Measurement Science and Standards to Support the Development of Safe and Effective Protein Therapeutics

3rd Annual Biopharmaceutical Summit on PAT and QbD in the Biopharmaceutical Industry

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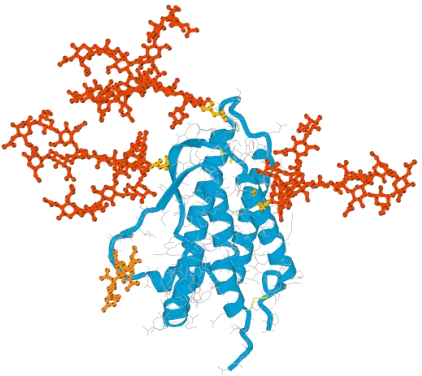
NIST Program in Biomanufacturing



Measurement science, standards, and data to support development, manufacturing & regulatory approval of biologic drugs

Developed from Over 5 Years of Stakeholder Input:

AMGEN, Janssen Pharmaceutical Companies of Johnson & Johnson, MedImmune, FDA, Genentech A Member of the Roche Group, biogen idec, Bristol-Myers Squibb, Pfizer, Lilly, MERCK, gsk, SANDOZ Biopharmaceuticals

NIST Criteria for Priority Setting:

1. Magnitude/urgency of industrial need
2. Correspondence between need and NIST mission to develop infrastructural technologies
3. Potential impact of NIST involvement
4. Can NIST respond with a timely, high quality product

NIST Biomanufacturing Program Areas and Projects

Protein Stability

- Methods & Reference Materials for the Measurement of Protein Particles
- Broadband-CARS imaging for characterizing individual protein particles
- Bench Top Optical Method to Estimate Protein Stability in Solid Forms
- Microfluidic electrical sensing – optical imaging instrument for characterizing protein particles
- Microfluidic measurement of viscosity & rheology of protein drug products

Protein Structure

- Development of NIST mAb Reference Material
- MS Library of Peptides, Glycans and Glycopeptides for Therapeutic Antibodies
- NMR Multi-Lab Inter-Comparison of GCSF
- Neutron Measurements of Protein Therapeutics
- HDX-MS Multi-Lab Inter-comparison
- Raman spectroscopy and MVA for ID of protein therapeutics

Understanding Production Cells

- Optical microscopy of CHO cultures to assess clonal stability
- MS library of CHO and E. Coli bioreactor metabolites and compounds
- Determination of concentration of host cell DNA standards using dPCR

Role of Measurement Science and Standards in Supporting QbD

- Testing controls are key element of overall control strategy to ensure process consistently delivers correct product QAs

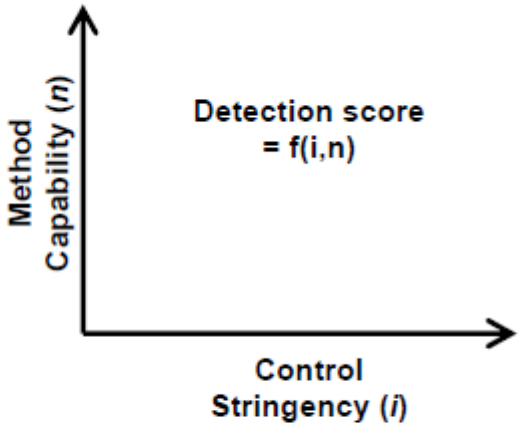
- Measurement science and standards can help:
  - Determine variability of test methods and setting test limits
  - Determine test methods are in control
  - Assess performance of new analytical technologies

Failure Modes and Effect Approach for Determining Overall Risk Level

PQRA (Overall Risk) $= f($ PQA Criticality Assessment *(Severity)* , Process Capability *(Likelihood of Occurrence)* , Testing Strategy *(Detection)* $)$

Detection scoring = f (method capability, control stringency)

- Detection scoring combines two concepts
  - **Method capability** considers limit of quantitation, precision, specificity and orthogonality
  - **Control stringency** accounts for frequency of testing and limits applied



AMGEN

Courtesy of Brent Kendrick, Amgen

Protein Particle Measurements and Standards Activities

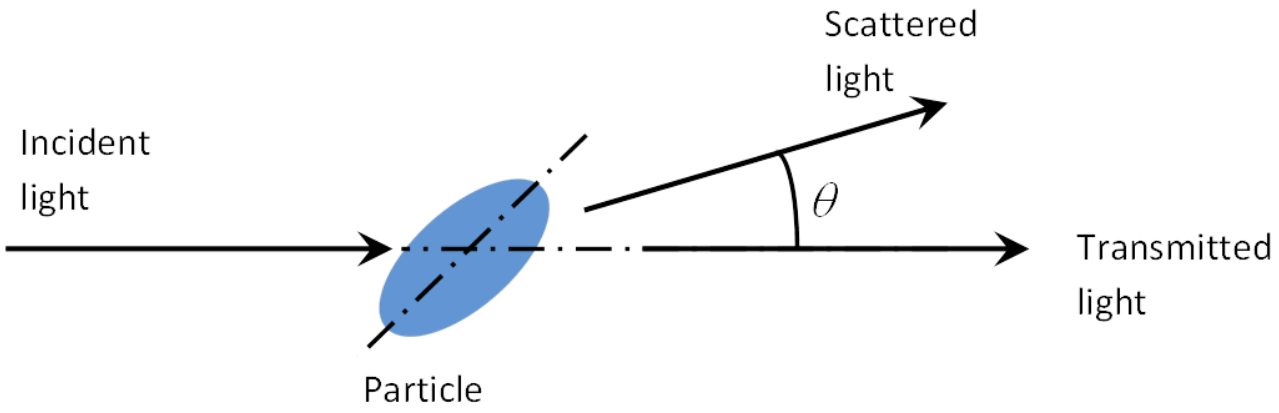
Goals:

- Reduce risks to safety and efficacy of biotherapeutics by supporting accurate counting and characterization of particles
- Support industry in understanding involvement of particles in biological pathways, e.g., immunogenicity

Activities:

1. **Measurement science:** appropriate models for instrument response
   - Identify and characterize physical properties of protein particles relevant to counting method considered
2. **Reference materials:** materials that mimic protein particles
3. **Measurement tools:** new orthogonal particle measurement technologies

Light Obscuration Scaling: Instrument Model



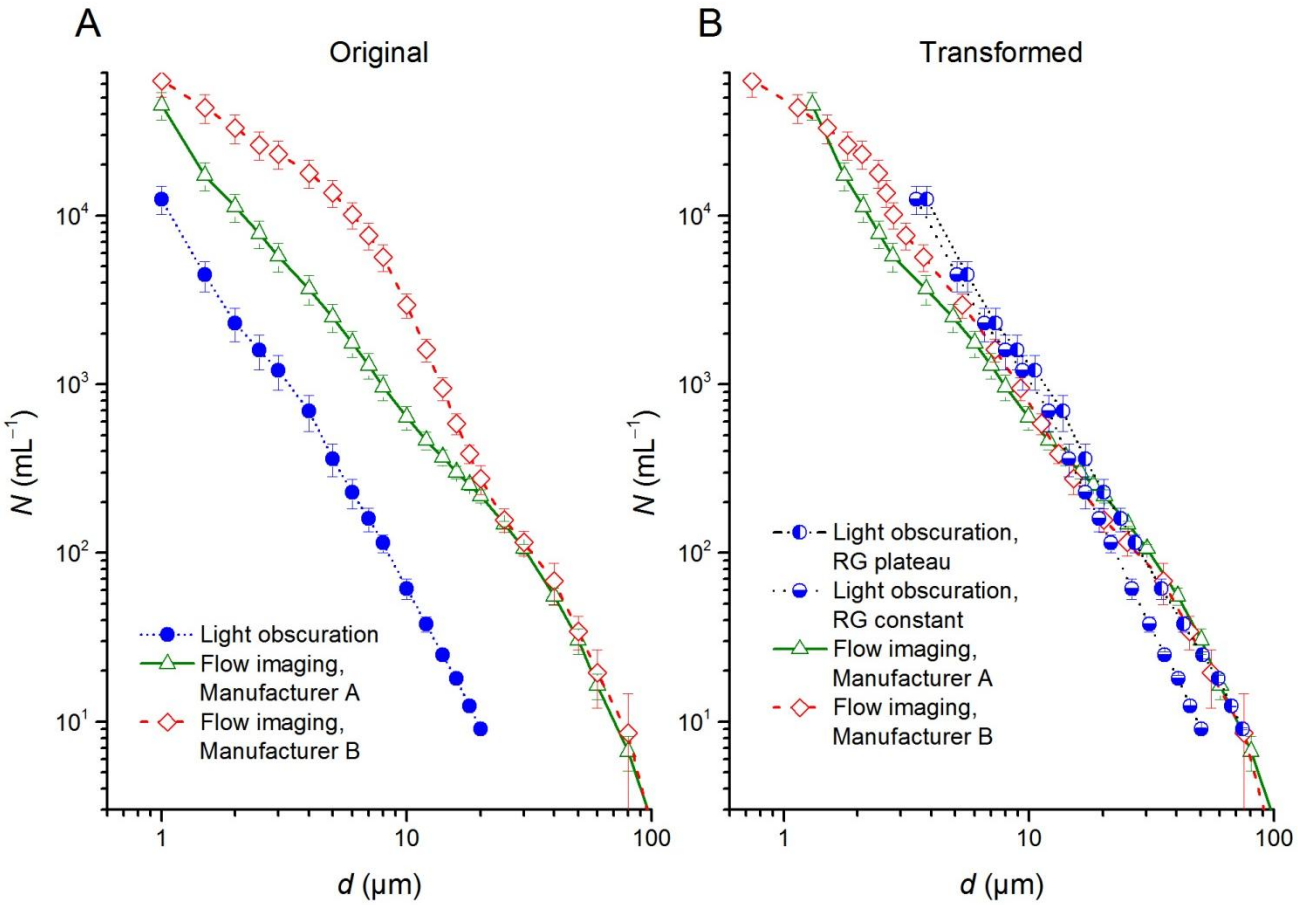
Approach:

