



# The qualification of reference standards for biochemical / biophysical assays

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September 20, 2011

IABS Conference on Reference Standards for Therapeutic Proteins: Their Relevance, Development, Qualification, and Replacement

# ABC Facility

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Abbott Bioresearch Center (ABC)  
Worcester, Massachusetts

*Center of Excellence in Biologics*



## Biologics Discovery & Development

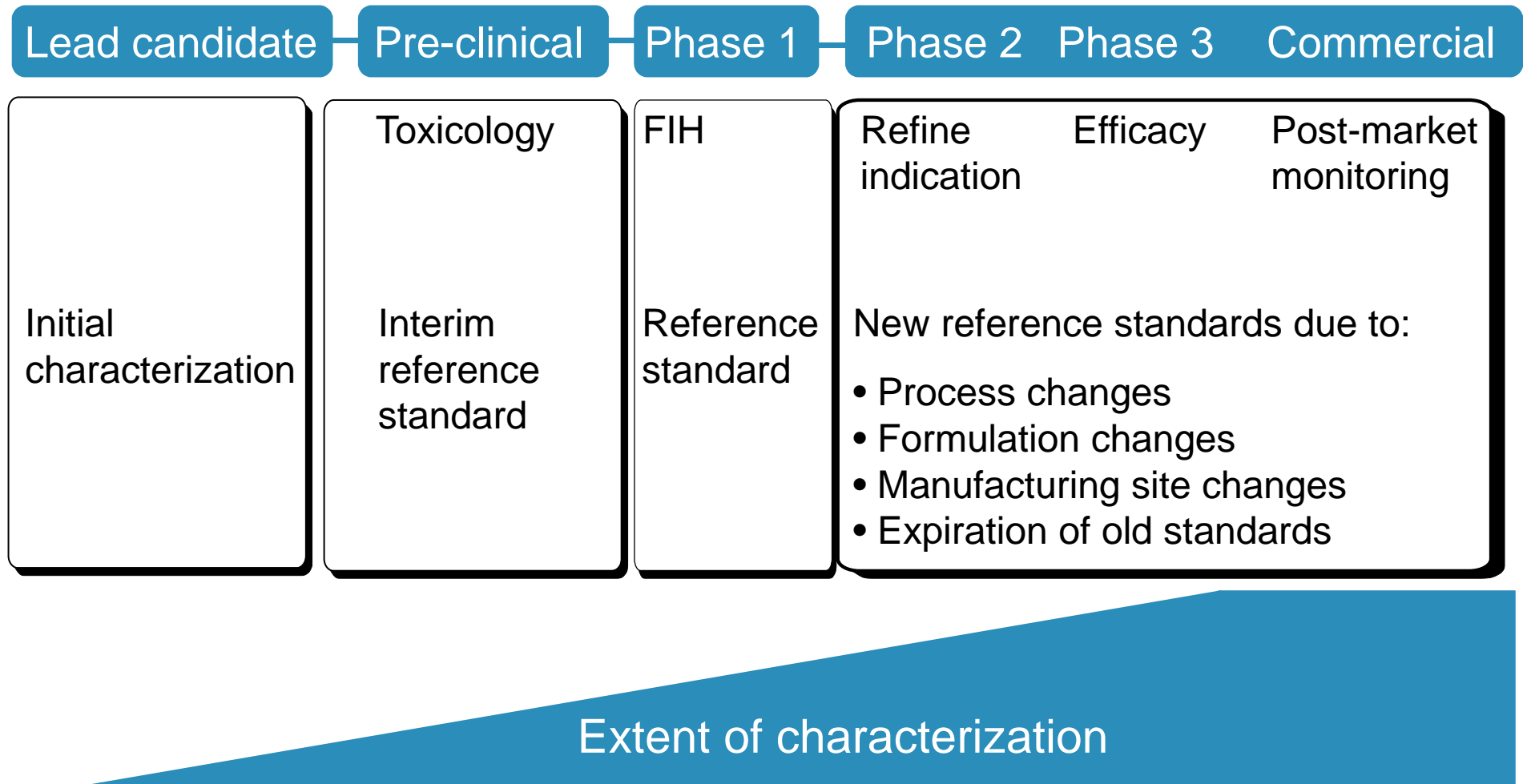
Clinical & commercial manufacturing of Abbott and Third Party products

20 years experience handling > 25 different biologics

~800 Employees on site with ~400 in Biologics Manufacturing

Licensed for commercial production of HUMIRA™ and Reoathrom™

# Reference standards in drug development and commercialization



# Reference standards are used ...

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- To confirm identity
- To ensure that the manufacturing process gives a consistent product
- To ensure consistency in test methodology

System suitability

Stability

Release

- To ensure that the clinical material is representative of the pre-clinical material
- In building the foundation of analytical / biophysical knowledge of the product

# What are the essential parameters for qualification?

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- Characterization
  - Confirmation of desired features of the molecule (primary sequence, higher order structure, function)
- Testing for purity
  - DNA, HCP, other process-related impurities
- Testing for product-related impurities (heterogeneity)
  - Charge, size, glycosylation, other post-translational modifications

# Heterogeneity in protein therapeutics

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“Wine and biotech drugs are both made by growing cells, usually in stainless-steel tanks. But rather than using yeast to convert sugar to alcohol, biotechs use genetically engineered animal or bacterial cells to make proteins from nutrients.

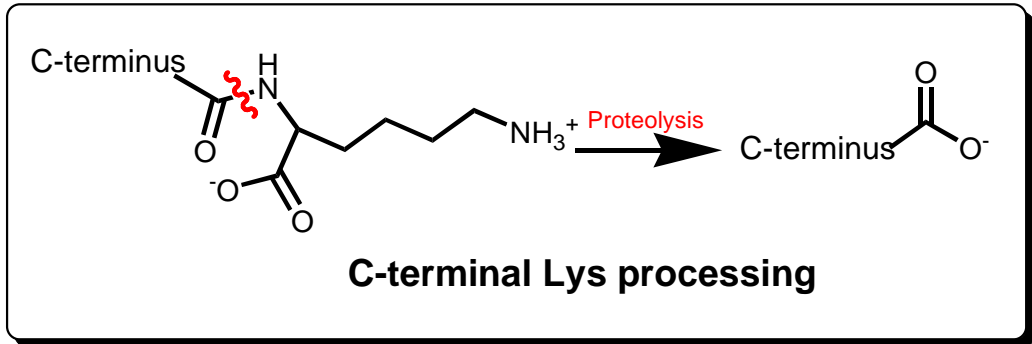
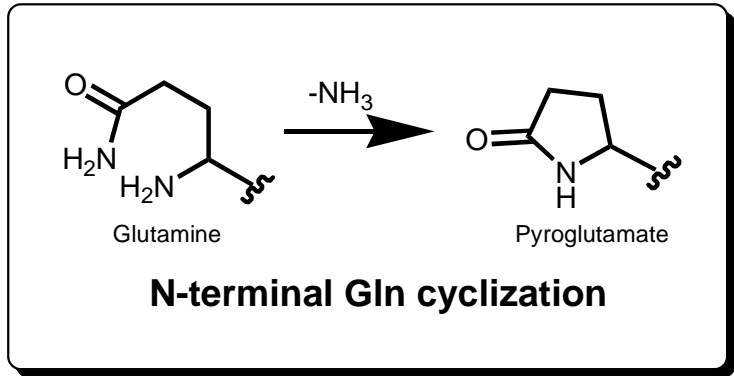
The process for both is part art, part science. Results can differ from factory to factory, or even between lots in a single site. While wine lovers savor the differences among vintages, patients could be hurt by inconsistencies in drugs... “

