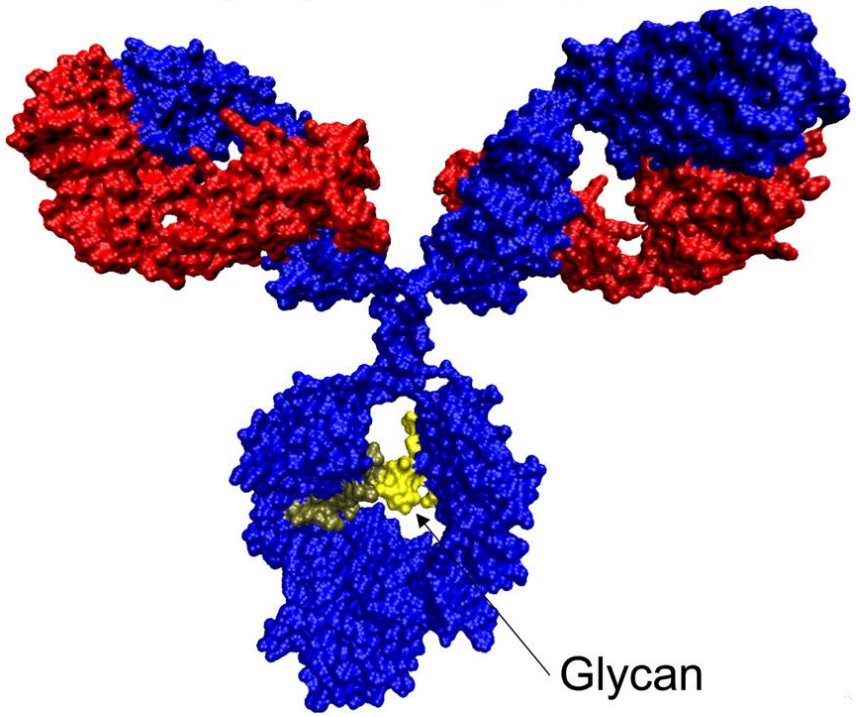
Biopharmaceutical Characterization

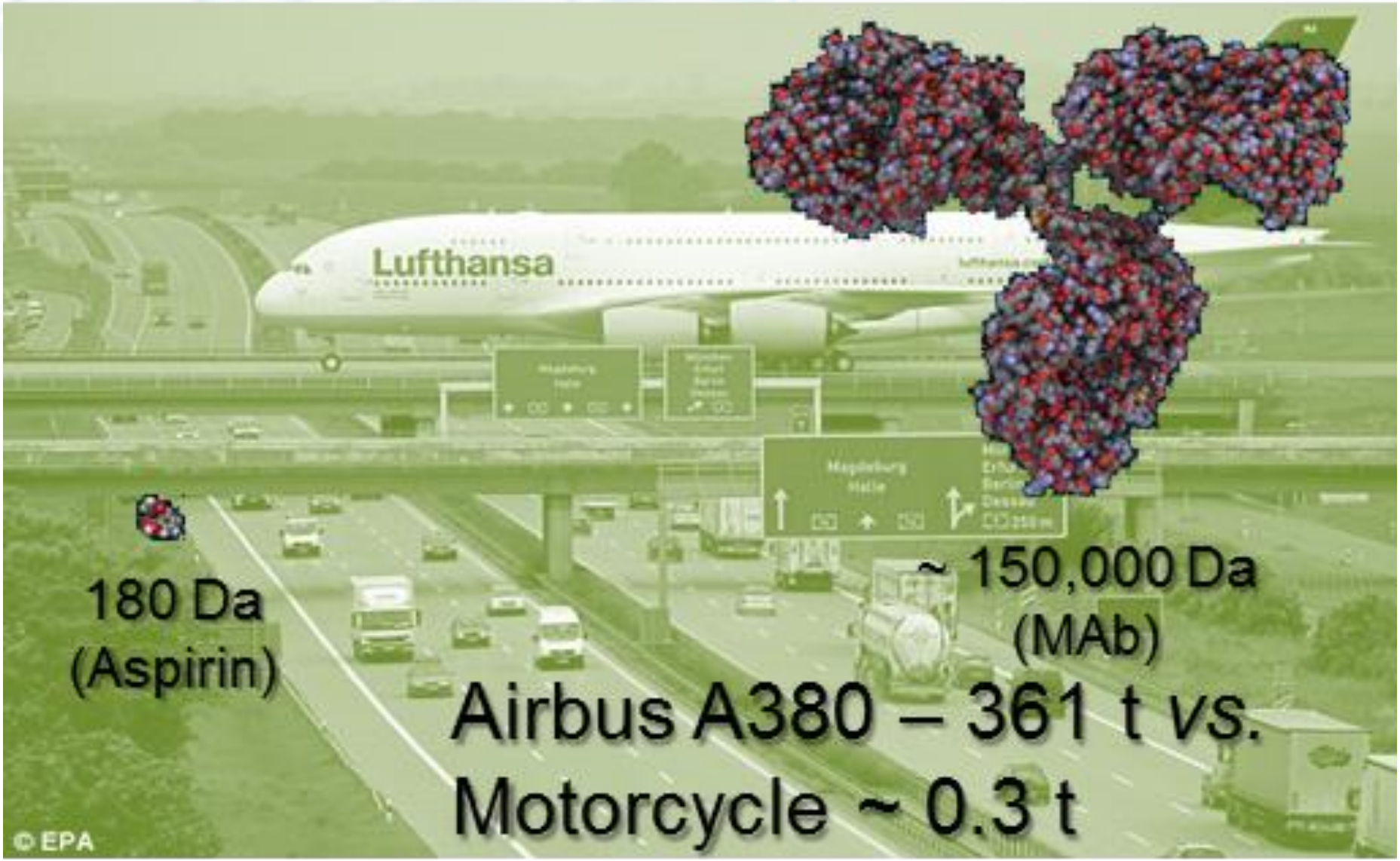
DEVELOPMENT OF NIST mAb REFERENCE MATERIAL

John Schiel, Trina Formolo, Lisa Kilpatrick, Meiyao Wang, Mark Lowenthal, Karen Phinney, Michael Tarlov (NIST)



National Institute of Standards and Technology
U.S. Department of Commerce

Recombinant mAb Therapeutics



Need for a Well Characterized Reference Material

Standard Measurements and Standard Materials

Testimony before the U.S. House of Representatives Committee on Science and Technology (2009) on the need for measurement standards by S. Kozlowski (CDER) , A. Mire-Sluis (Amgen), and Willie E. May (NIST).

