Mass Accurate Analysis of Protein Therapeutics: Assessment of Top Down Approaches

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M-CERSI

Top-Down Analysis of Antibodies

The ideas, findings, and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

The Office of Testing and Research Mission

- To improve the FDA's capacity for evaluating and monitoring drug quality, safety and efficacy
- To help modernize current regulatory pathways, or indicate a new regulatory pathway where there is currently none
- To address regulatory science issues that are mission critical, and/or cannot or will not be done by other government agencies or industry
- To maintain a state of 'research readiness' that anticipates potential regulatory needs while allowing for rapid response to emergent regulatory issues

Modern Analytics for Complex Drugs

1. Complex drugs require information rich techniques to fully describe their critical quality attributes
   - The key element to measure is structure
   - Structure (1^0,2^0, 3^0 or 4^0) is essential for function

2. Generate structural "patterns" that can fully describe the active pharmaceutical ingredient(s)
   - Techniques being assessed include: MS, CD, NMR, MALLS, and DUVRR
   - These physicochemical studies need a biological readout (bioassay) to be relevant to function

3. Knowledge gained by these studies prepares the agency to evaluate and respond to questions about these drug products during the review process, in response to adverse events, and eventually strengthen regulatory guidance documents

Modern Analytics for Complex Drugs

1. Identification and Characterization of Active Pharmaceutical Ingredient (API)

2. Chemical Comparability or Similarity Assessments

3. Surveillance for Counterfeiting/Adulteration

4. Process Monitoring and Drug Development

Characterization of Protein Therapeutics and Monoclonal Antibodies

Physicochemical

- Structure (primary and higher order)
- Molecular weight
- Degree of heterogeneity (chemical, conformational, etc)
- Functional, receptor binding and immunochemical
- Biological activity (e.g. potency)
- Functional domain characterization
- Enzyme kinetics
- Receptor binding studies
- Protein-target binding

Impurity profiles

- Product-related impurities
  - Inactive protein variants generated during manufacture or storage
- Process-related impurities
  - Host cell proteins & DNA
  - Media components
  - Column leachates

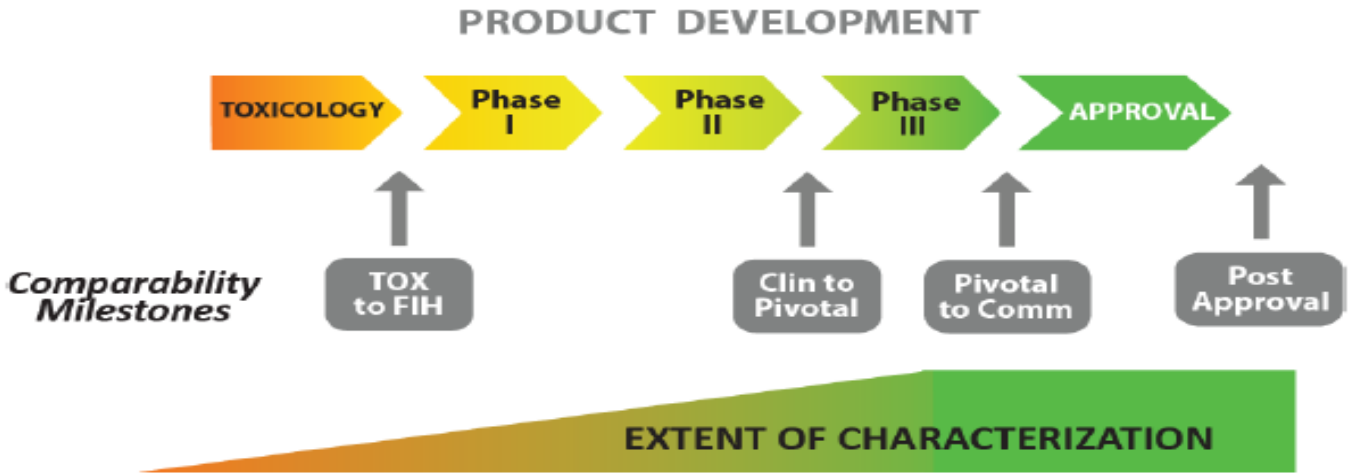
Stability

- Degradation profiles under real time, accelerated, stress (freeze-thaw, light high temperature exposure, agitation) conditions

Collectively, these quality attributes define identity, purity, potency, and stability of the product and if critical, they correlate with efficacy and safety

Characterization

- Main purpose of characterization is to provide product understanding to guide process control
- Extensive characterization is performed during development
  - Extent of characterization depends on the stage of product development
  - At the time of IND submission, sufficient characterization data to ensure safety and to demonstrate comparability between the clinical lots and toxicology lots



Characterization Methods

Characterization methods do not necessarily need to be validated for routine quality control purposes but should be:

