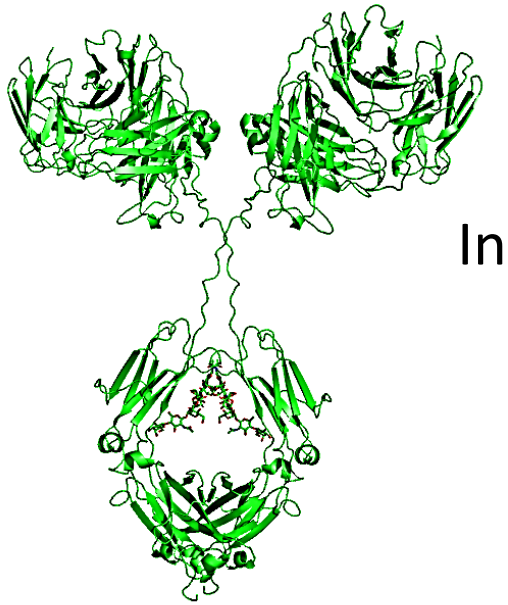
Quality assessment of biologics: higher order structure analysis using NMR



John P. Marino

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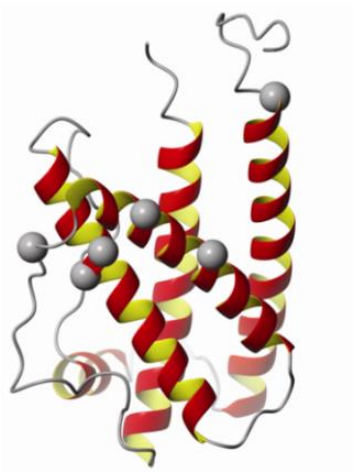
Institute for Bioscience and Biotechnology Research (IBBR)

National Institute of Standards and Technology &
University of Maryland
john.marino@nist.gov



European Pharmaceutical Review Webinar
September 27, 2018

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

UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH

Top 10 Drugs by Sales, 2017

Product	2017 Sales	% Δ v. 2016	Used For	Type
Humira (Adalimumab)	$18,427,000,000	14.6%	Anti-inflammatory	mAb
Rituxan (Rituximab)	$9,238,000,000	2.0%	Anti-Cancer	mAb
Revlimid (Lenalidomide)	$8,187,000,000	17.4%	Anti-Cancer	Small molecule
Enbrel (Etanercept)	$7,885,000,000	(11.1)%	Autoimmune diseases	Protein/IgG
Herceptin (Trastuzumab)	$7,441,000,000	3.4%	Anti-Cancer	mAb
Eliquis (Apixaban)	$7,395,000,000	46.3%	Anticoagulant	Small moleule
Remicade (Infliximab)	$7,152,000,000	(13.1%)	Autoimmune diseases	mAb
Avastin (Bevacizumab)	$7,096,000,000	(1.4)%	Anti-Cancer	mAb
Xarelto (Rivaroxaban)	$6,589,000,000	11.3%	Anticoagulant	Small molecule
Eylea (aflibercept)	$6,034,000,000	9.4%	Macular degeneration	Fusion protein

Statistics from Genengnews.com, Mar 12, 2018

Motivation for NIST's Measurement and Standards Program in Biomanufacturing

Biopharmaceuticals represent a significant and growing part of the health care economy

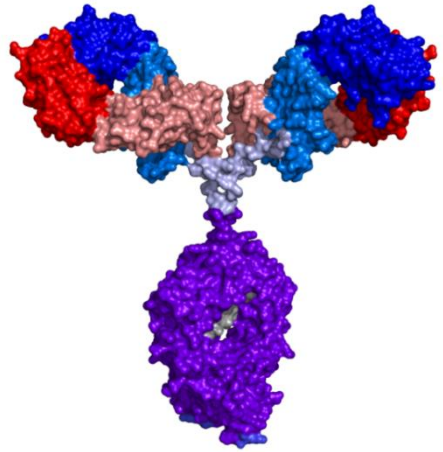
Patent Expiry of Innovator Products is leading to a rapid growth in biosimilars

The complexity of biopharmaceuticals is driving development of new analytical tools and standards for confident characterization and comparability assessment

Challenge to manufacture or make a copy (biosimilar) of a biopharmaceutical that is not adequately defined by measurement

Lipitor

Small Molecule Drugs
MW < 500 g/mol
Synthetic/homogeneous



Monoclonal antibody

- **Large, complex (~150,000 kDa)**
- **Heterogeneous product**

Application of 2D NMR Methods to Measure the Quality Attribute of ‘Higher Order Structure’ (HOS) of a Biopharmaceutical

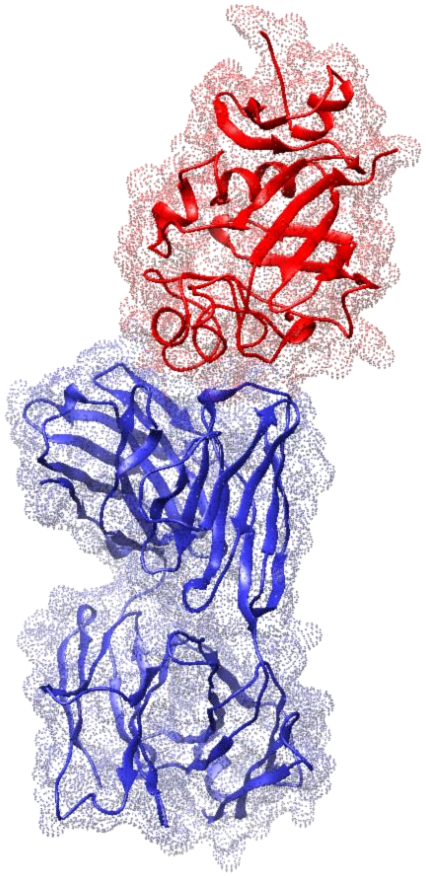
HOS of Biotherapeutics: Primary → Quaternary Structure

MET
GLN
ILE
PHE
VAL
LYS
THR
LEU
THR
GLY
LYS
THR
ILE
THR
LEU
GLU
VAL
GLU
PRO
SER

Primary Structure
(Amino Acid Sequence)

Secondary Structure
(Helix, Sheet, Turn, Coil)

Tertiary Structure
(Protein Fold)



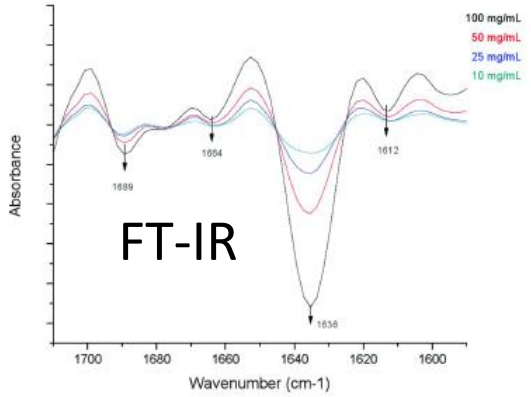
Quaternary Structure
(Complex or Aggregate of Two or More Proteins)

Harder to Measure →

Current Methods for HOS Assessment of Biotherapeutics

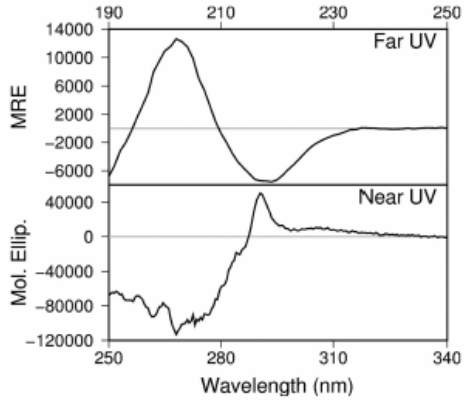
Number of methods used/proposed for HOS measurement:

- Chromatography: SEC/IEC/HIC
- CD, FT-IR, Raman
- HDX-MS
- Fluorescence
- Calorimetry (e.g. DSC)
- AUC
- Light Scattering, DLS
- 1D NMR (e.g., PROFILE)



FT-IR

CD



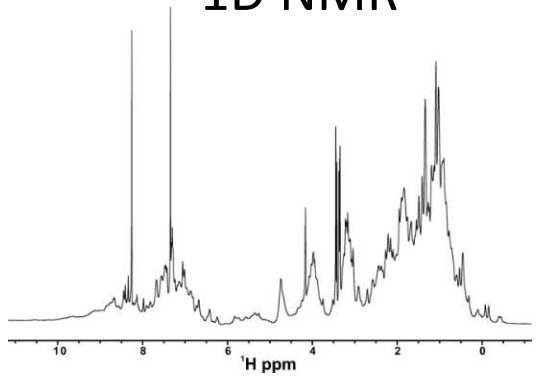
2D NMR: Advantages

- Atomistic Assignment
- Measurement/Instrument reproducibility
- Direct assessment ‘as provided materials’

2D NMR: Challenges

- Sensitivity
- Resolution (Spectral Complexity)

1D NMR



NMR is a Precise and Robust Approach for HOS Measurements

NMR can provide highly detailed fingerprint-like information about the structure (dynamics and environment) of a biopharmaceutical an atomic resolution.

NMR can find applications in:

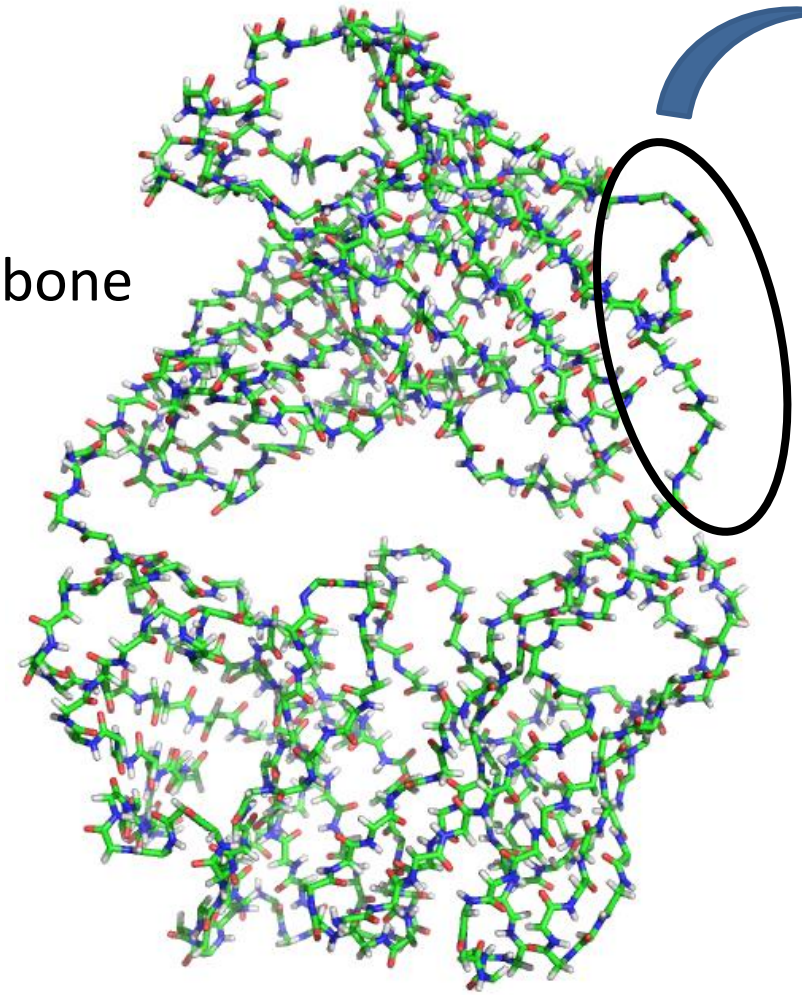
- Biopharmaceutical development
- Formulation studies
- Biomanufacturing – comparability
- Quality Control
- Biosimilarity