"With the development of new analytical methods comes the need for new standards to evaluate them." S. Kozlowski

- Well characterized and certified standard is an ideal means to:
  - Assess precision and accuracy across methods and labs.
  - Identify potential gaps and develop new technologies to fill them.
  - Adoption of new technology by correlating existing data with that from new technologies in a standardized fashion.
  - Assist reviewers and sponsors by allowing them to provide/assess methods and historical data for the standard.

NIST mAb Reference Material + Data (SRM/D)

NIST mAb Attributes:

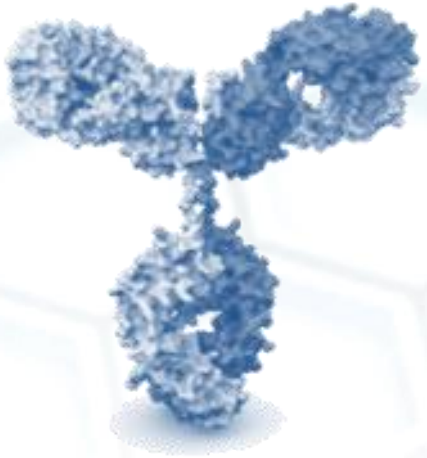
- Humanized mAb (IgG1κ) expressed in murine suspension culture
- Frozen bulk "Drug-like substance"
  - 100 and 10 mg/mL, ≥ 98% purity
  - 12.5 mM L-His, 12.5 mM L-His HCl (pH 6.0)

Definitions:

- **In-House Standard**: Manufacturer-specific drug substance
- **Reference Material**: Issued under NIST trademark and established to be fit for intended use in measurement of nominal property values.
- **Standard Reference Material**: Issued under NIST trademark and assigned one or more specified property values with associated uncertainties and traceability.

Approach for IgG SRM:

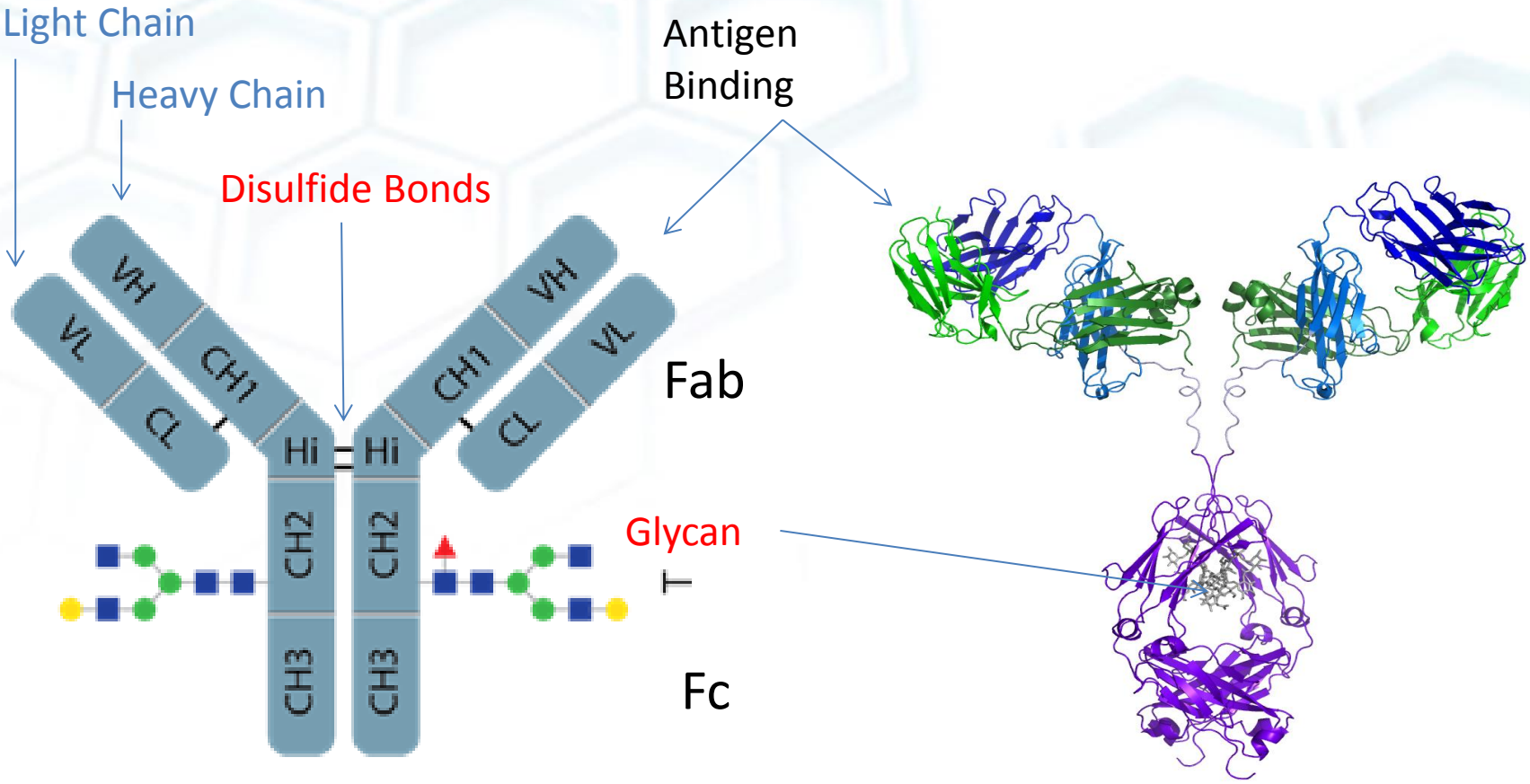
- Complete rigorous interlaboratory characterization
  - Results used for book compilation
- Compile reference data, methods, etc.
  - End user accessibility to http://igg.nist.gov/
- Certify for concentration traceable to the kg
- SRM made available to biopharmaceutical community