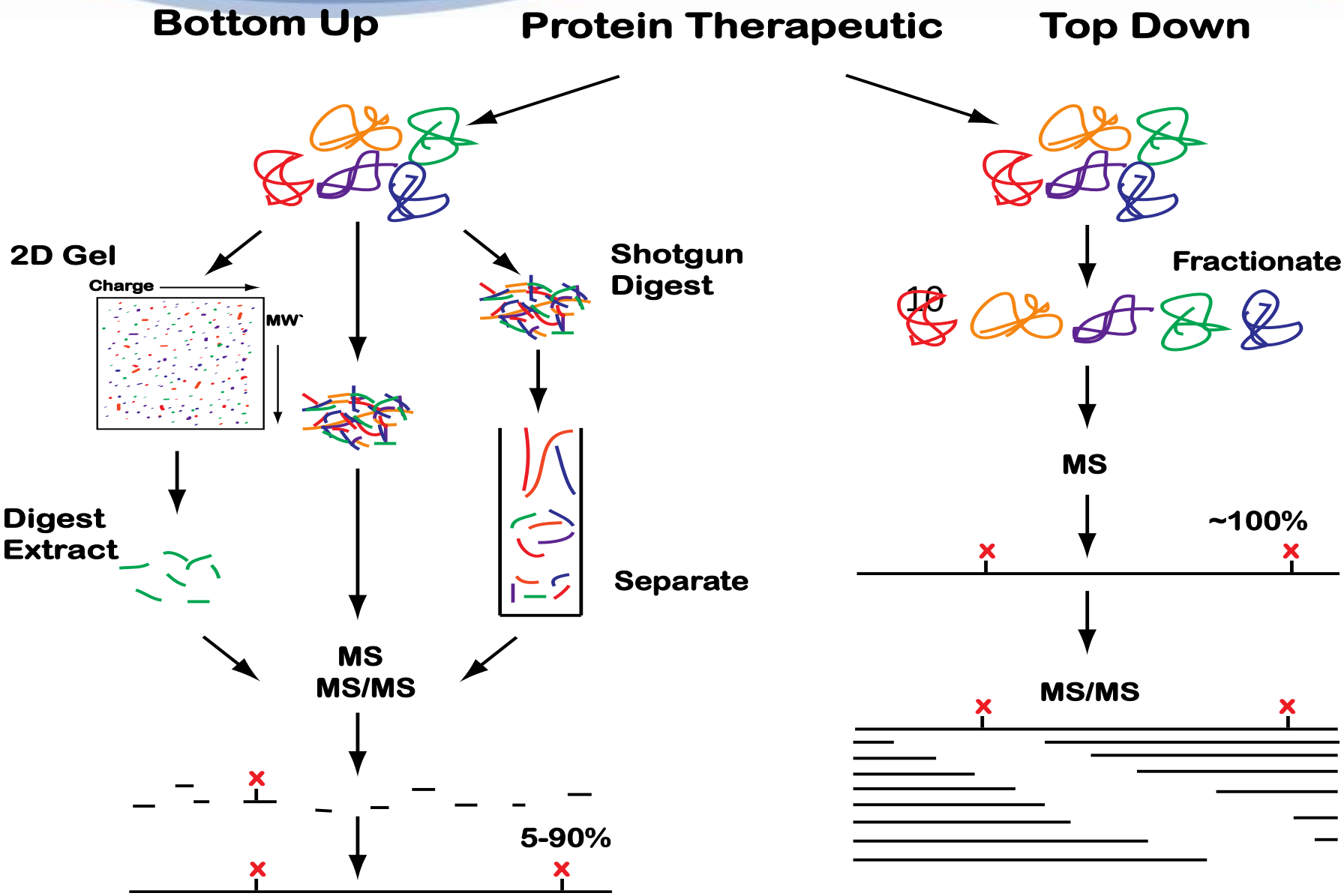
- Fit for the intended use and qualified
- Scientifically sound
- Produce results that are reproducible and reliable
- Capable of identifying product differences
- Qualitative & Quantitative
- Sensitive

Typically, the assay performance is demonstrated through the analysis of intentionally stressed or spiked samples

QC Methods

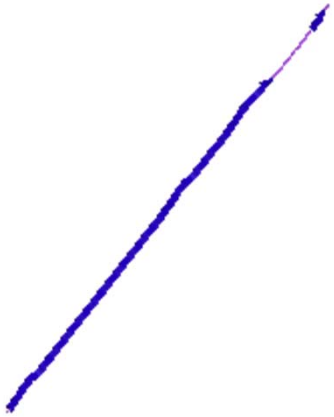
Should be validated for intended purpose as per ICH Q2(R1) and FDA guidance (licensure)