Applied in different modes to solve the specific measurement need:

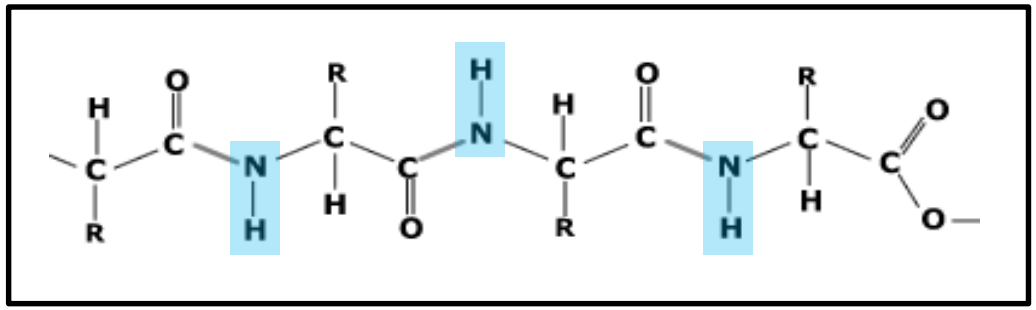
- from ‘pass/fail’ 1D screening to detailed 2D amino acid level analysis.

NMR Spectroscopy provides High-Resolution and Potential for Atomistic Assignment of Signals

Fab
Backbone



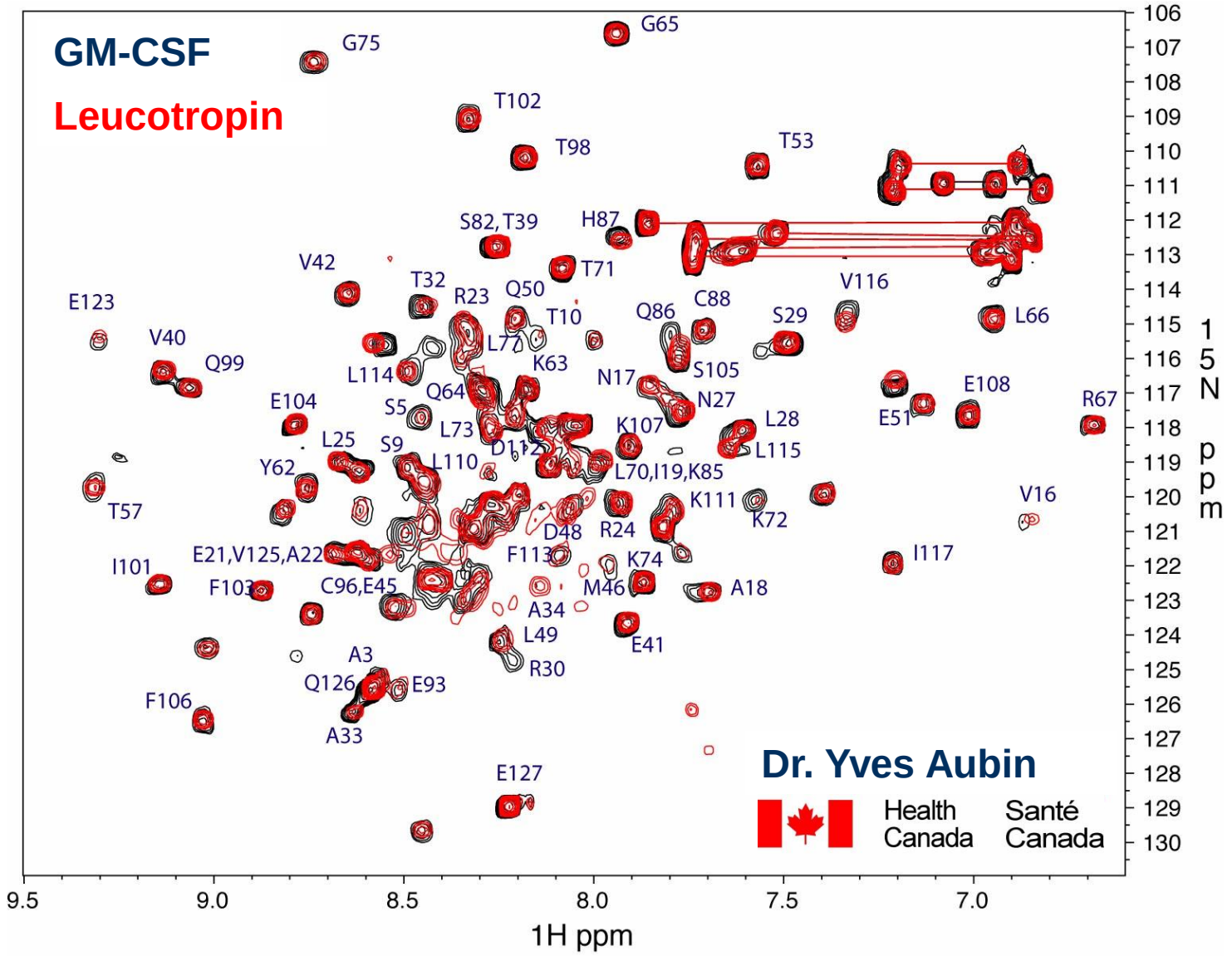
$^{1}H_{N}$-^{15}N HSQC – ‘Gold Standard’ for assessing protein ‘foldedness’



$^{1}H_{N}$-^{15}N backbone amide correlation for each amino acid in a protein

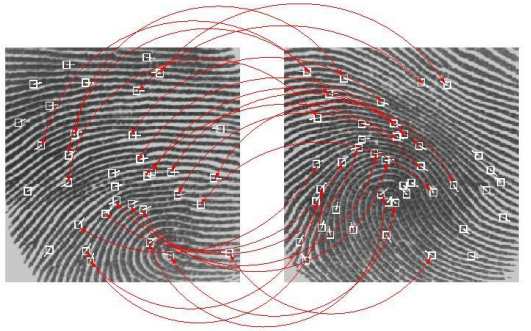
Sequence specific assignment of resonances with heteronuclear correlation methods

2D ^{1}H-^{15}N NMR 'Fingerprints' of the HOS of Protein Biologics



Y. Aubin, G. Gingras, and S. Sauvé *Anal. Chem.*, 2008, 80 (7), pp 2623–2627

2D NMR of Formulated Biopharmaceuticals



NMR is robust and applicable to formulated products

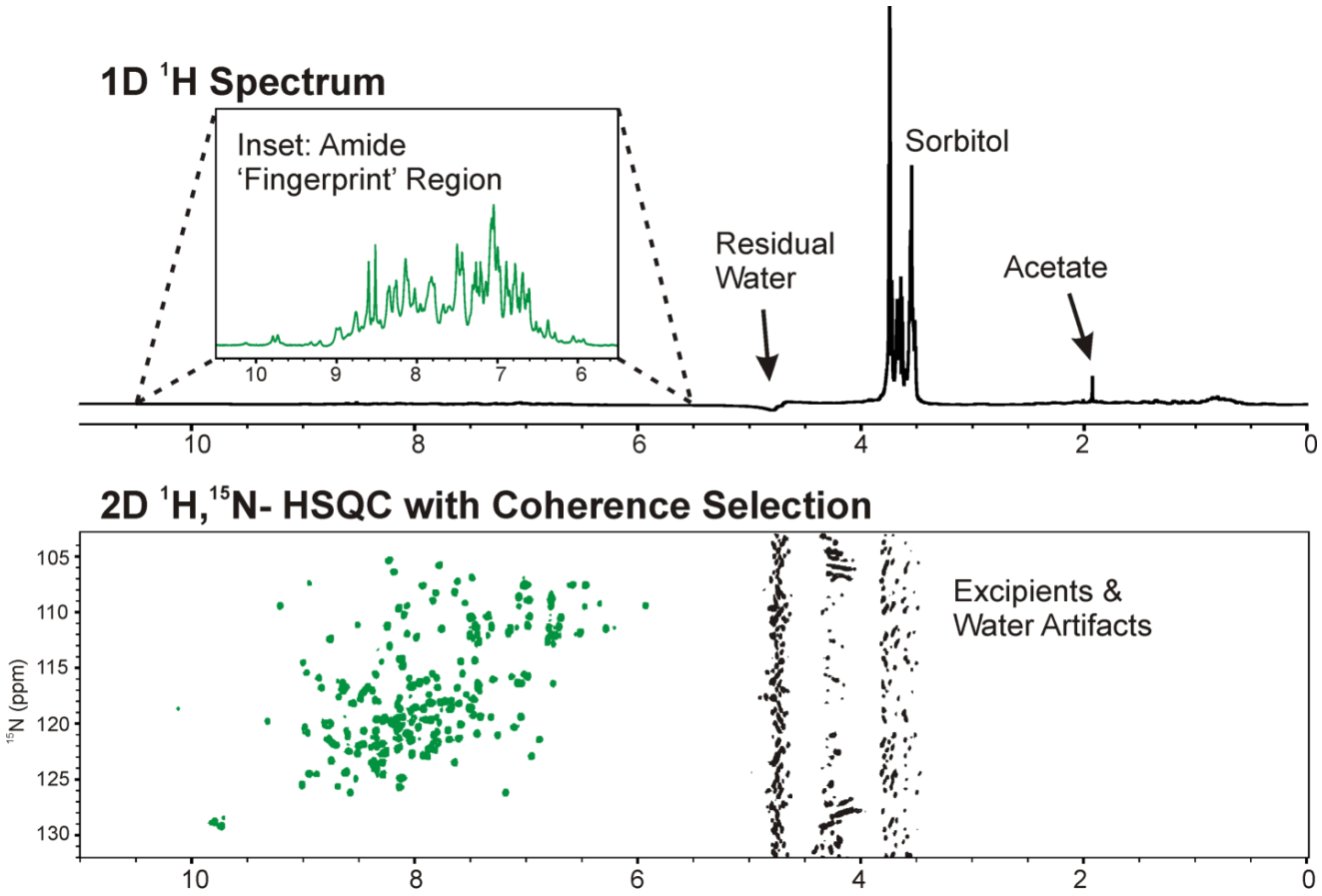
Isotope Labeling (^{15}N-labeling) while possible is **NOT** an option for **PRACTICAL** application

