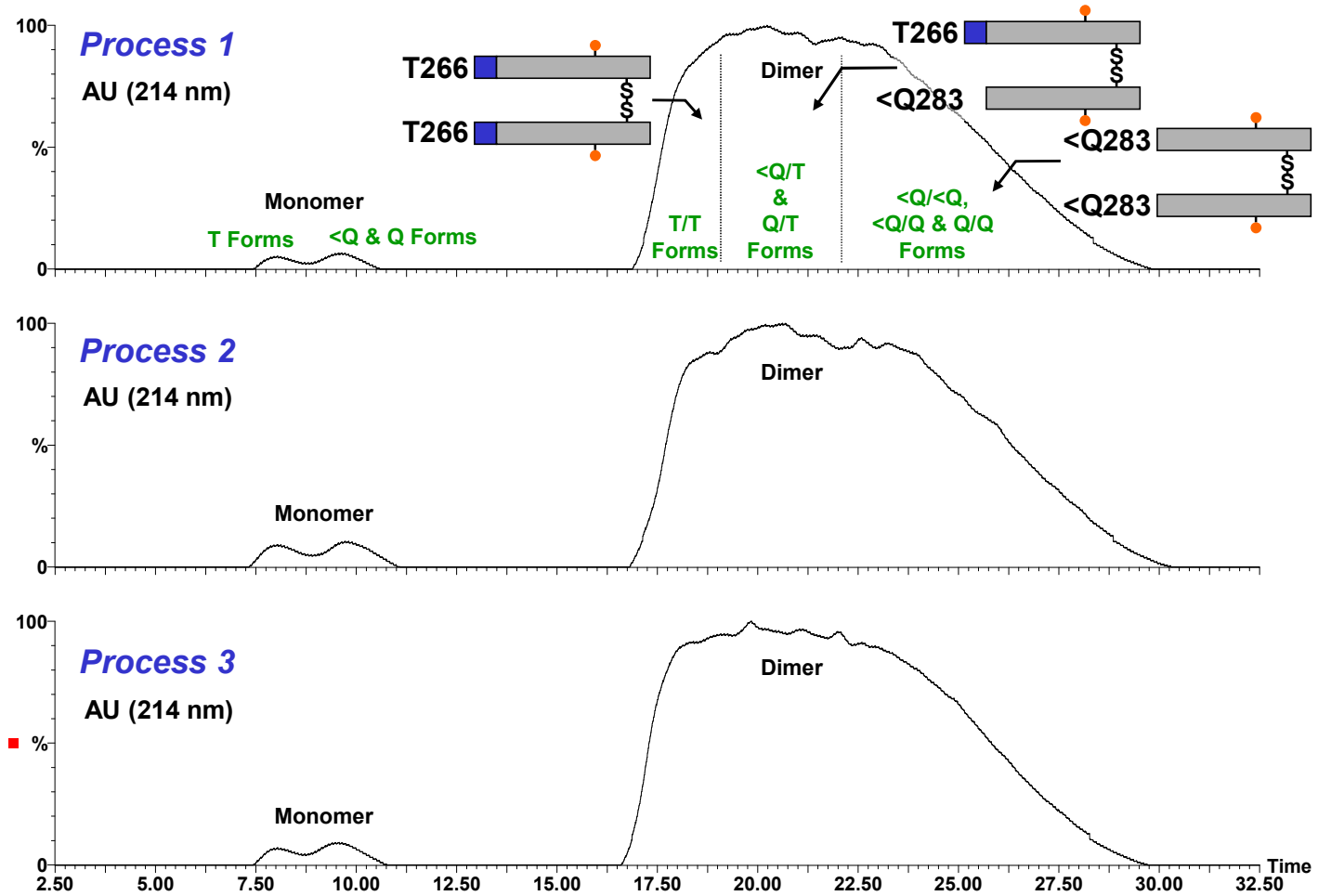
Rouse, Abbatiello, Haq, Marzilli, Nemeth-Cawley, Patel, Rathore, Jankowski, Porter, & Scoble, ACS NERM 2001, University of NH