1. Model small particles as spheroids; use scaling approximations for large particles
2. Obtain the average refractive index of the particles from Quantitative Phase Imaging
3. Calculate the instrument response using Rayleigh-Gans light scattering models
4. Transform the LO data using the instrument response curve to estimate the actual particle diameter corresponding to the measured diameters.

Transformation scales particle <u>diameter</u>, not <u>count</u>

Results of Adjusting Diameters

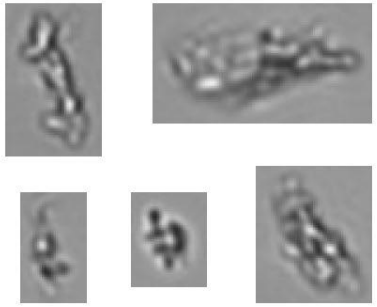
- Two flow imaging instruments, one light obscuration instrument
- Particles formed by agitation of polyclonal human IgG



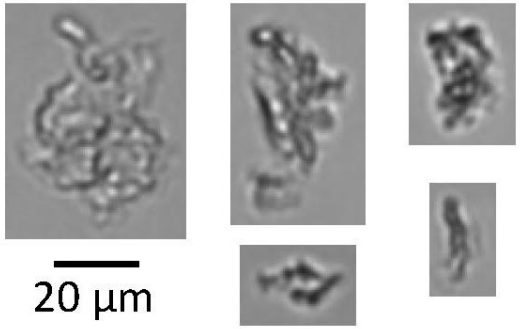
Candidate Particle Reference Material: Abraded Fluoropolymer

ETFE polymer (tetrafluoroethlyene/ethylene copolymer) has desirable properties:

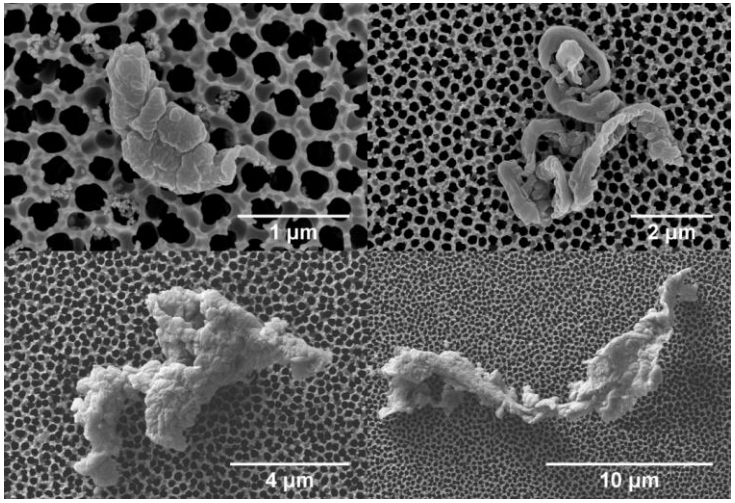
1. Rugged, with refractive index of 1.40—close to that of protein
2. Appears like protein with mechanical abrasion process—oscillatory motion pulls off irregular, tangled particles
3. Producing polydisperse suspension as reference material, 1 to 25 µm