- From **In Biotech Brews, Questions of Consistency**

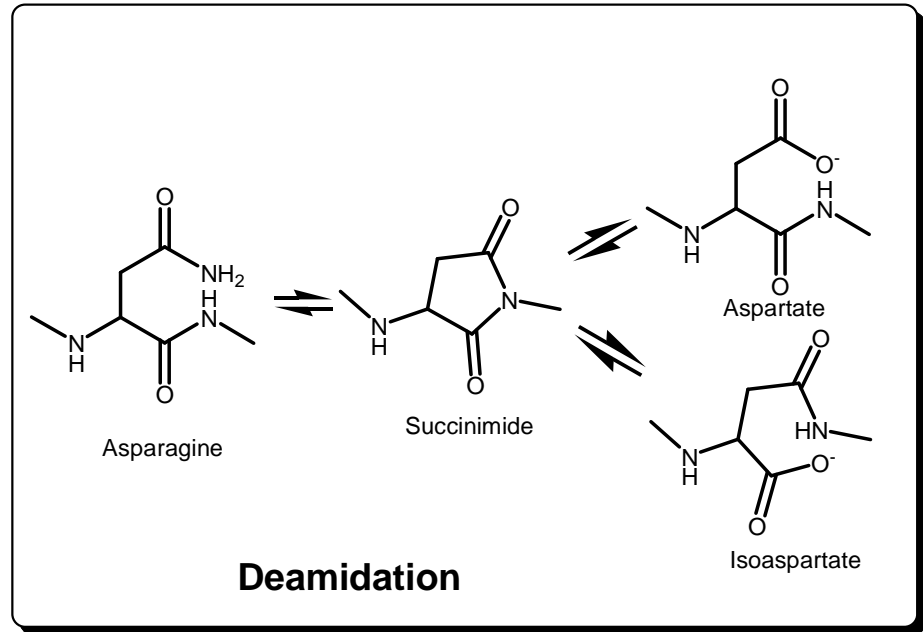
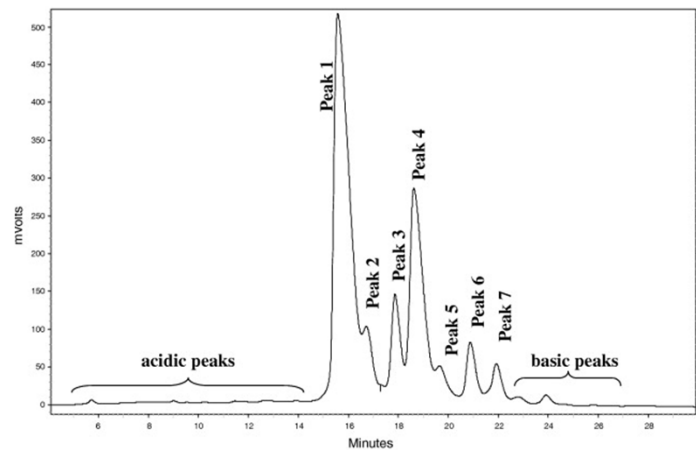
By Andrew Pollack, New York Times, June 11, 2007

<http://www.nytimes.com/2007/06/11/business/businessspecial3/11vat.html>

# Examples of heterogeneity in protein therapeutics: charge variants



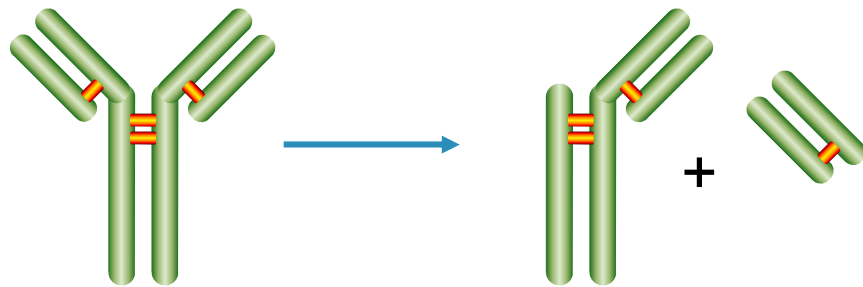
Common analytical methods: IEX, IEF



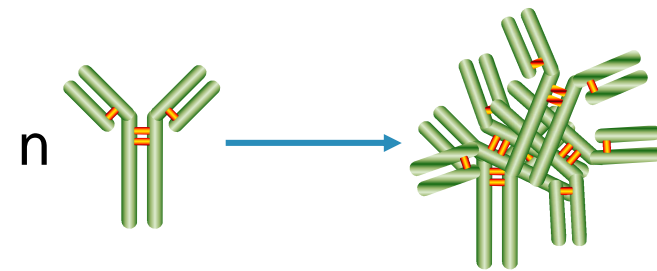
Correia and coworkers, Anal. Biochem. 2010, 397, 37-47

# Examples of heterogeneity in protein therapeutics: size variants

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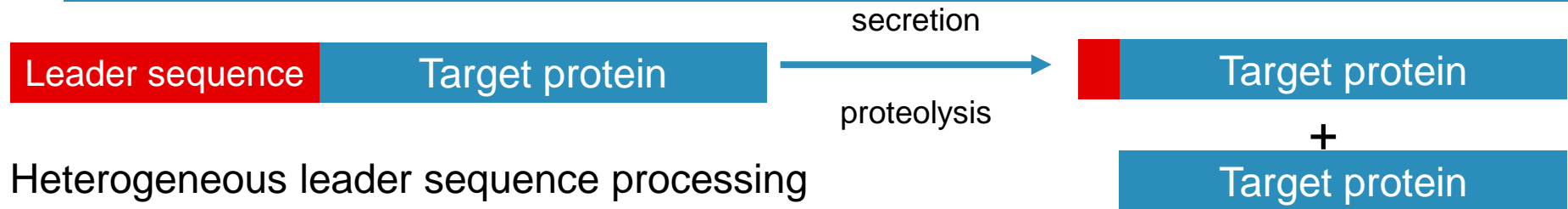
Fragmentation



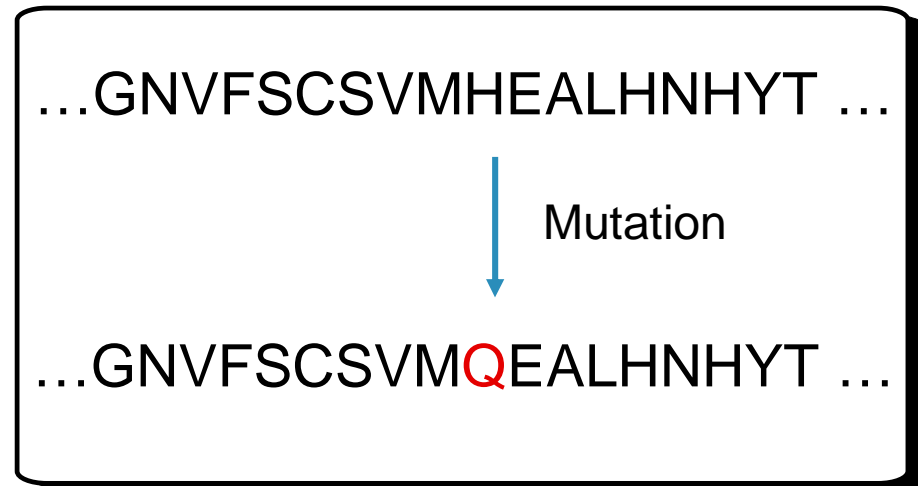
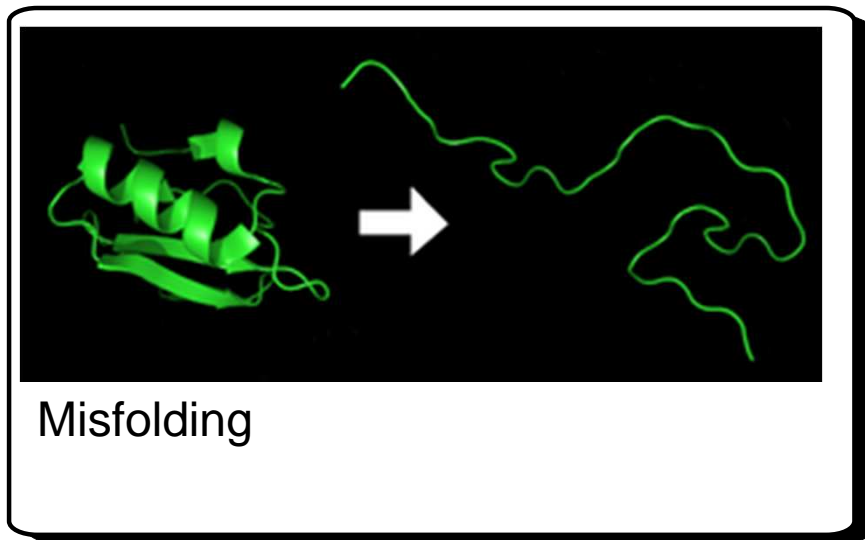
Aggregation