Comparison of ESI-QTOF & MALDI-TOF MS for Covalent rhBMP-2 Dimer



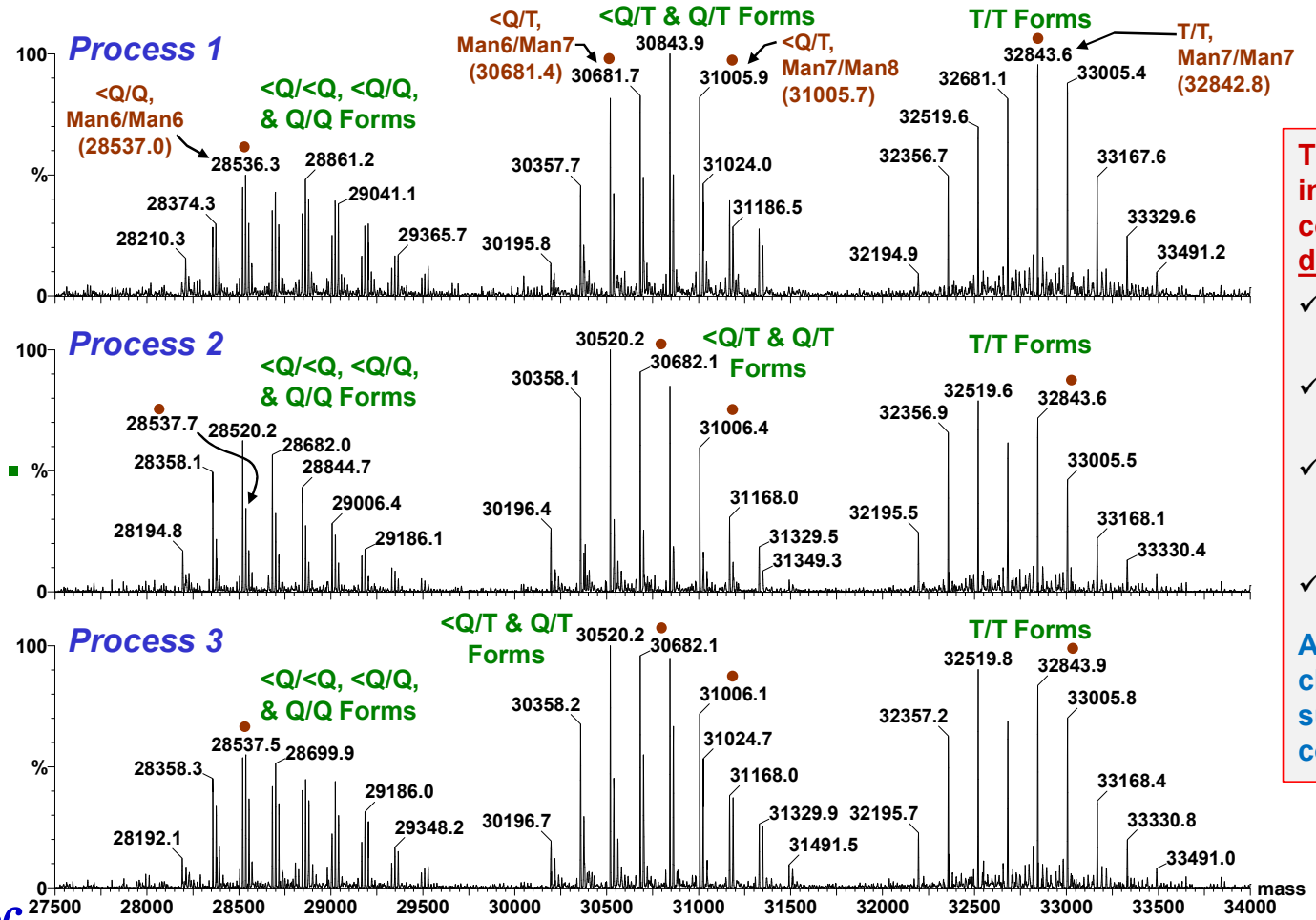
Rouse, Abbatiello, Haq, Marzilli, Nemeth-Cawley, Patel, Rathore, Jankowski, Porter, & Scoble, ACS NERM 2001, University of NH

rhBMP-2 Comparability Study: Phenyl RP-HPLC-UV / ESI QTOF MS



Rouse, Abbatiello, Haq, Marzilli, Nemeth-Cawley, Patel, Rathore, Jankowski, Porter, & Scoble, ACS NERM 2001, University of NH

rhBMP-2 Comparability Study: Zero-Charge Mass Spectra



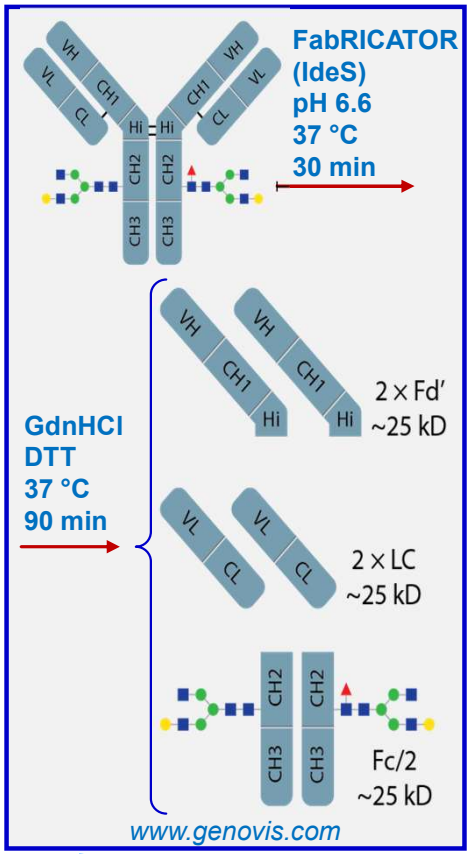
The mass spectra of intact rhBMP-2 indicated the 3 processes produced comparable DS (according to pre-determined acceptance criteria):