Protein particles



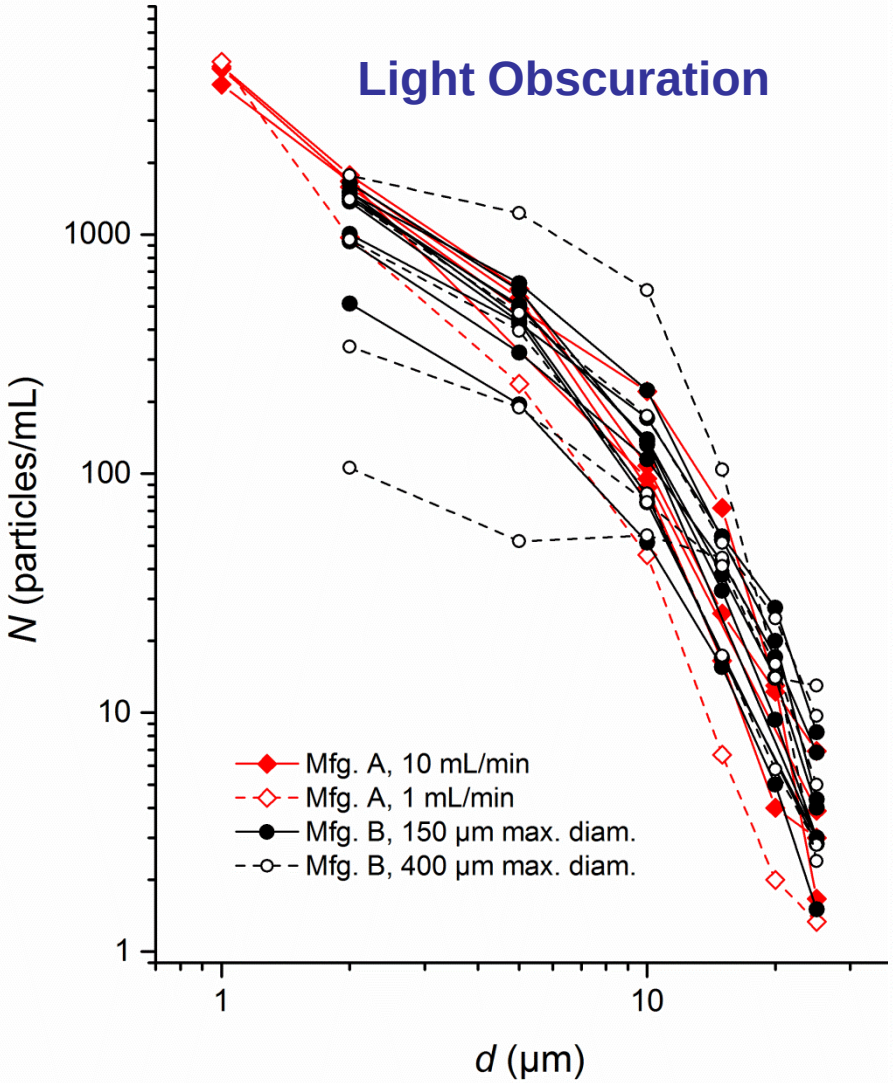
ETFE particles

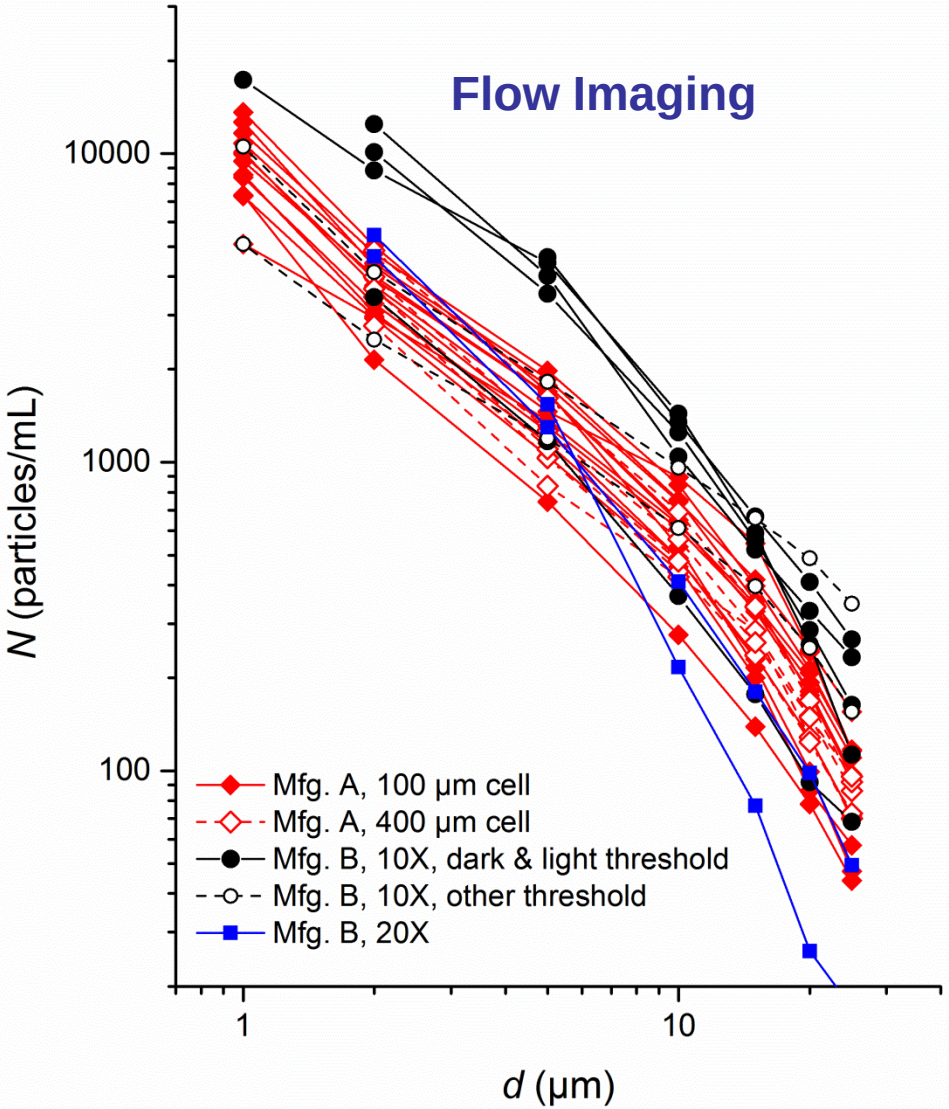


SEM images of ETFE particles on alumina filters

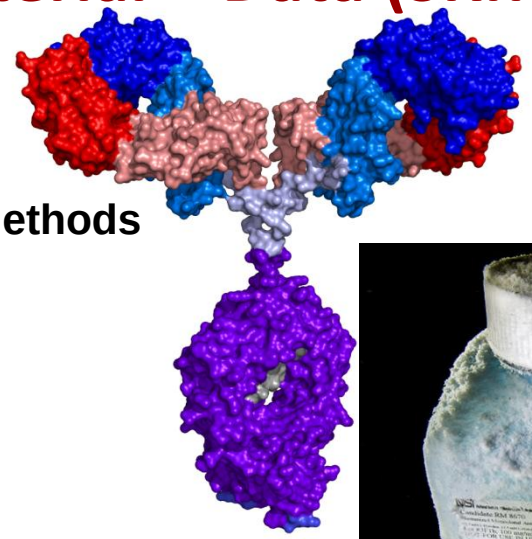
Interlaboratory Comparison of ETFE Particles

- 24 participants: biopharma, instrument vendors, academia, government
- Diameter range 1 to 25 µm
- Paper submitted to J. Pharm. Sci.





NIST mAb Standard Reference Material + Data (SRM/D)



A mAb (IgG1) reference material could be useful for:

- System suitability material or cross-checking test methods
- Testing new measurement technologies
- Comparing changing analytical test methods

NIST mAb attributes:

- Humanized mAb (IgG1κ) expressed in murine 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)

"Crowd-Sourcing" approach for IgG characterization:

- Complete rigorous interlaboratory characterization
- 65+ Biopharma, Regulatory, and Academic participants
  - Results used for ACS book "State-of-the-Art and Emerging Technologies for the Analysis of Monoclonal Antibodies"
- NIST will certify concentration traceable to the kg
- Compile reference data (MS library), methods, etc.
  - Publically available: http://igg.nist.gov/

Possible uses for IgG SRM:

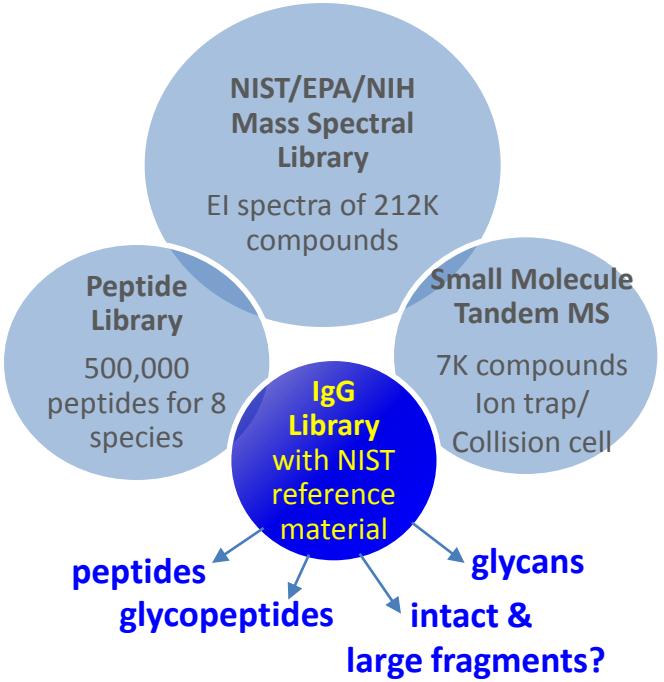
Amino Acid Sequencing
Amino Acid Analysis
N-terminal Sequencing
C-terminal Sequencing
Peptide Mapping by MS
S-S Bridge Analysis
Glycosylation Analysis
Molecular Weight Information
Isoelectric Focusing
SDS-PAGE
Extinction Coefficient
Post-Translational Modifications
Spectroscopic Profiles: CD, NMR
LC: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

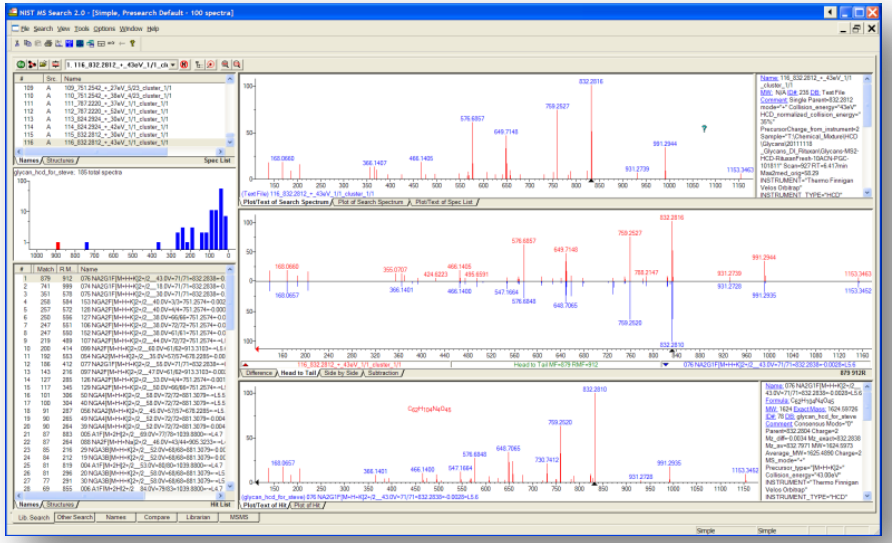
NIST CHARACTERIZATION

- **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 Total Protein Concentration**
  - AAA
  - Peptide IDMS
- **Future potential certified values**
  - Extinction coefficient
  - Monosaccharide content
- **Higher Order Structure**
  - NMR
  - XRD
  - HDX
  - Small angle neutron scattering (SANS)
  - Small angle x-ray scattering (SAXS)
- **Biophysical Measurements**
  - AUC
  - SEC-MALS/DLS
  - CD
  - FTIR
- **Fc binding assays**
- **Rheology**