Type of analytical procedure characteristics	IDENTIFICATION	TESTING FOR IMPURITIES quantitat.	 limit	ASSAY - dissolution (measurement only) - content/potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm.Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+



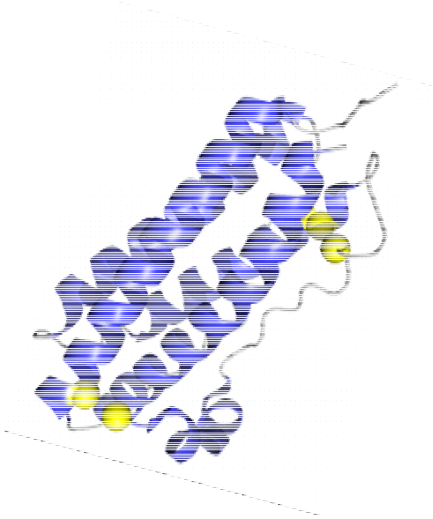
Protein and Peptide Mass Spectrometry in Drug Discovery

Intact Molecule Measurements - Top Down



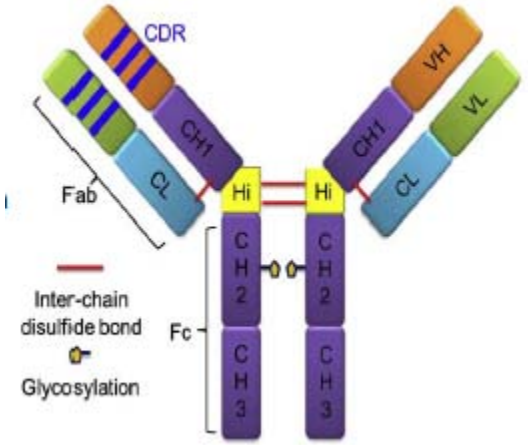
Protamine
4 kDa

Primary Sequence Analysis



GCSF
18.8 kDa

As a part of Similarity Assessment



Monoclonal Antibody
150 kDa

TBD

Protamine

Protamine Sulfate is a purified mixture of simple protein principles obtained from the sperm or testes of suitable species of fish, which has the property of neutralizing heparin. Each mg of Protamine Sulfate, calculated on a dried basis, neutralizes NLT 100 USP Heparin Units.

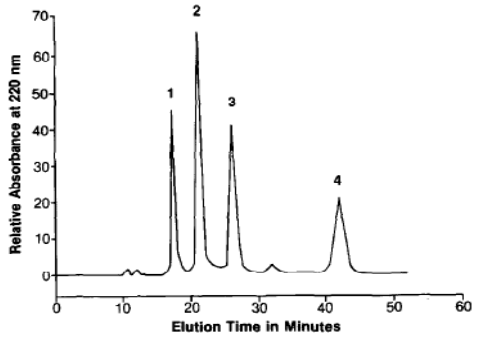
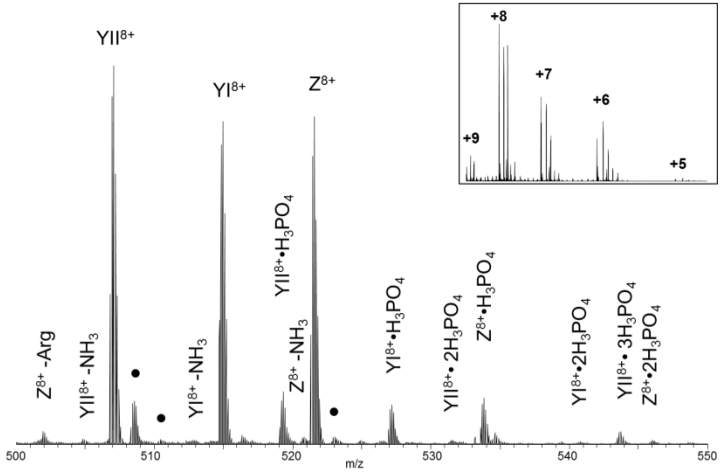


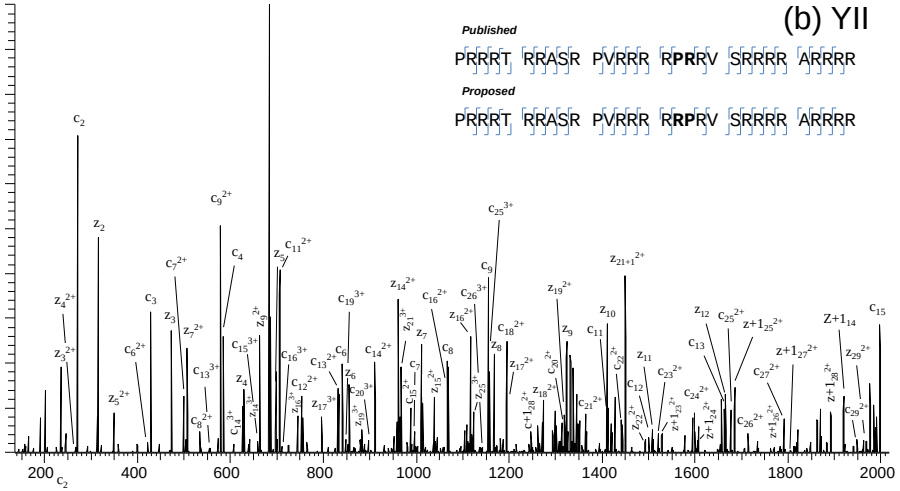
FIG. 2. An analytical HPLC profile of a 200-μg load of chum protamine. Peak resolution gradually decreased with column use.