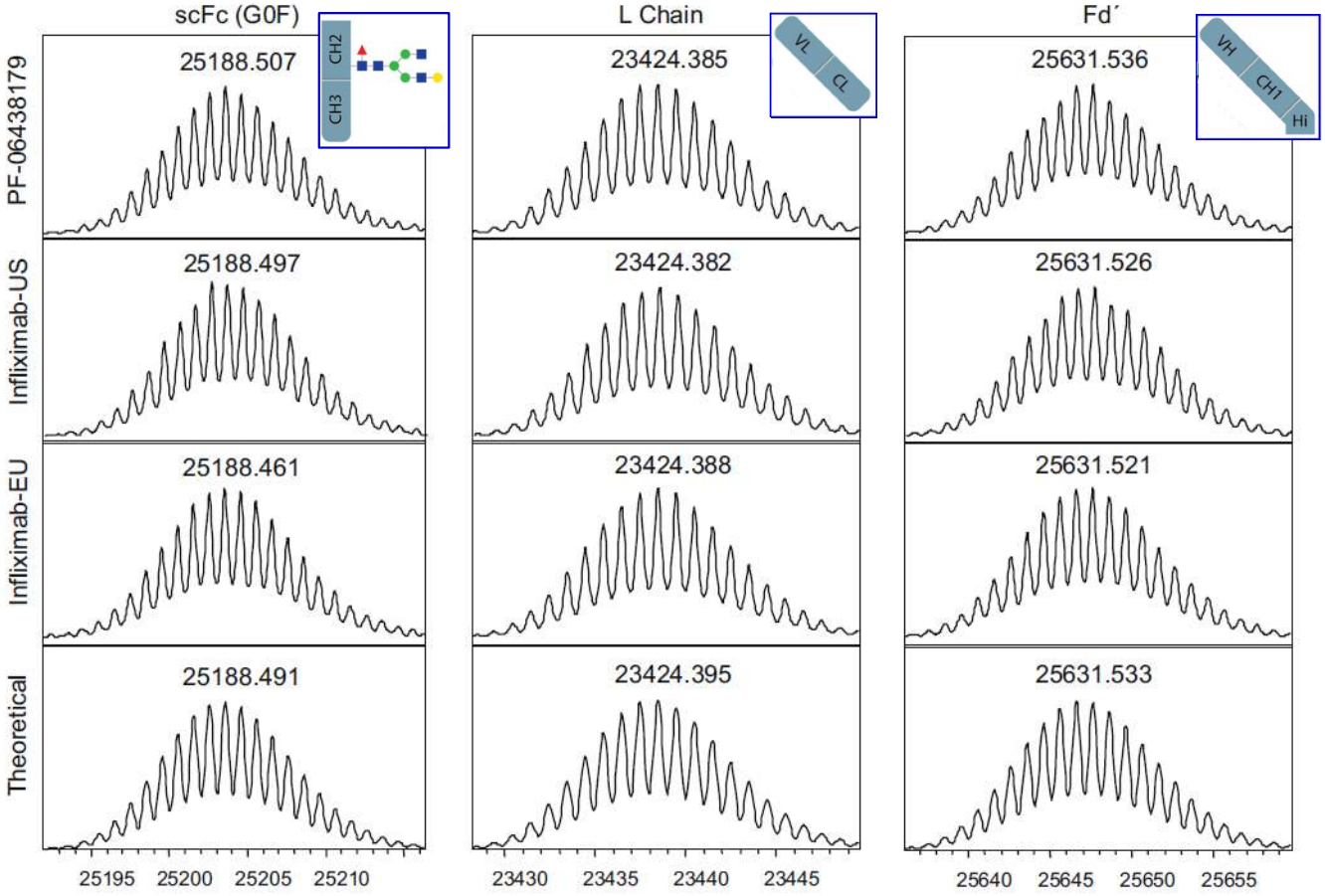
- ✓ **Mass differences between the same isoforms were < 1.3 Da**
- ✓ **All isoform masses were < 1.6 Da (50 ppm) from theoretical values**
- ✓ **Similar isoform distributions were observed (slight redistribution in process 2)**
- ✓ **No new isoforms were detected**

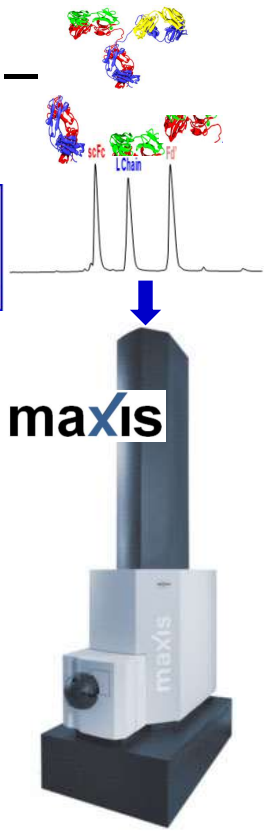
All routine testing and characterization studies together supported structural and functional comparability of rhBMP-2 DS

Rouse, Abbatiello, Haq, Marzilli, Nemeth-Cawley, Patel, Rathore, Jankowski, Porter, & Scoble, ACS NERM 2001, University of NH

Biosimilarity Assessments: Zero-charge Mass Spectra from LC/MS – Subunit Analysis of PF-06438179, Infliximab-US, and Infliximab-EU







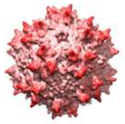
UHR QTOF MS
- ✓ 40k FWHM resolution
- ✓ <2 ppm mass accuracy

Derzi et al. Adv. Ther. (2016) 33:1964–1982.