Building a Comprehensive MS Reference Library of Peptides, Glycans, & Glycopeptides of the NIST mAb

- NIST MS reference libraries most widely used in world
- No comprehensive MS spectral library of mAbs exists
- Build integrated IgG MS library of:
  - Tryptic peptides – all modifications
  - Glycans – all forms
  - Glycopeptides
- Future mAb Standard Reference Material will include MS reference data (SRM/D)





NA2G1F from Rituximab Matches Library

High quality MS reference data

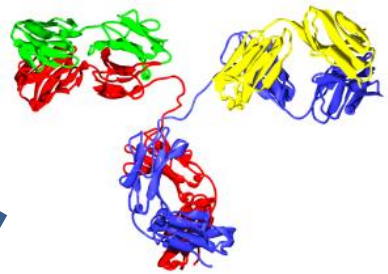
- Spectra for ion trap and collision cells (range of energies) for various precursor ions
- Consensus spectra: peak voting; intensity averaging; reject contaminants & noise
- Quality control: % explained peaks, proper energy dependence, precursor purity

From IgG to MS Library

Enzymatic Fragmentation – LC Separation - MS Identification

IgG Fragmentation:

- Break 'hinge' (papain,…)
- Break S-S (reduce)
- Stabilize S (alkylate)



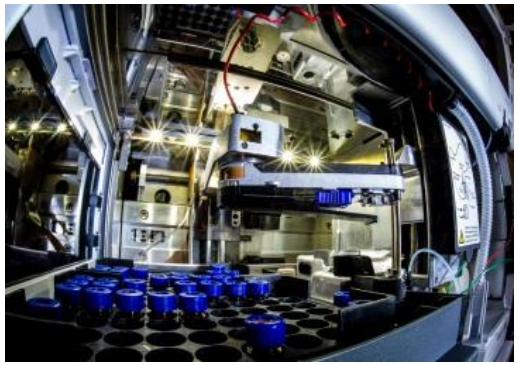
Proteolysis:

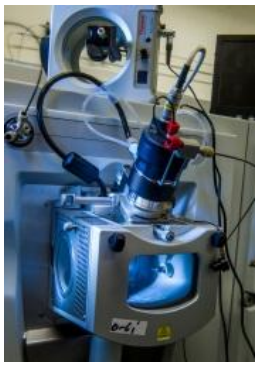
- Trypsin
- Asp-N
- Glu-C
- Lys-C

Deglyco-sylate

Big Fragments | Glycans | Peptides

Separate by LC, identify by MS/MS





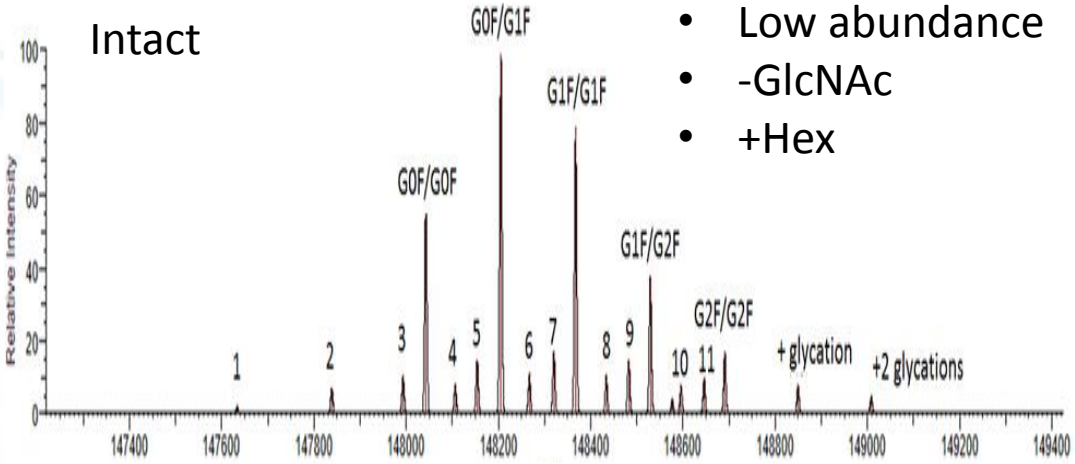
http://promega.wordpress.com/;
Photos courtesy of Matt DeLorme

Intact *m/z*

- Support primary sequence
- High abundance variants
  - PTM's, truncation, etc.
  - Intact analysis shows major glycoforrms
- Deglycosylated verifies
  - C-terminal truncation
  - N-terminal pyro-glu
  - Glycation

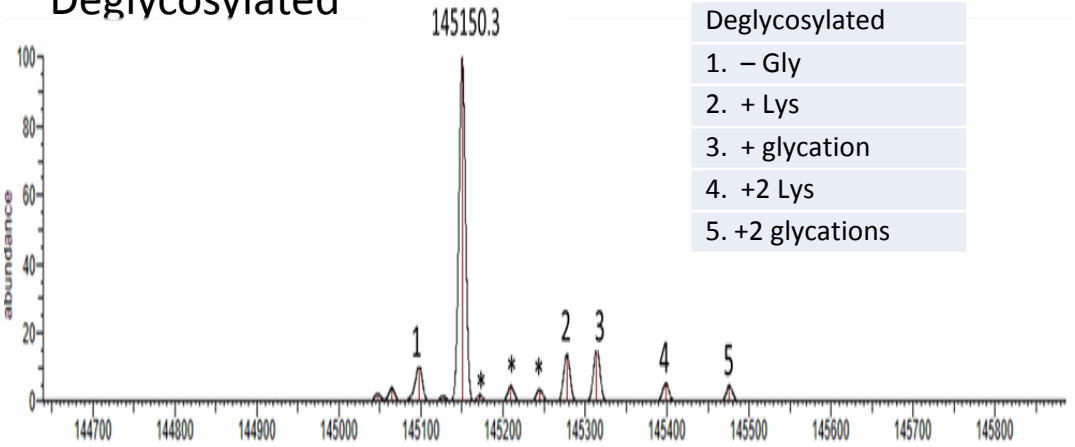
Consistent with Expected Primary Sequence