Common analytical methods: SEC, CE-SDS, SDS-PAGE, AUC, MALS

# Examples of heterogeneity in protein therapeutics: structural variants

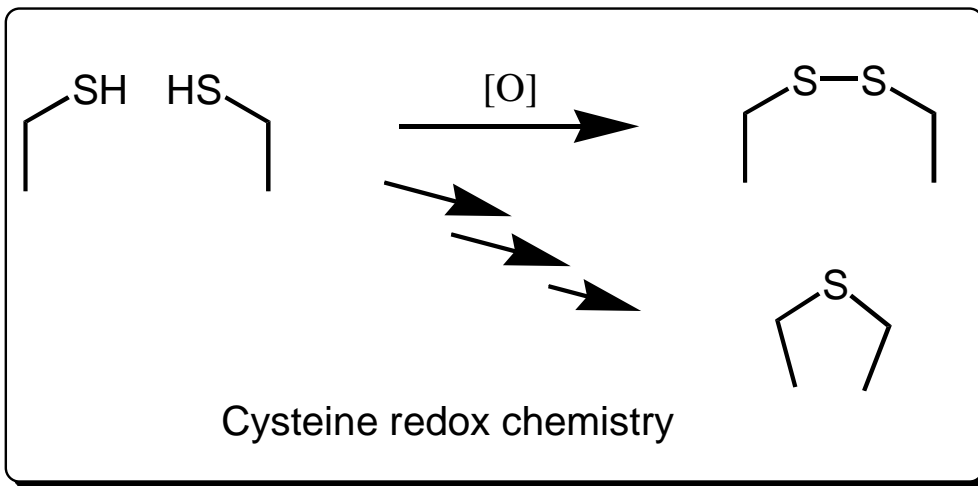
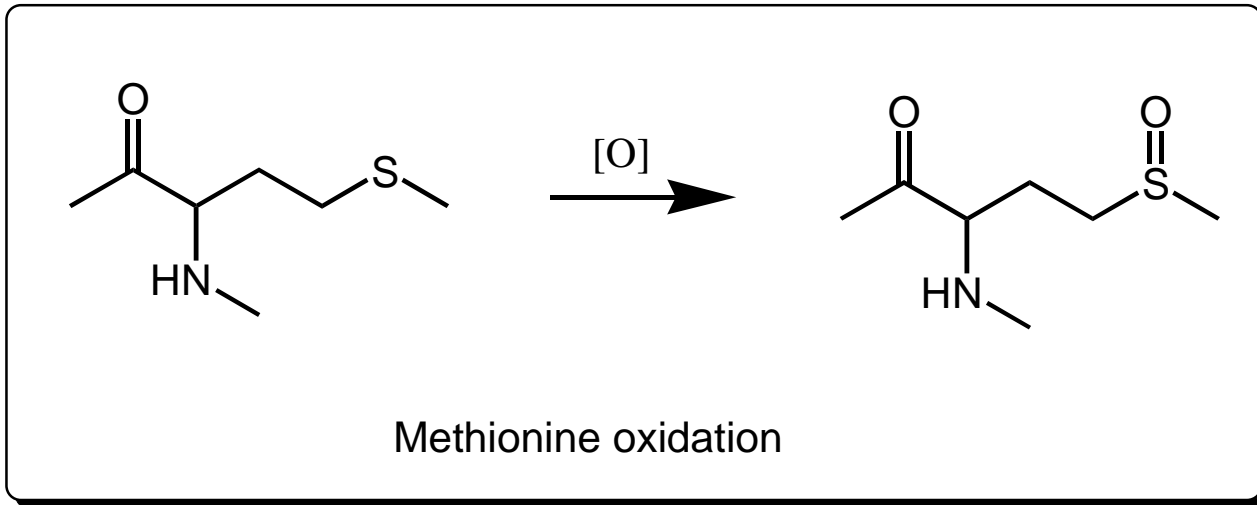


Heterogeneous leader sequence processing



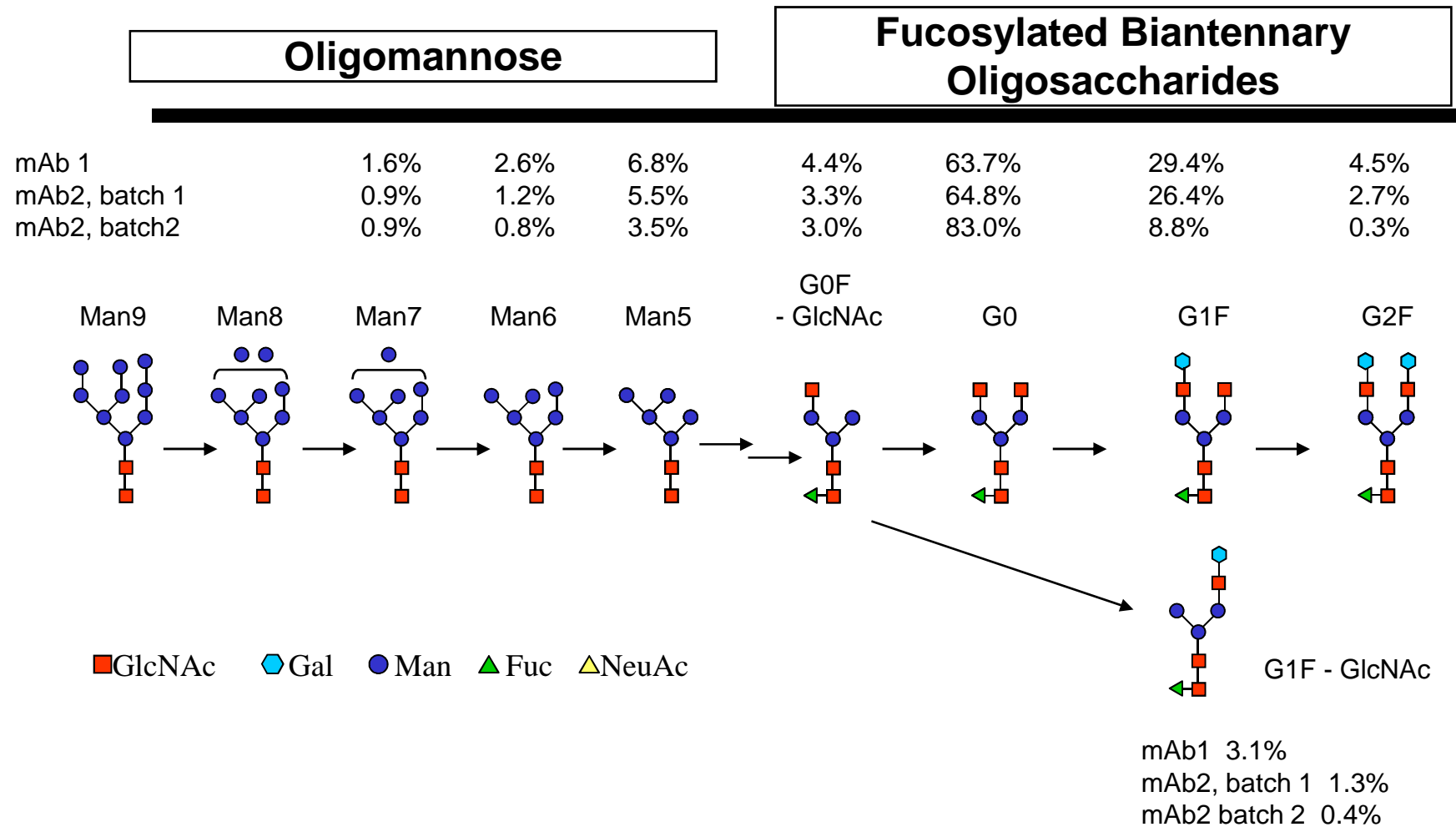
Common analytical methods: MS, peptide map, Edman degradation  
Indirect measures of higher order structure: functional assays, DSC