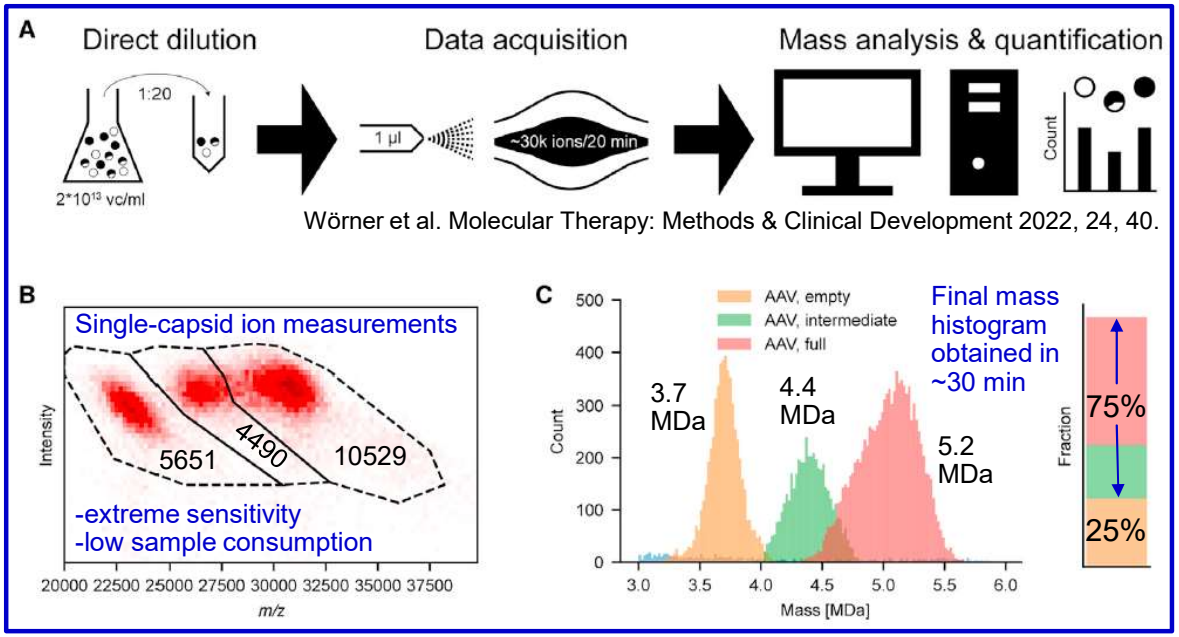
Emerging Ultrahigh Mass Measurement Techniques (2022)

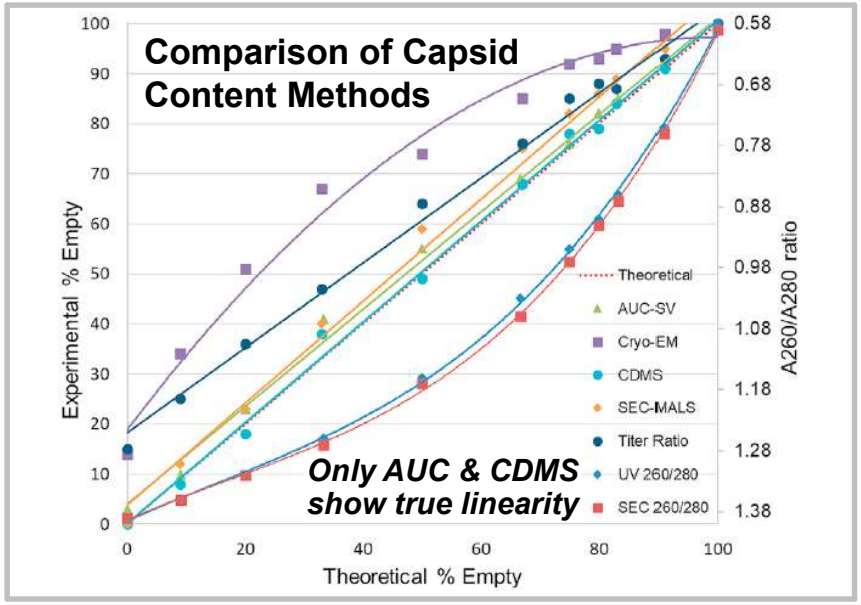
Werle et al. Molecular Therapy: Methods & Clin. Dev. 2021, 23, 254.