Intact



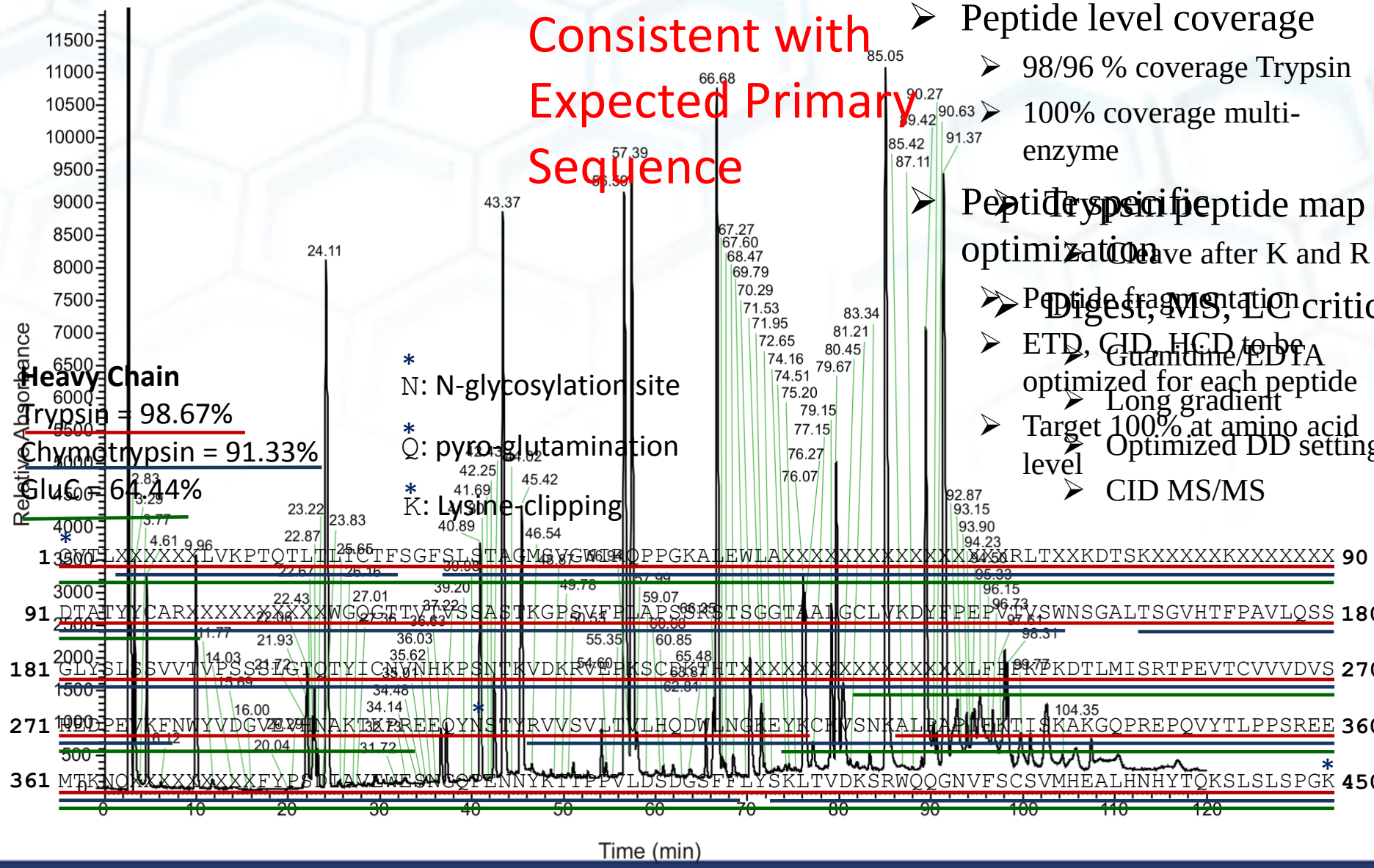
- Low abundance
- -GlcNAc
- +Hex

Deglycosylated



Proteoform
Deglycosylated
1. – Gly
2. + Lys
3. + glycation
4. +2 Lys
5. +2 glycations

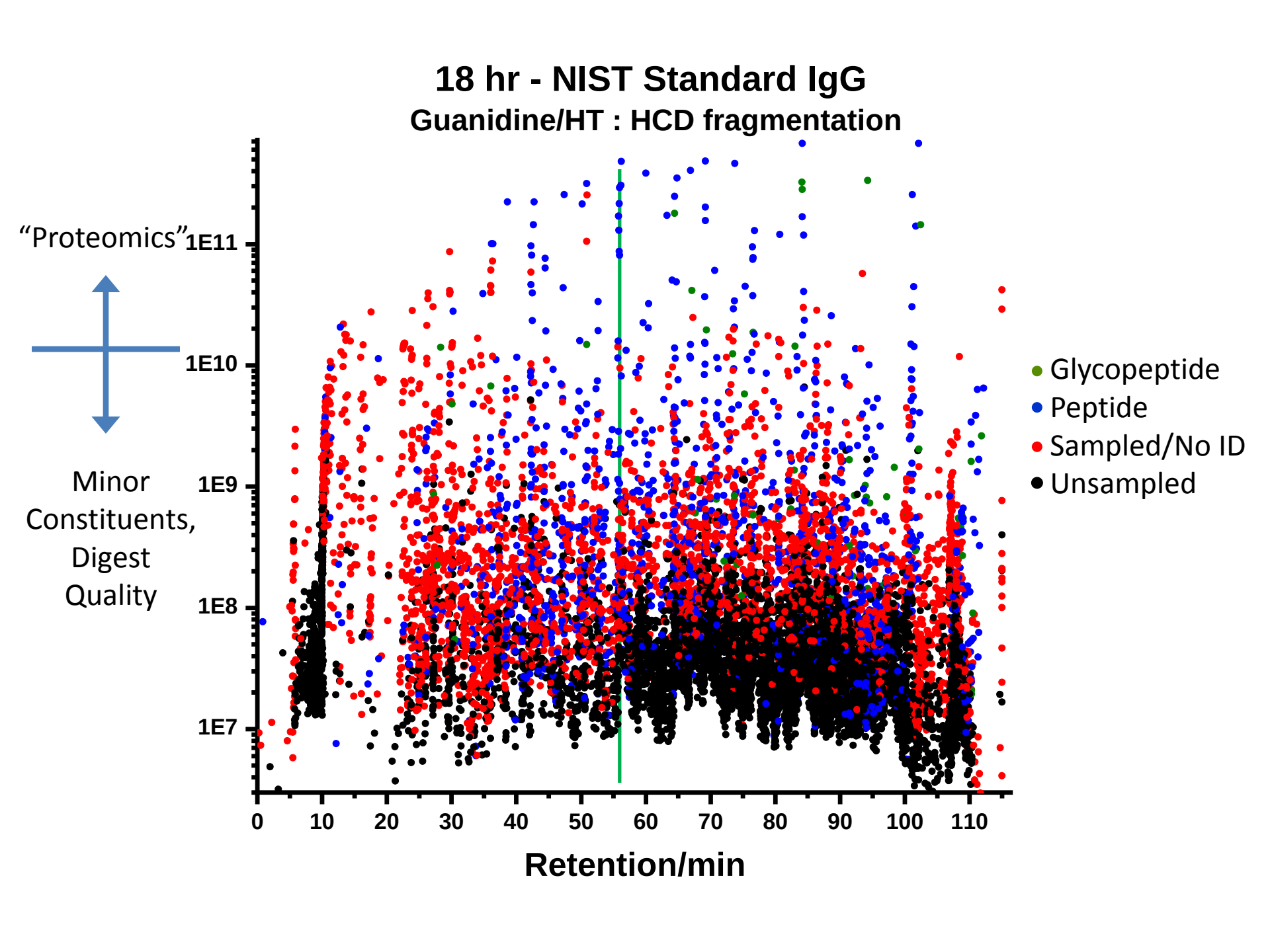
LC-UV-MS/MS Peptide Map



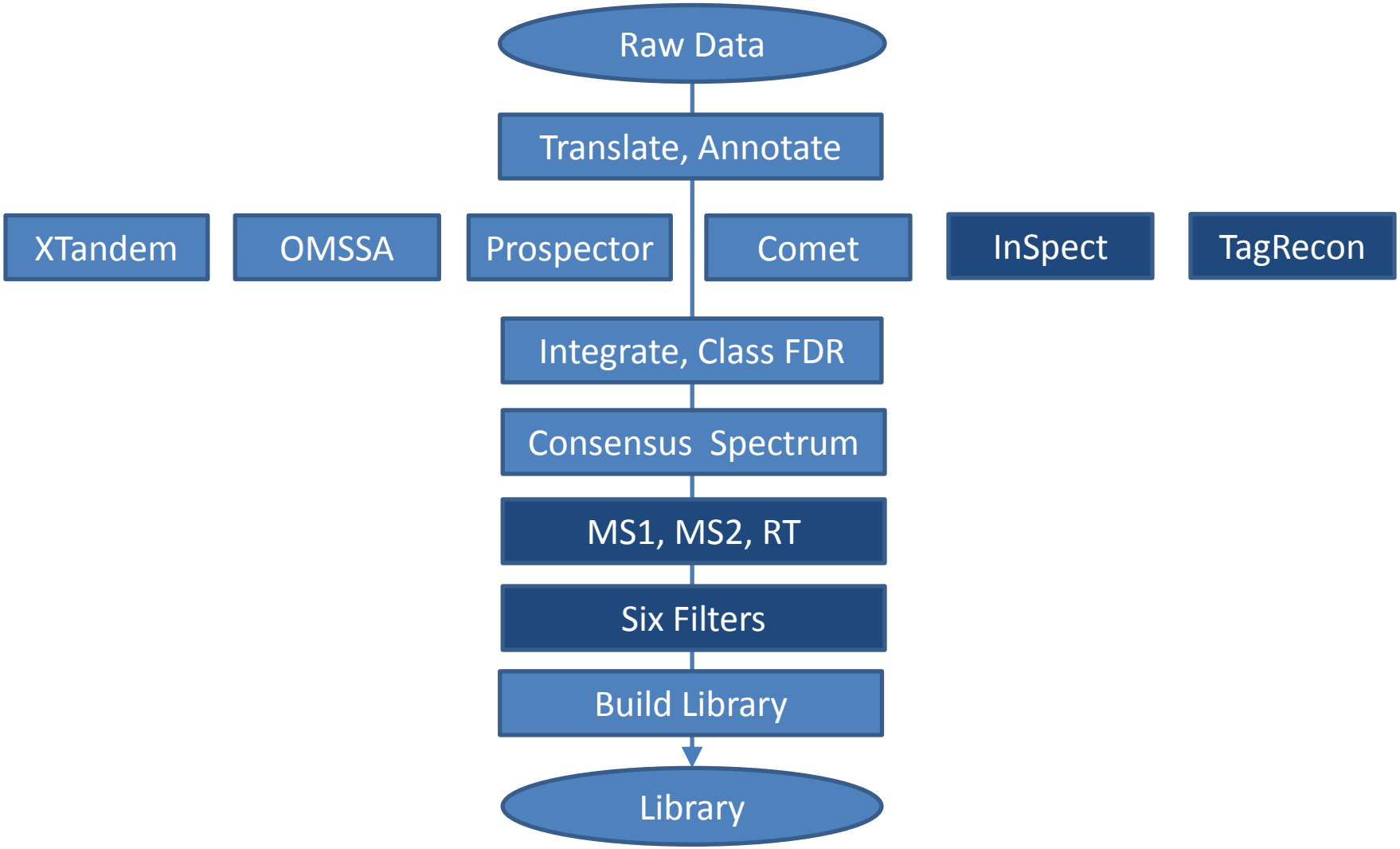
- Peptide level coverage
  - 98/96 % coverage Trypsin
  - 100% coverage multi-enzyme
- Peptide specific peptide map optimization
  - Trypsin
    - Cleave after K and R
  - Peptide fragmentation
    - ETD, CID, HCD to be optimized for each peptide
    - Target 100% at amino acid level
      - CID MS/MS

