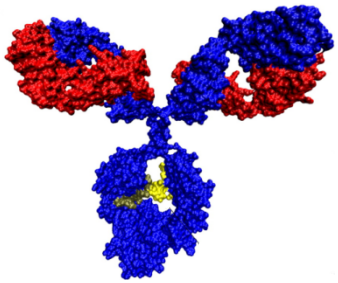
NIST Activities Supporting the Development and Manufacture of Biologic Drugs

November 3, 2014

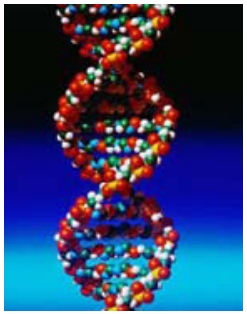
Innovations in Science, Technology, Engineering and Math

Dean C. Ripple
Leader, Bioprocess Measurements Group
Biomolecular Measurement Division
Material Measurement Laboratory

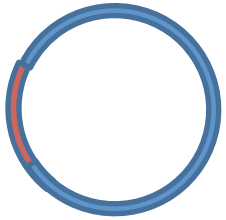
Background: Biotech Industry & Biologic Drugs



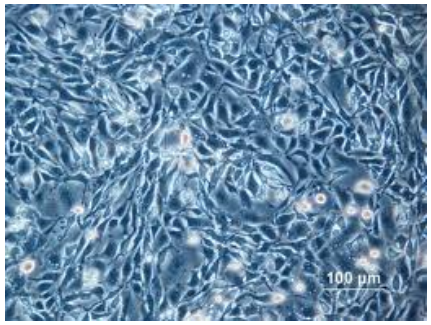
Protein therapeutic candidate



Gene of interest



Clone into expression vector



Transfer into host cell

Fermentation

Purification

Fill & formulation

Biologic drugs: US economic driver & health care cost issue

- Biologic drugs are now/future of pharma (~$50B, 180,000 jobs, US 2009)…
- But, fastest growing category of US health care costs: increased use & high cost

By 2016, 7 of the top 10 pharmaceuticals worldwide will be biologics[1]

Monoclonal Antibodies (mAbs) dominate biologics:

	Product	Type	2016 Rev. (USD bn)	2010 Rev. (USD bn)
mAbs	1. HUMIRA	Biologic	10.0	6.7
mAbs	2. AVASTIN	Biologic	7.7	6.2
mAbs	3. RITUXAN	Biologic	7.6	6.1
mAbs	4. ENBREL	Biologic	7.1	7.3
	5. CRESTOR	Small molecule	7.5	6.0
	6. SERETIDE/ADVAIR	Respiratory / device	6.7	7.9
mAbs	7. REMICADE	Biologic	6.2	6.5
mAbs	8. HERCEPTIN	Biologic	6.3	5.2
	9. REVLIMID	Small molecule	6.1	2.5
	10. LANTUS	Biologic	5.3	4.7

[1] Source: Evaluate Pharma, Sandoz analysis

SANDOZ

Used with Permission: Mark McCamish, Head Biopharmaceutical Development, Sandoz

Inadequate Measurement Infrastructure Is A Factor for High Cost of Biologic Drugs

Problem

- For regulatory approval need to demonstrate safety, efficacy, and consistent manufacturing process…
- But protein drugs cannot be completely defined by measurement
- Product safety & efficacy determined by clinical trials ($$$)

"API"

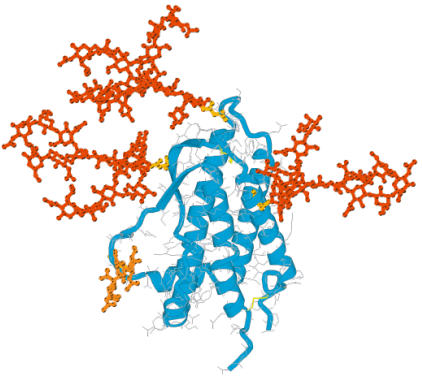
"Drug substance"

DNA specifies amino acid sequence, but not chemical modifications

Consequence: high cost

- Costly & inefficient manufacturing:
  - — Manufacturing changes require regulatory review
  - — Products & processes remain frozen
- Monopoly pricing: no US "biosimilars"
  - — Biologics Price Competition Innovation Act 2010
  - — First biosimilar application to FDA July, 2014

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, FDA, MedImmune, 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. NIST mission is to develop infrastructural technologies for an industry
3. Potential impact of NIST involvement
4. Can NIST respond with a timely, high quality product

NIST Biomanufacturing Program

1. **Protein Stability**

   - Tools/models for measuring/prediction of protein stability
   - Protein particle measurements → **Protein particle reference material**

2. **Protein Structure**

   - Primary structure: sequence of amino acids
   - Key modifications: sugars, i.e., glycans or oligosaccharides
   - Higher order structure: complex folding of protein drugs

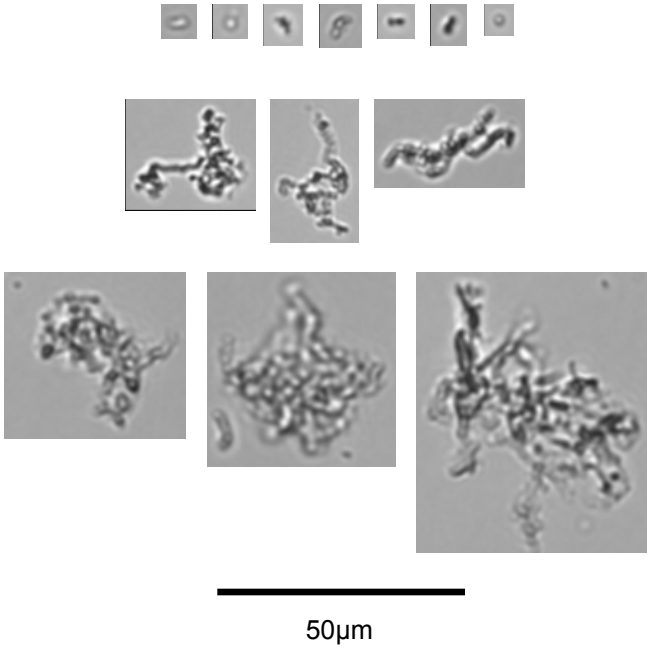
   } **NISTmAb**

3. **Understanding Production Cells**

   - Tools to enable improved understanding of production cells to reduce product variability

Protein Particulates in Biotherapeutics
PI: Dean Ripple

- Proteins in solution partially denature and subsequently agglomerate
- Highly hydrated (≈ 95% water)
- Evidence of immunogenic properties
- Particulate size from 10s of nm to 100 µm



Current state-of-the-art

- Differing optical methods disagree by 10X
- No means of standardizing instruments for response to protein particulates

Limitations of existing standards:

- No particles of similar morphology or shape
- No particles with low optical contrast
- Existing standards have high density

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

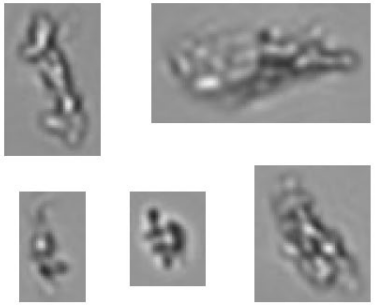
Activities:

