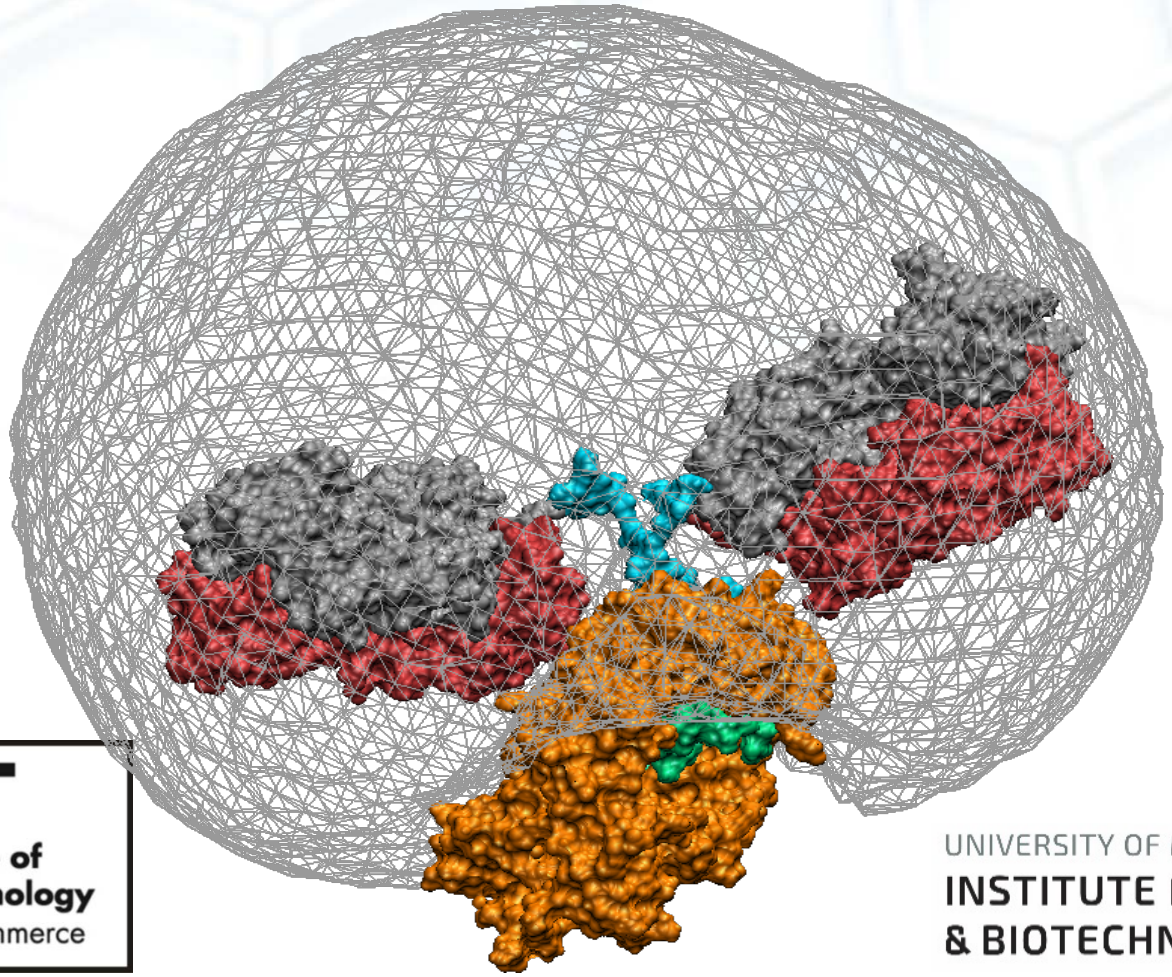
Innovative Analytical Technologies and Biopharmaceutical Reference Materials

John Schiel, John Marino, Zvi Kelman, Jeff Hudgens, Robert Brinson, Luke Arbogast, Frank Delaglio, Trina Mouchahoir, Thomas Cleveland, Kyle Anderson, Michael Tarlov