Companion Reference Data

Amino Acid Sequencing
Amino Acid Analysis
N-terminal Sequencing
C-terminal Sequencing
Peptide Mapping by MS
S-S Bridge Analysis
Glycosylation Analysis
Molecular Weight Information
Isoelectric Focusing
SDS-PAGE
Extinction Coefficient
Post-Translational Modifications
Spectroscopic Profiles: CD, NMR
LC: SEC, RP, IEX

mAb Structure



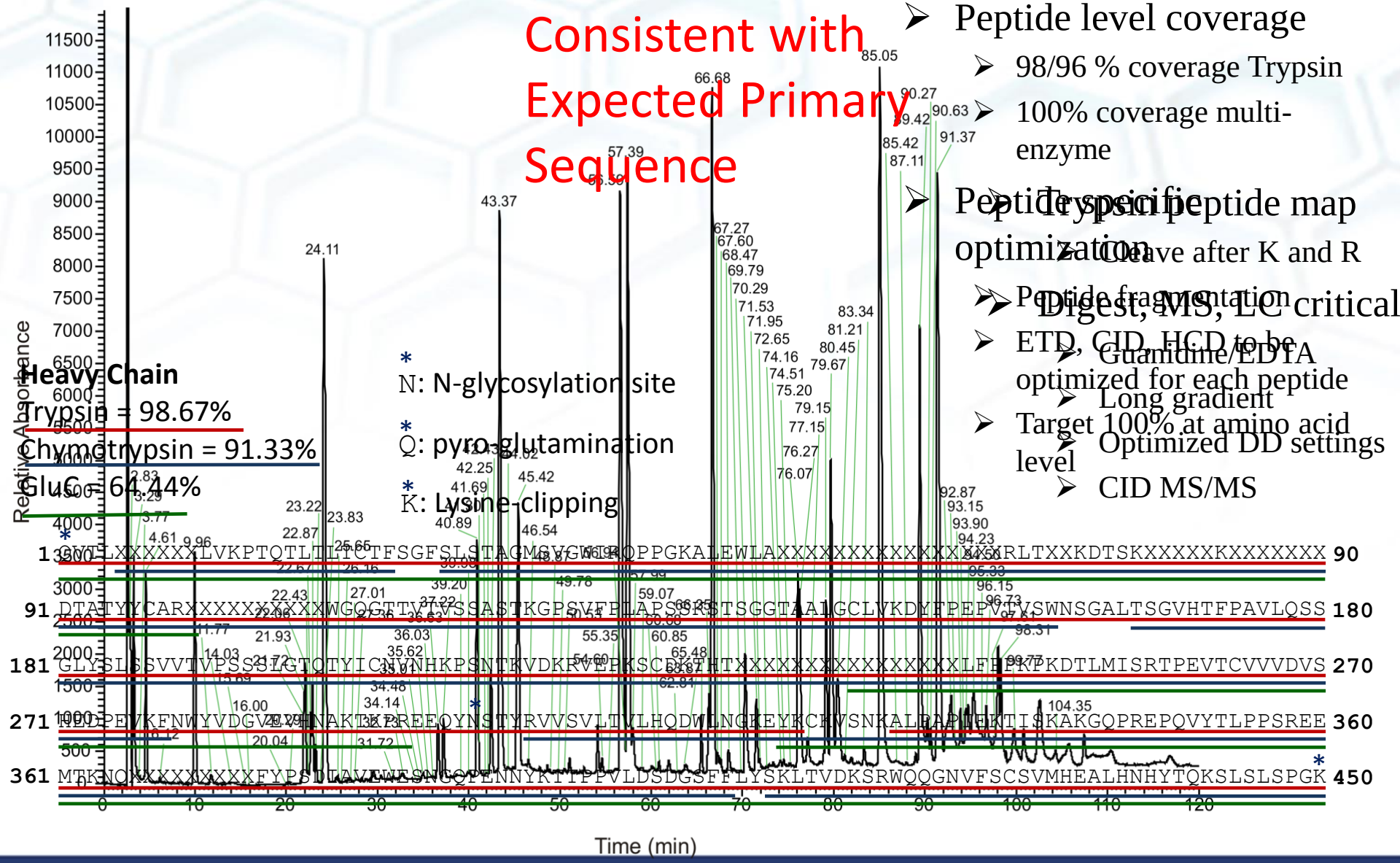
Heavy Chain

```
  1 QVTLXXXXXXLVKPTQTLTLTCTFSGFSLSXXXXXXXWIRQPPGKALEWLAXXXXXXXXXXXXXXXXXRLTXXKDTSKXXXXXKVXXXXXX  90
 91 DTATYYCARXXXXXXXXXXWGXGTTVTVSXASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS 180
181 GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCXXXXXXXXLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS 270
271 HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE 360
361 MTKNQXXXXXXXXXFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 450
```

Light Chain

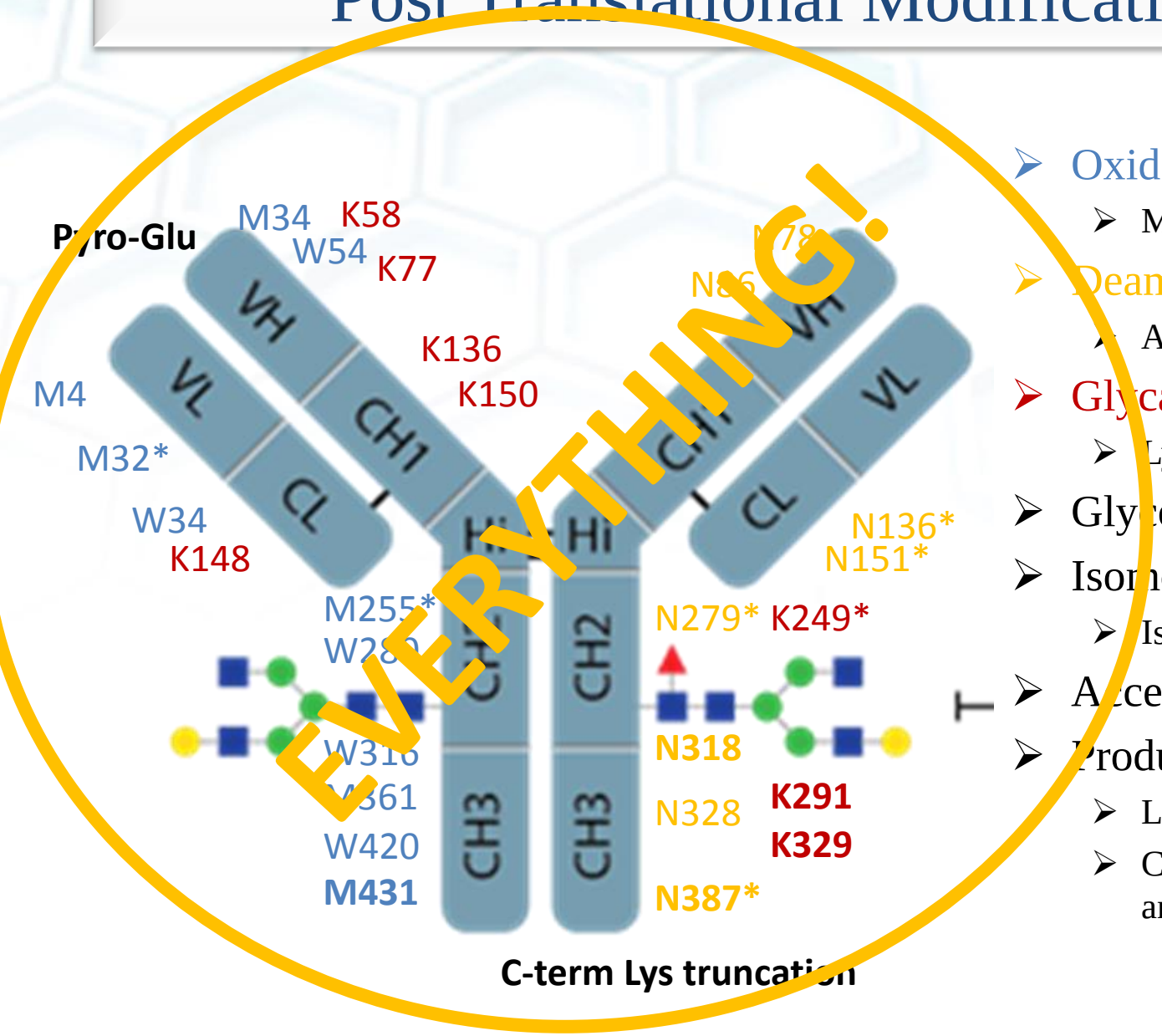
```
  1 DIXXXQSPXXLSASXGDRVTXTCXXXXXXXXXXWYQQKPGKXPKLXIYXXXXXXXGVPXRFSGSGSGTXXXLTISXXXXXDFATYYCXXX  90
 91 XXXXXXFGGGTKXEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSXXXXXXXTEQDSKDSTYSLSSTLTL 180
181 SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC                                                            213
```