Digest, MS, LC critical

- Guanidine/EDTA
- Long gradient
- Optimized DD settings


18 hr - NIST Standard IgG
Guanidine/HT : HCD fragmentation
"Proteomics"
Minor Constituents, Digest Quality
1E11
1E10
1E9
1E8
1E7
0
10
20
30
40
50
60
70
80
90
100
110
Retention/min
Glycopeptide
Peptide
Sampled/No ID
Unsampled

Single Protein Digest Data Analysis Pipeline For MS Library Construction



NIST mAb HCD Tryptic Peptide Library

Peptide Classes

Class	Common or Uncommon	Peptide Class	Spectra
1	C	Simple Tryptic	567
2	C	Tryptic with Expected Missed-Cleavage	461
3	C	Common Modifications	369
4	C	In-Source Semitryptic	1010
5	U	In-Solution Semitryptic	
6	U	Artifacts and PTMs	372
7	U	Unexpected Missed-Cleavage	689
8	U	Under/Over Alkylation	42
		Total	3510

Peptide Modifications

Modification	Delta mass	Modified site	Spectra
Oxidation	+15.9949	M, H, W	778
Deamidation	+0.9840	N, Q	96
Cation:Na	+21.9819	D, E	84
Cation:Fe[II]	+53.9193	L, G, S, T, P, V…	62
Formyl	+27.9949	N-terminus, K, S, T	50
Pyro-carbamidomethyl	+39.9949	C at N-terminus	24
Gln->pyro-Glu	-17.0265	Q at N-terminus	21
Dehydrated	-18.0106	D, S, T	15
Glu->pyro-Glu	-18.0106	E at N-terminus	14
Cation:Ca[II]	+37.9469	I/L, P, S, T, G…	10
Methyl	+14.0157	K, H	9
Dioxidation	+31.989829	M,W	8
Carbamyl	+43.0058	N-terminus, K,T,M	8
Trioxidation	+47.984744	W	3

Preliminary: More Mods to Come!

Conclusions

- NIST measurement science and standards support QbD for protein biologics through improved characterization of CQAs and development of methods specific for CQAs

- Particle measurement science and standards aid in more accurate sizing and counting of protein particles

- NIST monoclonal antibody reference material will find use in:
  - Assessing method variability, utility, etc.
  - Determining performance of new technologies
  - System suitability testing/method qualification
  - Source of widely available historical data

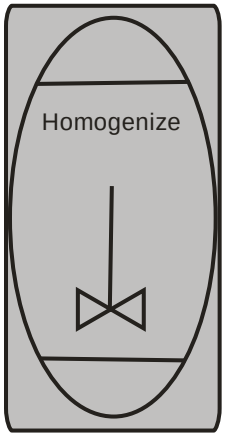
Acknowledgements

- NIST
  - **John Schiel (NIST mAb PI)**
  - **Steve Stein (Director, NIST MS Data Center)**
  - **Dean Ripple (Protein Particles)**
  - Catherine Formolo
  - Lisa Kilpatrick
  - Meiyao Wang
  - Karen Phinney
- ACS Book Co-Editors
  - Darryl Davis, Janssen
  - Oleg Borisov, Novavax
  - Other participants
- FDA
  - Kurt Brorson
  - Erik Reed
  - Michael Boyne
  - Cyrus Agarabi
  - Scott Lute

Collaborators/Stakeholders

Storage and Vialing Plan

Multiple Production Runs
(12.5 mm l-HIS, 12.5 mm l-HIS HCl, pH 6.0, currently at -20°C)



- 1 L x 80
- 100 mg/mL
- (-80°C)

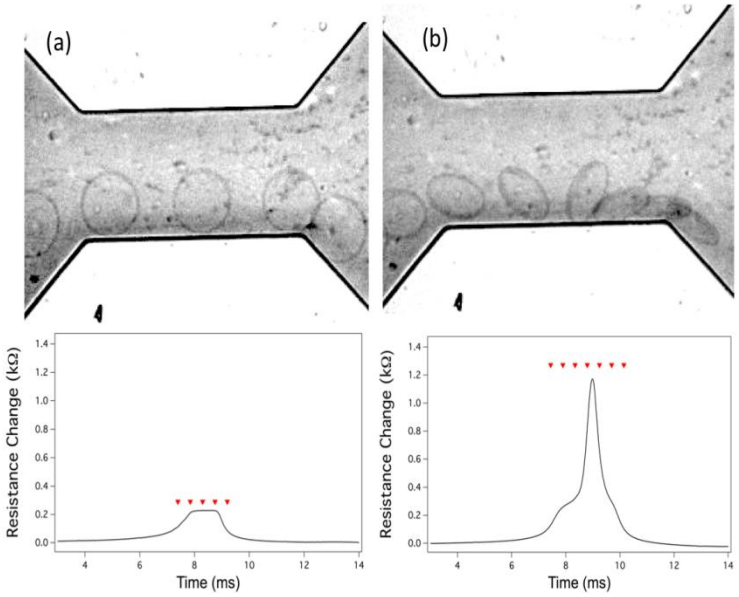
Dilute to 10 mg/mL and vial at 1 mL

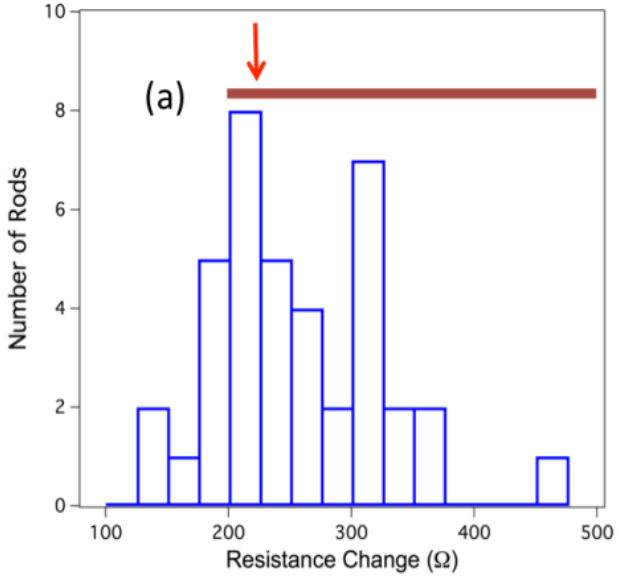
Performed as needed

- Initial Fill 16,000 vials (-80°C)
  - 10,000 as RM/SRM
  - 6,000 reserved as primary standard

Engineered Particles I: Understanding Effects of Tumbling in Coulter Counters

Image and electrical signal for tumbling and non-tumbling discs in NIST Micro-Coulter Counter.