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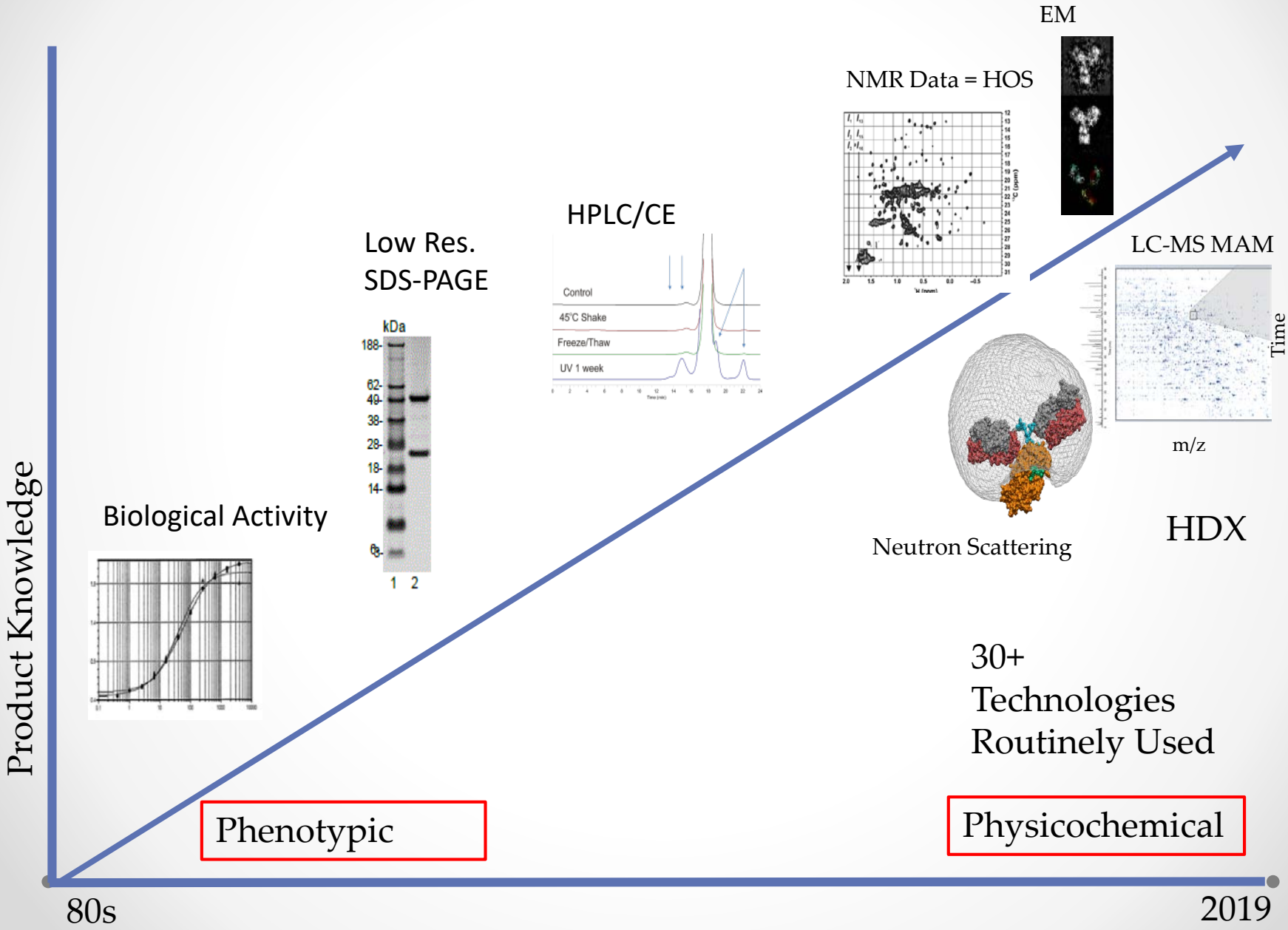
Evolution of mAb Analytical Methods



The toolbox is getting full

- Trend toward
  - Attribute-specific analytics (e.g MAM, CZE/cIEF-MS)
  - Higher-order structure with high resolution (NMR, HDX, etc.)
  - Conformational Ensembles (EM, Neutron and X-ray scattering, etc.)
- Replacement vs. addition = lower cost and speed
- Requirements for successful lifecycle appropriate implementation
  - Deep fundamental knowledge of analytical figures of merit
    - Nuances of validation, information content
  - Data analytics to completely harness information content of complex methods
    - Visual conformance to expectation can be a thing of the past
  - Ability to critically evaluate method suitability/fitness for purpose
    - A very good analytical technique can be performed sub-optimally

Pre-competitive Reference Materials valuable to define state-of-the art and support evolution of Analytics and Control

National Institute of Standards and Technology

- Non-regulatory agency within U.S. Department of Commerce
- Founded in 1901 as National Bureau of Standards
- NIST responsible for US physical standards, test methods, & calibrations