LC-UV-MS/MS Peptide Map



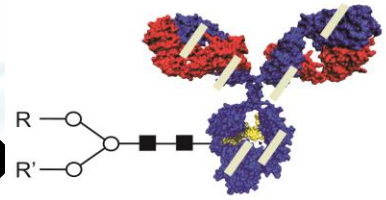
- Peptide level coverage
  - 98/96 % coverage Trypsin
  - 100% coverage multi-enzyme
- Peptide specific optimization
  - Trypsin peptide map
    - Cleave after K and R
    - Peptide fragmentation
    - ETD, CID, HCD to be optimized for each peptide
    - Target 100% at amino acid level
      - CID MS/MS
  - Digest, MS, LC critical
    - Guanidine/EDTA
    - Long gradient
    - Optimized DD settings

Post Translational Modifications

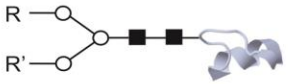


- Oxidation
  - Met, Trp, His
- Deamidation
  - Asn, Gln
- Glycation
  - Lys
- Glycoprofile
- Isomerization
  - Iso-Asp, pyro-Gln
- Accelerated stability
- Product-related substances
  - Low abundance in native mAb
  - Critical Quality Attributes of an RM?

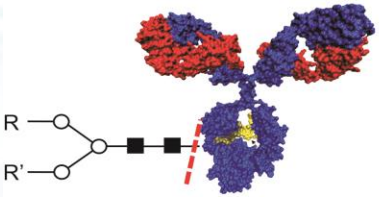
Glycosylation



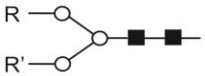
Proteolysis



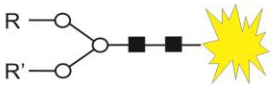
- Glycan Sequence
- Glycan Position
- Relative Quantification*