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

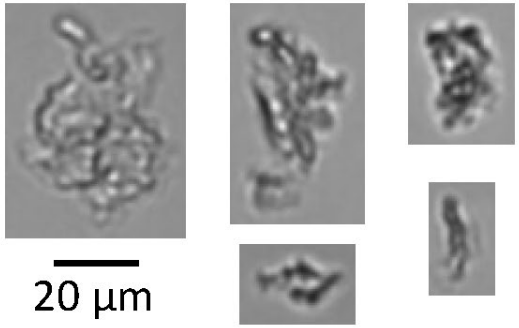
Candidate Particle Reference Material: Abraded Fluoropolymer

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

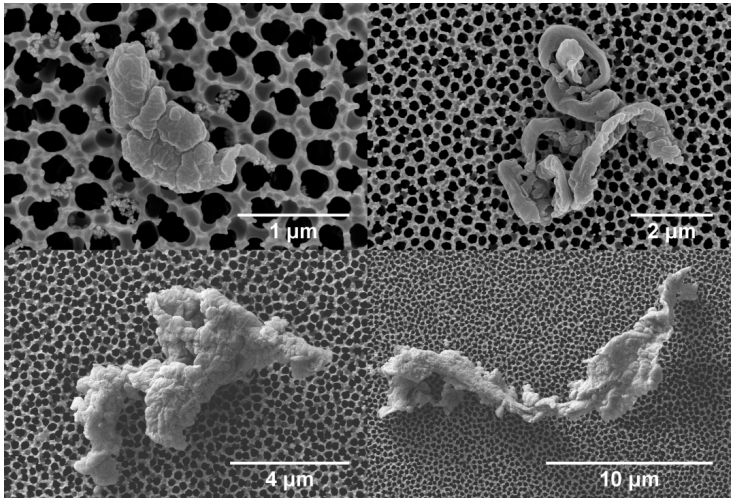
1. Rugged, with refractive index of 1.40—close to that of protein
2. Appears like protein with mechanical abrasion process—oscillatory motion pulls off irregular, tangled particles
3. Producing polydisperse suspension as reference material, 1 to 25 µm