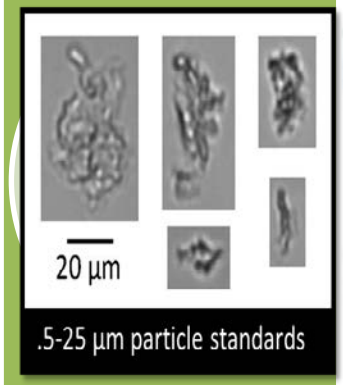
Unique Mission within the Federal Government

to promote innovation and industrial competitiveness by advancing

measurement science, standards, and technology

in ways that enhance economic security and improve our quality of life

Example of NIST Reference Materials



Surrogate Protein Particle

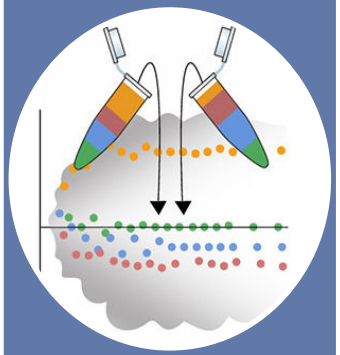
SRM 1989

NIST Monoclonal Antibody

RM 8671

Genomic DNA Standard Standards for HER2 Measurements Reference Material

SRM® 2373



DNA Sequence Library for External RNA Controls

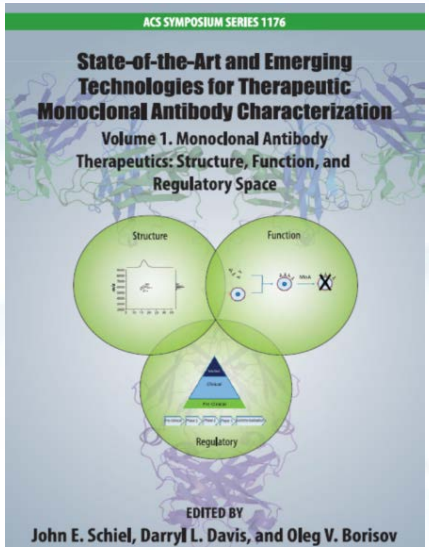
SRM 2374

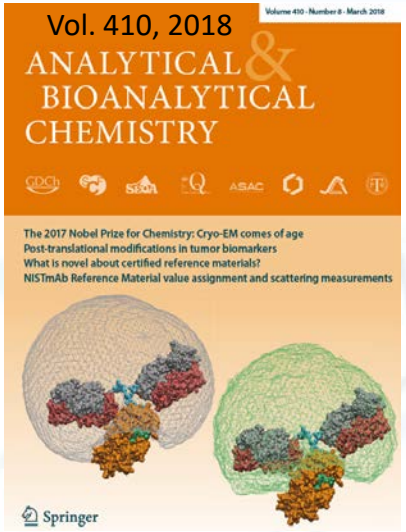
GIAB: Human DNA for Whole-Genome Variant Assessment

RM 8398
RM 8391
RM 8392
RM 8393
RM 8375

Measurement Assurance

NISTmAb RM 8671





NISTmAb Attributes:

- Publicly available "biopharmaceutical grade"
- Open Innovation Humanized mAb (IgG1κ)
  - Voluntary, pre-competitive, product neutral standard
  - Exhaustively characterized for physicochemical and biophysical attributes
  - Reference Values, stability, homogeneity assured through lifecycle plan

Provides an industry-wide shared resource

- Novel technology development and de-risking
- Industry wide collaborative studies to
  - Address shared challenges
  - Advance analytical capabilities
  - Evaluate "regulatory readiness" and appropriate implementation points

- LC-MS Multi-Attribute Method
- HOS: NMR, HDX, XRD
- EM
- Neutron scattering
- Intact, middle down MS
- Glycosylation Analysis
- LC: SEC, RP, IEX, HIC
- CE: cIEF, cSDS, CZE
- SDS-PAGE
- MS/MS library compilation
- Biophysical: CD, FTIR, DSC, DLS, AUC, SLS, DSF
- Protein particulates

NIST Inter-laboratory studies and analytical R&D have targeted emerging analytics expected to have broader impact in the coming 5-10 years

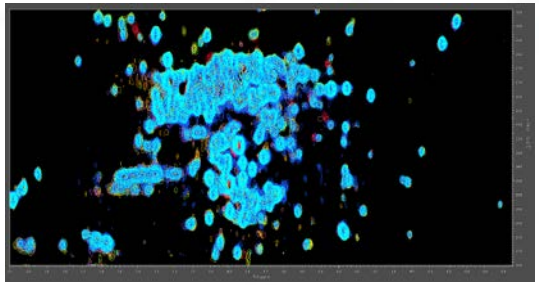
http://pubs.acs.org/isbn/9780841230262, http://pubs.acs.org/isbn/9780841230293 , http://pubs.acs.org/isbn/9780841230316