Data collected using isotopes **at NATURAL ABUNDANCE**

^{15}N = 0.37 %

^{13}C = 1.1%

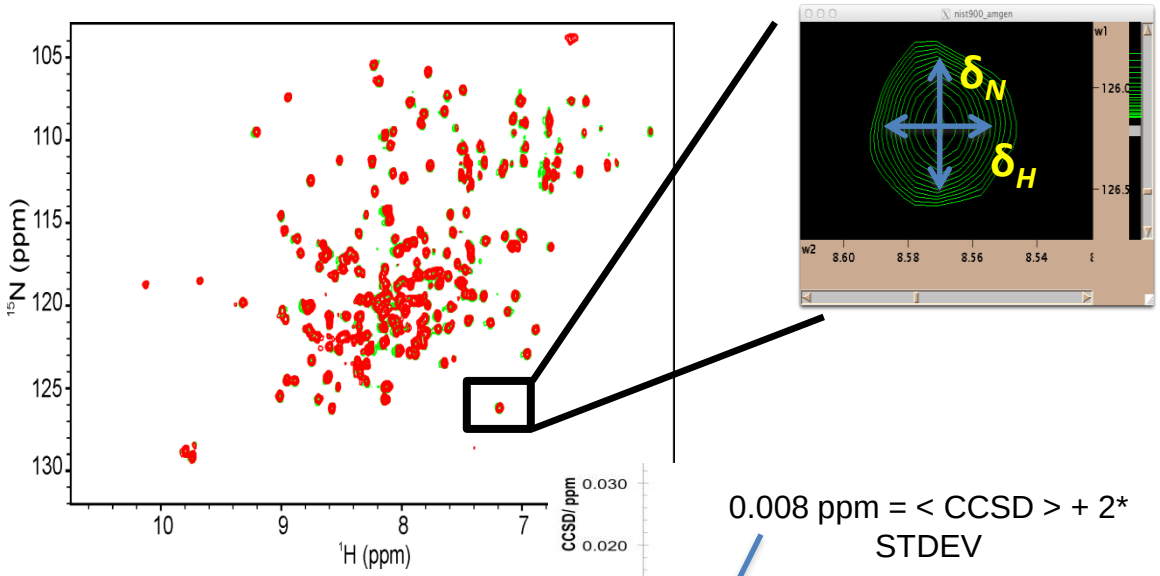
Example: Formulated NUFIL Safe™



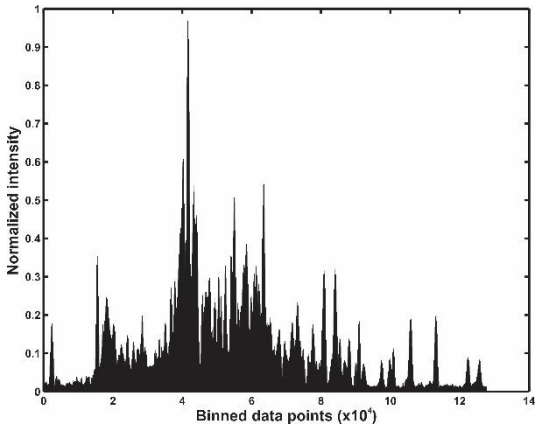
How Can We Correlate 2D NMR Spectral Fingerprints?

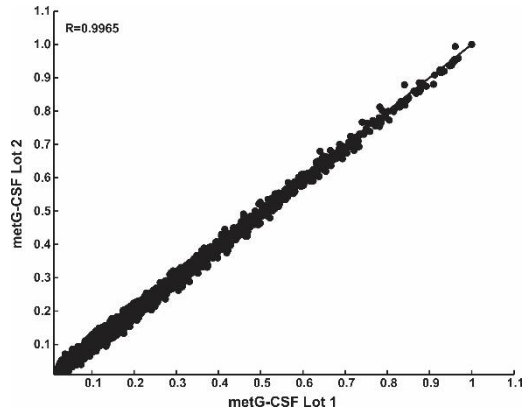
Data Analysis:

- Visual Inspection
- Combined Chemical Shift Deviation (CCSD)
- Point-by-point comparison
  - Linear Correlation plots
- If many spectra, a full multivariate analysis (e.g., PCA) can be done

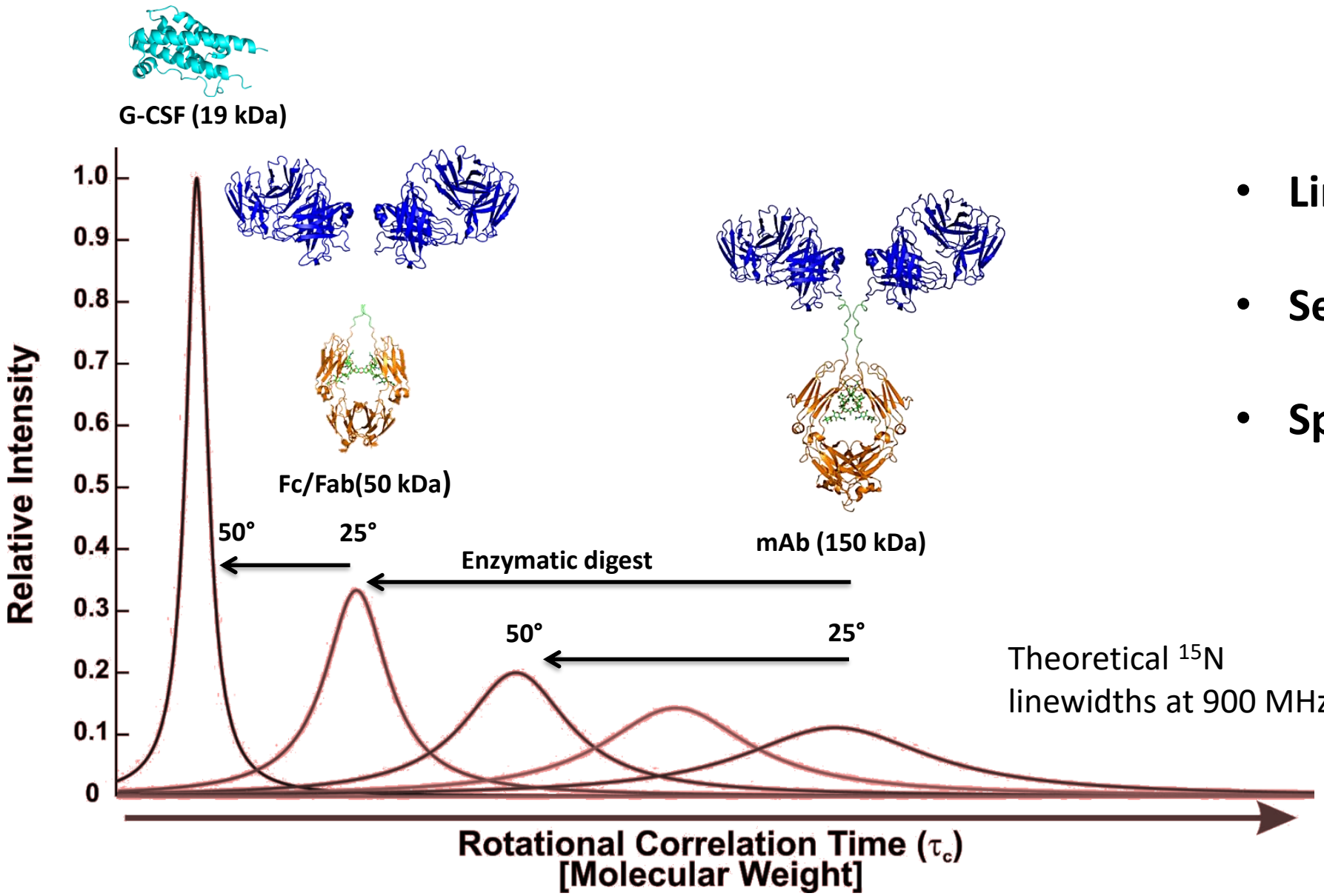


$(I_1, I_2, I_3 \ldots\ldots I_n)$





Challenges in Applying 2D ^{1}H-^{15}N NMR 'Fingerprinting' to mAbs



- **Line Width**
- **Sensitivity**
- **Spectral Overlap**

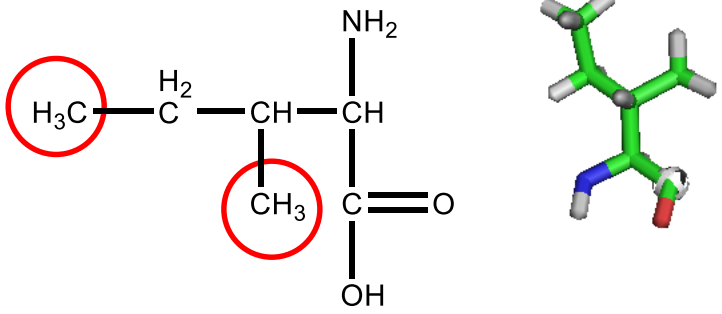
Theoretical ^{15}N linewidths at 900 MHz