# Examples of heterogeneity in protein therapeutics: redox variants



Common analytical  
methods: MS, peptide map,  
Ellman's test

# Examples of heterogeneity in protein therapeutics: glycosylation



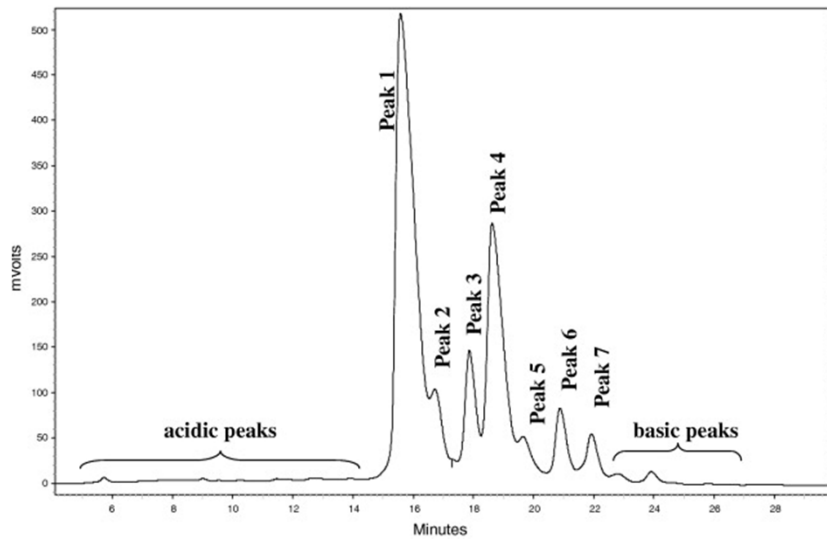
# From where are acceptance limits derived?

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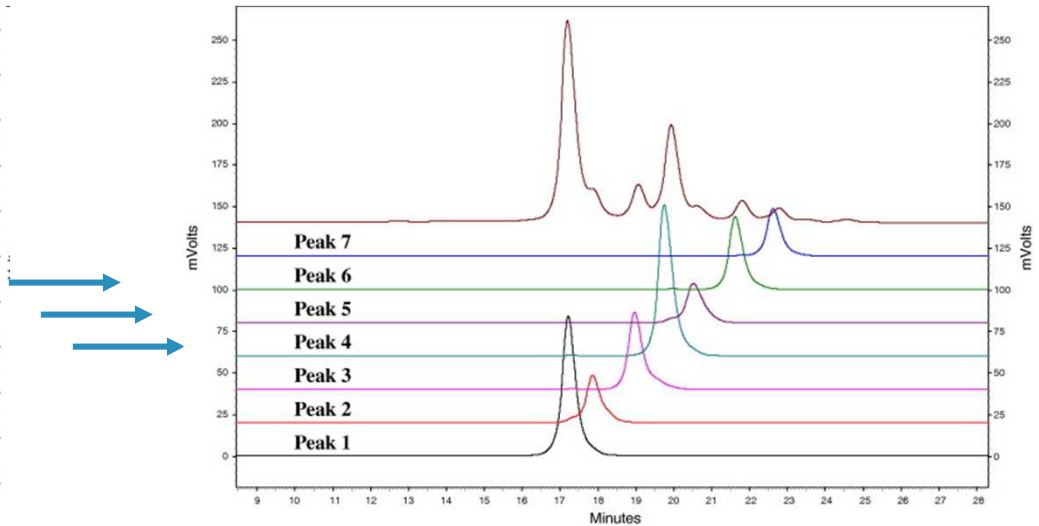
- Few qualification parameters have safety requirements
  - Process-related impurities (eg. DNA, HCPs)
  - Peptide map / primary structure
  - Aggregates
- Acceptance limits may be defined by mechanism of therapeutic
  - Significance of afucosylated oligosaccharides for soluble vs cell surface targets
- Often derived from consistency of manufacturing process
  - Historical
- Ideally, acceptance limits would be based on knowledge of specific nature and context of heterogeneity, not broad categories
  - Lysine mutant / pyroglutamate vs. % basic species
  - Oxidation of Met in Fc vs CDR

# Deep knowledge of the protein often not linked to characteristics of assay until late in development

## Early development



## Late stage



Species	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
LC, % pE	91	86	52	85	68	49	83
HC, % pE	100	95	92	50	50	49	6