Interlaboratory Measurement Comparisons Using NISTmAb

- Assess variability between labs and analytical technologies
- Identifies potential technology gaps
- Informs development of potential NIST reference materials
- Fosters collaboration across global biopharma community

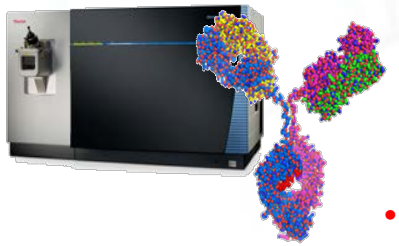
2D-NMR of NISTmAb Fab

- 30 participants, ~ 11 industrial
- Rob Brinson



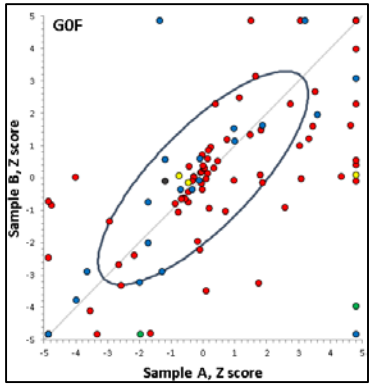
Multi-Attribute Method Consortium

- 30 industrial participants
- submitted



Glycoanalysis of NISTmAb

- 108 participants, ~ 50 industrial
- Lorna DeLeoz

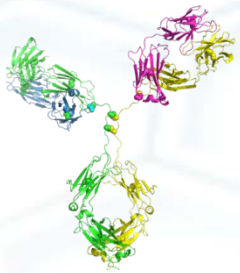


HDX-MS of NISTmAb Fab

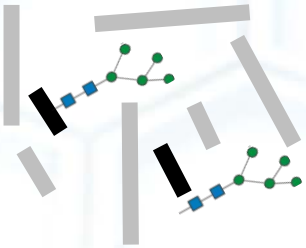
- 22 participants, ~ 8 industrial
- Jeffrey Hudgens

Multi-Attribute Method

- Enables attribute-specific monitoring
- Enables comprehensive detection of process/product impurities