2D ^{1}H-^{13}C NMR (Methyl): An Approach for mAb HOS Fingerprints

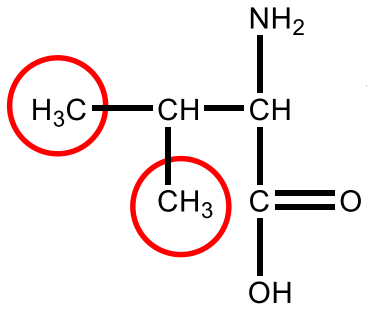
Side Chain Methyl Groups

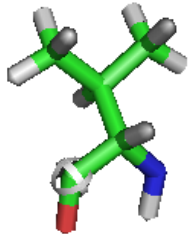
Ala, Ile, Leu, Met, Thr, Val

Isoleucine

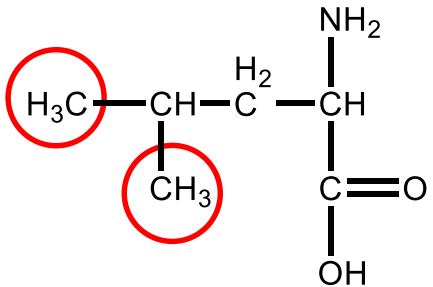


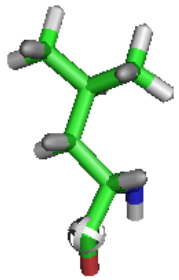
Valine

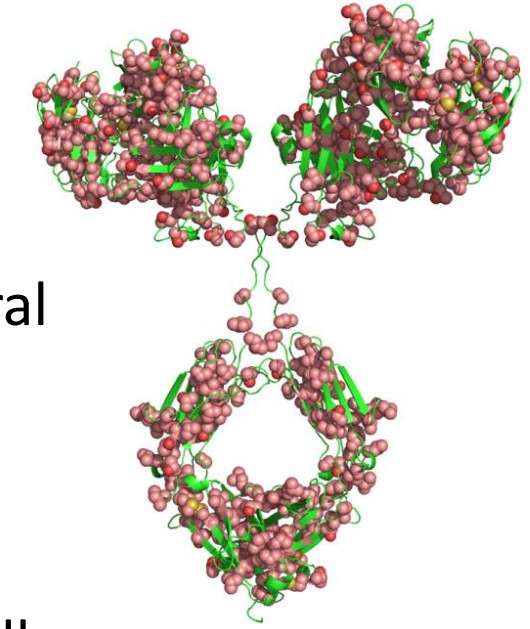




leucine



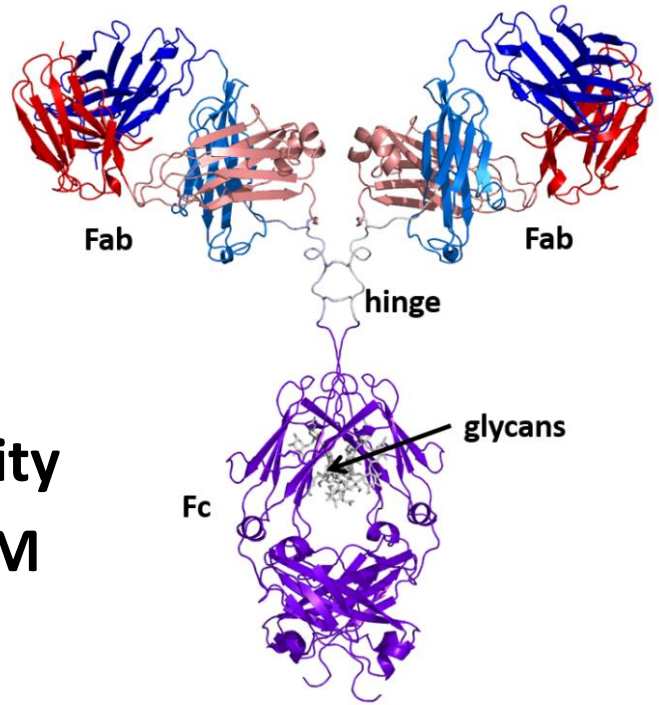


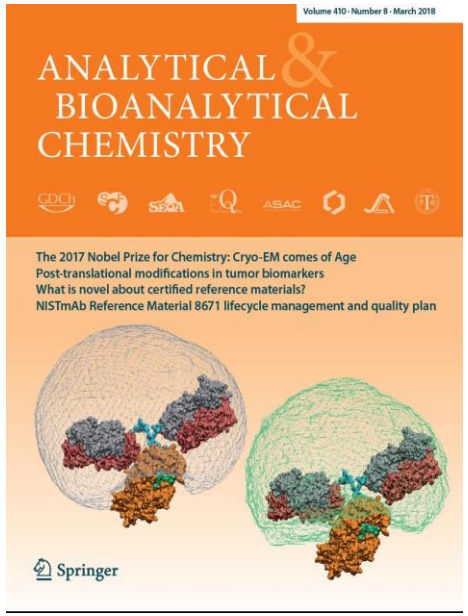


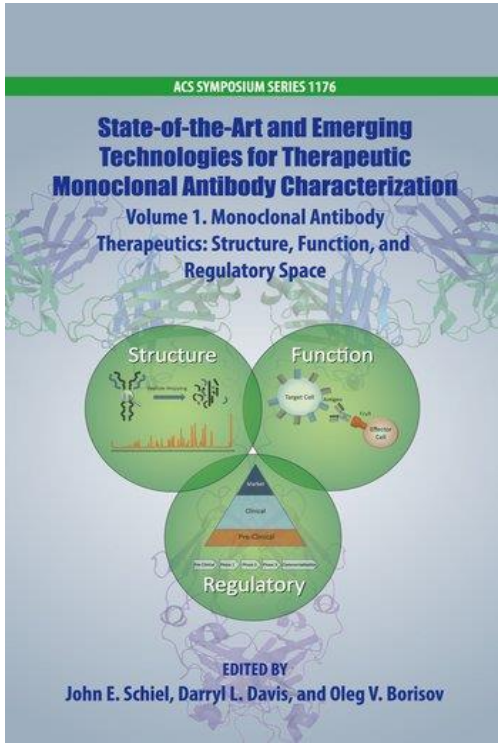
- ^{13}C at natural abundance is more sensitive than ^{15}N natural abundance: ^{15}N = 0.37 % versus ^{13}C = 1.11%
- Methyl groups have intrinsically favorable relaxation
- Non–uniform sampling (NUS) of data can cut experimental time by a further 50 % for 2D data collection

NISTmAb Reference Material: A Platform for Measurement Innovation and Benchmarking

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



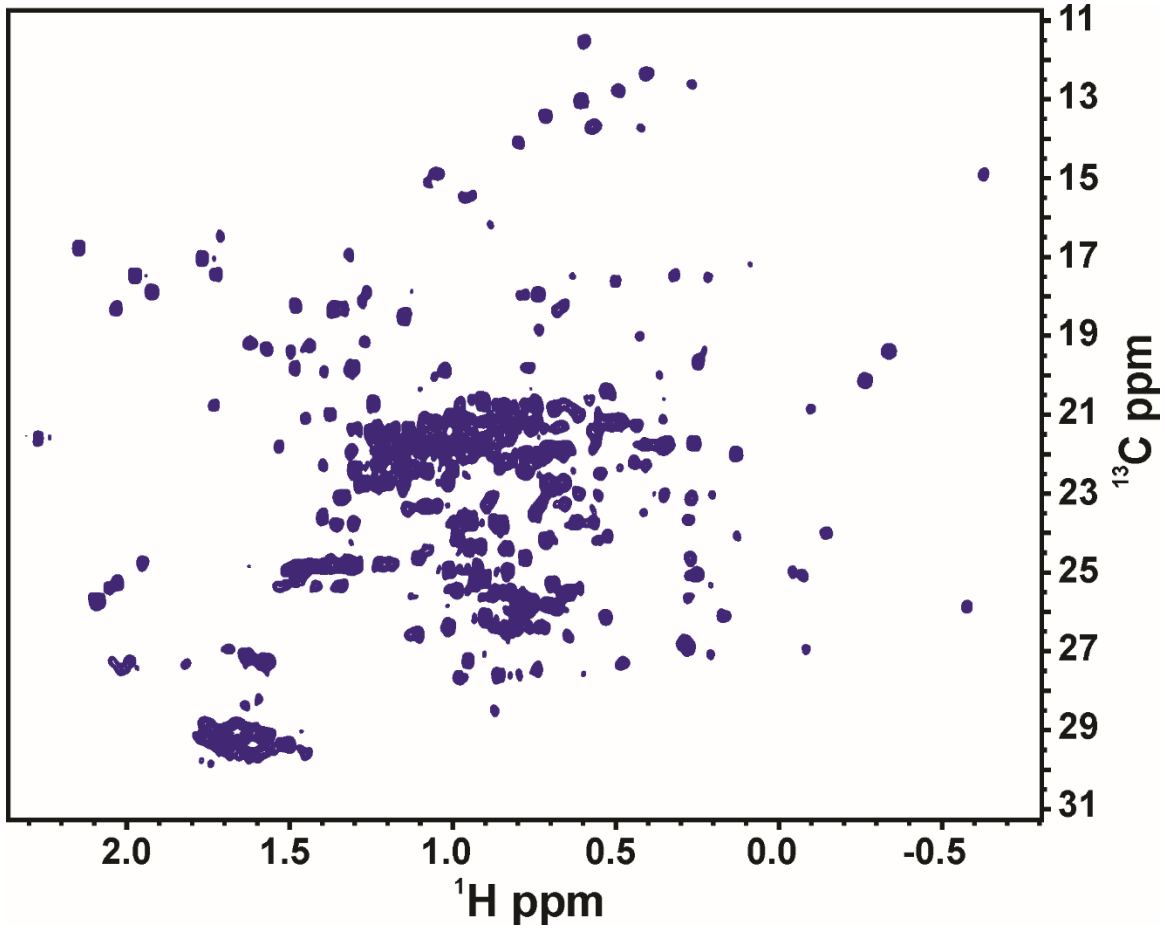


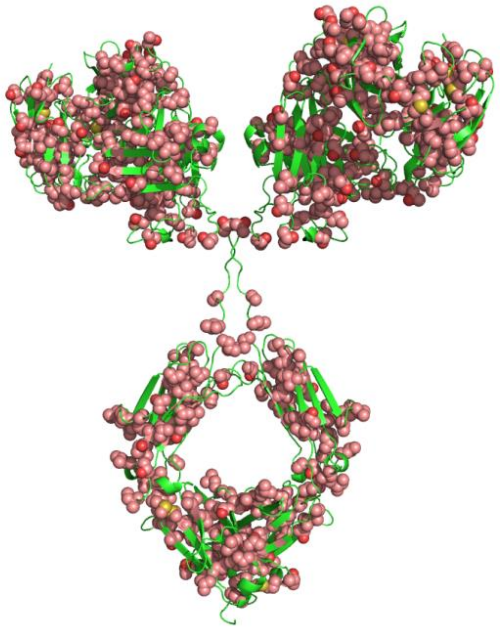


NISTmAb contact: john.schiel@nist.gov

NISTmAb: 2D Methyl Fingerprinting at Natural Isotopic Abundance

^{1}H-^{13}C SOFAST-HMQC



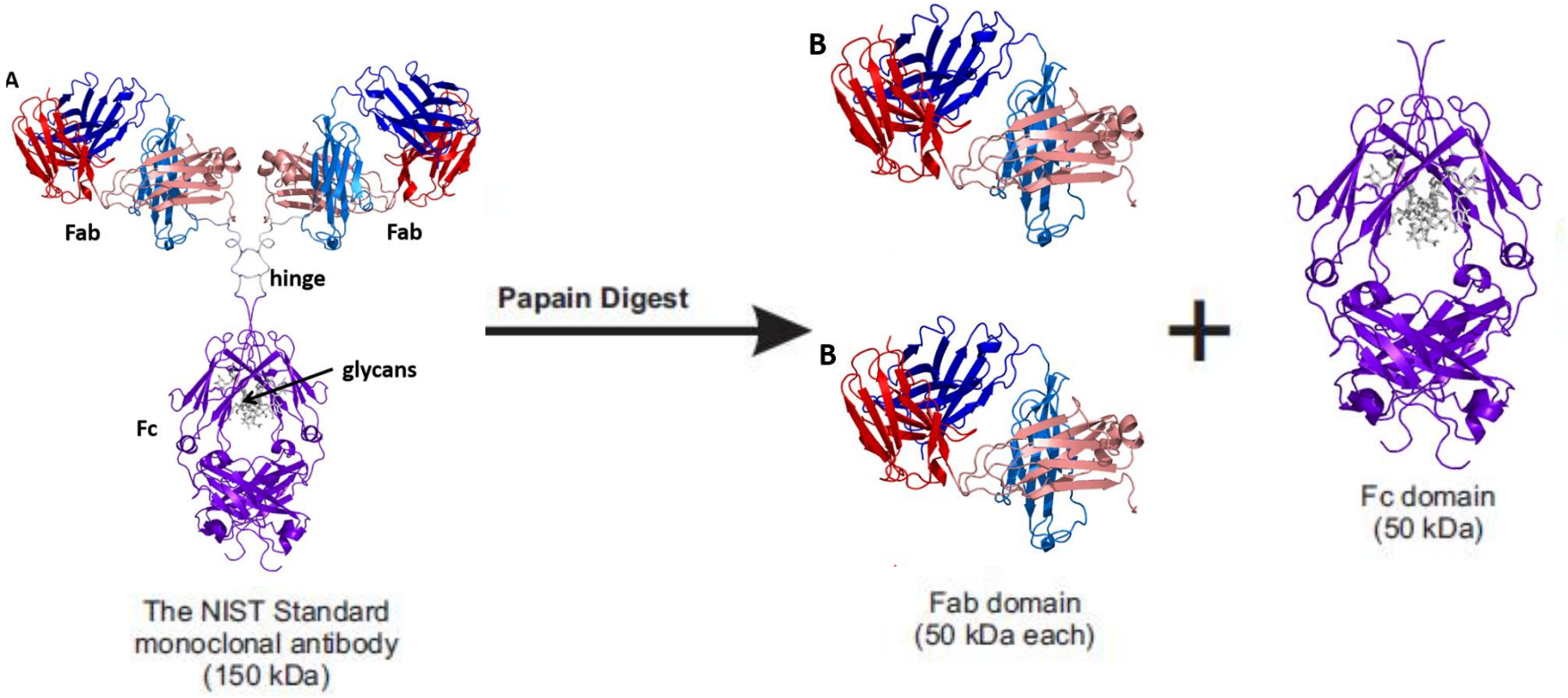


0.5 mM NIST standard
mAb at 50 °C at 900 MHz

Using a cutoff of peak S/N ≥ 10:1,~ 210 peaks of the 221 expected signals (95%) can be observed.