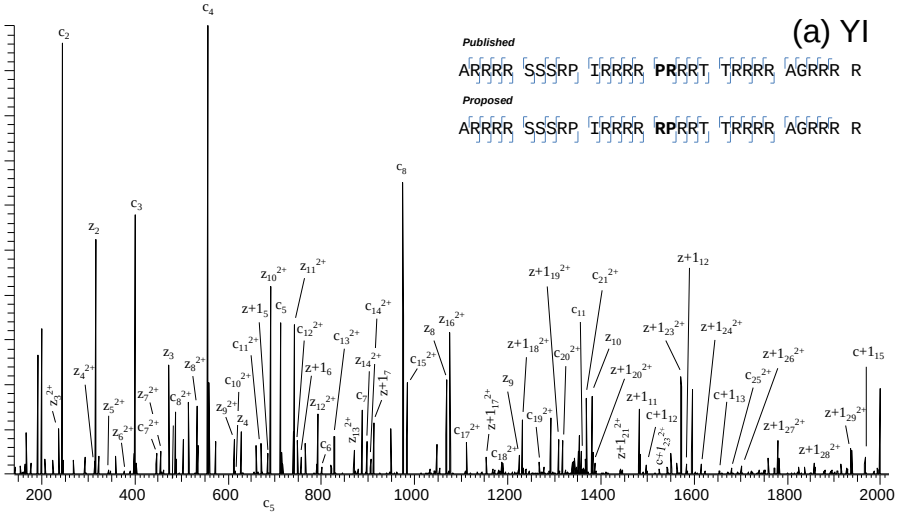
In 1990, Eli Lilly (original NDA holder) published a characterization of the major components of chum salmon protamine.

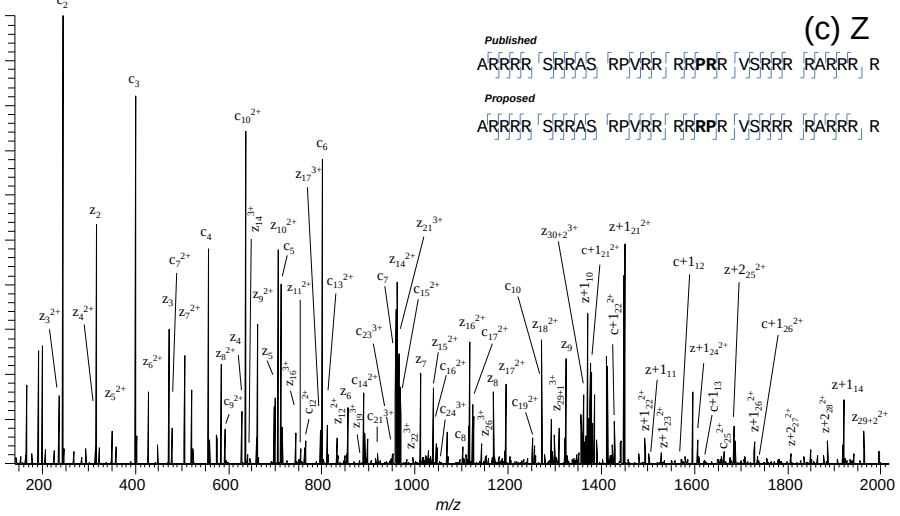
[1] Hoffmann JA, Chance RE, Johnson MG (1990) Purification and analysis of the major components of chum salmon protamine contained in insulin formulations using high-performance liquid chromatography. Protein Expr Purif 1:127-33