Charge Detection MS (CDMS)

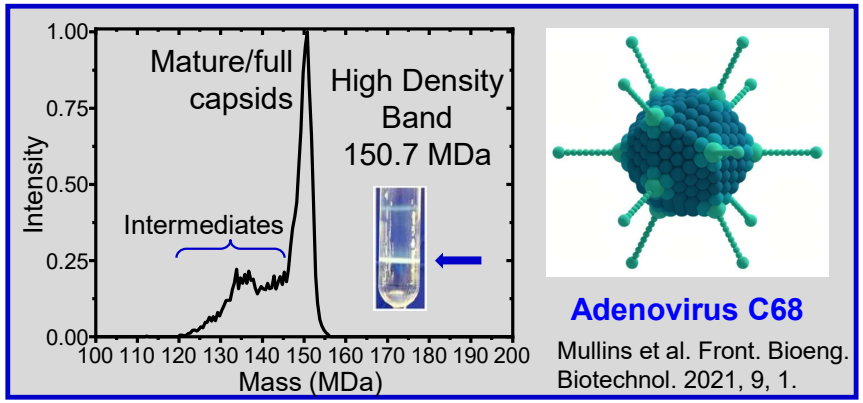


Adeno-associated Virus (AAV)





- ✓ 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 →



Adenovirus C68

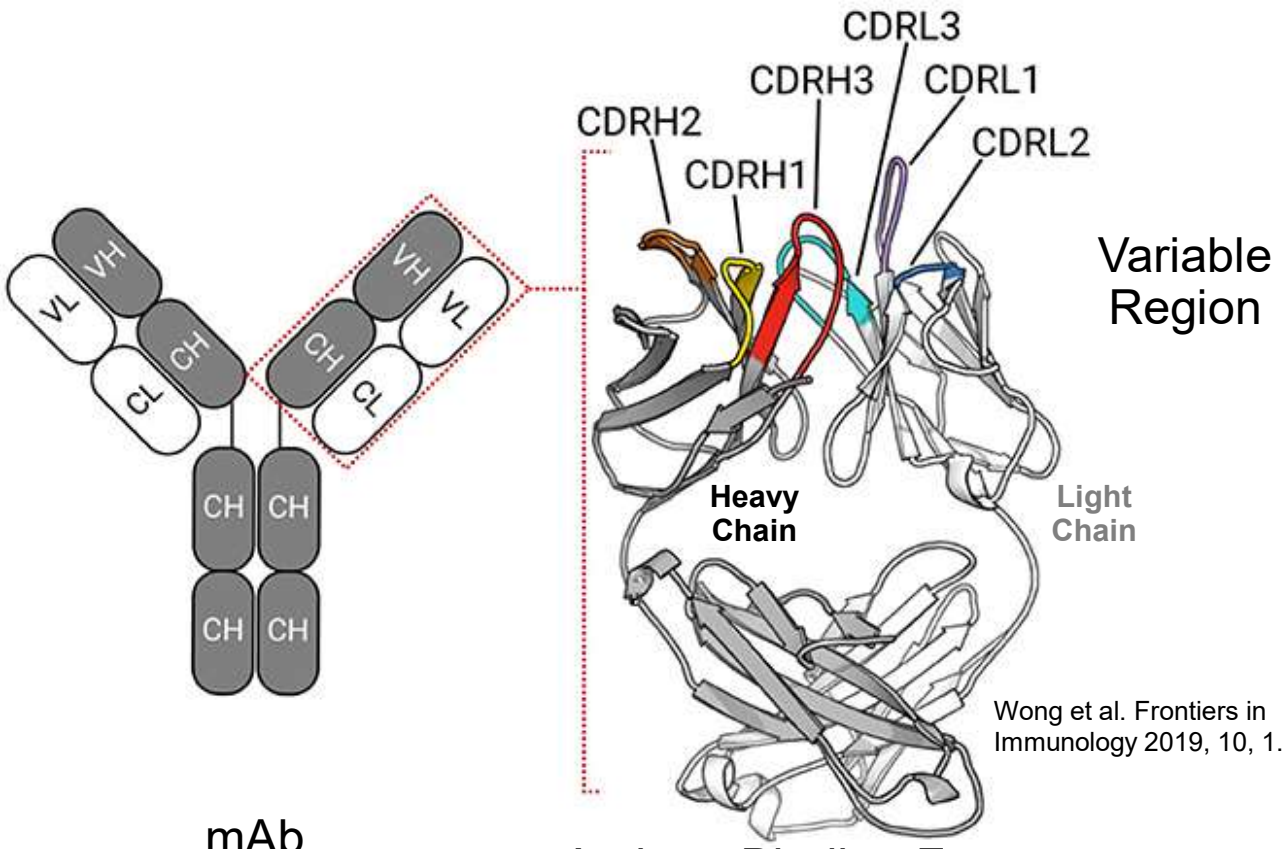
Mullins et al. Front. Bioeng. Biotechnol. 2021, 9, 1.

Pfizer

Biotherapeutics Pharmaceutical Sciences – Analytical Research & Development

Thomas Powers & Olga Friese, Pfizer

Chemical Modifications in Complementarity-Determining Regions (CDRs)



mAb

Antigen Binding Fragment (Fab) Region

Variable Region

Wong et al. Frontiers in Immunology 2019, 10, 1.