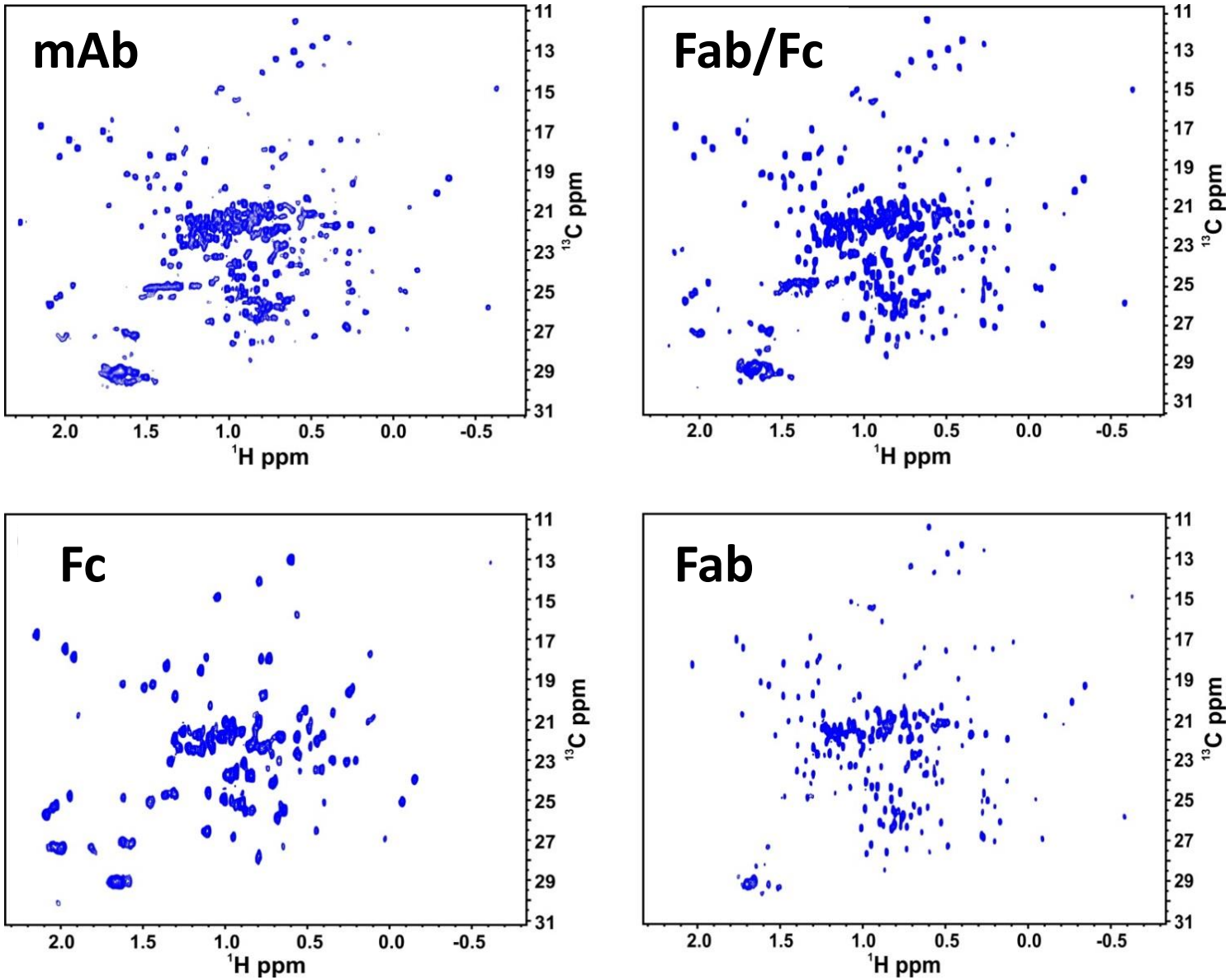
Arbogast, L.W.; Marino, J.P.; Brinson, R.G. *Analytical Chemistry*, 2015, *87*, 3556-3561.

Fingerprinting the Fab/Fc Fragments of a mAb

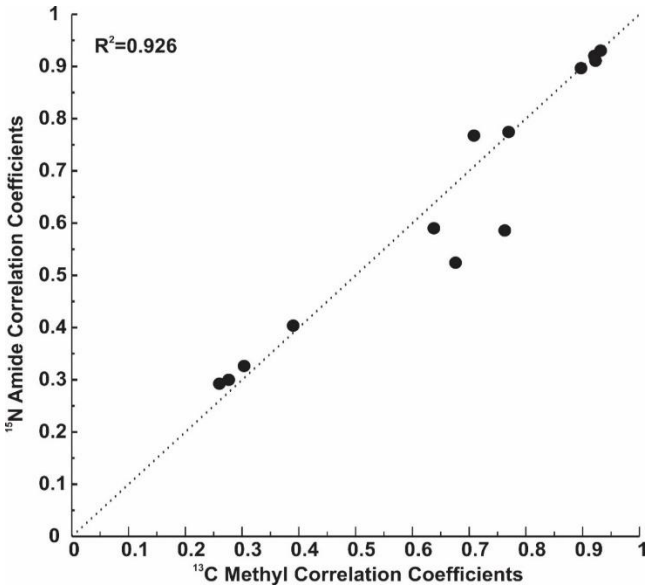


- In language of mass spectrometry, a “middle down approach”
- Use the protease Papain to cleave at the hinge region

NISTmAb, Fab and Fc Methyl Fingerprints

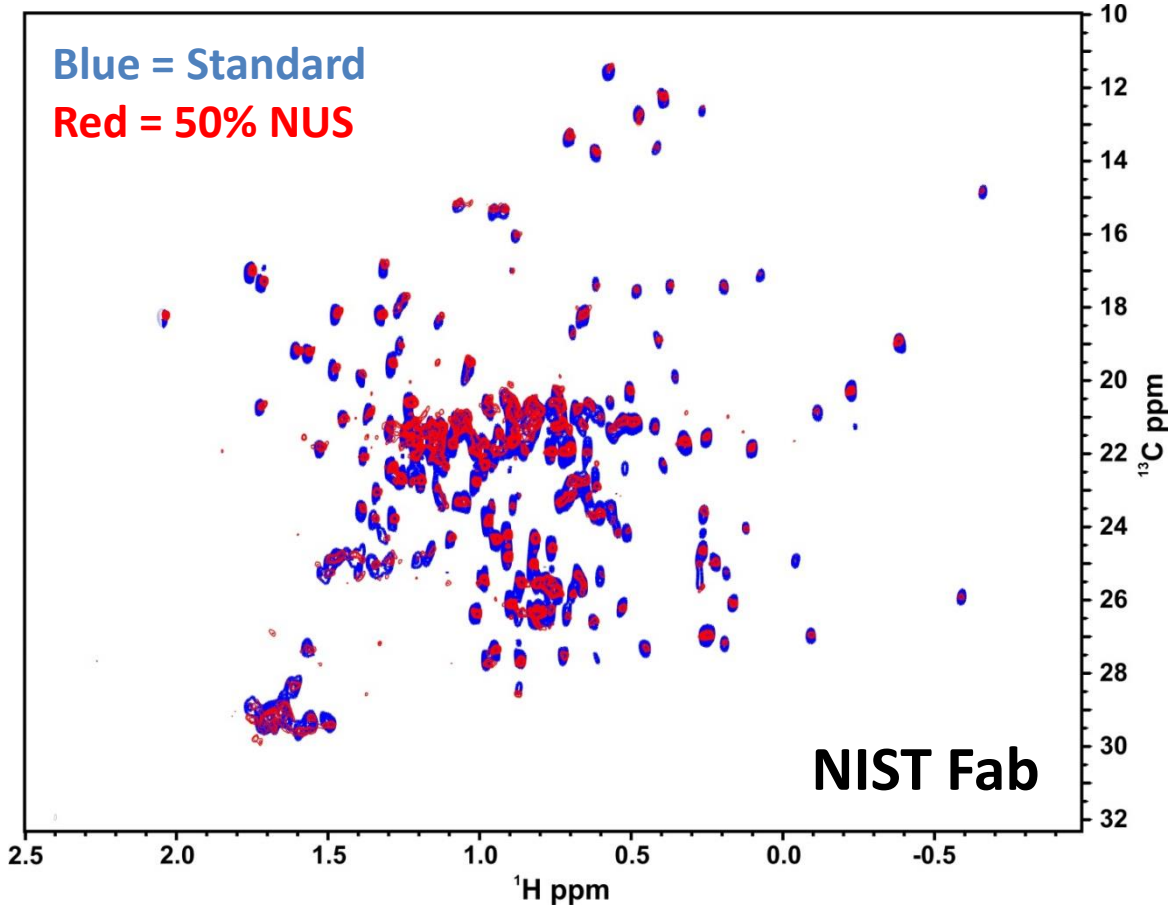


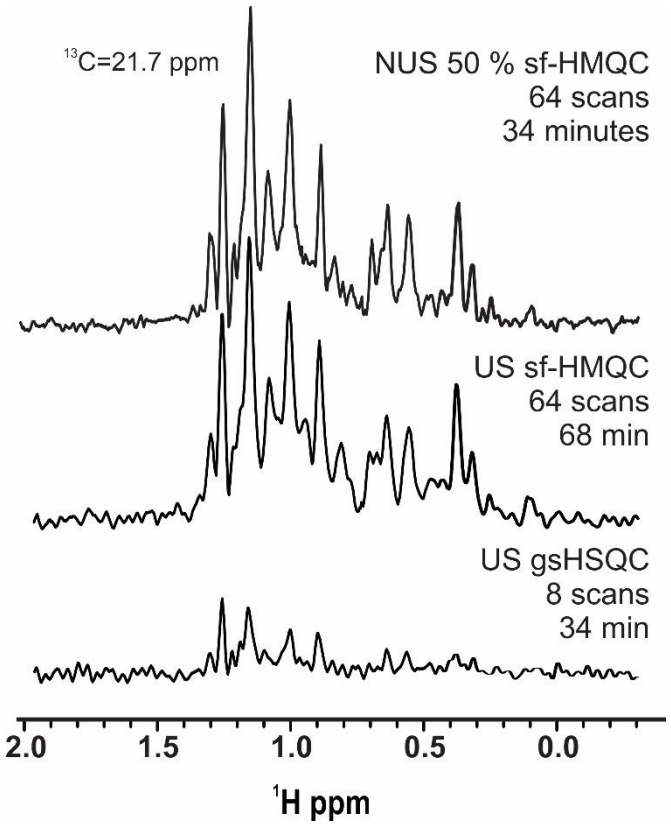
^{13}C-Methyl and ^{15}N-Amide Datasets Yield Highly Similar Statistical Correlations



Arbogast, Brinson, Formolo, Hoopes, Marino. *Pharm Res.* 2016, *33,* 462-475.