Protein particles



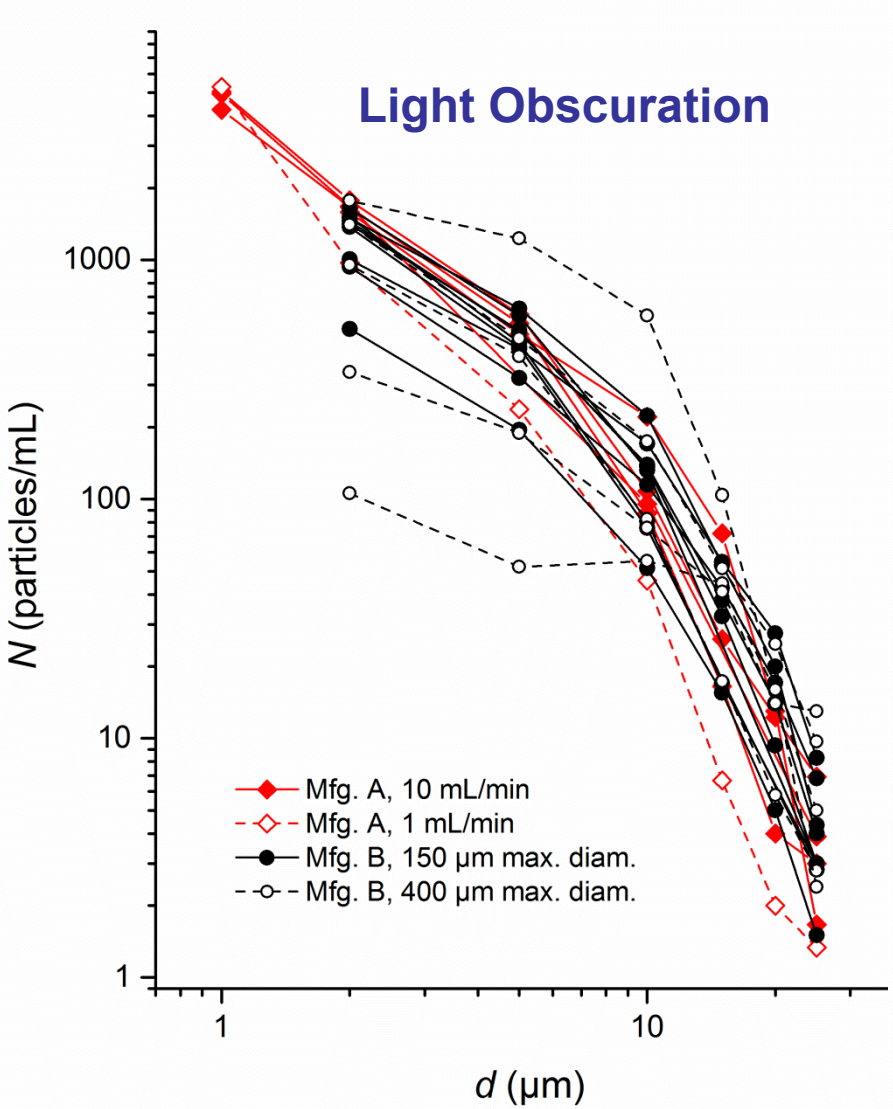
ETFE particles

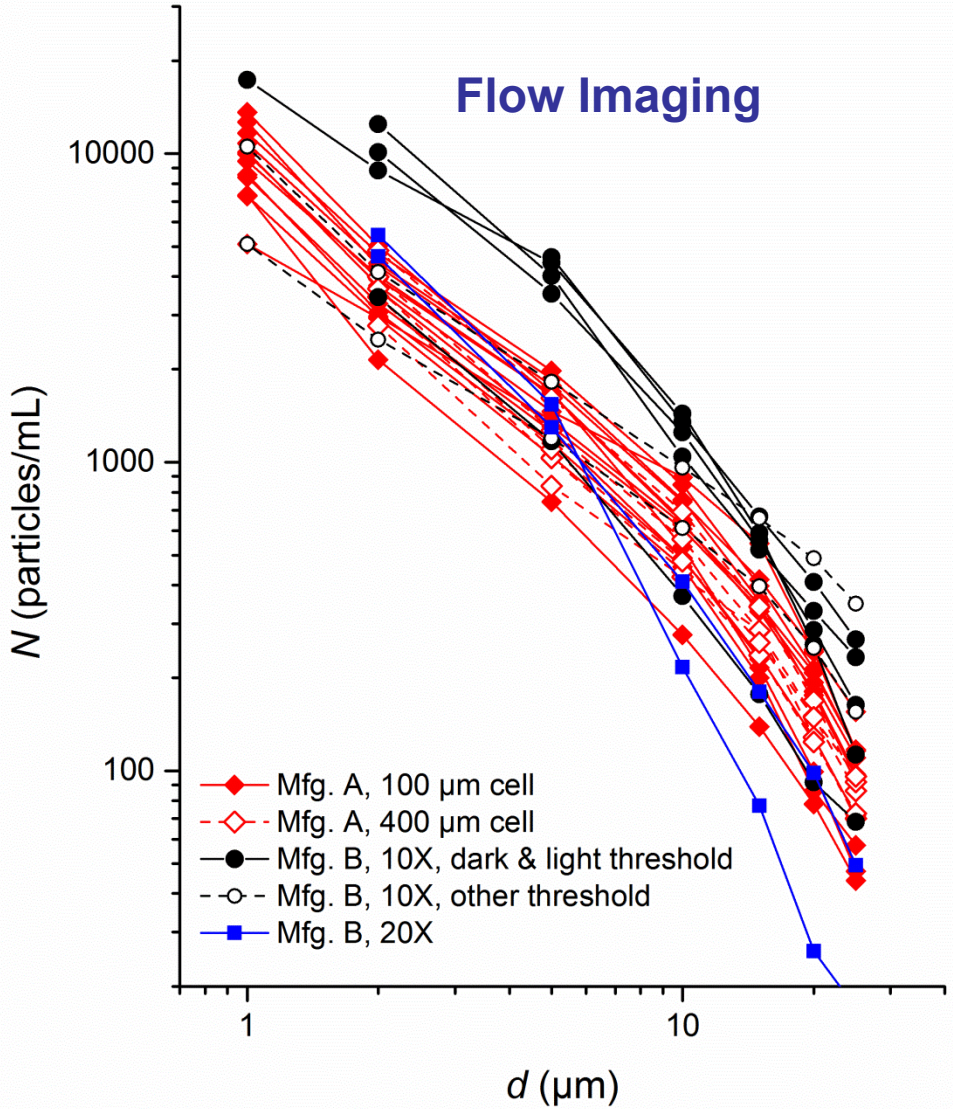


SEM images of ETFE particles on alumina filters

Interlaboratory Comparison of ETFE Particles

- 24 participants: biopharma, instrument vendors, academia, FDA
- Diameter range 1 to 25 µm



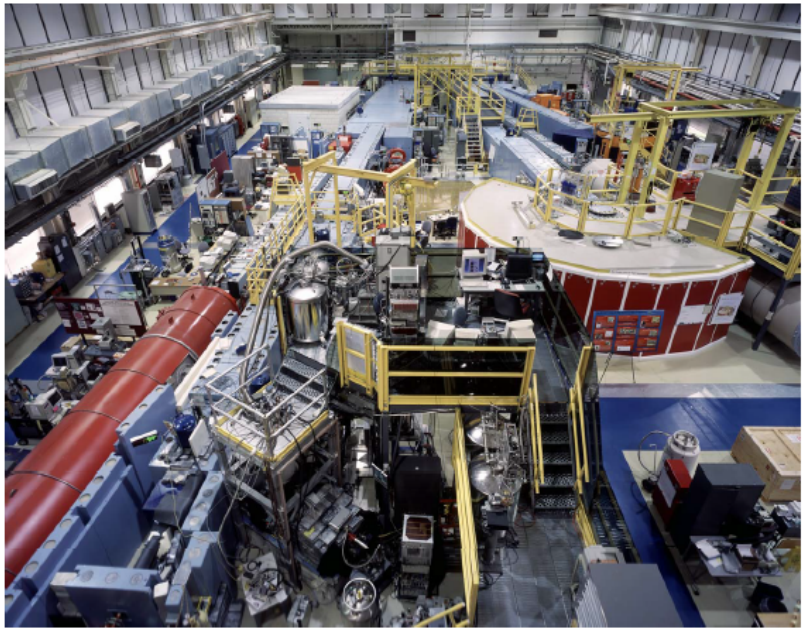


Neutron Measurements of Protein Therapeutics

- Ability to measure energy and momentum transfer:
  - geometry & dynamics of motion
  - length scale of structures (10 nm - μms)
- Neutrons scatter by nuclear interactions that are well understood: easier interpretation and modeling of scattering data
- Can analyze concentrated solutions, lyophilized, or frozen products
- nSoft: New NIST led industrial consortium to enable access to neutron facilities and expertise (Members: MedImmune, Genentech, Amgen), nSoft Director: Ronald Jones

nSoft
A consortium for the advancement of neutron-based measurements for manufacturing of soft materials.

The NIST Center for Neutron Research



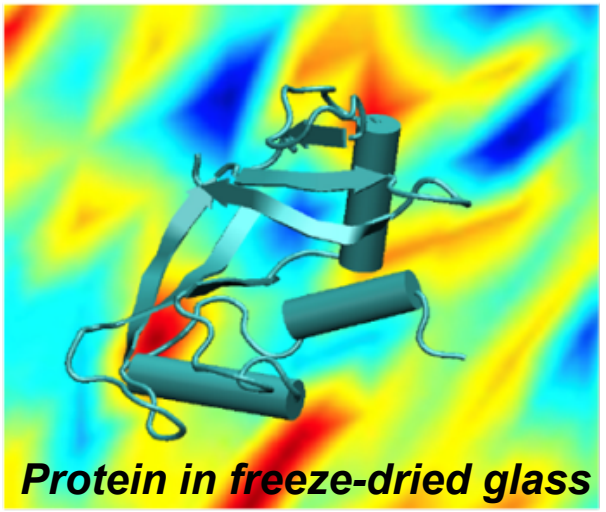
NIST Guide Hall

Neutron Measurements for Predicting & Understanding Protein Stability

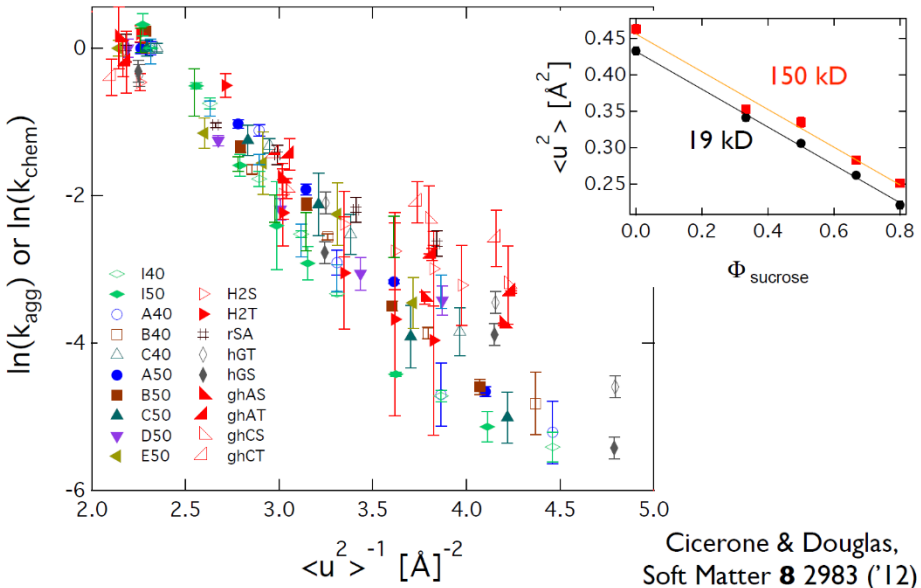
PI: Marcus Cicerone

Protein Stability During Storage

- 1/3 of therapeutic proteins are freeze-dried, but formulation for freeze-drying is empirical with 60% success rate
- Neutron scattering discovers new metric, fast β relaxation, correlating with long-term protein stability
- Bench-top optical method developed at NIST to measure β relaxation