Glycan Release

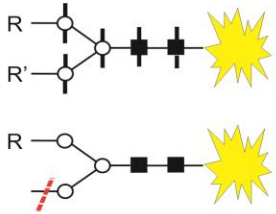


Label



Permethylate

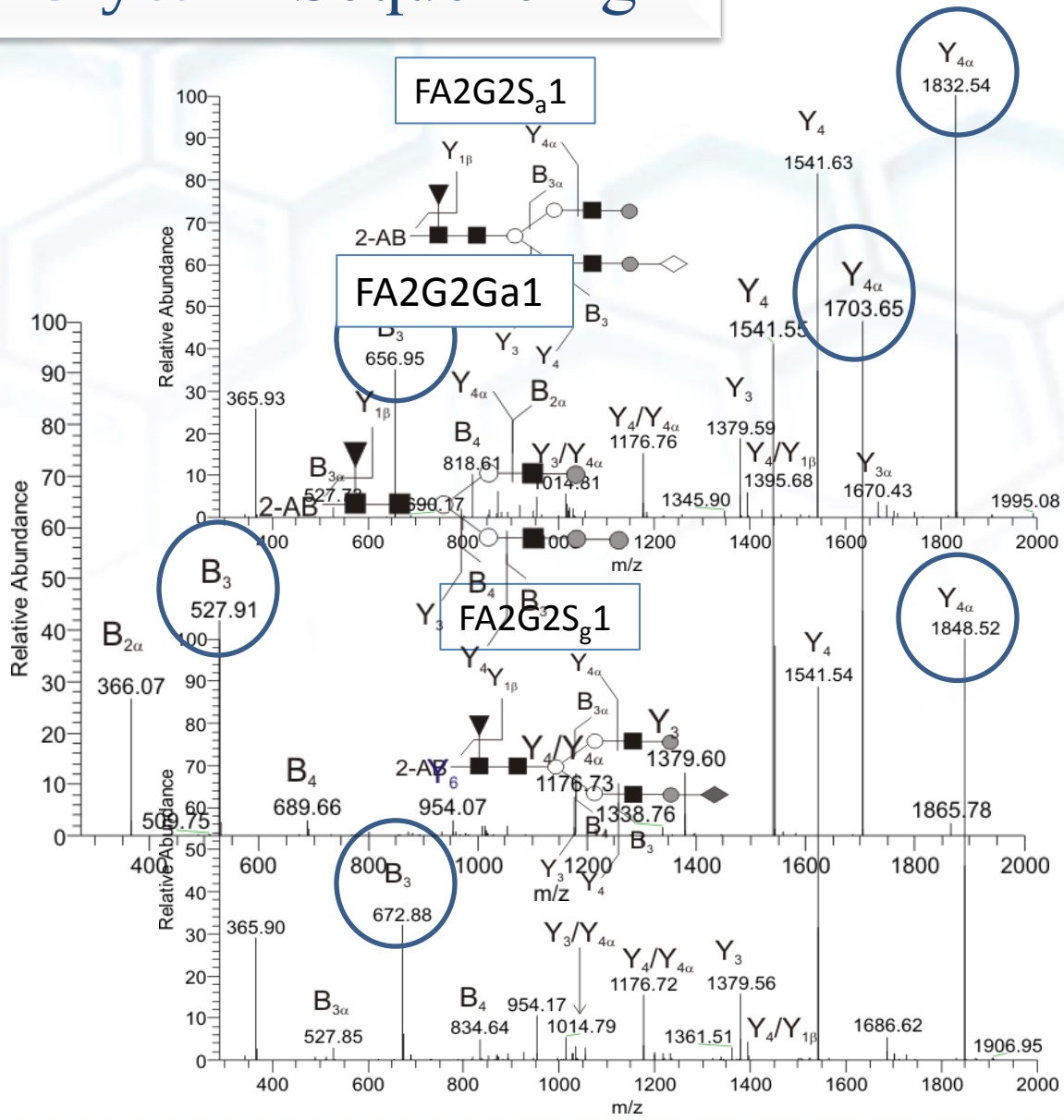
Exoglycosidase



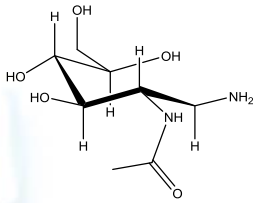
- Glycan Sequence
- Relative Quantification
- Linkage

LC-F-MS/MS IgG Glycan "Sequencing"

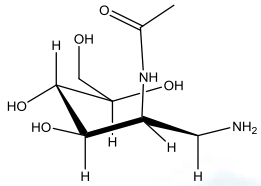
- Murine suspension hydbridoma
- Monoclonal $IgG_1\kappa$
- Potential glycan immunogens easily identified
- Not all linkage verified
  - Permethylation MSn
  - Exoglycosidase
  - NMR