Rapid Data Acquisition through Non-Uniform Sampling (NUS)





SOFAST/NUS Spectra in <u>< 1 hour (9x faster than standard experiment)</u>

Wagner Lab (Harvard) NUS Protocols

Aliphatic Excipient Abound and Can Interfere with the NMR HOS Fingerprint

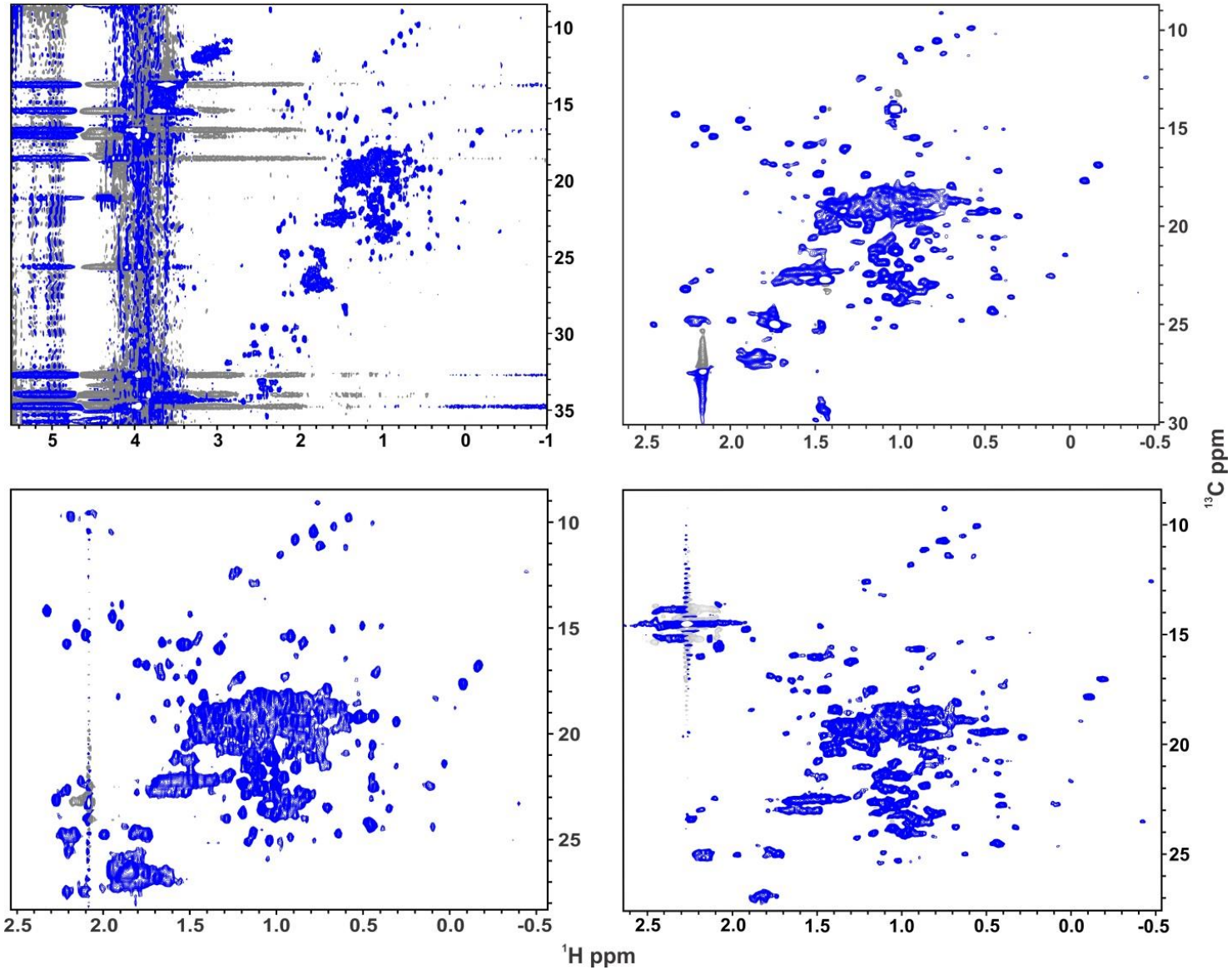
Common Aliphatic mAb Excipients

Excipient	Polysorbate-80	Polysorbate-20	Poloxamer-188	Mannitol	Sorbitol	Sucrose	Trehalose	Dextrose	Dextran-40	Arginine	Glycine	Methionine	Ascorbic Acid	Acetate	Tris	Succinate	Histidine
All (%)	57	19	3	8	3	35	14	3	3	8	8	3	3	19	3	3	35
Lyo (%)	45	36	0	9	9	82	18	9	9	0	0	0	0	0	0	9	27
Liquid (%)	62	12	4	8	8	15	12	0	0	12	12	4	4	27	4	9	38

Kang, J; Lin, X; Penera, J. *Bioproc. Intl.* 2016.

Examples of Aliphatic Excipient Interference with ^{1}H-^{13}C Methyl Spectra

10 % w/v Sucrose



0.3 % w/v Polysorbate-80

10 mM NaOAc

50 mM L-methionine

Optimized Filters for Excipient Reduction and Removal (SIERRA): Case Study with 50 mM L-methionine

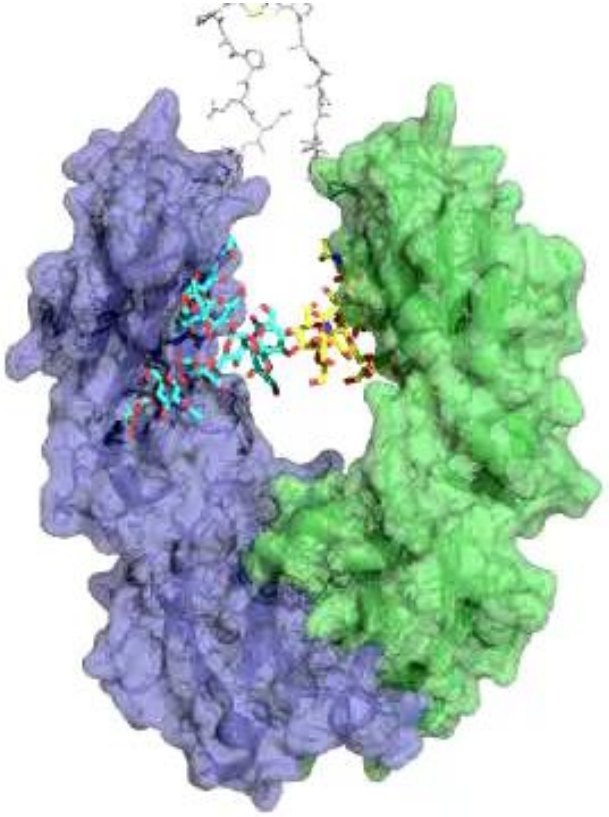
Experiment*	S/N	S/N Ratio	S/N-sel Ratio
gsHSQC	22.938	1	N/A
gsHSQC-sel	21.260	0.927	1
SIERRA-gsHSQC CP off	20.232	0.882	**0.951**
SIERRA-gsHSQC CP on	19.390	0.845	**0.911**
PFGSTE-gsHSQC 5 ms	5.079	0.221	N/A
PFGSTE-gsHSQC 30 ms	3.765	0.164	N/A

Selective pulse techniques combined with SMILE-based signal subtraction can mitigate interference from commonly employed aliphatic excipients with minimal sensitivity loss

Ying, J.; Delaglio, F.; Torchia, D.A.; Bax, A. *J. Biomol. NMR*. **2017**, *68*, 101-118; Arbogast, et. al., **2018** *J. Biomol. NMR*, in submission

Application of 2D NMR Methods to Detect, Quantify and Assign HOS Variation

Simulating Structural Variation in mAbs: Glycan Remodeling



Structure → Function

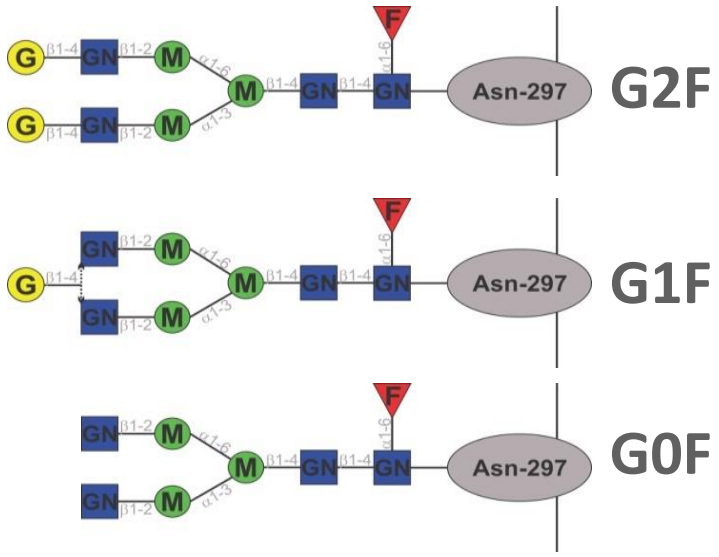
Asparagine(N)-linked carbohydrate chain (glycan) on Fc is required for cell surface Fc γ receptors (FcγRs) interactions

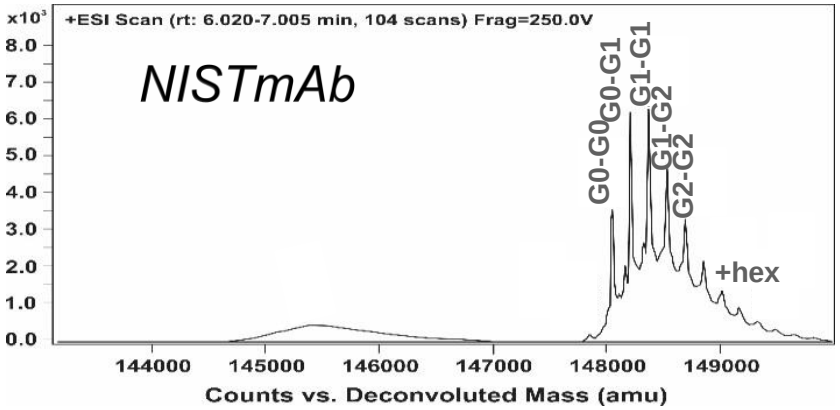
Crystal structure of NISTmAb Fc with G0F/G1F glycans
2.1 A resolution (PDB 5VGP)

Structure & Image provided by D.T. Gallagher (NIST)