Protamine – Sequence Assignment of Peptides



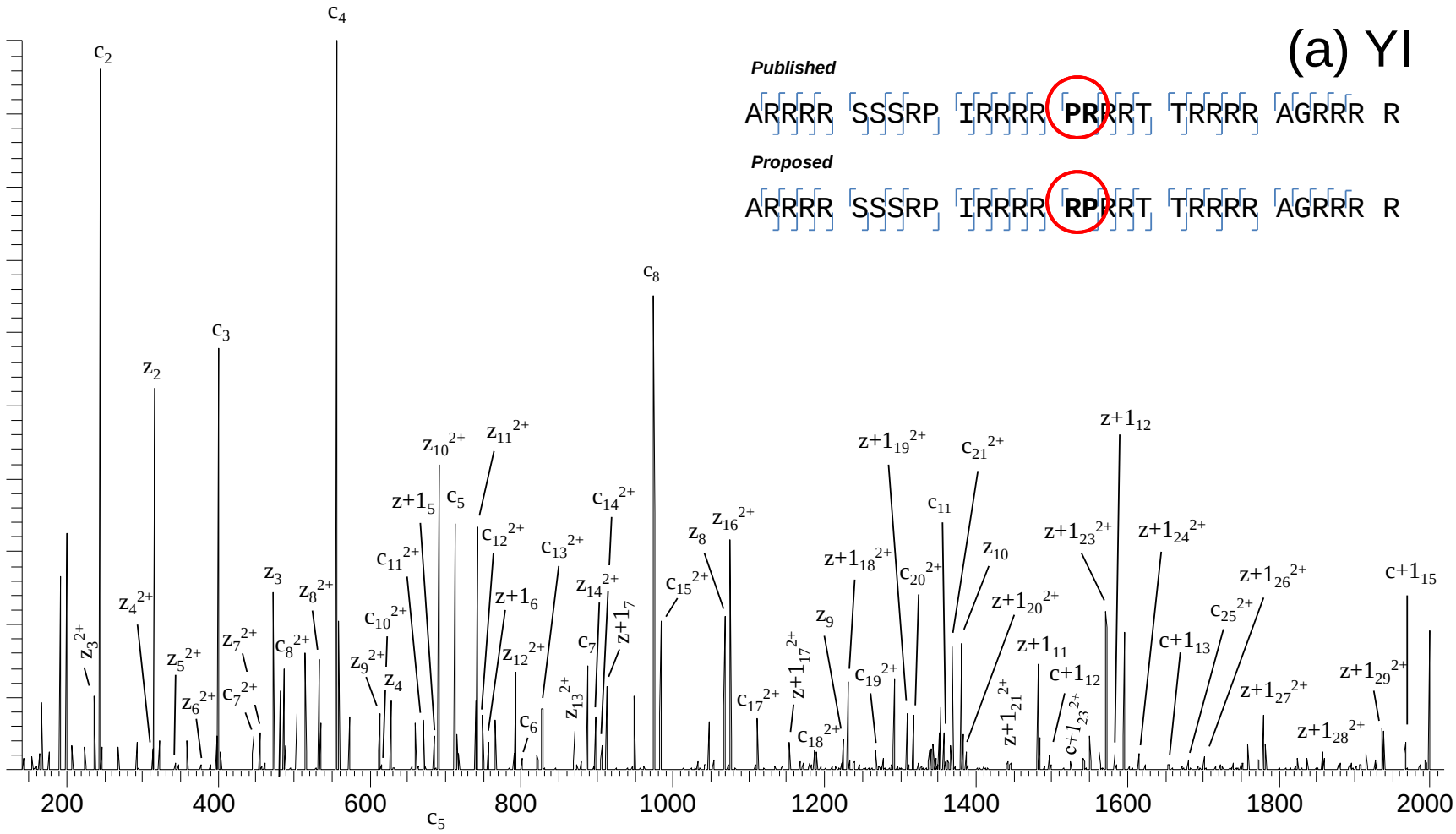






Gucinski, A. C., Boyne, M ., RCMS, In press

Protamine – Sequence Assignment of Peptides



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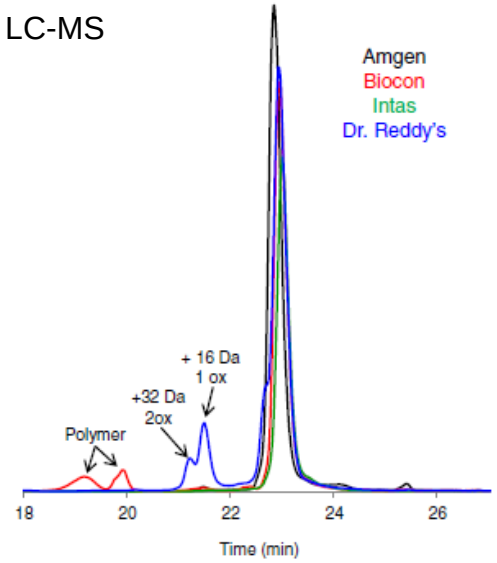
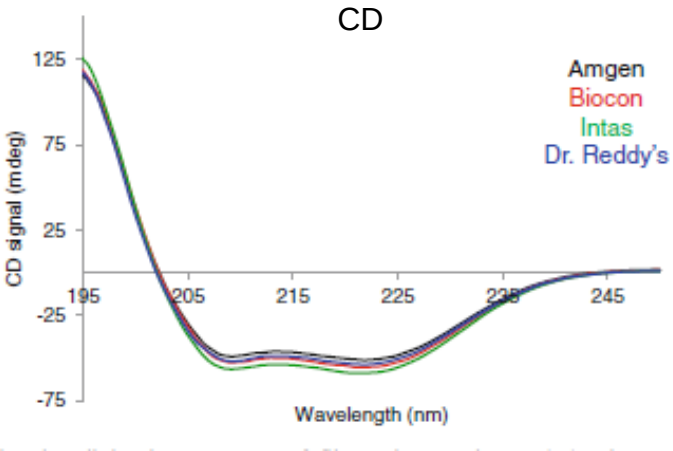
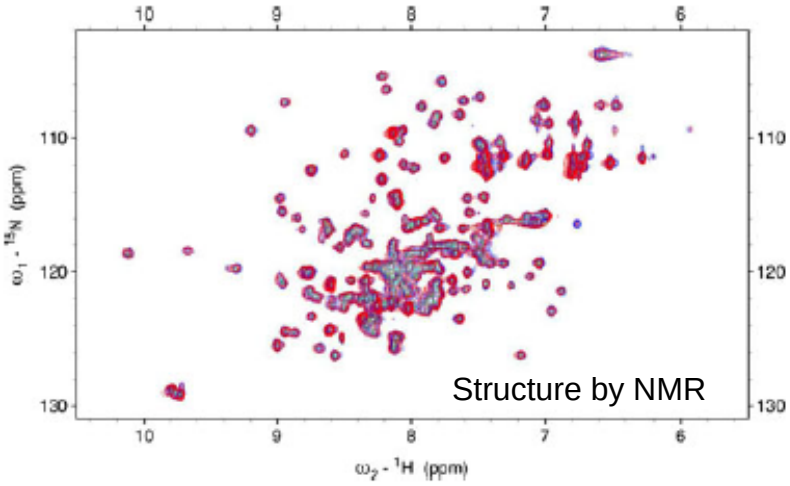
Granulocyte-Colony Stimulating Factor (G-CSF)

- Acidic glycoprotein recombinantly produced in *E. coli*
  - Native form is glycosylated
- Used in conjunction with many cancer treatments since 1991
- Analysis of protein structure by analytical techniques gained insight into 1º, 2º, and 3º structure differences between US and foreign produced G-CSF