Digestion

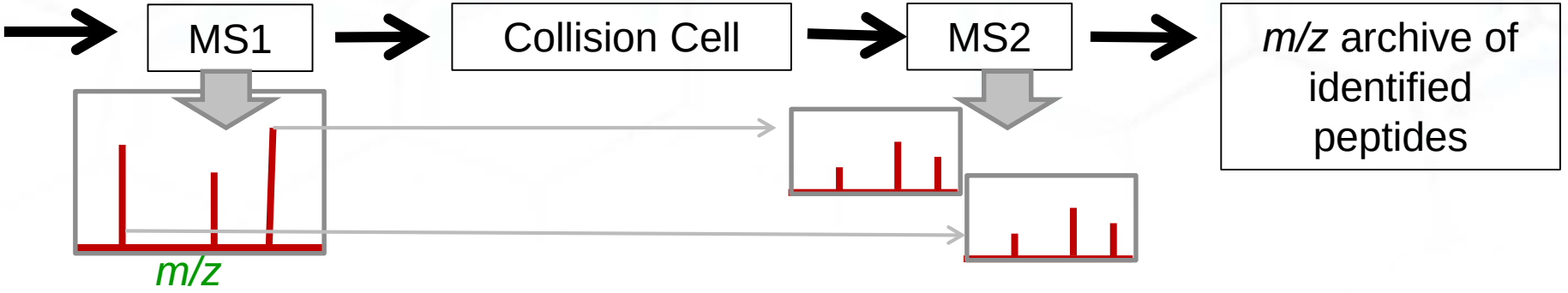


LC-MS/MS

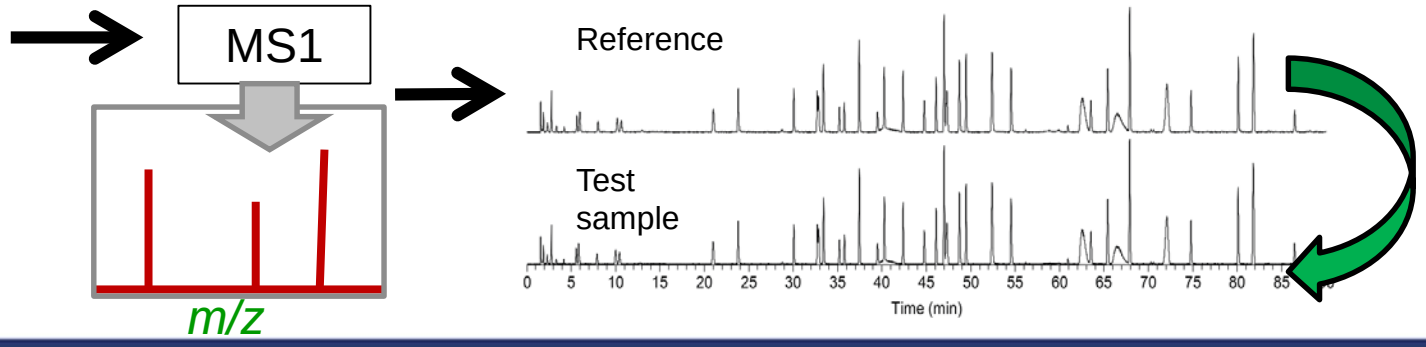
High Resolution LC-ESI-MS

- Primary sequence confirmation
- PTMs
- Site-specific glycan identification

MAM Development



MAM Implementation



- *m/z* -based specifications
- New/changed peak detection

MAM Consortium NPD Round Robin

Trina Mouchahoir, Rich Rogers, John Schiel

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Biosciences and Health

Purpose

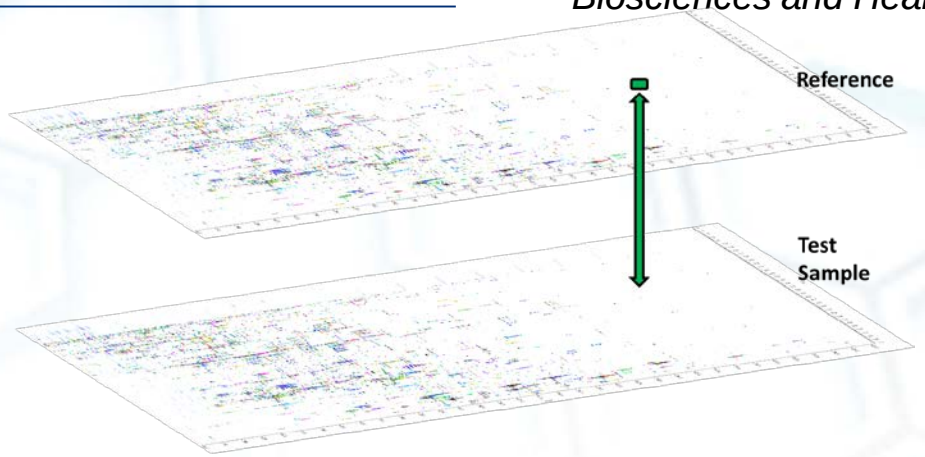
- Evaluate industry-wide new peak detection performance metrics

Results

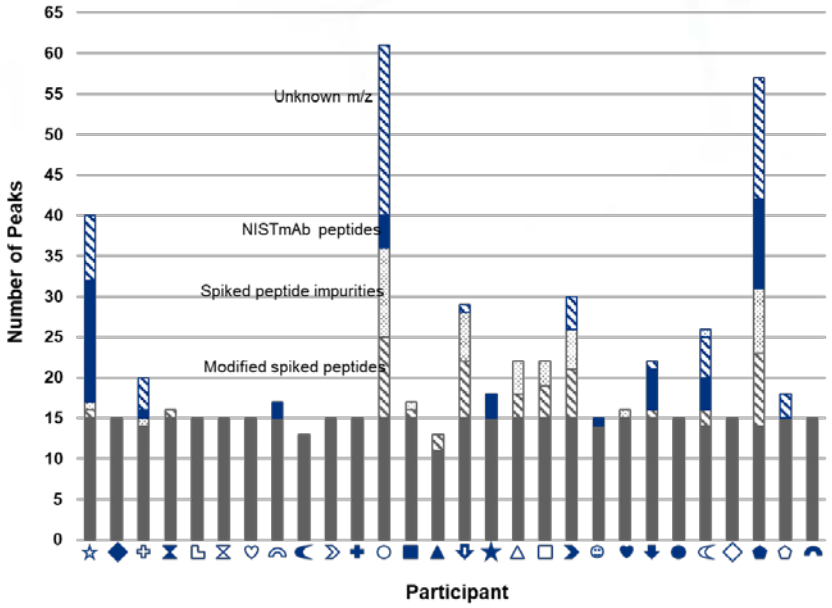
- 28 Industry/Instrument Vendor/Government Participants
- New peak detection is sensitive impurity test
- NPD criteria are being set within performance metrics of current instrumentation
- False positives and false negatives can be mitigated with proper controls. Common pitfalls were identified and explained