Orthogonal Detection

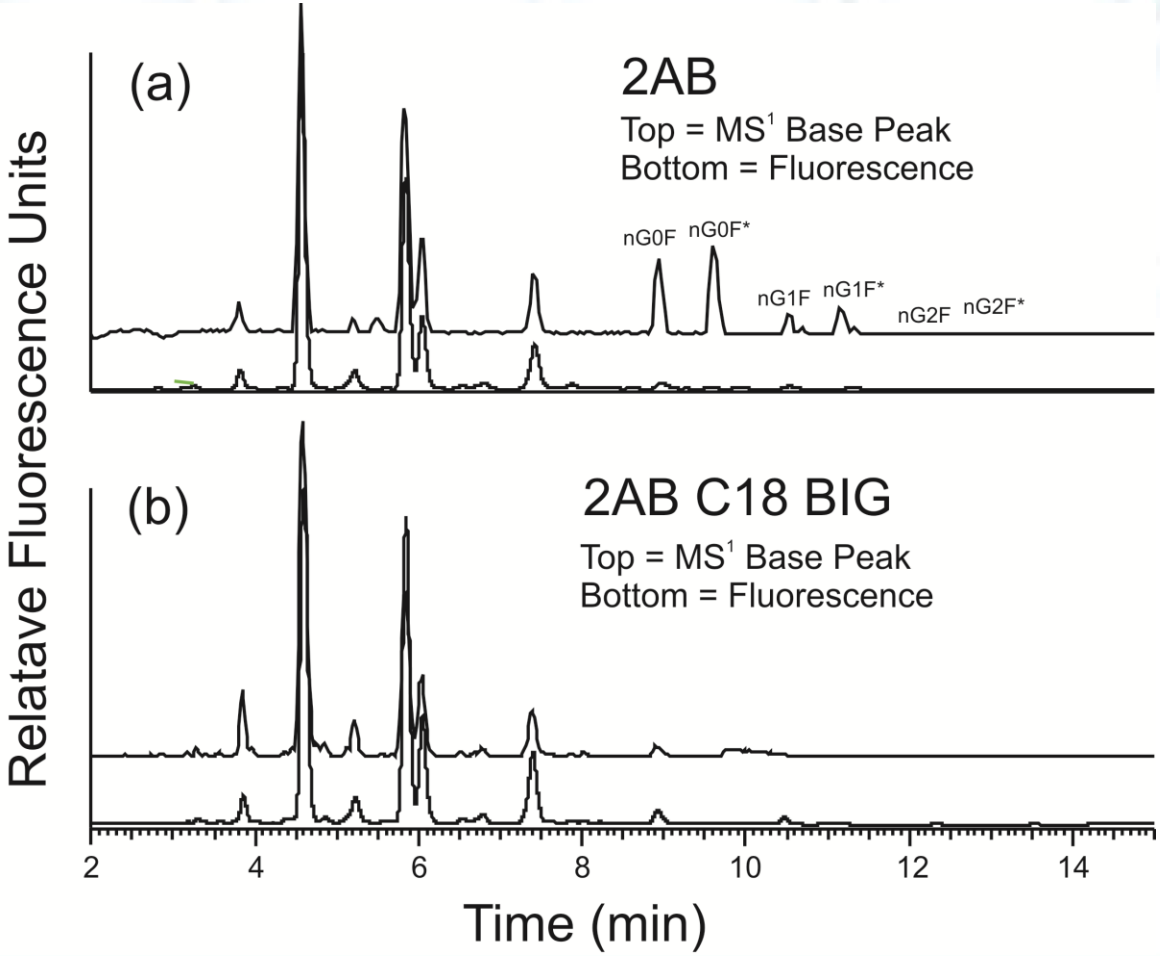


GlcNAc



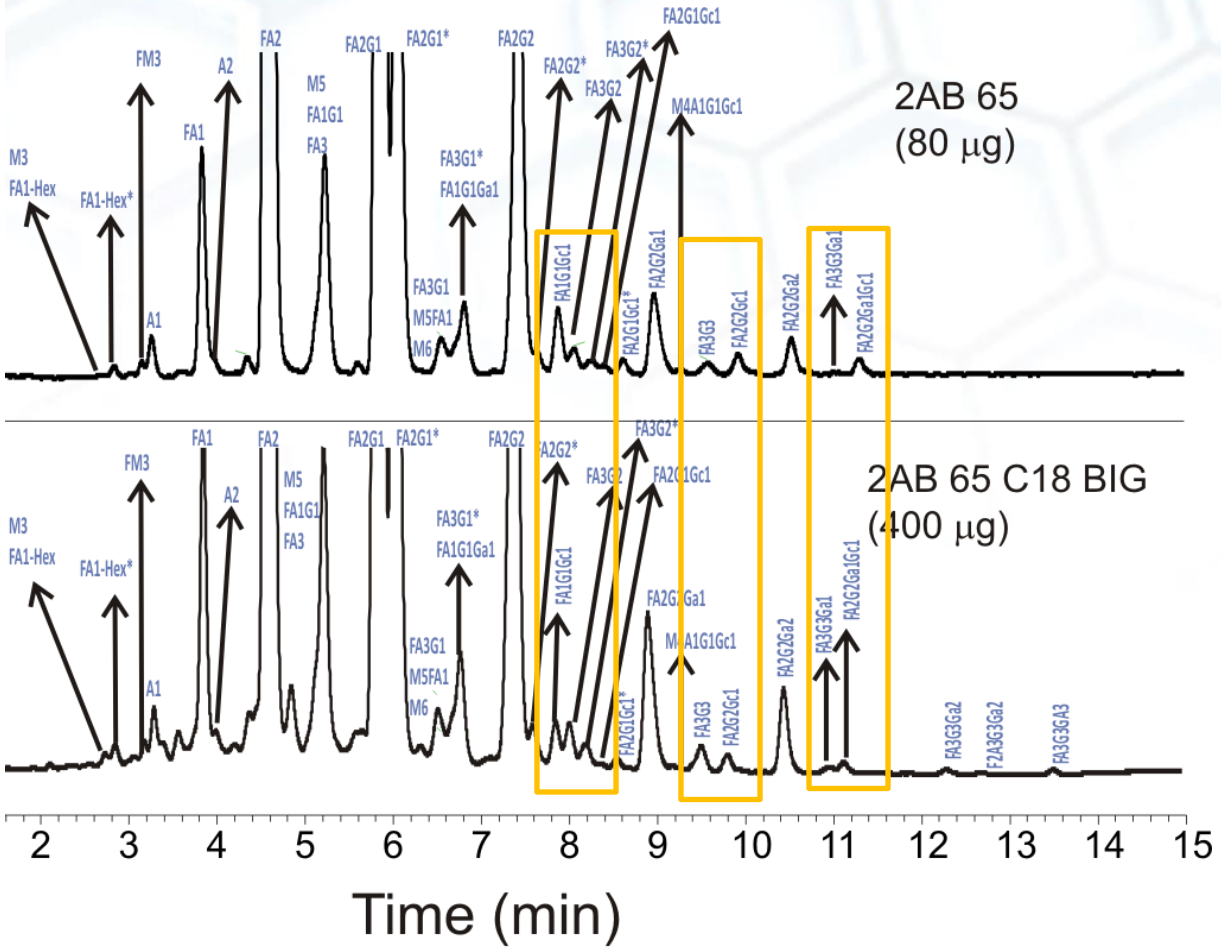
ManNAc

- Accurate *m/z* identified Glycamine byproduct
  - C2 Epimers
- Only detected when use MS
  - Monitor labeling efficiency
  - Unwanted byproducts
  - Co-eluting glycans
  - Process impurities
- MS useful during early process/method development



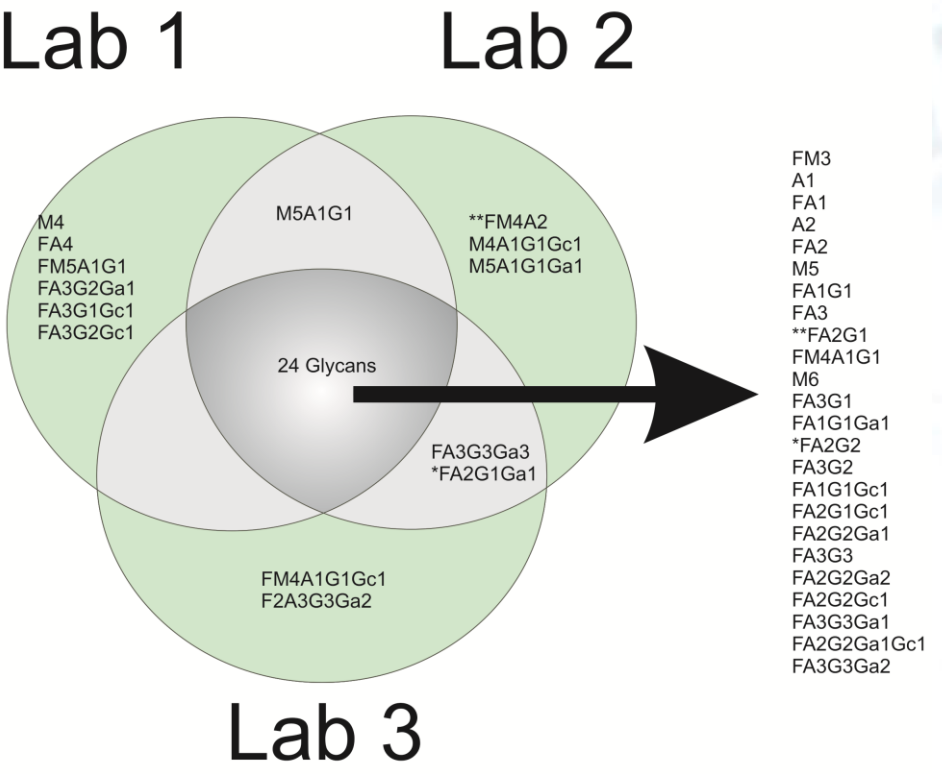
Sample Preparation Artifacts

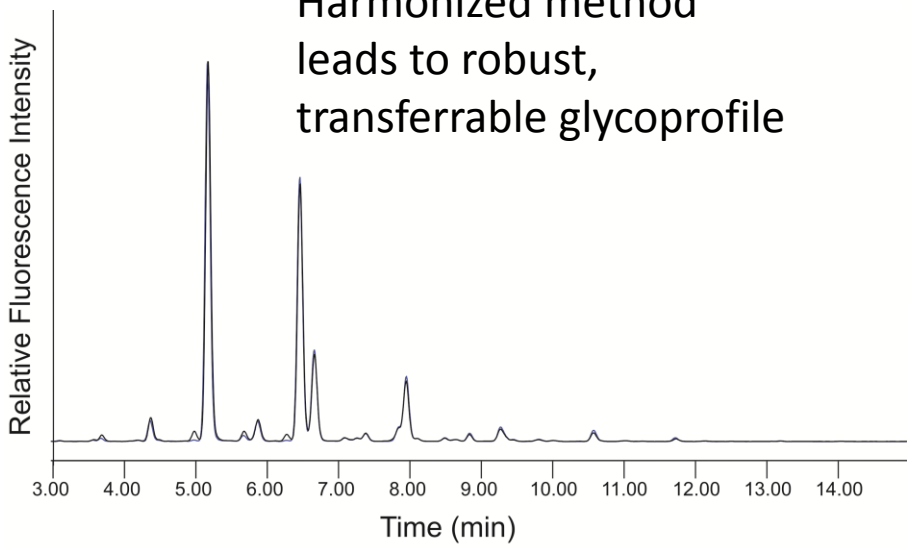
- C18 after HILIC
  - Decrease in sialic
  - Improved MS^2 low abundance
- Larger inj. quantity
  - No effect on resolution
- Apparent glycoprofile method-dependent
  - Biosimilar implications
  - Regulatory review
- Numerous sample prep conditions optimized