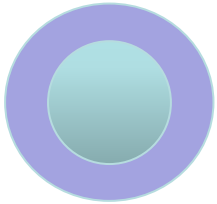


Histogram of measured resistance changes for rods. Red bar indicates tumbling region. Arrow indicates expected diameter.

Engineered Particles II: Understanding Biases in Flow Imaging

- Diffraction effects result in oversizing of fibrous particles
- Lithographic “rods” produced
- We developed a simple algorithm that uses the measured perimeter to give an improved measurement of particle area

Instrument algorithm: correct diameter by a constant value

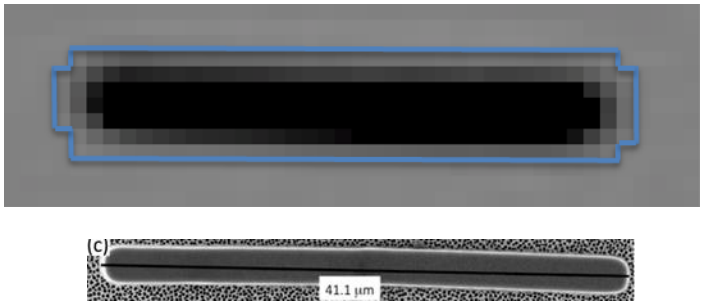


$D_m - 6.7\mu m$

Method	Equiv. dia. (µm)
SEM	13.4
Instrument algorithm	17.9
NIST algorithm	13.1

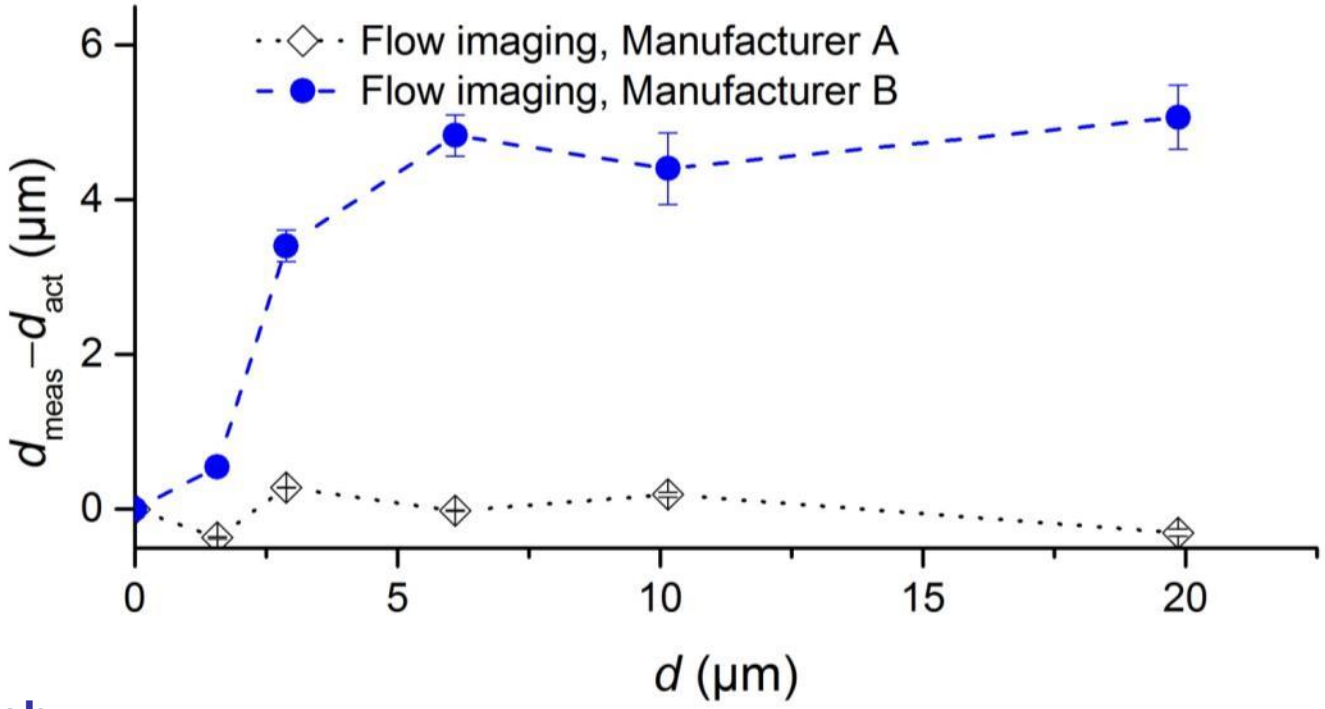
NIST algorithm: correct area by an amount proportional to perimeter

$$[D_m^2 - \delta D \cdot P_m(2 - \delta D/D_m)/\pi]^{1/2}$$



Flow imaging (top) & SEM (bottom)

Flow Imaging: Instrument Model



Approach:

1. Measure diameters of silica beads suspended in water/glycerol mixtures
2. Adjust the measured diameter for protein particles by the bias measured for silica beads

Transformation scales particle <u>diameter</u>, not <u>count</u>

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."

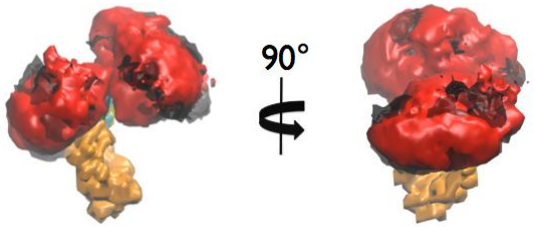
S. Kozlowski, FDA

- 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
  - Assess capabilities of new analytical technologies
  - Ease regulatory burden on reviewers and sponsors by allowing them to assess methods and demonstrate system suitability by evaluation of the standard

Neutron Measurements of Protein Therapeutics

Why neutrons?

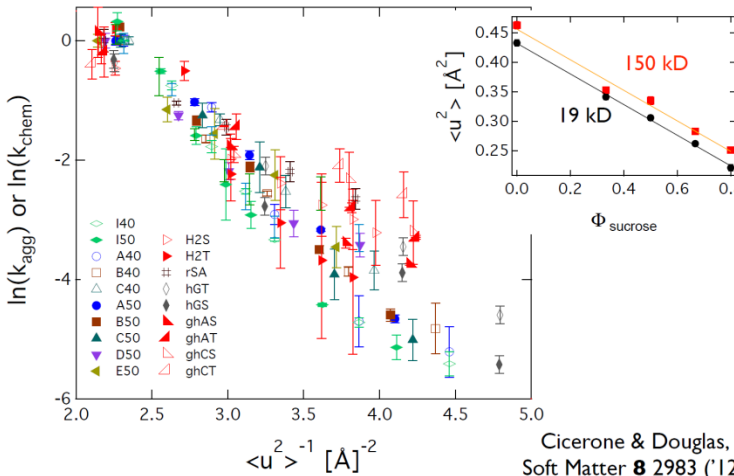
- Neutron spectroscopies provide information on geometry of motion and length scale (nm - μm)
- Simplicity of the interaction allows easy interpretation of intensities & comparison of theory and models
- H & D scatter differently, many materials transparent to neutrons
- Neutrons can probe high conc. liquid, solid, & frozen formulations, & interfaces



Protein therapeutic projects

- Antibody structure and interactions: what causes high viscosity?
- Dynamics in freezed-dried formulations
- Protein association & aggregation
- Adsorption of proteins at surfaces & interfaces:
  Air-water & ice-water interfaces

Degradation Tracks $\langle u^2 \rangle^{-1}$



Cicerone & Douglas, Soft Matter **8** 2983 ('12

nSoft
A consortium for the advancement of neutron-based measurement technologies for soft materials.