Future Perspective

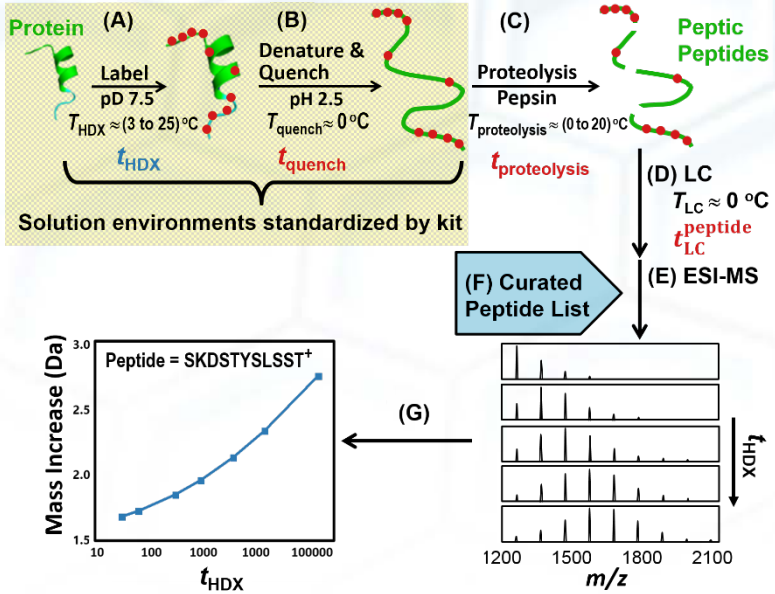
- MAM-specific validation criteria, including NPD analysis, are the next step toward robust QC
- Empirical process/product-specific NPD criteria will be critical to success
- Likely to see broader implementation of MAM
  - QC, process development, comparability, biosimilarity



NISTmAb digest spiked with 15 synthetic peptides (0.5 pmol each)



Protein Structural Dynamics Measured by H/D Exchange Mass Spectrometry (HDX-MS)

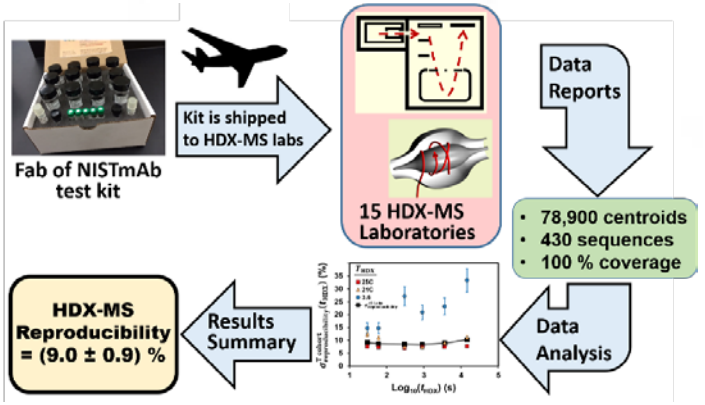


Method

- HDX-MS measures H/D exchange rates that are governed by protein 3D structure and ligand-protein interactions.
- Until recently, HDX-MS is mostly used for epitope mapping and for protecting IP.
- HDX-MS is very sensitive to PTMs.
- HDX-MS can resolve dynamics of IgG1 glycoforms

NIST Interlaboratory Comparison Project

- The study analyzed 78,900 measurements on Fab of NISTmAb, reported by 15 labs in 4 countries, resulting in the first determination of HDX-MS reproducibility
- HDX was demonstrated to be reproducible, potential areas for improvement identified



HDX-MS Reproducibility Determined by Interlaboratory Comparison Project

Hudgens, et. Al. Anal Chem. 2019 Jun 4;91(11):7336-7345

HDX-MS Technology Advancements

Drs. Kyle Anderson, Jeffrey W. Hudgens

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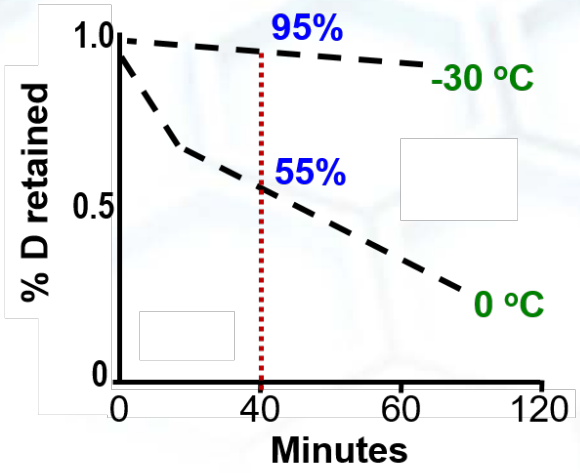
Future Perspective