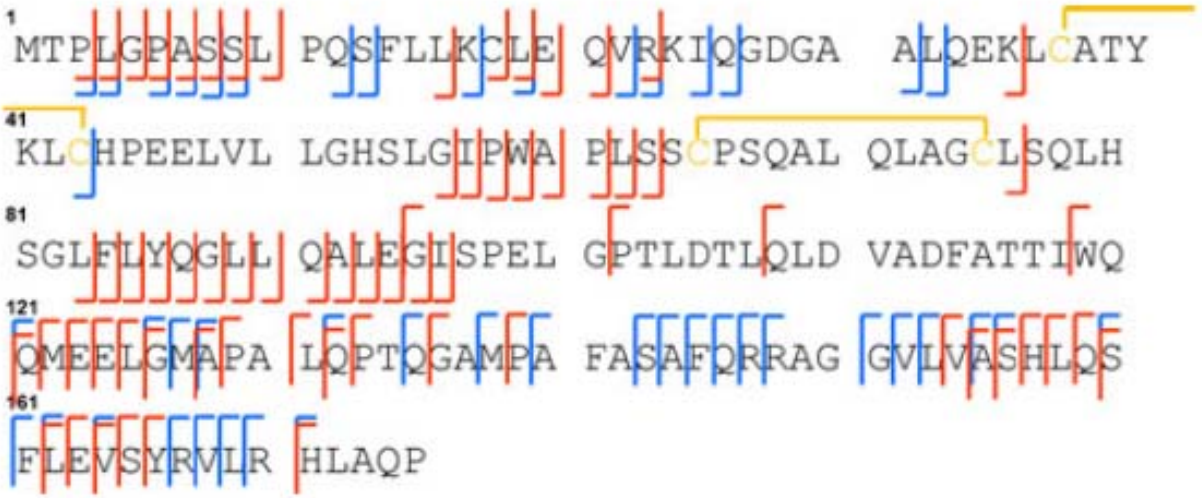




Levy, M.J., Boyne, M *et al*, *Anal. Bioanal Chem*, 2013

Top-down Mass Spectrometry of G-CSF

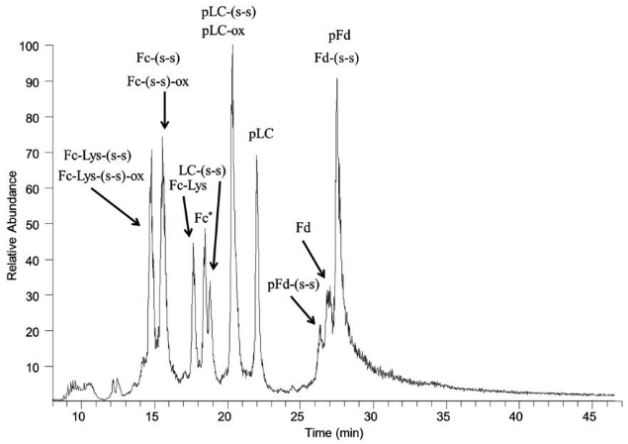
- Multiple fragmentation modes gave 26% (CID) or 39% (ETD) coverage with ~56% of the inter-residue bonds being broken.
- Identified the location of the two disulfide bonds



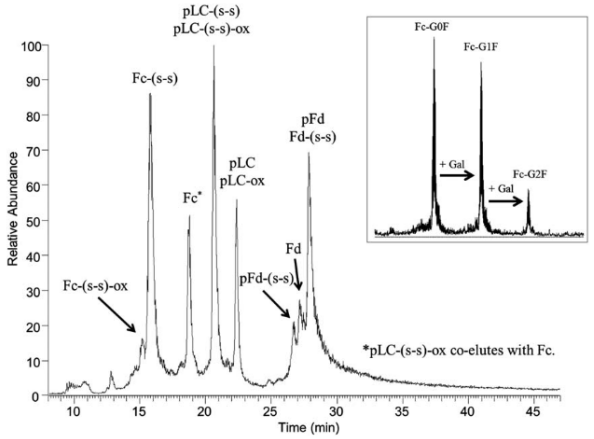
Levy, M.J., Boyne, M *et al*, *Anal. Bioanal Chem*, 2013