Interlaboratory Comparison

- Diff. sample prep and method
  - Different labels and method
  - Retention and/or MS ID
- Unique ID's less than 1% total glycan
- Quantitative comparability good
  - Minor differences due to chromatographic selectivity and ID





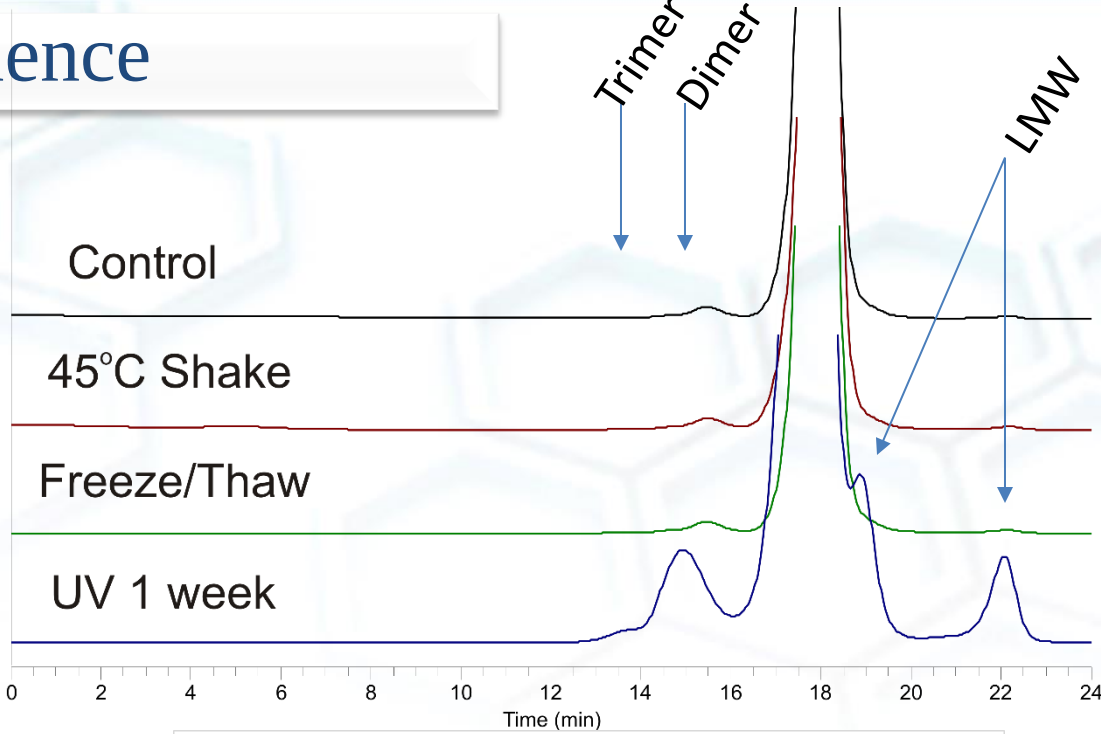
Harmonized method leads to robust, transferrable glycoprofile

Expanded Interlaboratory Study Planned

Separation Science

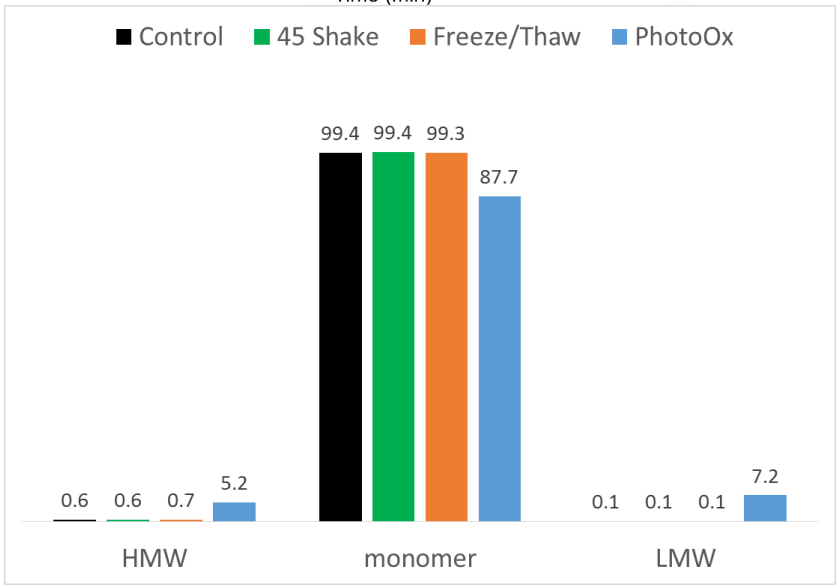
Representative Methods

- SEC
  - Aggregation
- WCX and cIEF
  - Charge variants
- RP and HILIC
  - Hydrophobic variants
- cSDS
  - Purity