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.
- Sydow et al. PLoS ONE 2014, 9, e100736.
- 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

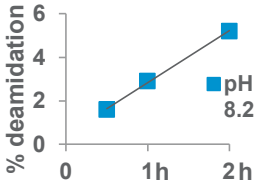
Used the latest LC-MS/MS technologies…

Reduce (DTT)	Alkylate (IAA)	Desalt (BioSpin6)	Trypsin/Lys-C (pH 8.2, 37C)

Marzilli et al. LC/GC Supp. 2017, 15, 6.

Orbitrap Lumos (HCD & EThcD)

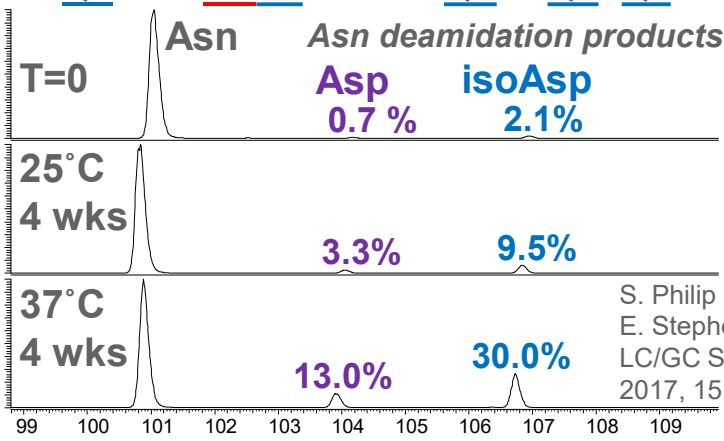
High selectivity



Low-artifact digests

…to separate, identify & quantify hotspots

^{25}SSQSLVHSNGNTFLYWYLQKPGQSPQLLIYR^{55}

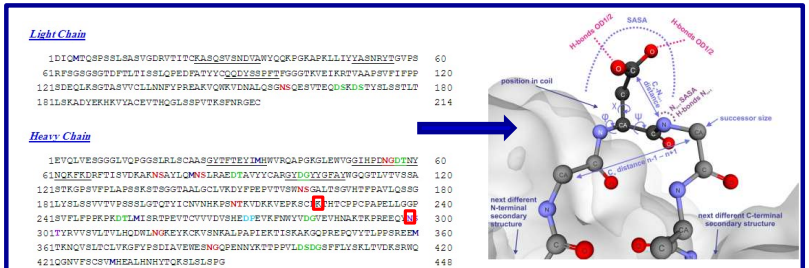


S. Philip E. Stephens LC/GC Supp. 2017, 15, 6.

Compiled a hotspot database→shared across orgs

mAb1 L Chain CDR Hotspot Database			Rel. Abund.
1	DIVMTQTPLSLSVTPGQPASISCRSSQSLV	30	High (>5%)
31	HSNGNTFLYWYLQKPGQSPQLLIYRVSNRF	60	Low (1-5%)
61	SGVPDRFSGSGSGTDFTLKISRVEAEDVGV	90	Potential
91	YYCFQATHVPWTFGGGTKVEIK		Framework

…and developed a structure-based hotspot prediction algorithm for deamidation, isomerization & oxidation