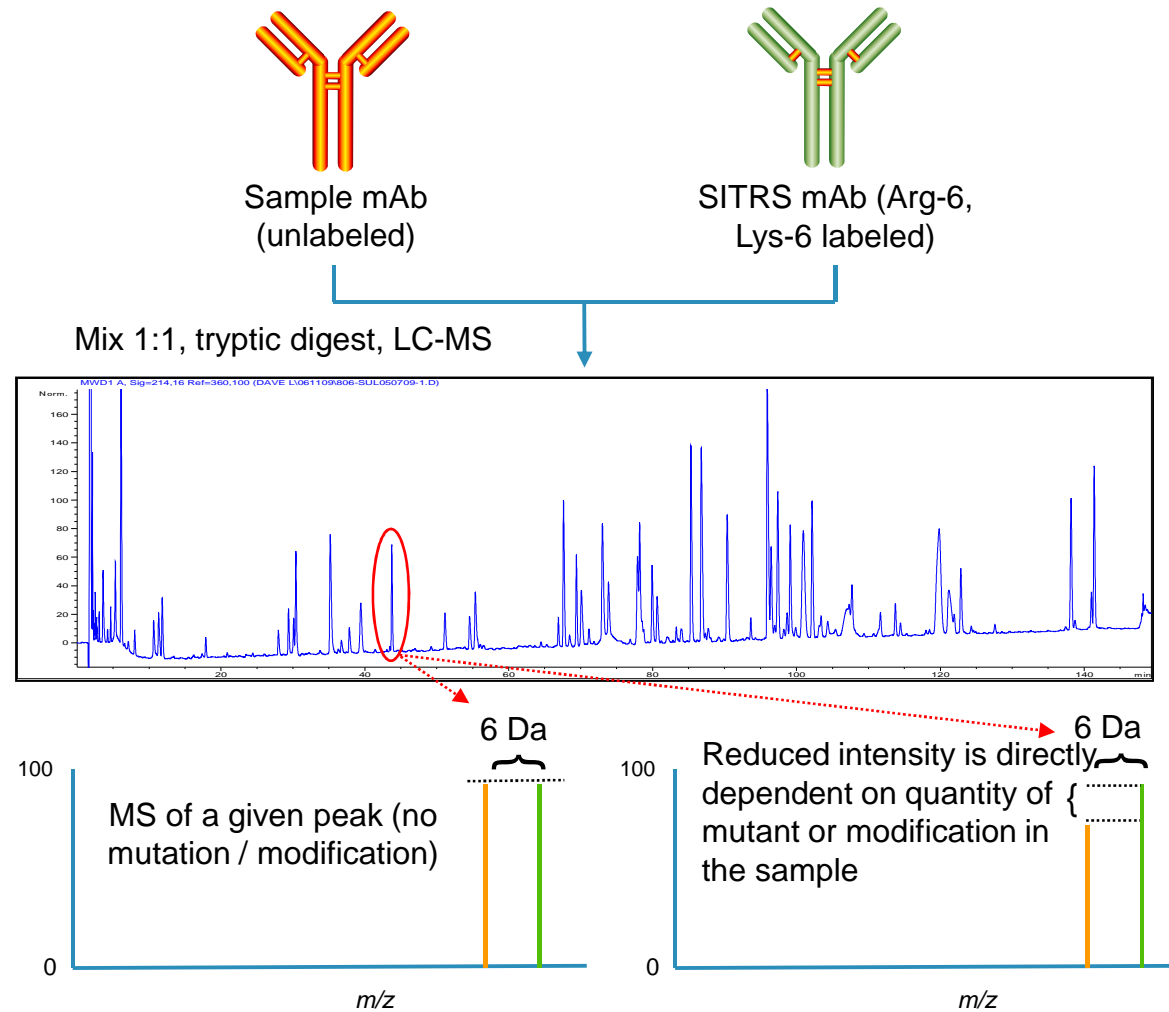
Unpaired cysteine residues in Peaks 2 and 5

Correia and coworkers, Anal. Biochem. 2010, 397, 37-47

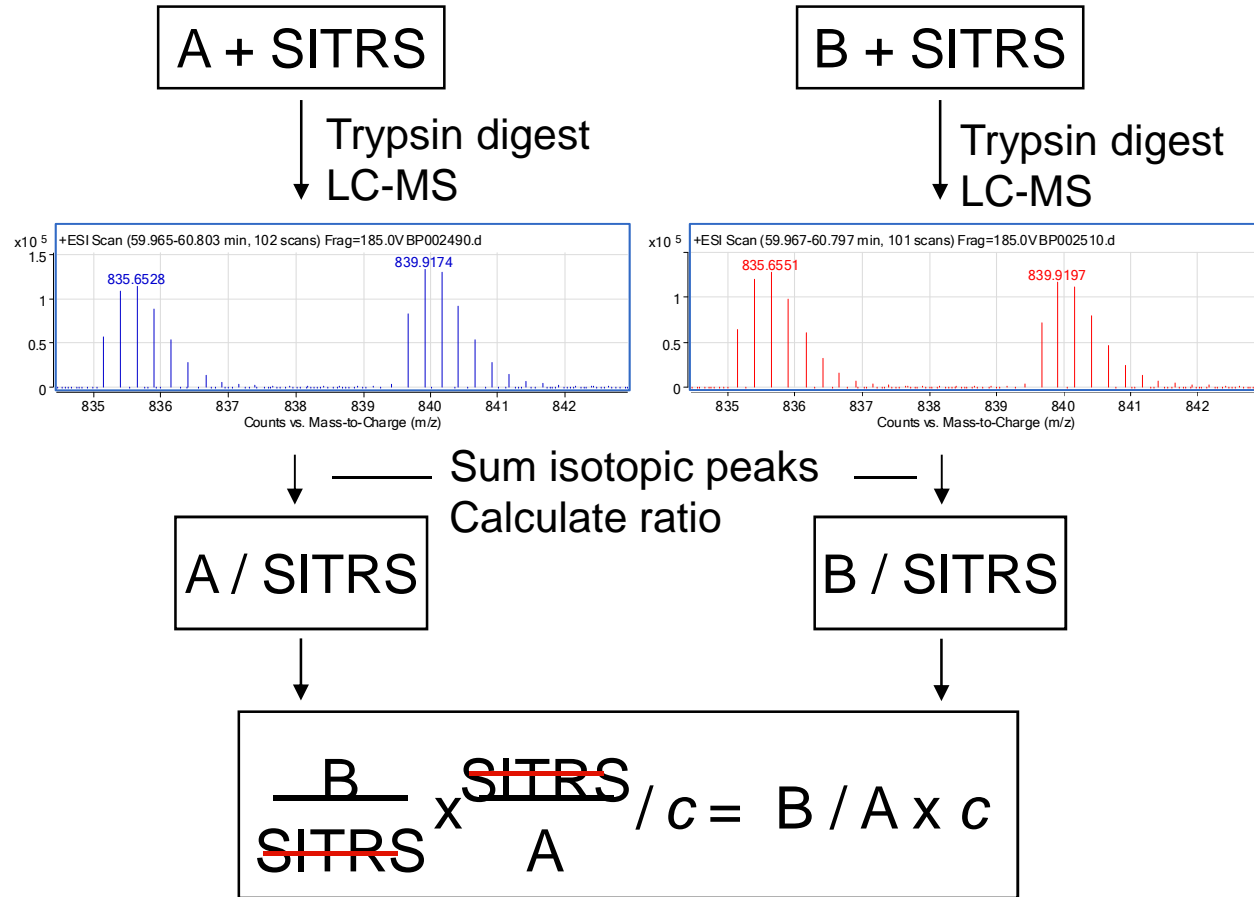
# Paucity of quantitative methods with high structural information

	Qualitative	Quantitative
High structural resolution	<p>Qualitative results</p> <p>High resolution method</p> <p><b>Peptide map with MS</b></p>	<p>Quantitative results</p> <p>High resolution method</p> <p><b>Oligosaccharide analysis</b></p>
Low structural resolution	<p>Qualitative results</p> <p>Low resolution method</p> <p><b>Circular dichroism</b> <b>FT-IR</b> <b>Fluorescence</b></p>	<p>Quantitative results</p> <p>Low resolution method</p> <p><b>SEC</b>      <b>IEX</b>      <b>Binding ELISA</b> <b>CE-SDS</b>   <b>IEF</b>      <b>Bioassay</b> <b>AUC</b>                      <b>SPR</b></p>
	<b>Common characterization method</b>	<b>Common release test</b>