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

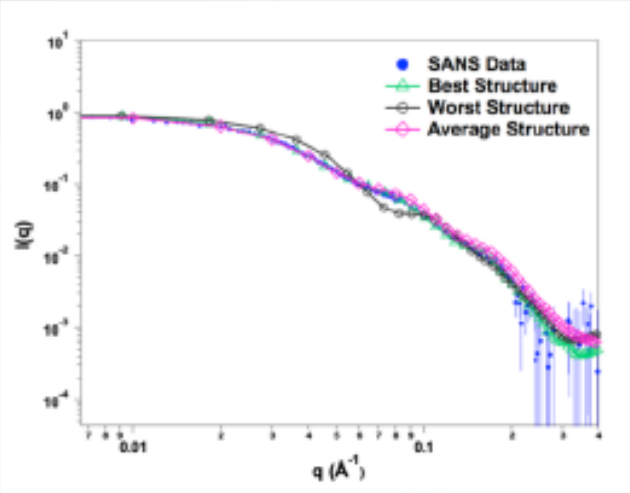


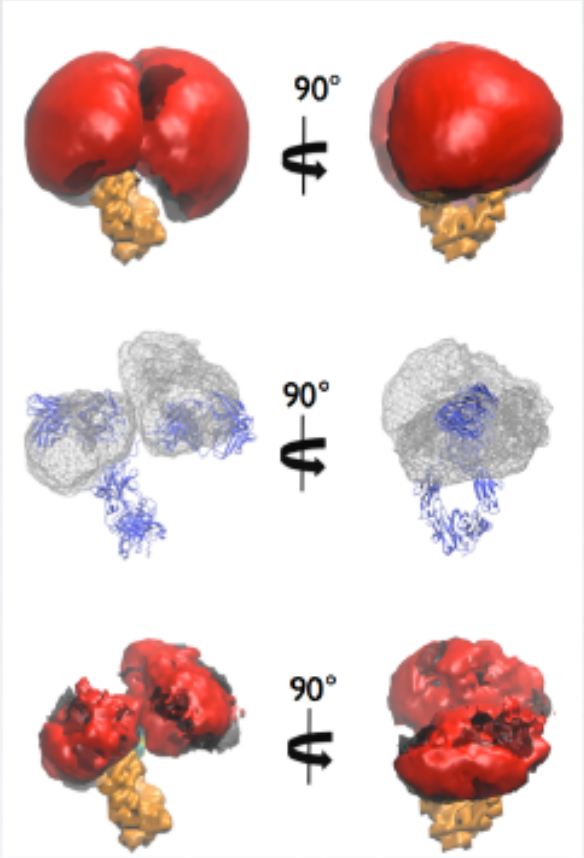
Cicerone & Douglas, Soft Matter **8** 2983 ('12)

MedImmune · Lilly · AMGEN · NIBIB · University of Connecticut · Pfizer · MERCK Be well · Wisconsin

ANTIBODY STRUCTURE AND INTERACTIONS

low concentration

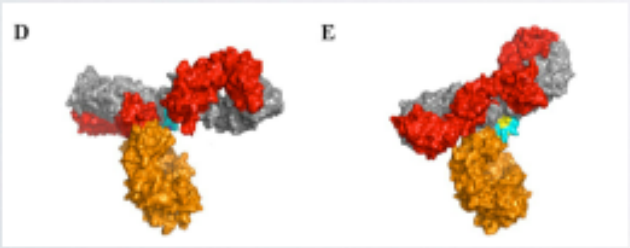




ensemble modeling:

SANS
0.5 to 5 mg/ml
< 1 hour / sample

Free-energy analysis



-16400 kcal/mol -16800 kcal/mol

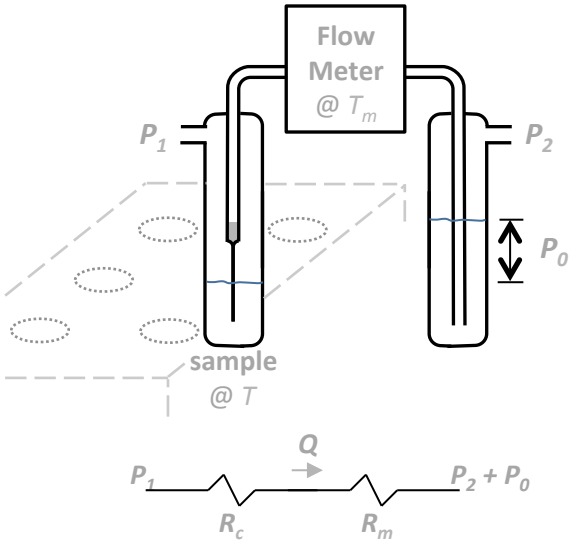
Future:
-Other mAbs
-Excipients
-Aggregate structure
-Structure/viscosity

AMGEN

PI: Joseph Curtis, NCNR

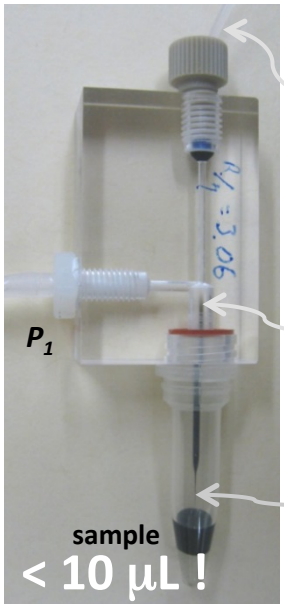
J. Phys. Chem. B 117 (2013) 14029

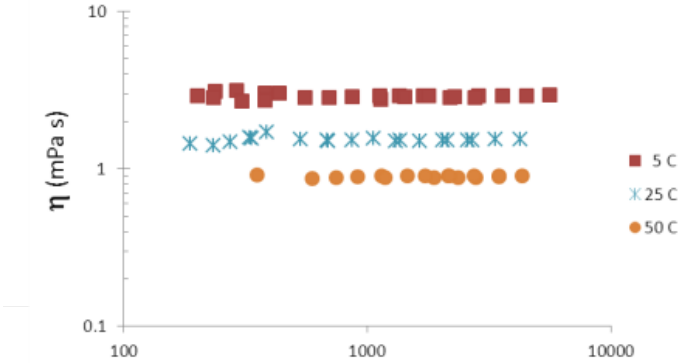
PROTEIN RHEOLOGY: Microcapillary Viscometer

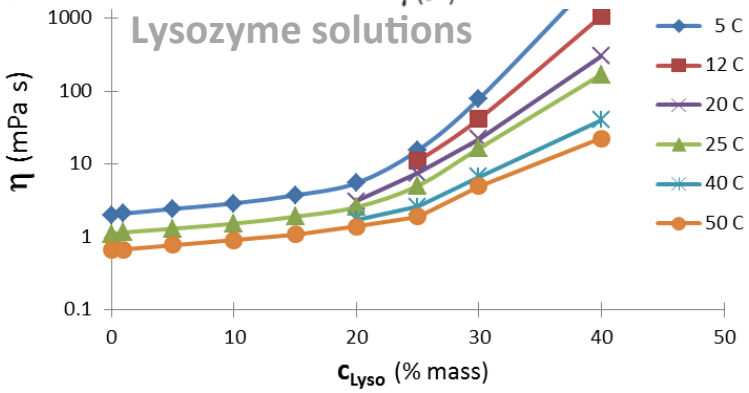


Importance of viscosity measurement for protein drugs

- **mAbs formulated at high concentration can be too viscous for processing/delivery by syringe**
- **High throughput viscometry methods needed for formulation development**
- **NIST microviscometer: potential high throughput, low sample volume (< 10 µL), broad range of shear and temperature**





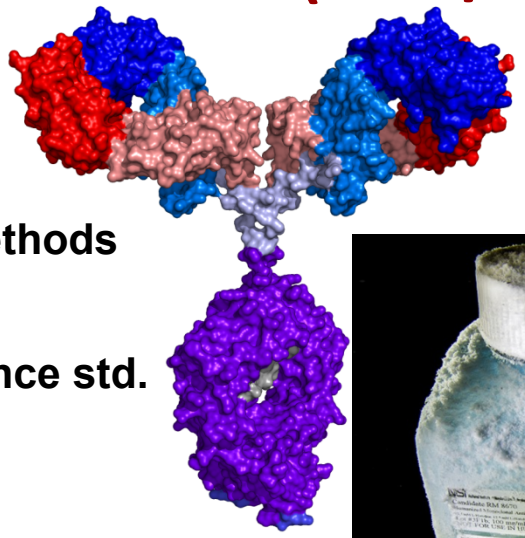


MedImmune

steven.hudson@nist.gov et al.
Polymers & Complex Fluids Group

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

PI: John Schiel, Trina Formolo



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

- System suitability material or cross-checking test methods
- Testing new measurement technologies
- Will not replace reference product or in-house reference std.