Middle Down Analysis of Monoclonal Antibodies

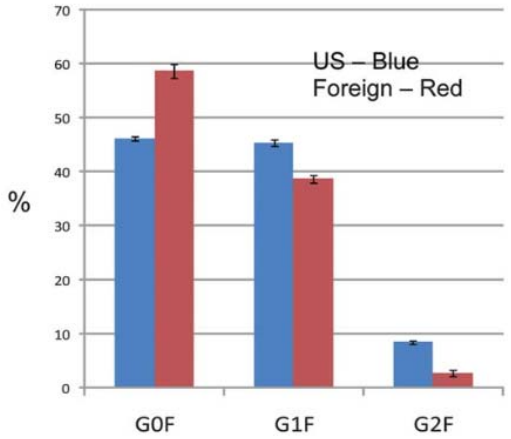
Digestion with IdeS followed by reduction and LC-MS allowed for the identification of the fragments of the intact monoclonal antibody



Foreign Product



US Product



Glycan Profiling

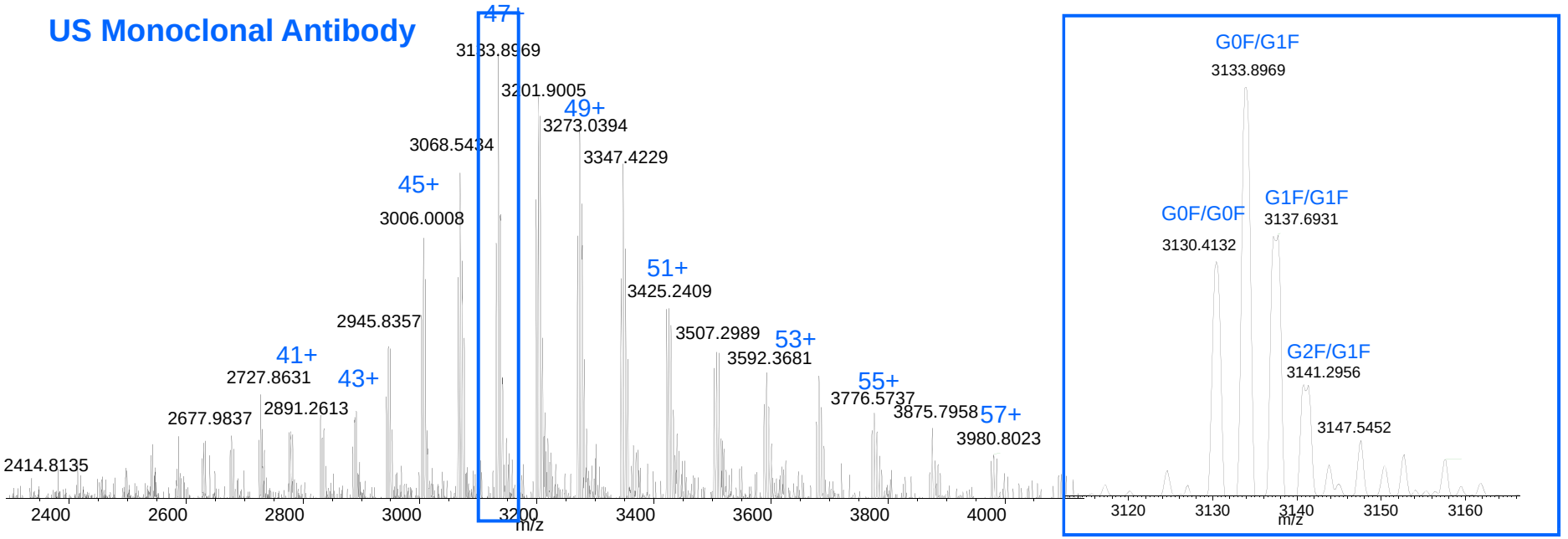
Different N- and C-terminal modifications, glycosylation profiles and disulfide bond patterns observed between the US and Foreign product with the same primary protein sequence