# Quantitative MS through use of a stable-isotope-tagged reference standard



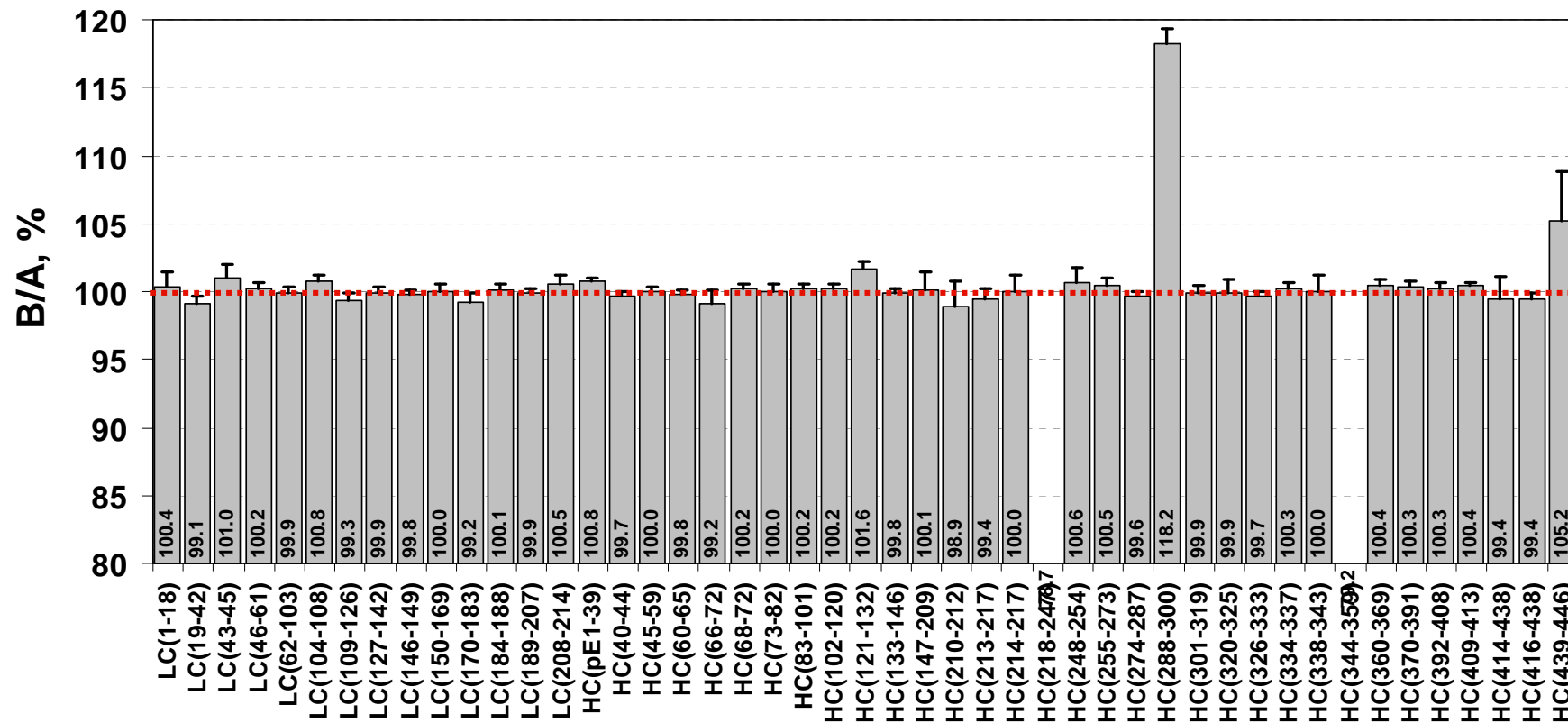
# Workflow of a SITRS experiment



c is a constant to normalize the result to %A

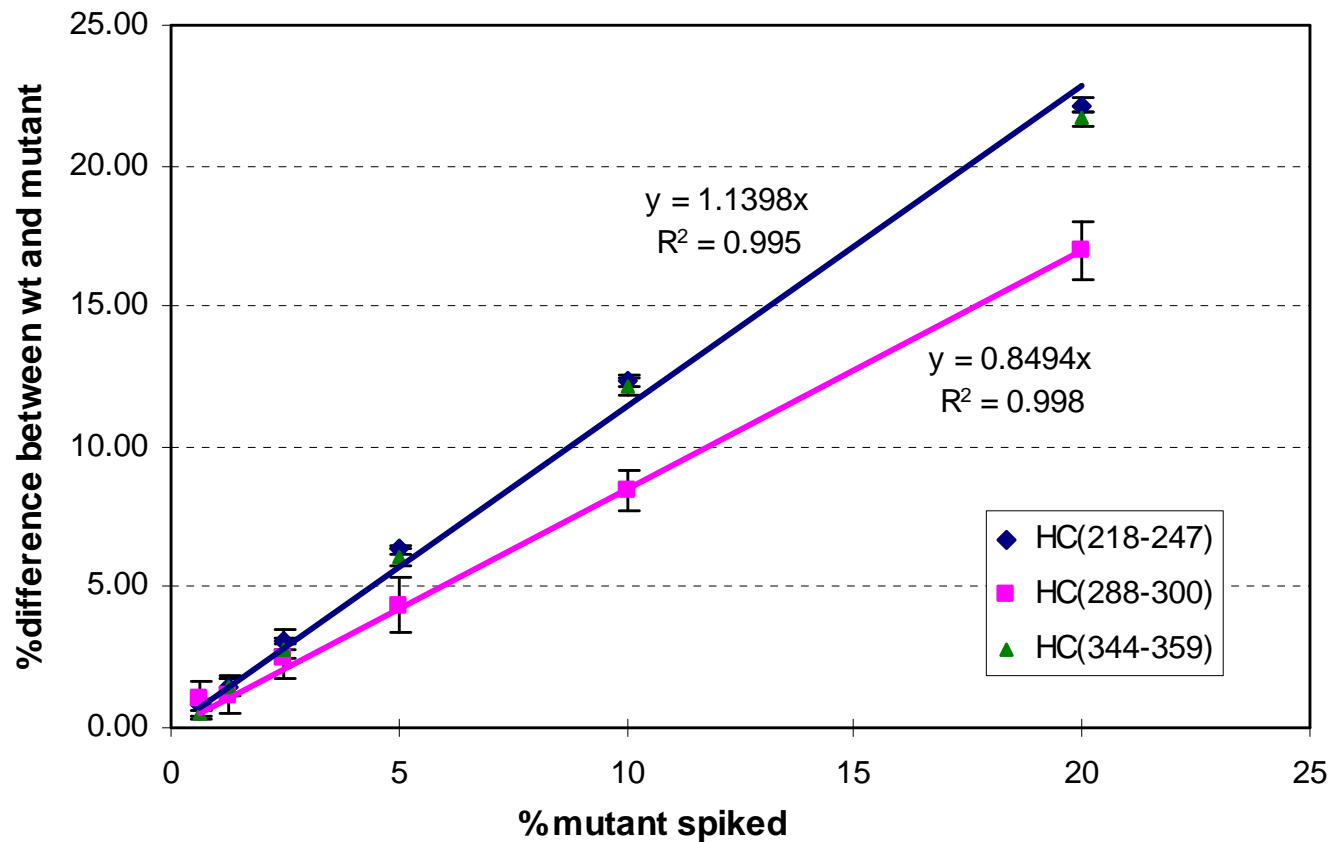
Manuilov et al. mAbs, 2011, 3: 387-395, US Non-Provisional 61/###,###