NIST mAb attributes:

- Humanized mAb (IgG1κ) expressed in murine culture
- Frozen bulk "Drug-like substance"

"Crowd-Sourcing" approach for IgG characterization:

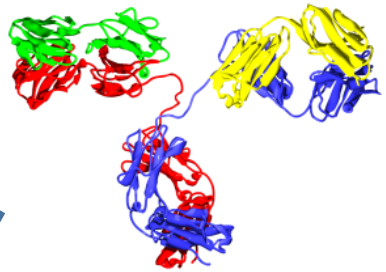
- Complete extensive interlaboratory characterization
- 65+ Biopharma, Instrument, Academic, FDA participants
  - Results used for ACS book "State-of-the-Art and Emerging Technologies for the Analysis of Monoclonal Antibodies" (published mid-2015)
- SRM: NIST will certify concentration traceable to the kg
- D: Compile reference data (MS library), methods, etc.
  - Publically available: http://igg.nist.gov/

MS Characterization of NISTmAb

Enzymatic Fragmentation – LC Separation – ES – MS/MS Identification

IgG Fragmentation:

- Break 'hinge'
- Break S-S
- Stabilize S

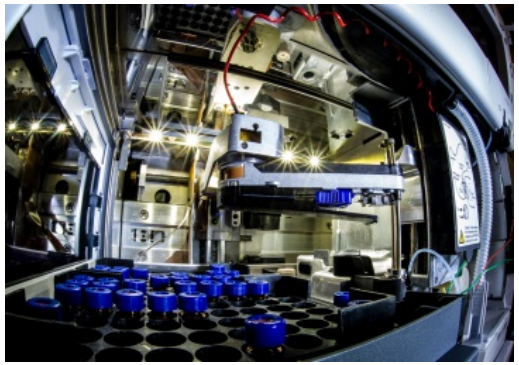


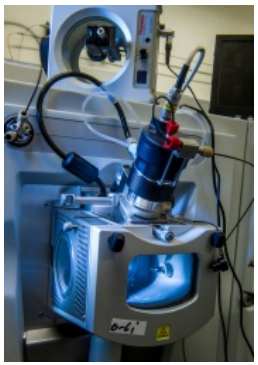
Proteolysis (multiple enzymes)

Deglyco-sylate

Big Fragments | Glycans | Peptides, Glycopeptides

Separate by LC, identify by MS/MS



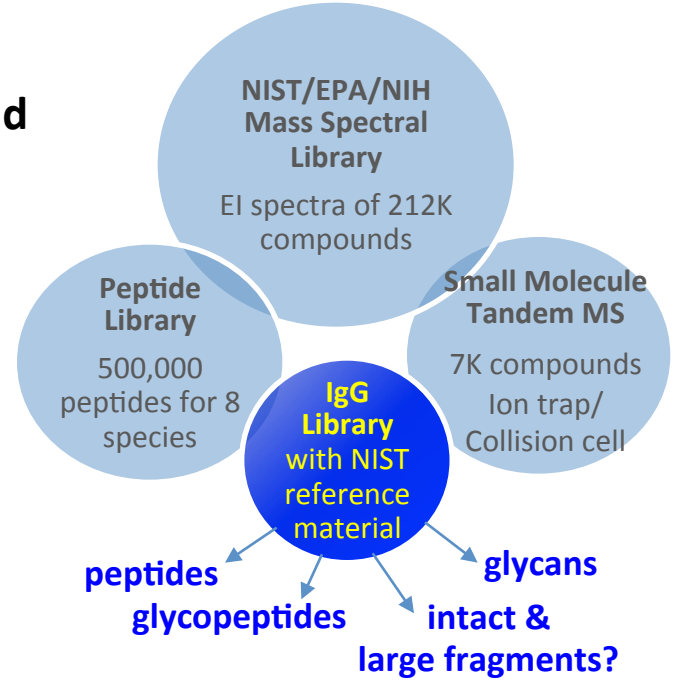


Photos courtesy of Matt DeLorme

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

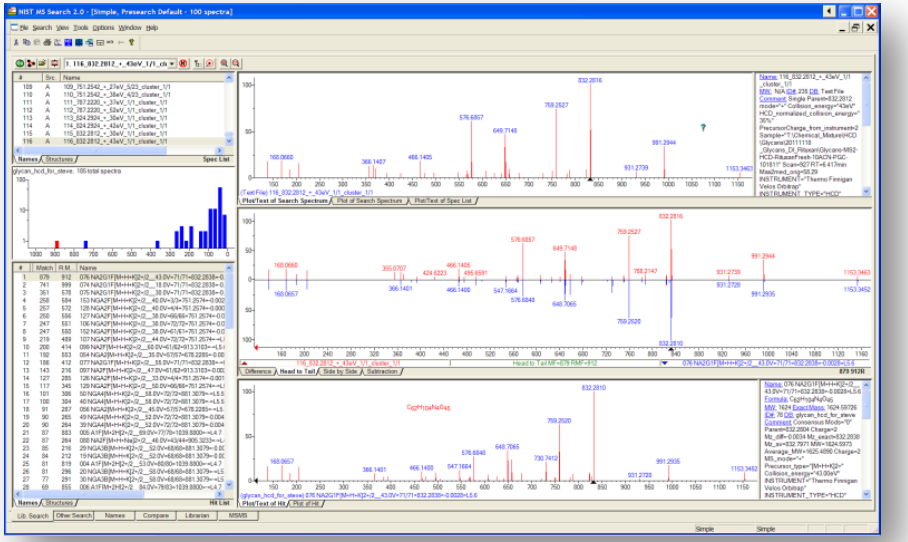
PI: Steve Stein

- NIST MS reference libraries most widely used in world: sold with over 5,000 instruments/year
- Gold standard for chem/biochem identification
- Quality control metrics for screening of all spectra
- Library includes software tools for spectral searching, matching, scoring of match, and library building