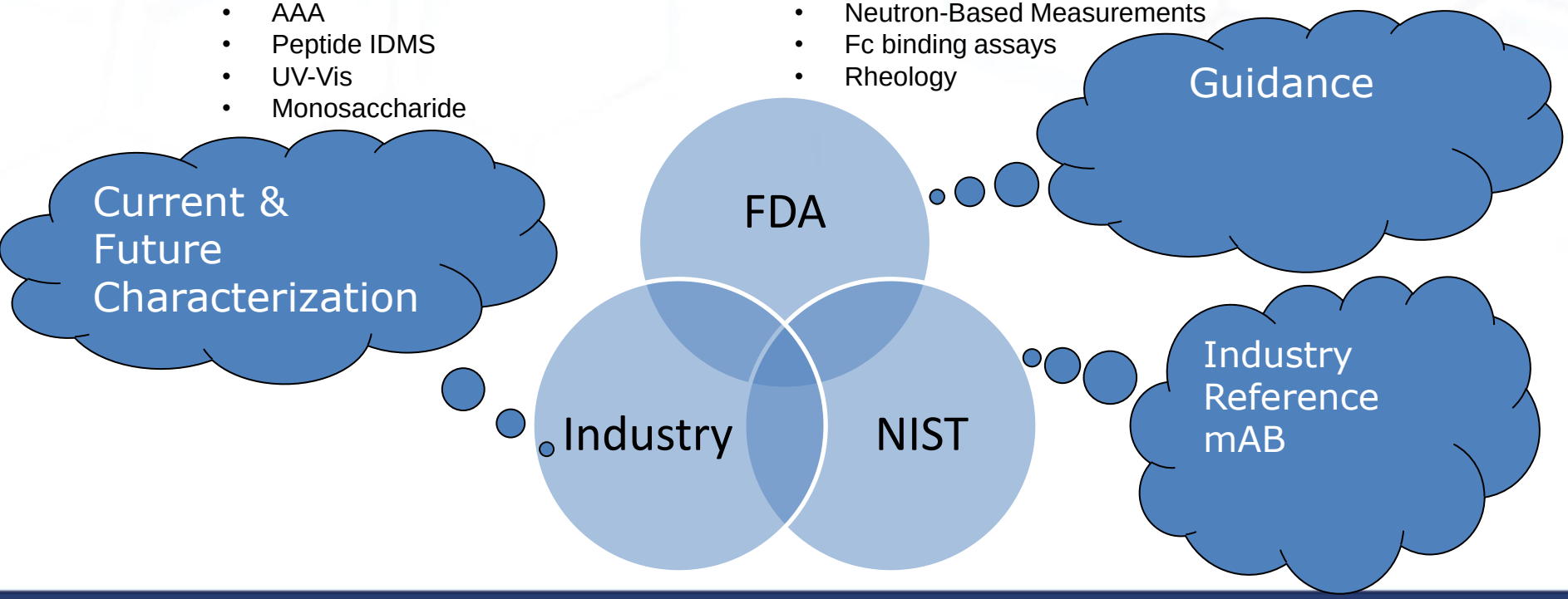
Fraction Collect

- Product variants for detailed analysis
- Forced degraded material for stability/identity/activity testing



NIST Characterization

- Separation Science
  - SEC, RP, HIC, CEX, WAX
- Mass Spectrometry and LC-MS
  - Peptide mapping, middle down, and intact
  - PTM analysis
  - Sequence Variant
  - Glycoanalysis
  - HCP's
- Mass Spectral Database
  - Peptide MS/MS
  - Glycan MS/MS
- Certification of Concentration
  - AAA
  - Peptide IDMS
  - UV-Vis
  - Monosaccharide
- Higher Order Structure
  - NMR
  - XRD
  - HDX
- Biophysical Measurements
  - AUC
  - SEC-MALS/DLS
  - CD
  - FTIR
  - AUC
- Neutron-Based Measurements
- Fc binding assays
- Rheology



ACS Book Project

"State-of-the-Art and Emerging Technologies for the Analysis of Monoclonal Antibodies"

John Schiel (NIST), Oleg Borisov (Novavax), Darryl Davis (Janssen)

Co-editors

Structure overview – three volumes

- mAb Function, Structure, Production and Regulatory Overview
- The Protein Characterization Lab of Today
- The Protein Characterization Lab of Tomorrow
  - Product-Related Technologies
  - Process-Related Technologies

Approach

- Characterization of NIST mAb as book topic
- Engage industry scientists to collaboratively demonstrate best practices round robin characterization NIST mAb
- Establish NIST mAb as Industry Standard

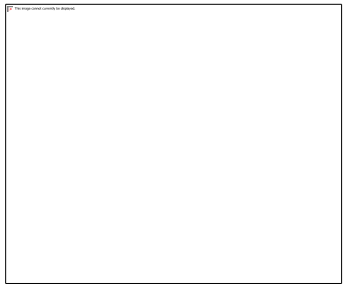
Industry Collaboration

Contributors

- 65+ Industry, Regulatory, and Academic participants confirmed
  - Global Biopharma Participation
  - Multiple NIST and FDA Chapters
  - Academia and OEM contributions
- 33 Chapters Confirmed

Progress and Activity

- Interim NIST mAb sent to collaborators for characterization and book composition
- Complete physicochemical and biophysical characterization
- Chapters currently in peer reveiw

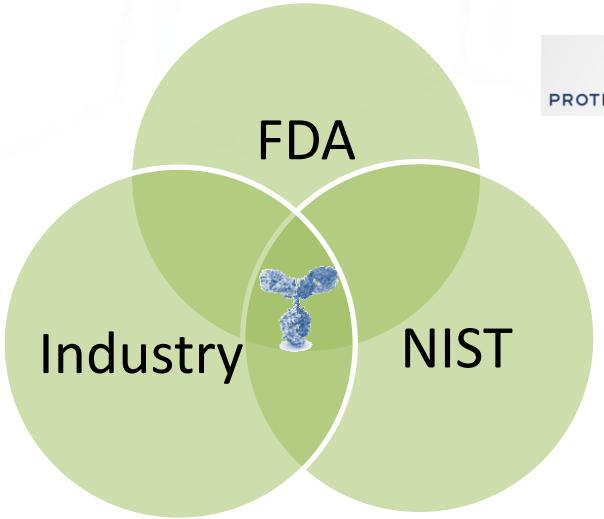


Conclusion

- RM to Supplement In-House Reference Standard Program
  - Streamline implementation of new technology
  - Assist method qualification
  - System suitability
  - Widely available historical data
  - Harmonize approaches to well characterize
  - Assess method variability, utility, etc.
  - Differentiate method vs. product related artifacts
- Physicochemical and Biophysical Data Generated with industry wide collaboration
  - ACS Book Project
  - ASMS Workshop
  - FDA/NIST Symposium
- Expected Impact
  - Underpin regulatory decisions
  - Higher-order characterization
    - Method accuracy, precision, comparability
    - Translate to product safety and efficacy

Acknowledgements

- ACS Book Co-Editors
  - Darryl Davis, Janssen
  - Oleg Borisov, Novavax
- Workshop
  - Ref. Mat. And Ref. Data
  - Tuesday, 5:45 Room 327
- Additional Posters
  - TP252, Middle Down
  - WP214, Stability Analysis



Collaborators/Stakeholders

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