# Sites of mutation are easily discernable by SITRS (20% spike)



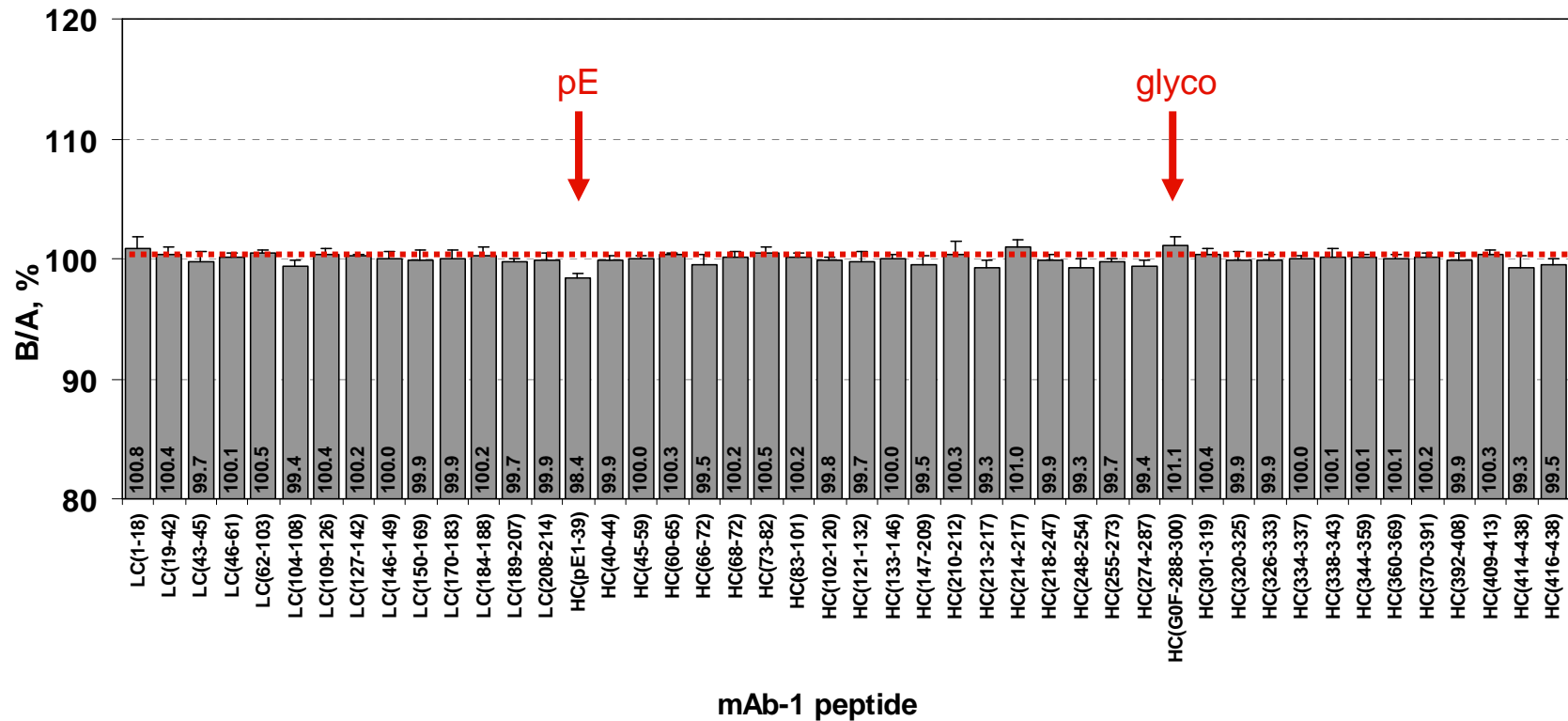
Manuilov et al. mAbs, 2011, 3: 387-395, US Non-Provisional 61/###,###

# Response of SITRS method to mutations is linear



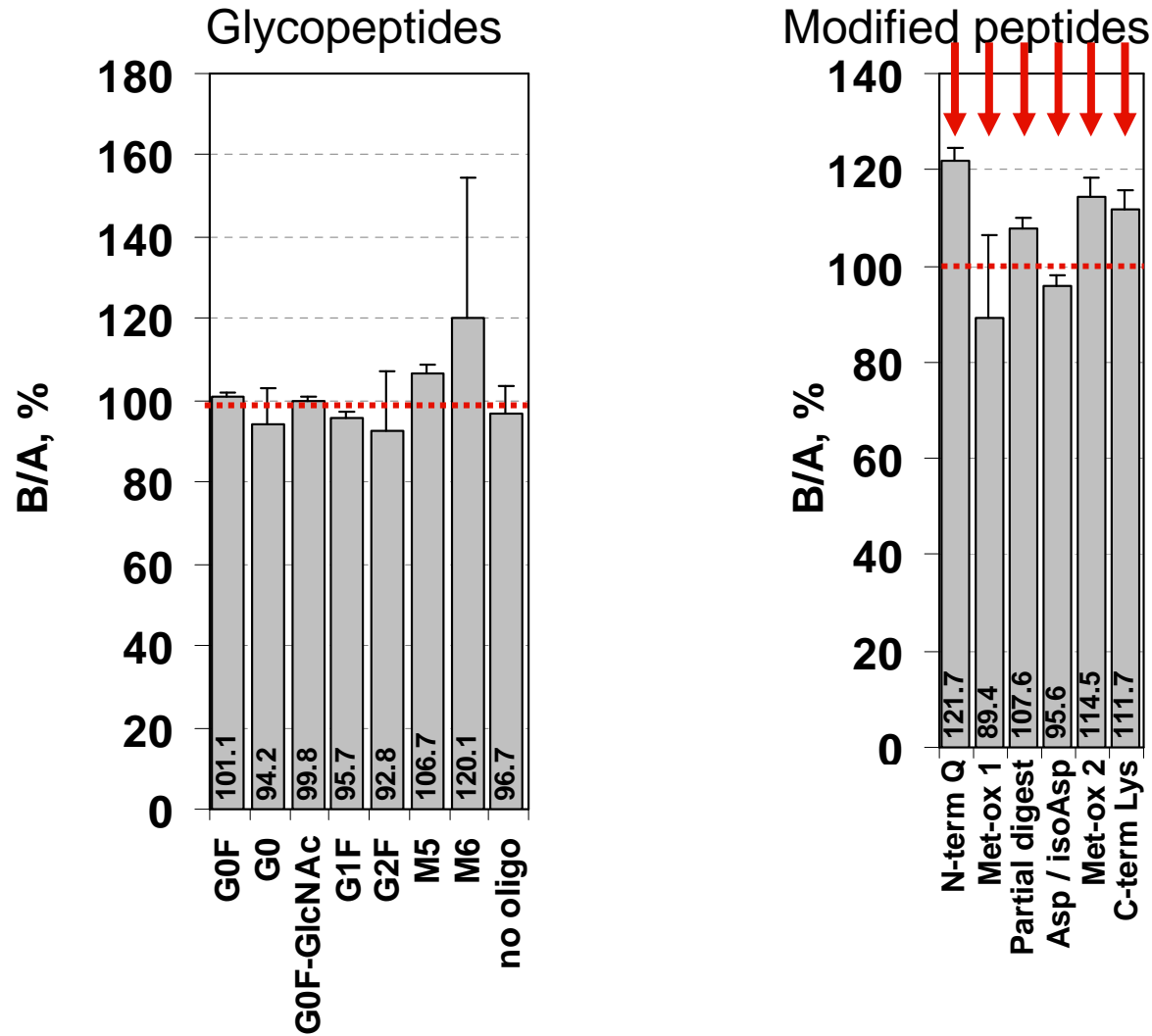
Manuilov et al. mAbs, 2011, 3: 387-395, US Non-Provisional 61/###,###