- Additional hardware/software innovations will furher improve resolution, dynamic range, and reproducibility of HDX-MS.
- HDX-MS implementation will continue to grow
  - Novel modalities
  - More routinely used throughout drug development lifecycle

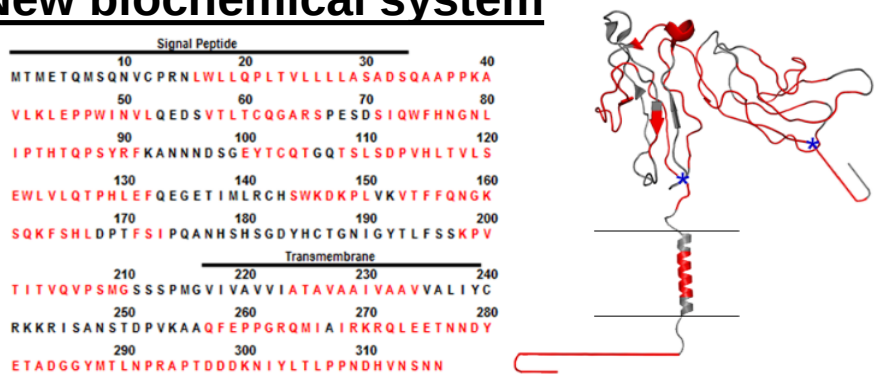
Reduced Back-exchange at -30 ºC



Ongoing Projects : Examples

- Developed an ultra-cold (-30 ºC) proteolysis-chromatographic system, which suppresses data-corrupting back-exchange by >7x.
- Reported a robotic lipid removal method for HDX-MS—promptly adopted by industry—that facilitates pharmaceutical studies of membrane proteins.

New biochemical system



Sequence coverage of FcγRIIa entrained in a bilipid matrix.

2D-NMR International Interlaboratory Study

Robert G. Brinson, Frank Delaglio, Luke Arbogast, John P. Marino

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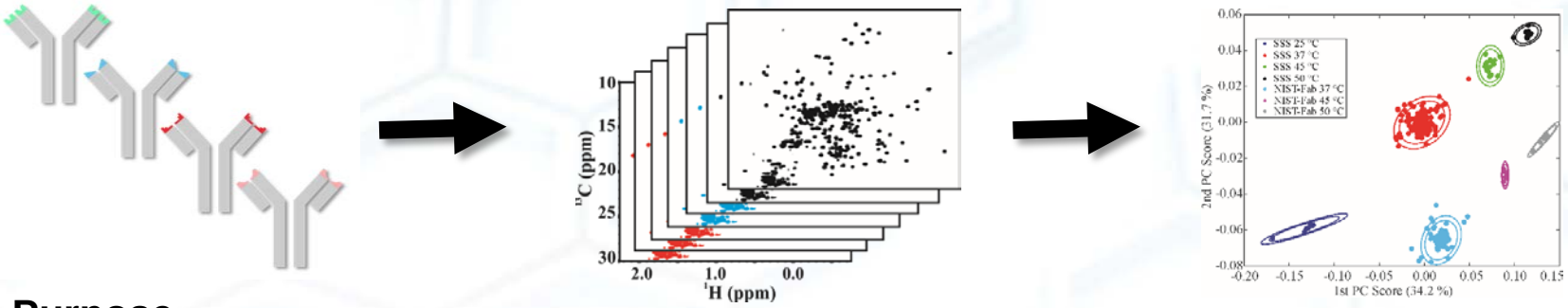
mAb Therapeutic Samples ***2D NMR Fingerprints*** ***Statistical Analysis***



Purpose

- Establish a community standard for the measurement of the higher order structure (HOS) critical quality attribute (CQA) by the 2D-NMR method at atomic resolution.

Results

- Database of spectra from 26 laboratories in nine countries, with equal representation from industry, government, and academia.
- 2D-NMR spectral fingerprinting method is both **repeatable** and **reproducible.**
- Peak position is a **robust** measurement. The same answer is obtained regardless of hardware, field strength, or experimental set-up.
- All data and scripts available at: https://www.ibbr.umd.edu/groups/nistmab-nmr

Future Perspective

- Assurances that the structures of two proteins are highly similar is generally considered to be highly indicative of similar function. Therefore, the ability to precisely and accurately compare structural attributes of two proteins could be key pieces of information that inform the degree of clinical data necessary to confirm safety and efficacy.

Brinson, et. Al. MAbs. 2019 Jan;11(1):94-105.

Measurement Science to Evaluate Conformational Ensembles

Tom Cleveland, John Marino

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Protein therapeutics do not have "a" static structure

Mechanism of Action: Structure contributes to understanding of drug mechanism

Potency: Dynamics may govern interactions

Harmful Side Effects: Structural flexibility correlate with stability?