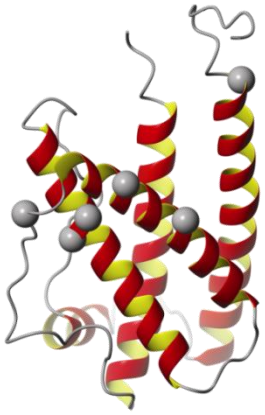
- NIST consortium enabling access to neutron facilities for soft materials manufacturers

AMGEN MedImmune Genentech A Member of the Roche Group

Comparability of Protein Therapeutic Structure using High-Resolution NMR: An Inter-laboratory Round Robin Study

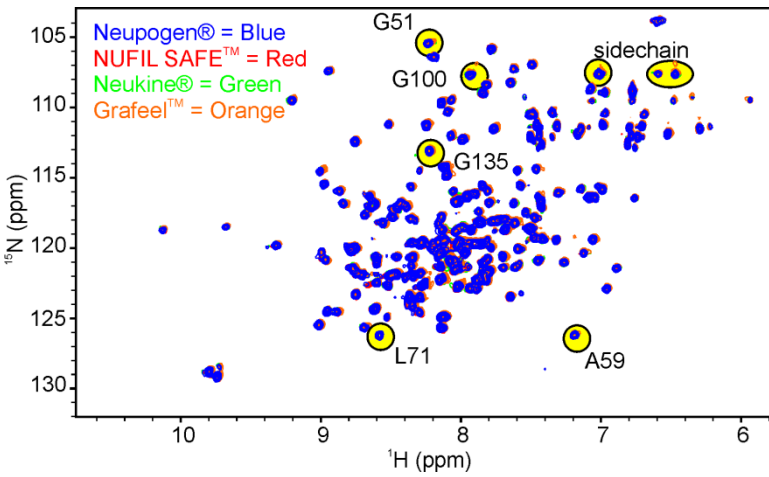
- **Project Goals**
  - Demonstrate NMR for assessing primary, secondary, and higher-order structure at atomic resolution through spectra 'finger-printing'
  - Measurements establish drug product consistency from manufacturing changes or for comparing a follow-on biologic to an innovator product
  - Filgrastim (Neupogen® and 3 foreign-sourced drugs) used in the study

- **Results of the Study**
  - Established system suitability standards and experimental protocols for robust comparability across labs
  - Combined Chemical Shift Differences and Multivariate Analysis methods demonstrated for comparative analysis
  - Highly similar structural finger-prints found between Filgrastim drug products

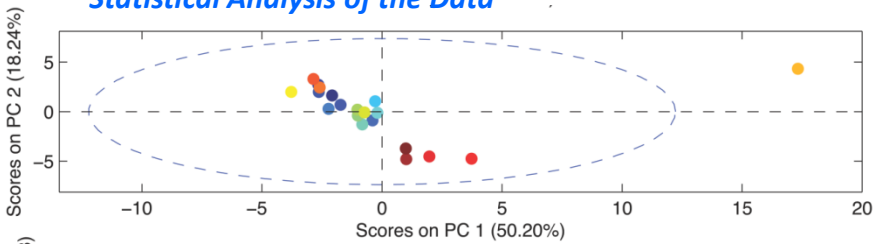


Filgrastim (Neupogen®)

2D NMR Spectral 'Finger-Printing'



Statistical Analysis of the Data



FDA

Health Canada Santé Canada

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

LÄKEMEDELSVERKET MEDICAL PRODUCTS AGENCY

Utility of a NIST mAb Reference Material

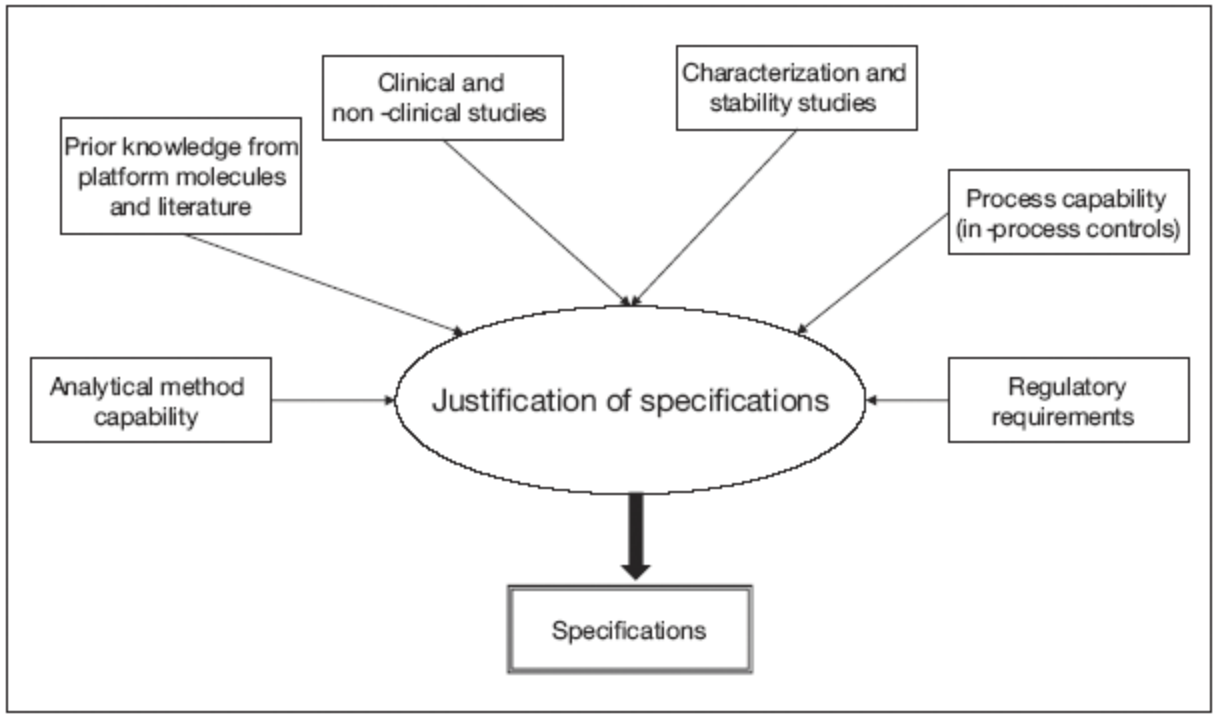
- Used to distinguish analytical variability from product variability and cross-check analytical methods
- Publically available, certified material with historical characterization data representative of a large class of biotherapeutic
- Used to reconcile differences between orthogonal methods measuring same attribute
- Used in qualification or assessment of changing analytical test methods
- Used to assess performance of new analytical technologies

Typical release tests used for monoclonal antibody products.15 Also shown are mock specifications and data for five lots used in the clinical trials.

Table 1. Typical release tests used for monoclonal antibody products.[15] Also shown are mock specifications and data for five lots used in the clinical trials.

Test	Purpose	Specification	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
Protein concentration by A280 absorbance (mg/mL)	Quantity	50–60	55	54	55	55	56
Percent purity by high-performance size-exclusion chromatography (HP SEC)	Purity (size)	≥98.0	99.5	99.1	99.7	99.8	99.5
Ion-exchange (IEC) purity	Purity (charge)	≥95.0	98.0	97.5	98.5	100.0	98.9
Percent deamidation by percent IEC	Purity (charge)	≤5.0	2.0	2.2	1.5	1.0	1.1
Capillary zone electrophoresis (CZE)	Identity	Conforms to standard	Yes	Yes	Yes	Yes	Yes
Peptide mapping	Identity	Conforms to standard	Yes	Yes	Yes	Yes	Yes
Antigen binding assay or other appropriate	Potency	80–120%	90	95	92	101	105
Host cell proteins (ng/mg)	Impurities	≤100	10	2	5	2	5
Residual DNA (pg/mg)	Impurities	≤20	2	2	3	2	2
Endotoxin (EU/mg)	Impurities	≤0.1	0.01	0.01	0.02	0.01	0.02
pH	General	6–6.5	6.2	6.2	6.2	6.2	6.2
Volume (mL)	General	≥15	15	15	15	15	15
Appearance	General	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless

Illustration of an approach for setting specifications for product quality attributes



Steps taken toward establishing critical quality attributes, specifications, design space, and control strategy

Detection scoring takes into account method capability and control stringency

Detection Scoring		Control Stringency				
		Routine testing with reject limits i=1	Routine testing with action limits i=3	Routine monitoring i=5	Periodic testing i=7	Characterization i=9
Method Capability	Qualitative n=9	5	6	7	8	9
	Low precision, quantitative n=7	4	5	6	7	8
	Not orthogonal, non-specific, precise n=5	3	4	5	6	7
	Orthogonal, non-specific, precise n=3	2	3	4	5	6
	Specific, precise n=1	1	2	3	4	5

AMGEN

Courtesy Brent Kendrick, Amgen