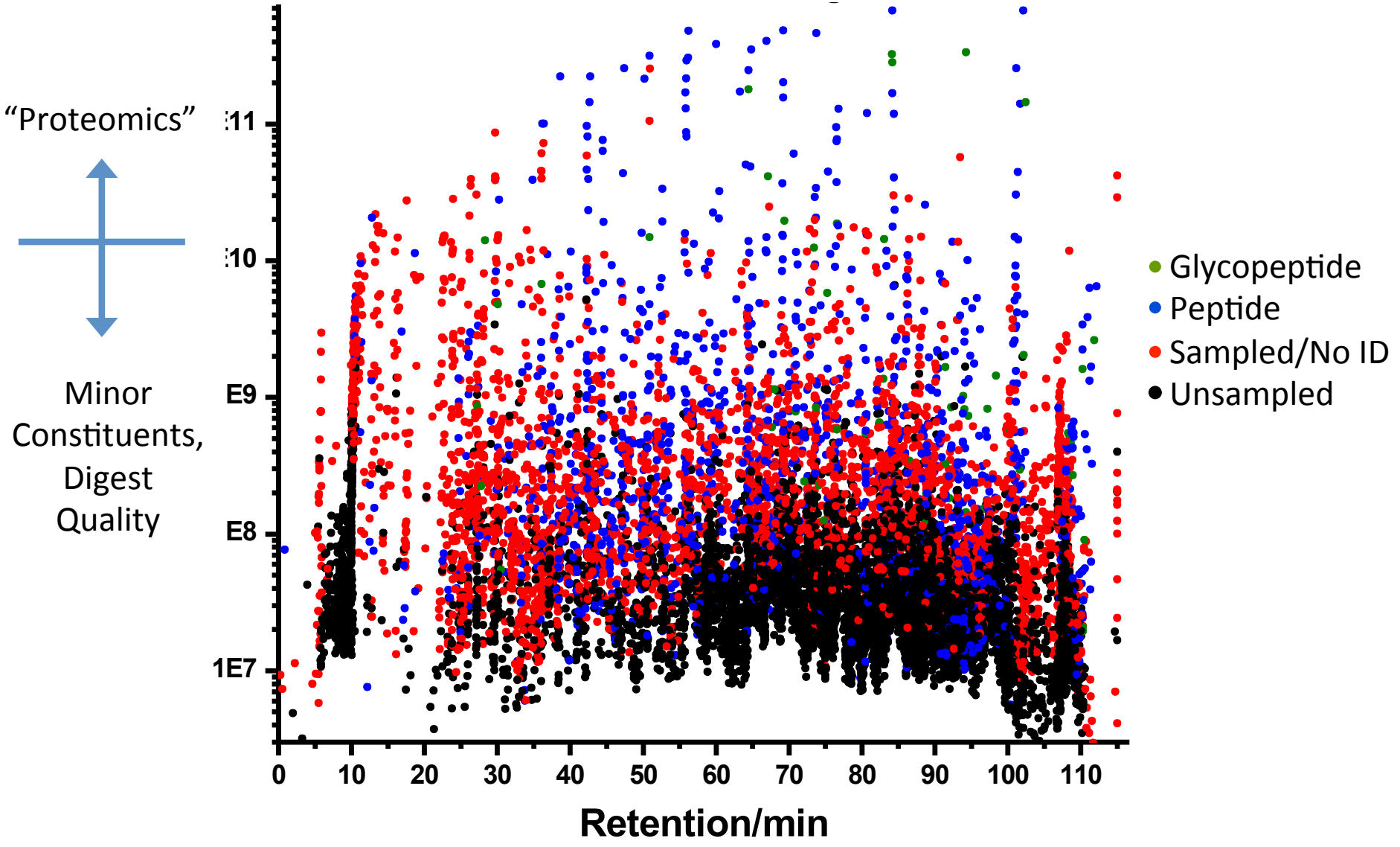
Developing IgG Library

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



NA2G1F from Rituximab Matches Library

Tryptic Digest of NISTmAb
LC-MS1, Ion Trap



Other Interlab Comparisons Using NIST mAb

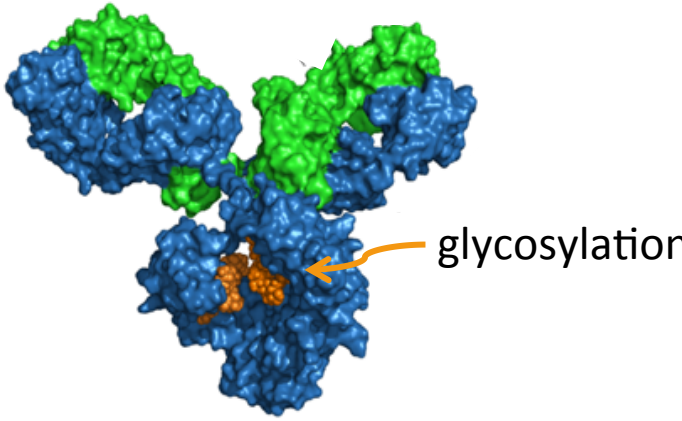
Hydrogen/Deuterium Exchange—Mass Spectrometry (HDX-MS) PI: Jeff Hudgens)

- HDX-MS measures water accessibility of peptide backbone
- 22 participants, 8 biopharma
- Oct. 2014, HDX-MS containing
- Fall 2015: NIST will collect data and publish evaluation of interlab study

NIST HDX-MS Interlaboratory Comparison Kit

IgG Glycosylation Intercomparison (PI: M. Lorna de Leoz)

- 108 participants, > 50 industrial
- Identify N-glycans in 2 samples & determine differences in their distribution (begin data phasemid-2015)
- NIST will collect data and publish results of interlab study

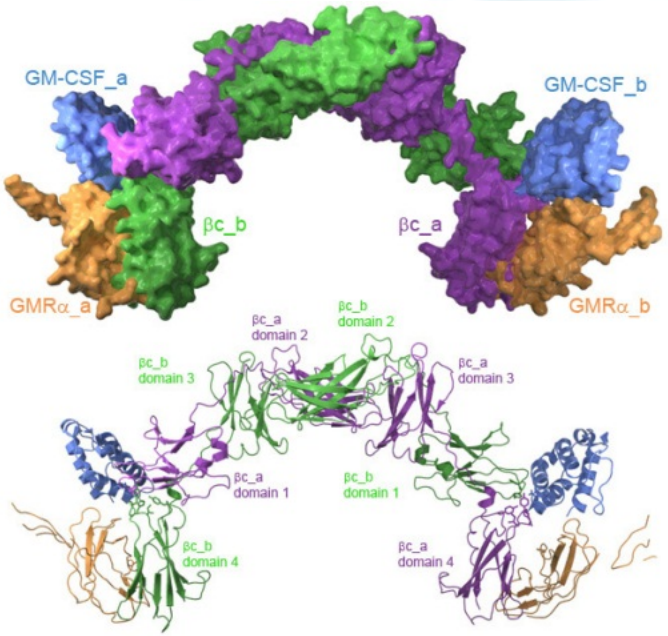


Higher-Order Structure is a Distinguishing Feature of Protein Therapeutics

Structure → Function

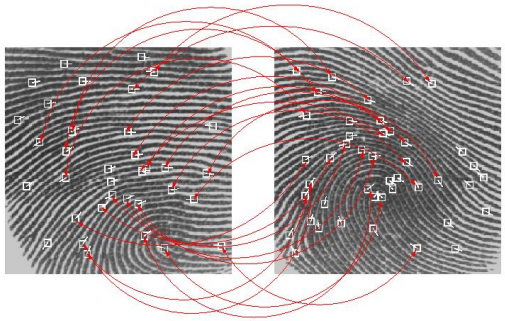
"Our current ability to **predict the potency of biologics** would be enhanced if we had improved ability **to measure and quantify** the correct (**major**) three-dimensional structure, aberrant three dimensional structures (**misfolding**), and the **distribution** of different three-dimensional structures".