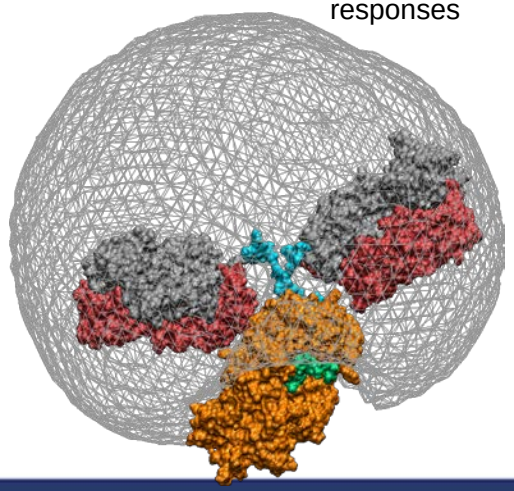
Safety and Efficacy

Manufacturing

Delivery/Formulation: Structure contributes to formulation stability and solution viscosity

Aggregation: Some structures can lead to drug aggregation and dangerous immune responses

NISTmAb Negative Stain EM



Lei, …, **Cleveland, Marino,** Ren. 2019. *Scientific Reports* 9:1-15.

Castellanos MM, Howell SC, Gallagher DT, **Curtis** JE. Anal Bioanal Chem. 2018 Mar;410(8):2141-2159.

Xu AY, Castellanos MM, Mattison K, Krueger S, **Curtis** JE. Mol Pharm. 2019 Oct 7;16(10):4319-4338.

Neutron Scattering and X-ray scattering combined with modeling offer insights into dynamics

NISTmAb non-originator cell lines

Zvi Kelman, Lila Kashi, William Odell

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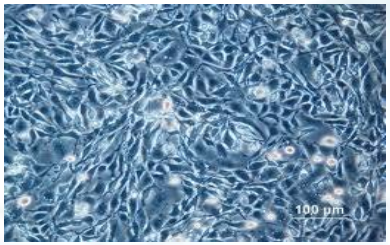
Biosciences and Health

Need

- Pre-competitive cell line to
  - Evaluate innovative process technology
  - Advance PAT, downstream, etc.
  - Continuous processing/real time release test case
  - Platform to demonstrate novel engineering biology approaches



Current Status

- Non-originator cell lines expressing NISTmAb under development
- Preliminary method development from NISTmAb to be ported to non-originators

Future Perspectives

- Data collected may extend open innovation concept to entirety of drug development lifecycle
- “Living” RMs may have substantial impact

Kashi, Yandrofski, Preson, Arbogast, Giddens, Marino, Schiel, Kelman. 2018 Aug/Sep;10(6):922-933.

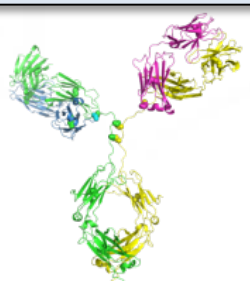
Public Partnership → Limitless Potential

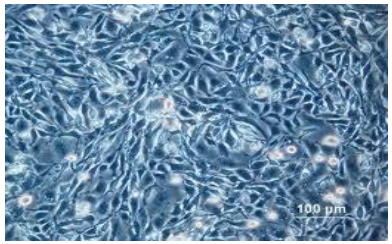
VISION: Utilize RMs to accelerate

Full utilization of emerging technology data for holistic product quality assessment

Emphasis on analytics for attribute-specific product optimization and control

Mechanisms for inter-assay data integration and/or replacement





Research Collaborations

Workshops and Public Meetings

RM and SRM Development

Predicting clinical performance and feedback to manufacturing

Computational Tools, Models & Analytics

Process Analytics & Manufacturing

Machine Learning/AI

Lifecycle appropriate analytical technologies

Advanced Analytical Technologies

Reference materials & qualified methods

Reference Materials

Maturation over time → Regulatory Opportunity!

Acknowledgements

- NISTmAb Team
  - Katharina Yandrofski
  - John Giddens
  - Trina Mouchahoir
- NPD Round Robin
  - Rich Rogers (JUST)
  - Trina Mouchahoir
- HDX Round Robin
  - Jeff Hudgens
  - Kyle Anderson
- Non-originator cell lines
  - Zvi Kelman
  - Lila Kashi
  - William O'Dell
- NMR Round Robin
  - Robert Brinson
  - John Marino
  - Frank Delaglio
  - Like Arbogast
- EM
  - John Marino
  - Thomas Cleveland
- Biomanufacturing Coordinator
  - Michael Tarlov