Glycan Remodeling Creates Defined Structural Changes in a mAb

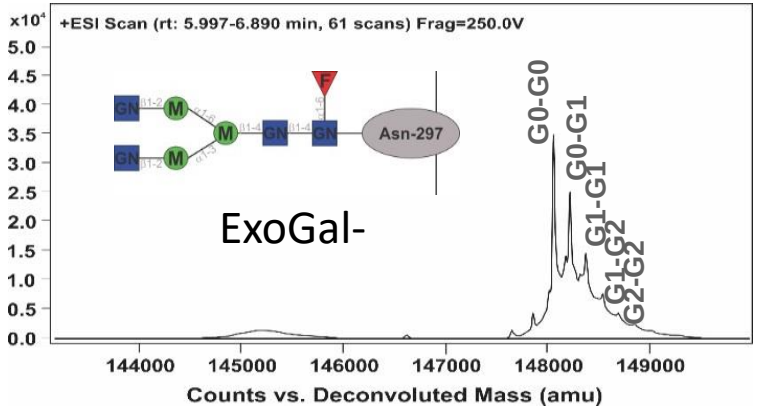
Intact Mass – Agilent 6545 QTOF





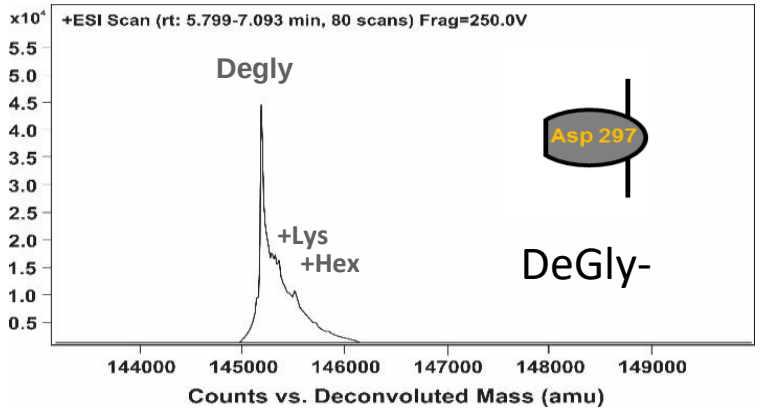
β1-4 galactosidase

Exoglycosidase Cleavage



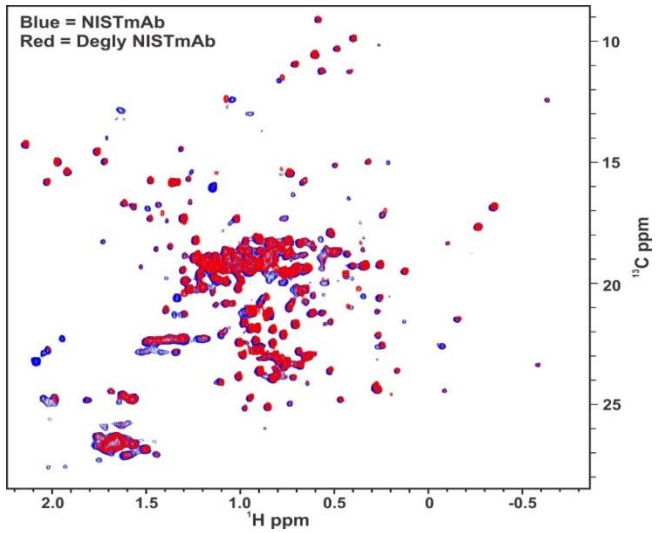
PNGase F

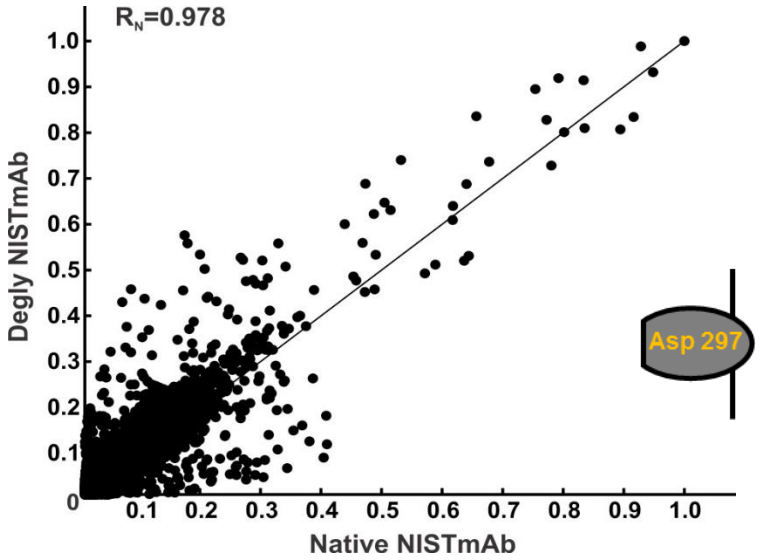
Deglycosylation



Arbogast, L.W; Delaglio, F; Schiel, J.E.; Marino, J.P; Analytical Chemistry, **2017**.