# Predominant peptides of GMP batch are highly comparable to interim reference standard by SITRS analysis



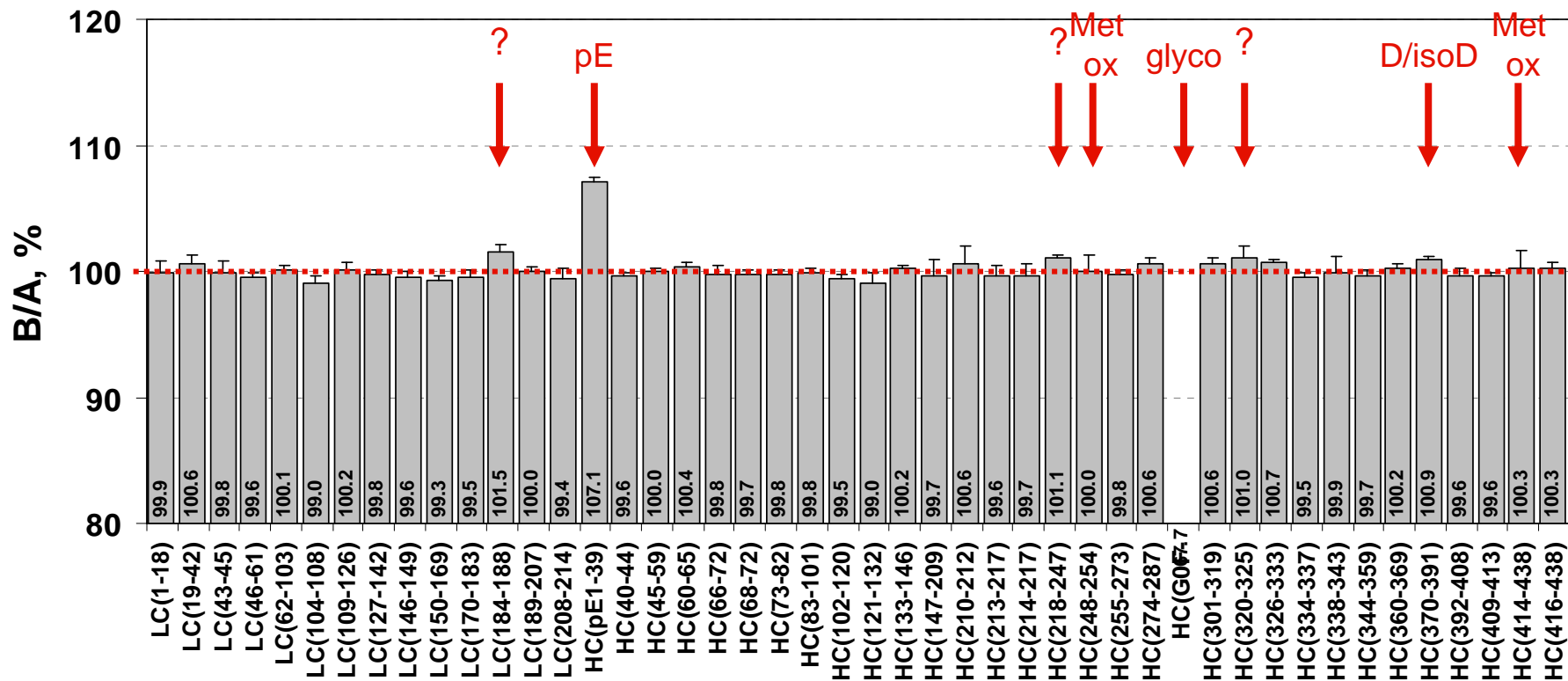
Manuilov et al. mAbs, 2011, 3: 387-395, US Non-Provisional 61/###,###

# Modified peptides of GMP batch are comparable to reference standard by SITRS analysis



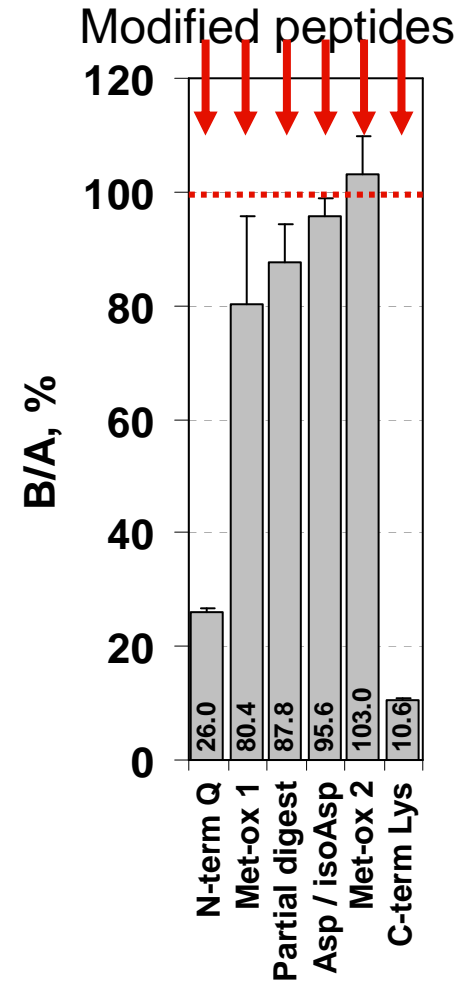
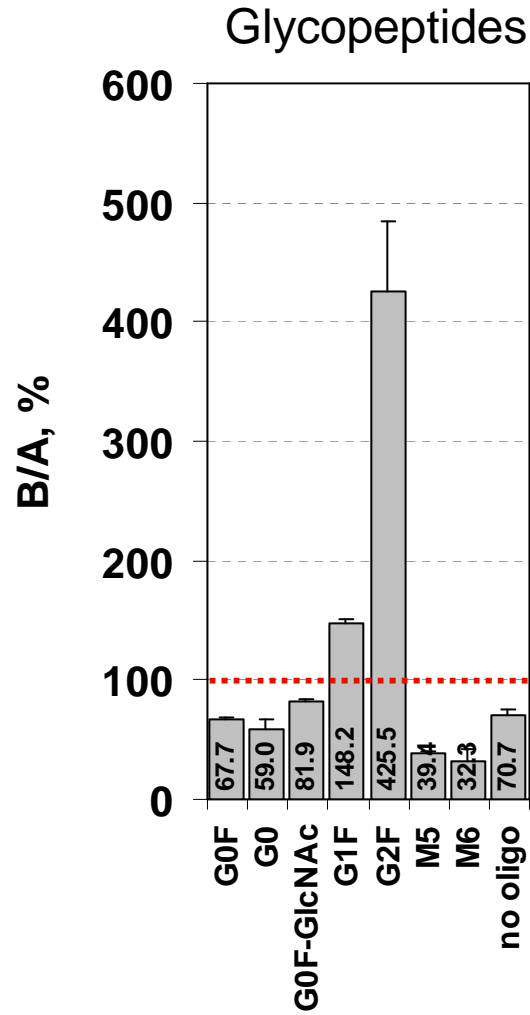
Manuilov et al. mAbs, 2011, 3: 387-395

# SITRS analysis quantitates differences amongst peptides for a mAb produced by two different processes



Manuilov et al. mAbs, 2011, 3: 387-395, US Non-Provisional 61/###,###

# SITRS analysis quantitates differences amongst peptides for a mAb produced by two different processes



# Summary

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- Essential qualification parameters for a reference standard should include
  - characterization that evaluates the quality of the protein structure / function
  - testing of process- and product-related impurities
- Acceptance criteria should originate from
  - the need for safety and efficacy (ICH Q6B)
  - As an added precaution, additional criteria that is derived from the manufacturing history
- Low resolution nature of most release tests and characterization studies do not enable early identification of significant process- or product-related impurities
- Quantitative MS methods such as SITRS
  - may be useful for sensitive and high resolution comparability studies
  - limited to mass with retention time differences
  - may enable setting of acceptance criteria based on specific modifications rather than broad categories early on in development

# Acknowledgements

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Judy Dudley  
Hal Hopkins

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