Can these differences be observed by direct analysis by top-down mass spectrometry?

Wang, B., Boyne, M *et al*, *Analyst*, 2013

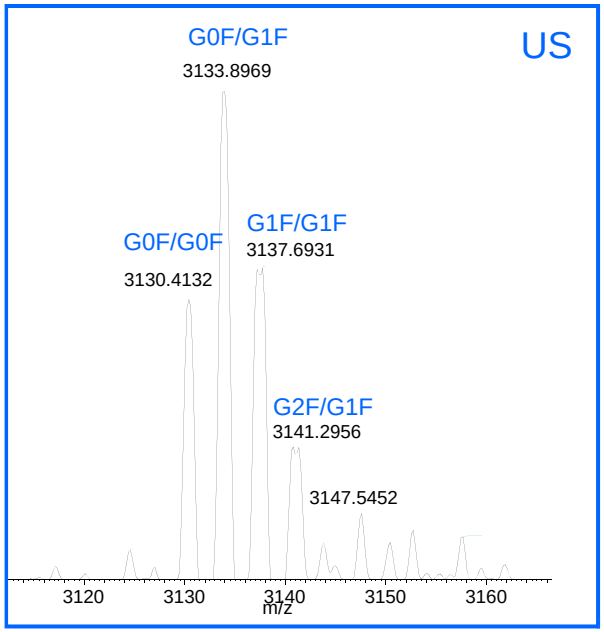
Intact Mass Spectrometry of Monoclonal Antibodies

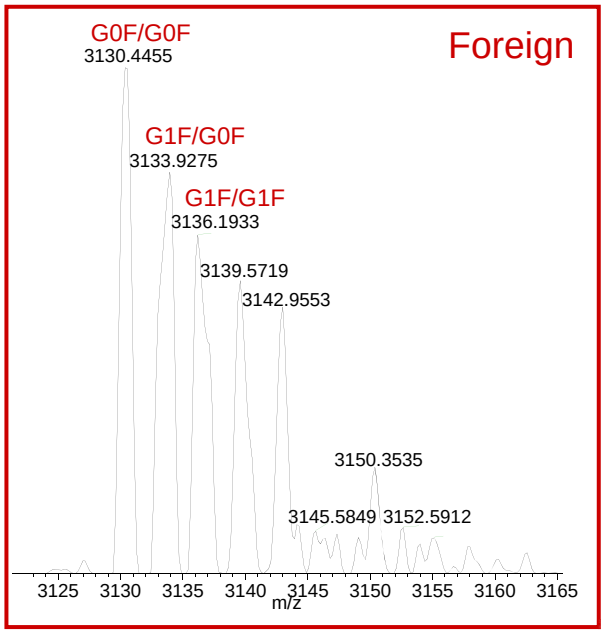
- The monoclonal antibody was desalted
- Diluted to ~1 µM in 20:79:1 ACN:H_2O:FA prior to direct infusion
- Data collected on a Thermo Fusion Tribrid in the high mass range



Intact Mass Spectrometry of Monoclonal Antibodies

Comparison of a single charge state from the US and foreign monoclonal antibody with the same primary sequence





Differences in glycosylation are observed in the intact analysis

Summary

- Product characterization is critical for building product understanding and for establishing relevant specifications as well as for supporting comparability and biosimilarity
- A comprehensive set of analytical methods, including orthogonal methods should be used to measure the full range of product characteristics

- Top Down Intact Approaches to Monoclonal Antibodies

Strengths
- Simplified Sample Prep
- PTM's in Combination
- Comparative Analysis

Weaknesses
- Expert Users
- Expensive Equipment
- Fragmentation Limitations

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OIP
Abi D'sa
Bruce Ross

Questions