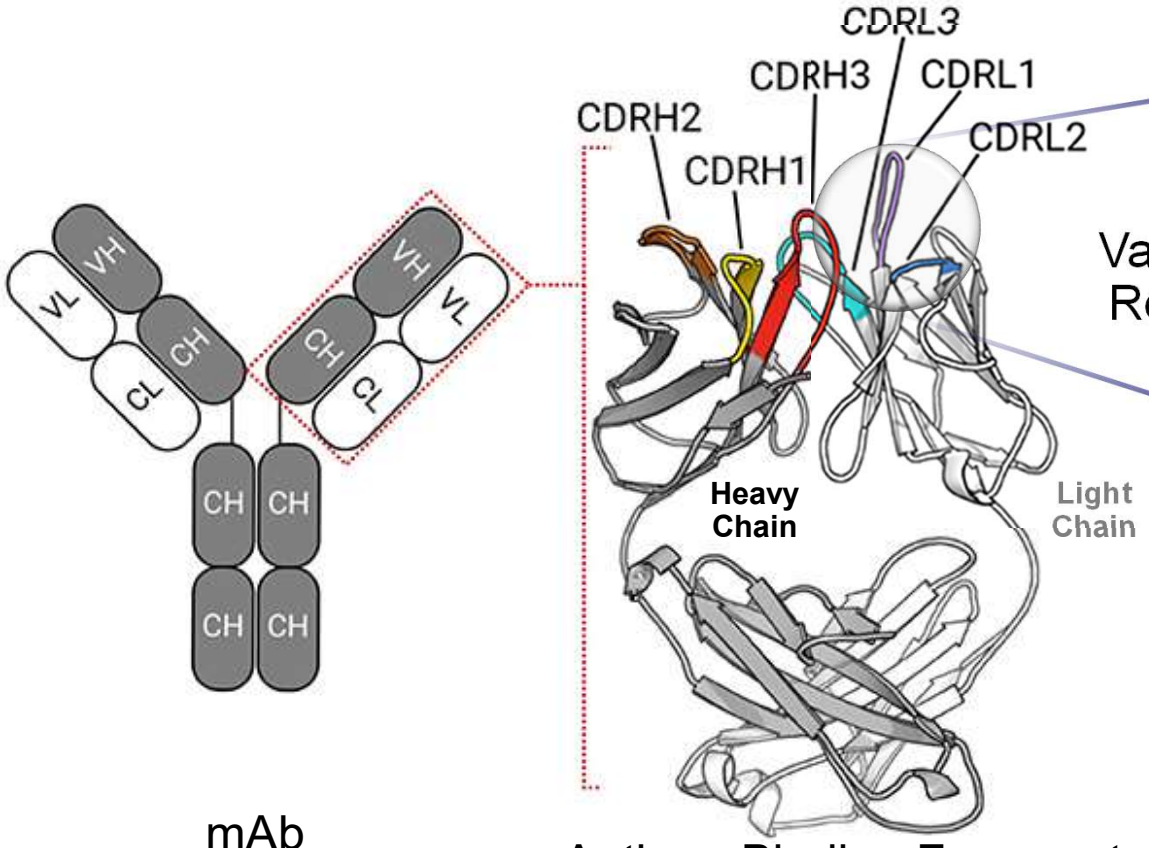
Sydow et al. PLoS One 2014, 9

Pfizer Structural & Computational Biology Team

Biotherapeutics Pharmaceutical Sciences – Analytical Research & Development

Elaine Stephens, Roger Theberge, Leah Wang, Mellisa Ly, Peilu Liu, Dennis Gessmann,
Lisa Marzilli, Jason Rouse, and Pfizer Structural & Computational Biology Team

Trastuzumab Light Chain CDR-1



mAb

Antigen Binding Fragment (Fab) Region

Hotspot Database

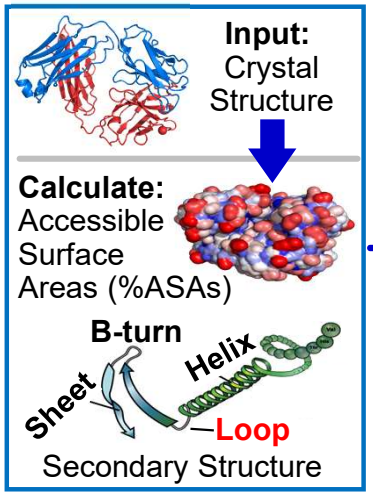
Trastuzumab Material	$D^{28}V$ (L-CDR1)	$N^{30}T$ (L-CDR1) Asn / Asp / isoAsp / Asu
T=0 control	ND	88.8 / 9.7 / 0.8 / 0.7
4w, 40C, Tris pH 7.5	ND	28.2 / 69.8 / 0.6 / 1.4
4w, 40C, His pH 5.8	ND	75.7 / 12.2 / 2.9 / 9.2
4w, 40C, Glu pH 4.5	ND	89.2 / 2.7 / 2.3 / 5.8
Harris et al. 2001 (%)	---	~15% (CEX-HPLC)
Sydow et al. 2014 (%)	---	11% → 24% (His, pH 6)

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, 40C,4wks)
  - ✓ His buffer (pH 5.8, 40C,4wks)
  - ✓ Glu buffer (pH 4.5, 40C,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



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

Trastuzumab Heavy Chain

```
  1EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRY  60
 61ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS 120
```

Trastuzumab Light Chain

```
  1DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPS  60
 61RFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPP 107
```

