NIST Activities Supporting the Development and Manufacture of Biologic Drugs

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Innovations in Science, Technology, Engineering and Math

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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 biologics¹

Monoclonal Antibodies (mAbs) dominate biologics:

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

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¹ Source: Evaluate Pharma, Sandoz analysis
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 ($$$)

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
FDA  Genentech  biogen idec
MedImmune  Bristol-Myers Squibb
Pfizer  Lilly  MERCK  gsk  SANDOZ

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
1. **Protein Stability**
   - Tools/models for measuring/prediction of protein stability
   - Protein particle measurements

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

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

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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 - µm)
- 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

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 $\beta$ relaxation, correlating with long-term protein stability
• Bench-top optical method developed at NIST to measure $\beta$ relaxation

Degradation Tracks $<u^2>^{-1}$

![Protein in freeze-dried glass](image)
ANTIBODY STRUCTURE AND INTERACTIONS

low concentration

Free-energy analysis

ensemble modeling:

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

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

PI: Joseph Curtis, NCNR
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

![Diagram of microviscometer](image)

**Lysozyme solutions**

- 5 C
- 12 C
- 20 C
- 25 C
- 40 C
- 50 C

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**Steven Hudson, NIST**

et al.

Polymers & Complex Fluids Group

Biopharmaceutical Measurement Roundtable, 1/27/14
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)

Big Fragments → Deglycosylate → Separate by LC, identify by MS/MS

Glycans

Peptides, Glycopeptides
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

- NIST/EPA/NIH Mass Spectral Library
  - EI spectra of 212K compounds

- Peptide Library
  - 500,000 peptides for 8 species

- Small Molecule Tandem MS
  - 7K compounds Ion trap/ Collision cell

- IgG Library with NIST reference material
  - peptides glycopes glycopeptides

- NA2G1F from Rituximab Matches Library

peptides intact & large fragments?
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

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 phase mid-2015)
- NIST will collect data and publish results of interlab study
Higher-Order Structure is a Distinguishing Feature of Protein Therapeutics

Structure \rightarrow 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)

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

Pls: John Marino and Rob Brinson

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

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

Met-G-CSF (19 kDa) – used in cancer patents with neutropenia.
900 MHz NMR NMR at IBBR
Comparison of 4 Filgrastim Products: $^1$H-$^{15}$N HSQC NMR Spectra at 4 sites

Amgen, NIST900
Biocon, FDA500
Dr Reddy’s, MPA600
Intas, HC700

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

CCSD (ppm) versus sequence

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
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