Steven Kozlowski, M.D. CDER, FDA (Congressional Testimony, 2009)



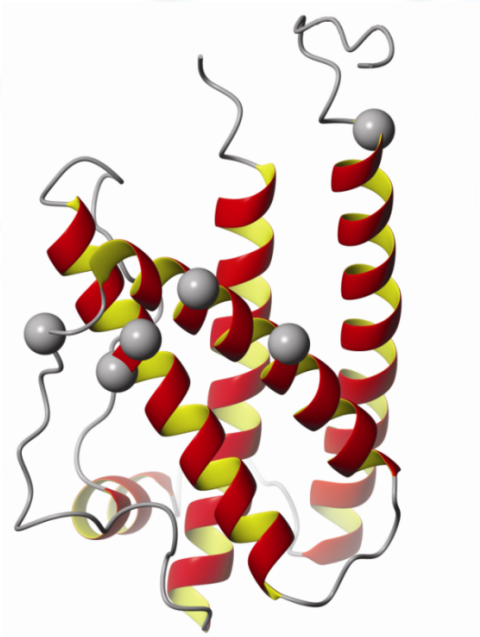
Receptor Bound GM-CSF

NMR can assess higher order structure of protein therapeutics at atomic resolution



Inter-laboratory Comparison: Harmonization and Validation of High-Resolution NMR as a Metric for Structure

PIs: John Marino and Rob Brinson



FDA

LÄKEMEDELSVERKET
MEDICAL PRODUCTS AGENCY

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

Health Canada Santé Canada

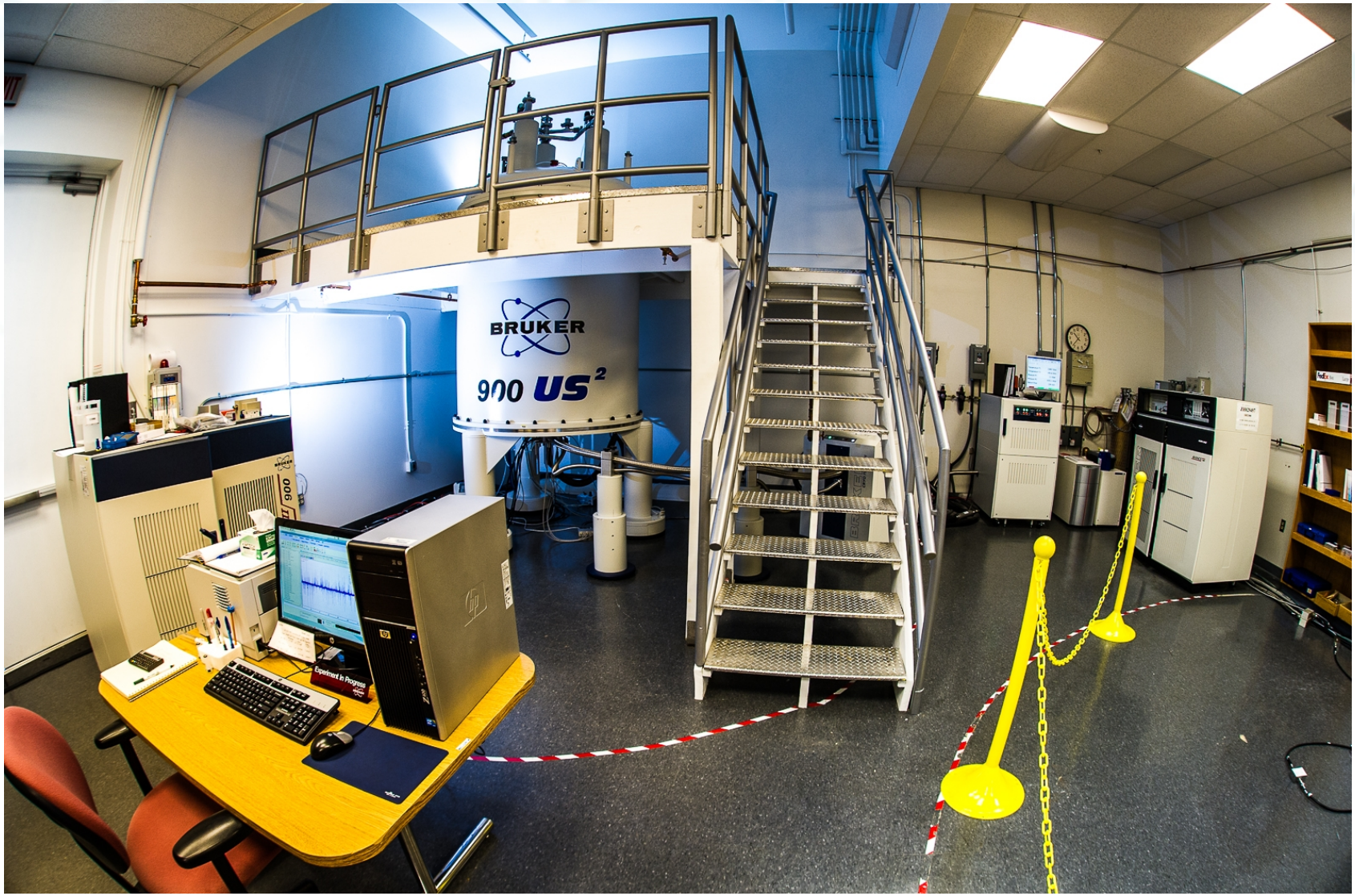
^{15}N-Labelled Filgrastim(^{15}N-GCSF)

Met-G-CSF (19 kDa) – used in cancer patents with neutropenia.

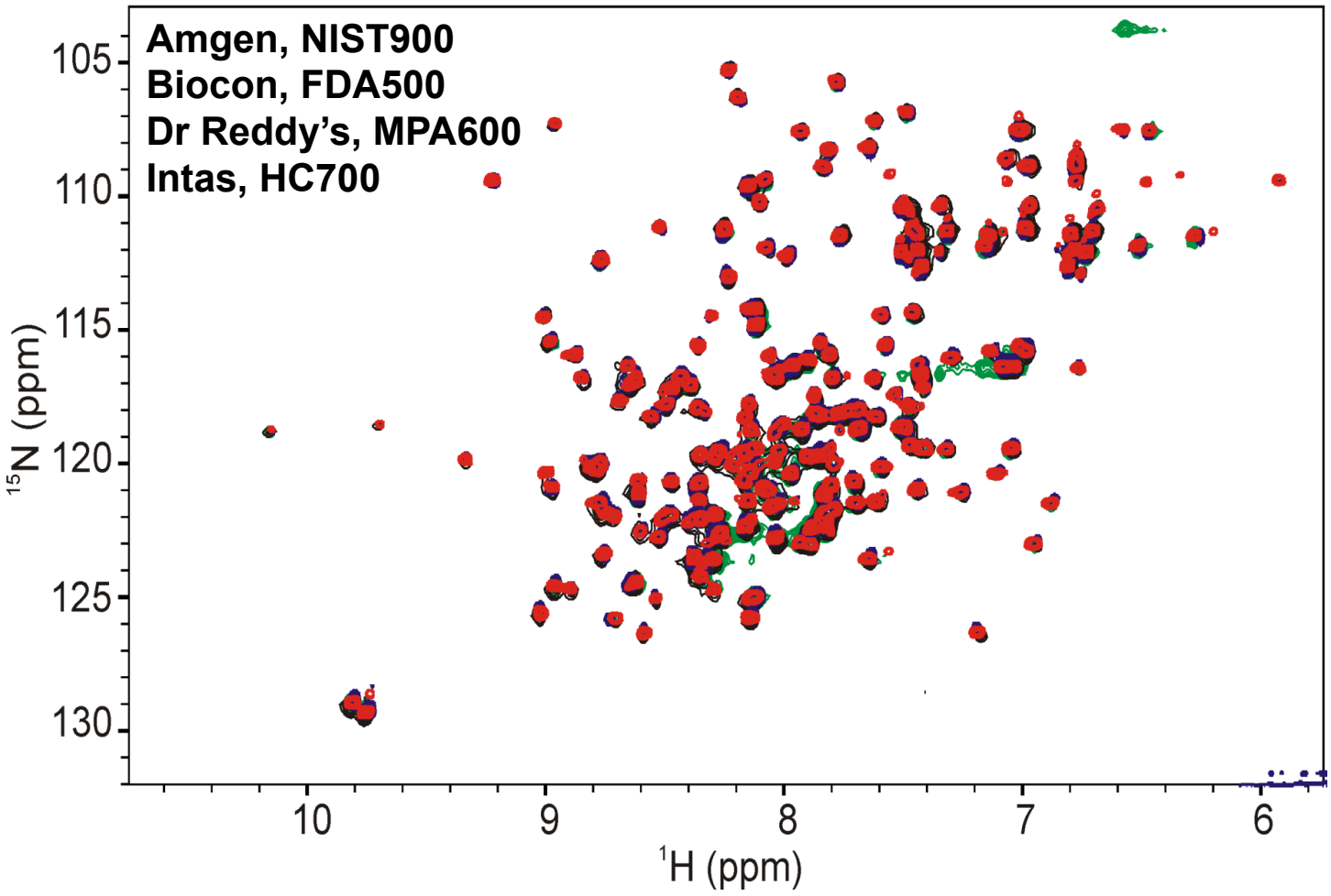
Comparability of NMR spectral 'finger prints' assessed using standardized NMR experiments & ^{15}N-Labelled Filgrastim sample