***Structure-based prediction** (96% accuracy rate; 68% false discovery rate)*

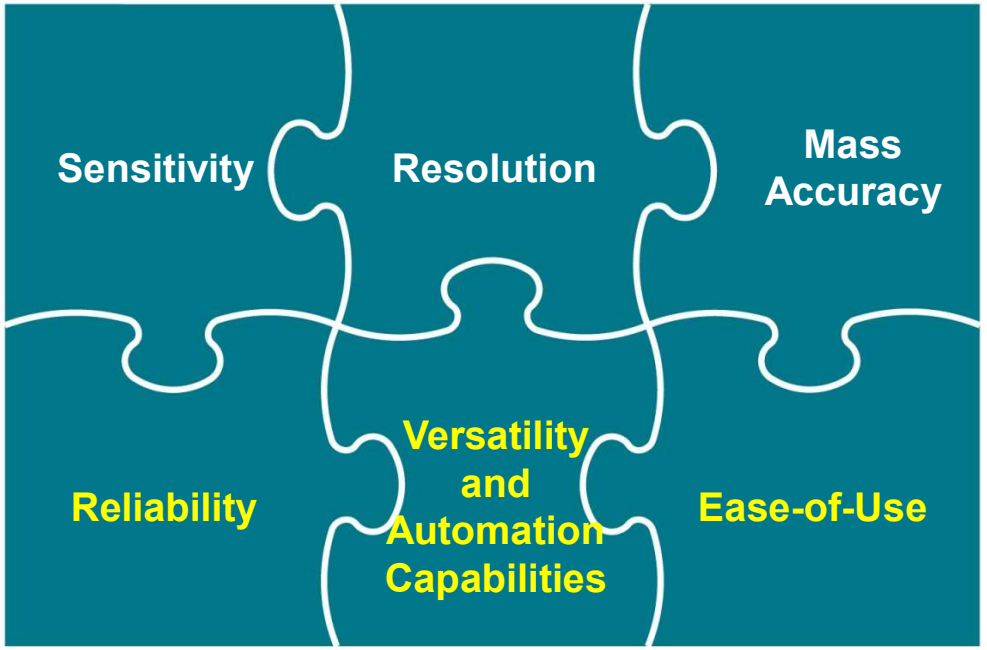
mAb	CDR	Site	Motif	%ASA (x)	%ASA (x+1)	B-turn Type	B-turn Position	Sec. Structure	Predicted Hotspot >5%	Exp. Hotspot Level (%) †
Trastuzumab	L-CDR1	28	DV	66.8	0	--	--	Loop	No	ND
(4hkz)		30	NT	70.6	48.5	II'	2&3	Loop	Investigate	71.8
	H-CDR1	28	NI	84.1	1.0	I	1&2	Loop	No	0.2
		31	DT	78.7	3.5	I	3&4	Helix	No	ND
	H-CDR2	55	NG	54.8	68.6	I	4&--	Loop	Investigate	7.3
		62	DS	92.1	64.3	I	2&3	Loop	Investigate	0.2
	H-CDR3	99	W	18.8	--	--	--	Sheet	No	0.6
		102	DG	83.0	84.0	I'	2&3	Loop	Investigate	43.3
		107	M	0.7	--	--	--	Loop	No	0.6
		108	DY	0.2	34.5	--	--	Loop	No	ND

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

Peilu Liu, Victor Beaumont, Omar Davulcu, Jason Rouse, and Pfizer Structural & Computational Biology Team

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!

- Research Grade Mass Spectrometers
- Smart UHPLC Mass Detectors

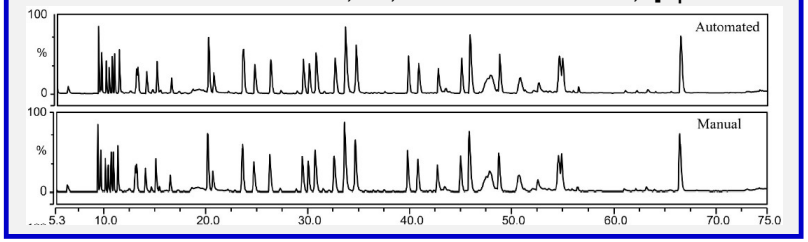
Modern View of Mass Spectrometry in Process & Product Dev. Labs

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

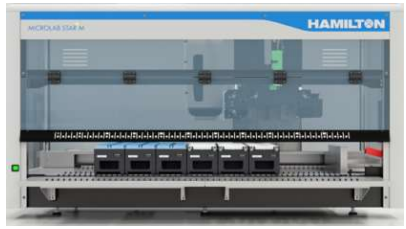
Direct analysis

Automated sample prep

Sitasuwan et al. mAbs 2021, 13, 1.: ↓hands-on time; ↑ precision



- 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

In-depth Characterization

Routine PQA monitoring (MAM)

UHPLC
LC – MS/MS

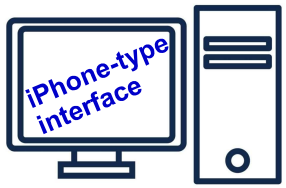
Research Grade Mass Spectrometer
(State-of-the-art, $$$)

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

Transfer key PQA characterization knowledge via attribute workbook

UHPLC
LC – MS

Mass detector



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

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

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 is 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!

Acknowledgements

Mass Spectrometry and Biophysical Characterization (MSBC)

- John Allison
- Jack T. Watson
- Hubie Scoble
- Steve Martin
- Jim Vath
- Wen Yu
- Anne Marie Strang
- Marta Czupryn
- Tom Porter
- Mike Jankowski
- Himakshi Patel
- Suman Shanker
- Smita Karnik
- Lisa Marzilli
- Meg Ruesch
- Karen Bertani
- Renee Olson
- Carolyn Slade
- Stephanie Flores
- Rohin Mhatre
- James Carroll
- Victor Fursey
- Robin Andreotti
- Weibin Chen
- Gary Valaskovic
- Chris Yu
- Eric Carlson
- Drake Zhang
- Mark Rogers
- Chris Ziegenfuss
- Rich Rogers
- Jonathan Josephs
- ...and many more

Pfizer

Biotherapeutics Pharmaceutical Sciences – Analytical Research & Development

Thank You