- **4 Sites in North America and Europe**
  FDA; Health-Canada; MPA-Sweden; NIST
- **4 Fields**
  500, 600, 700 and 900 MHz
- **Different Instrument vintages**
- **2 Vendors**
  Bruker Biospin, Varian/Agilent

900 MHz NMR NMR at IBBR



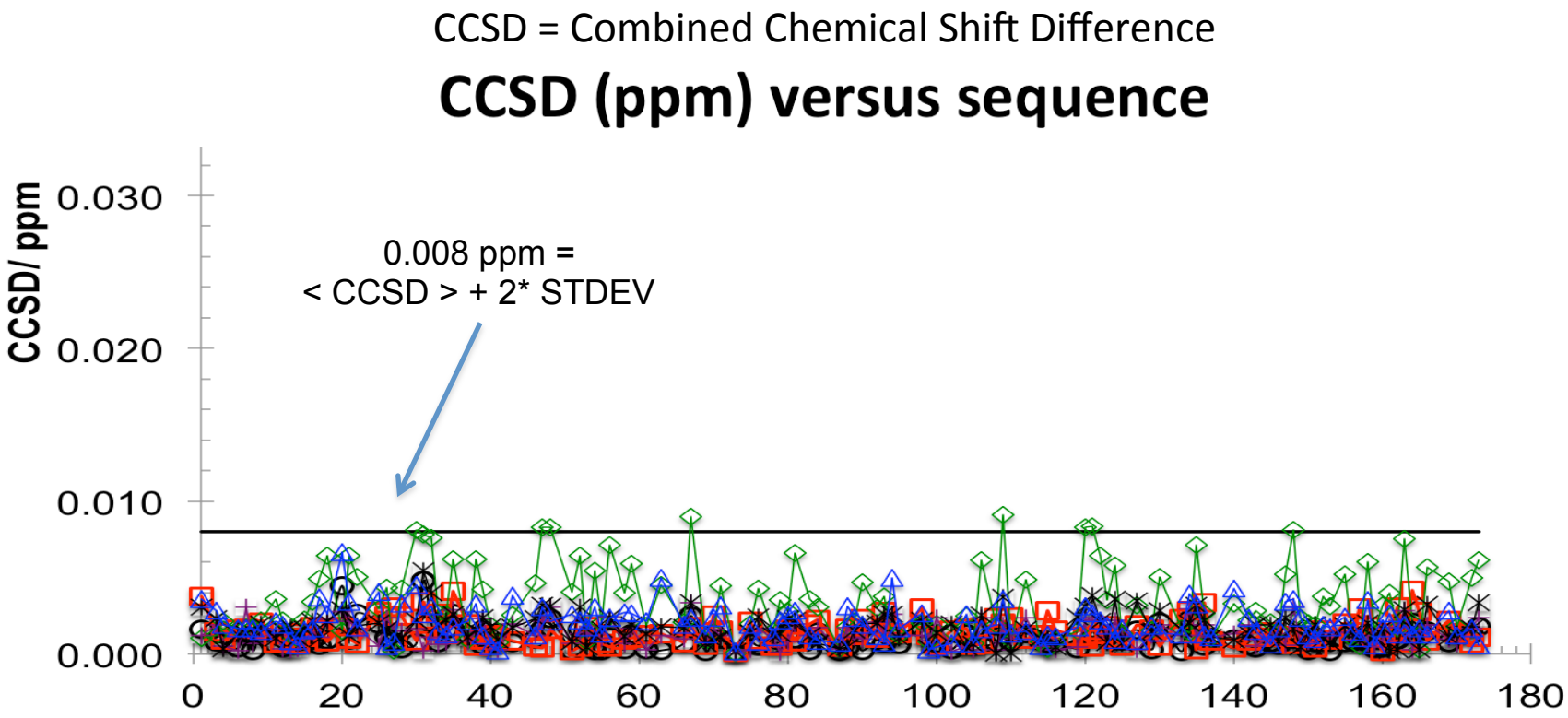
Comparison of 4 Filgrastim Products: ^{1}H-^{15}N HSQC NMR Spectra at 4 sites



Nearly identical 'finger print' map between the 4 samples/instruments/magnetic fields using comparable acquisition and processing parameters

CCSD Analysis for Comparability: Measurement Variation Observed for the ^{15}N-GCSF 'System Suitability' Sample

CCSD = Combined Chemical Shift Difference



Precision across labs is comparable to measurement precision of single instrument!

Conclusions

- NIST Biomanufacturing Program is developing improved measurement science, standards, and technologies to support development of protein drugs

- NIST develops fundamental measurement science for:
  - Protein particle measurements (instrument response functions)
  - Prediction of protein stability (neutron methods)
  - Structural measurements (neutrons methods and NMR)

- NIST monoclonal antibody reference material will find use in:
  - Assessing test methods and new technologies
  - As a publicly available source of historical data of a well-characterized mAb

- NIST facilitates cross-industry collaboration by sharing well-characterized material for measurement intercomparisons

Acknowledgements

- NIST
  - Marc Cicerone (Protein stability, neutrons)
  - John Schiel (NIST mAb)
  - Joseph Curtis (SANS of IgGs)
  - Ronald Jones (nSoft)
  - Steven Hudson (Rheology, viscometry)
  - Steve Stein (MS Library)
  - Dean Ripple (Protein particles)
  - Richard Cavicchi (Protein particles)
  - John Marino, Rob Brinson (NMR)
  - Jeff Hudgens (HDX-MS)
  - Lorna de Leoz (Glycosylation)
  - Trina Formolo (NIST mAb)
  - Lisa Kilpatrick (NIST mAb)
  - Meiyao Wang (NIST mAb)
  - Karen Phinney (NIST mAb)
- ACS Book Co-Editors
  - Darryl Davis, Janssen
  - Oleg Borisov, Novavax
  - Other participants

Collaborators/Stakeholders

MedImmune
Agilent Technologies
Waters
THE SCIENCE OF WHAT'S POSSIBLE
Janssen
AMGEN
BRUKER
Thermo SCIENTIFIC
NOVAVAX
Creating Tomorrow's Vaccines
FDA
IBBR
INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH
UNIVERSITY OF BIRMINGHAM
Genentech
A Member of the Roche Group
biogen idec
NATIONAL INSTITUTES OF HEALTH
nibrt
National Institute for Bioprocessing Research and Training
Pfizer Wyeth
Lilly
MERCK