Glycan Remodeling and Resulting Methyl Fingerprints of NISTmAb

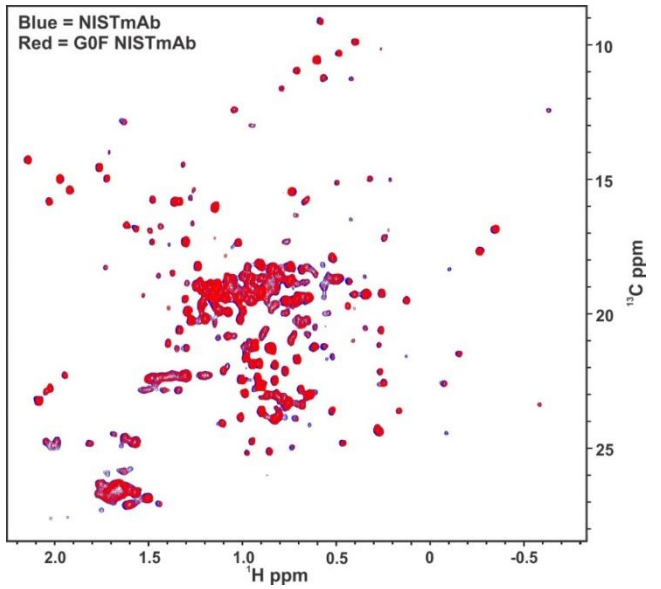


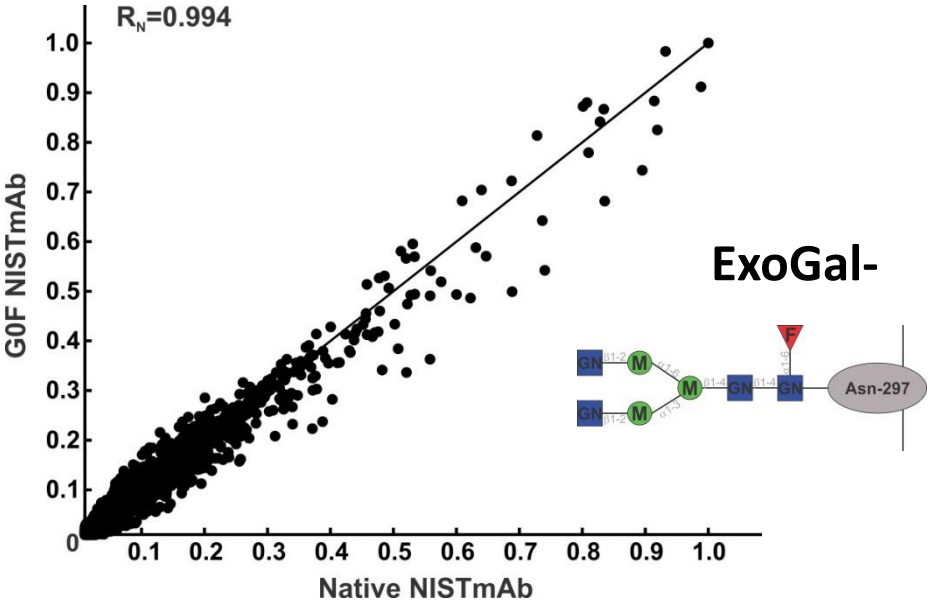


ECHOS

Amezcua and Szabo *J. Pharm Sci.* 102 2013

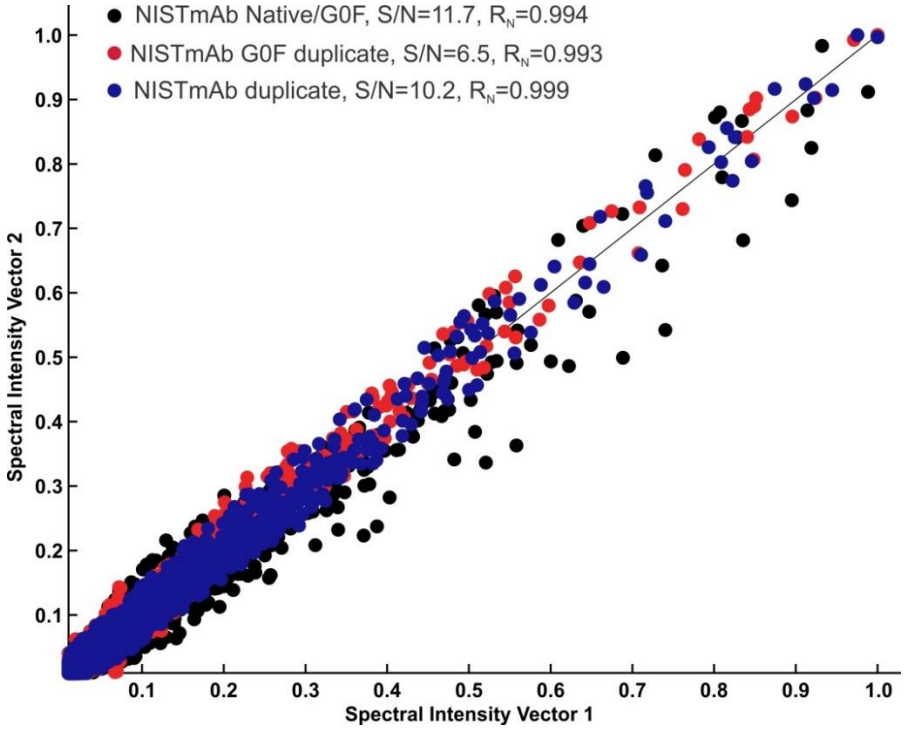
Degly-

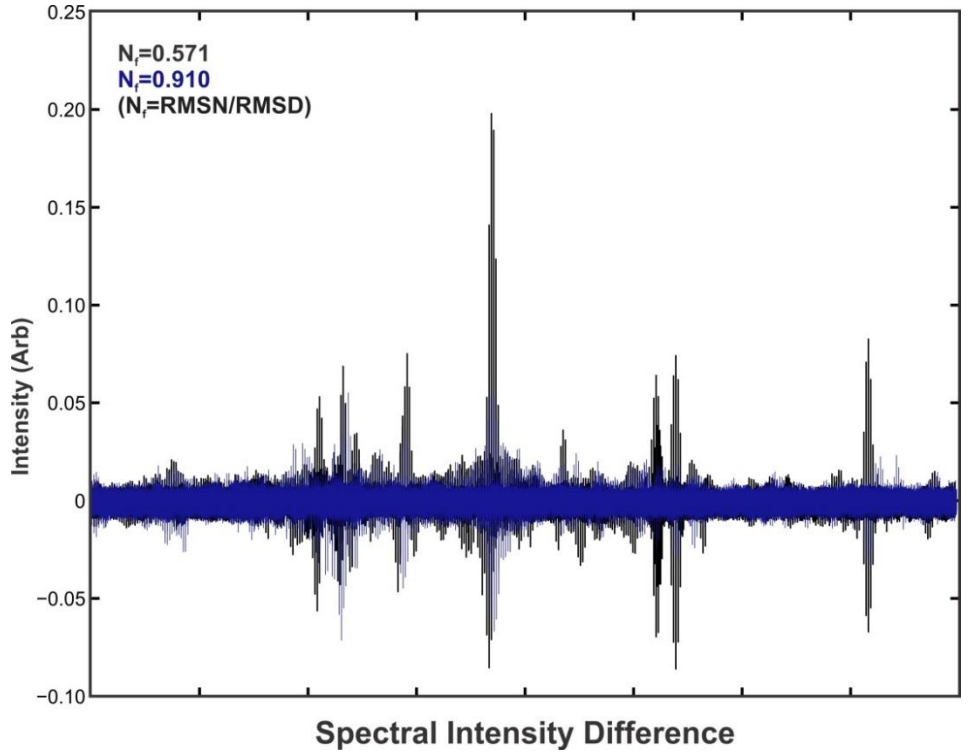




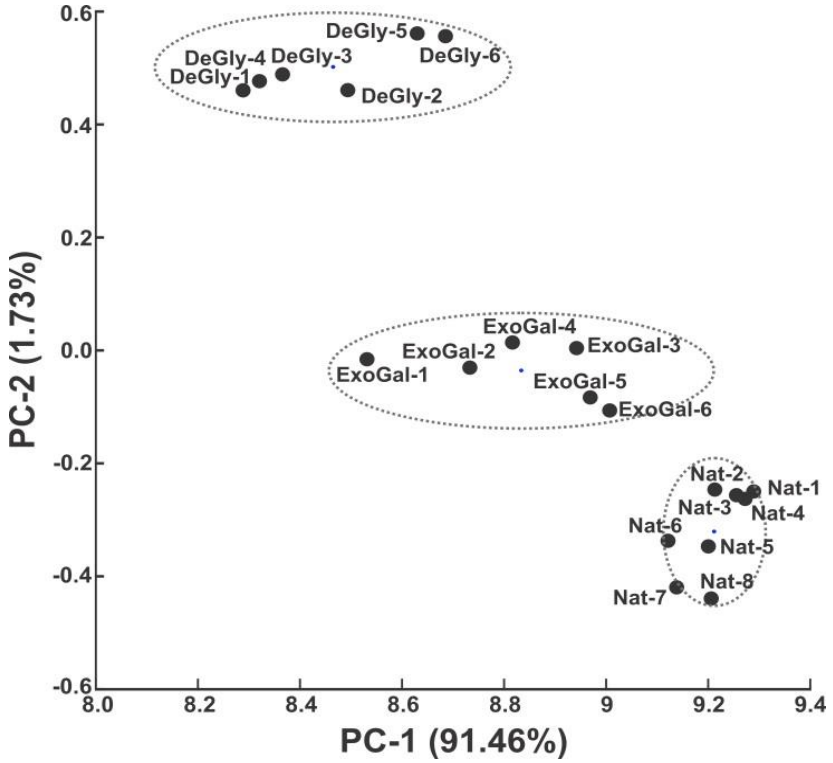
ExoGal-

Comparison of NISTmAb with ExoGal-NISTmAb

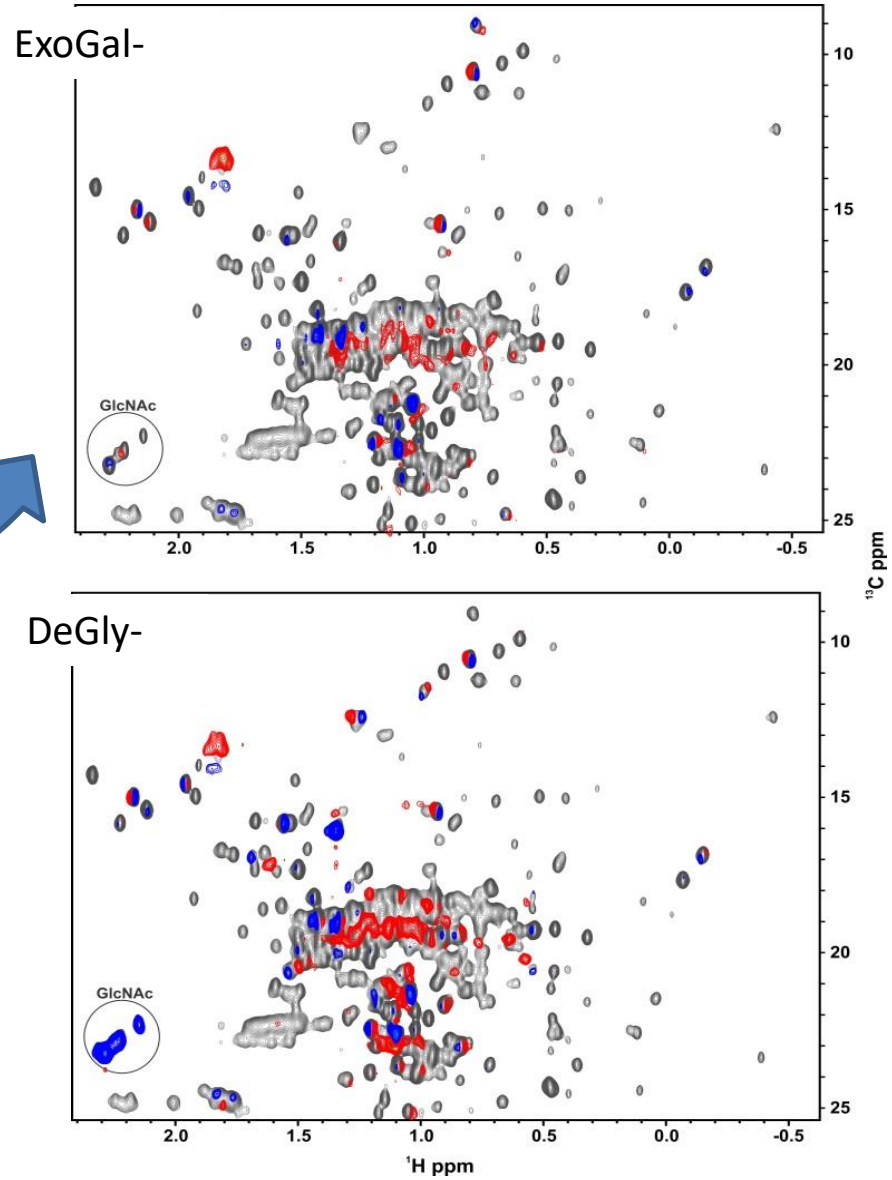




PCA Can Readily Differentiate Minor Structural Variation arising from Glycan Remodeling



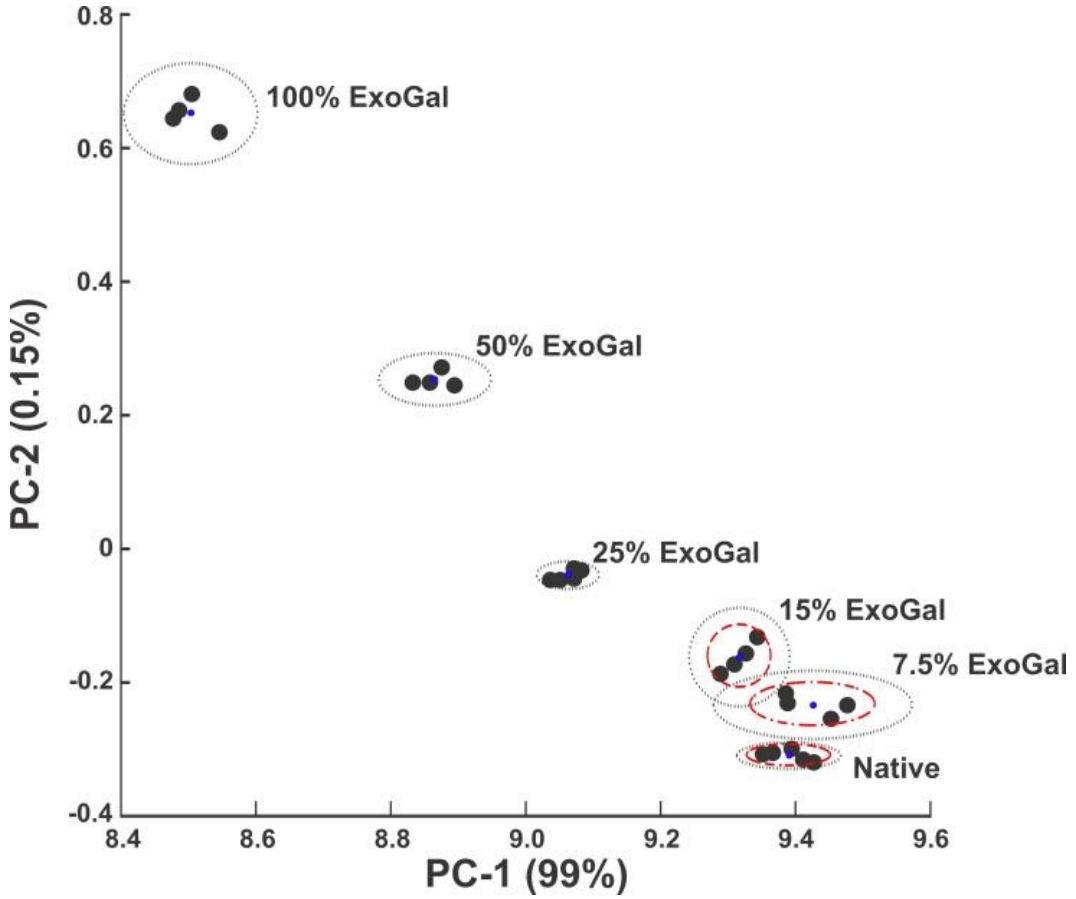
Corresponding PCA loading plots



PCA scoring plots from 20 NISTmAb glycoform ^{1}H-^{13}C methyl spectra. Dashed lines correspond to 95% confidence interval

Probing the Lower Limit for Detecting Structural Variation in a mAb Biopharmaceutical

PCA Scatterplot from 26 ^{1}H-^{13}C-methyl spectra of blended ExoGal-/Native-NISTmAb



At the 95% cluster confidence interval –

differentiation of 10% relative isoform composition

Grey lines correspond to the 95% confidence interval of the spread for each cluster.
Dashed red lines on the 15%, 7.5% and native clusters correspond to the 2σ interval

Application of Multivariant Analysis (PCA) Directly to the NMR Data without Spectral Processing

- PCA can applied to spectra or interferograms, conventional or NUS without the need for apodization, special reconstruction, baseline correction, or peak analysis.

- Since NMR fingerprinting can potentially be performed without the need to identify peaks, it might be possible to develop even more efficient measurement and processing strategies which do not produce spectra that can be analyzed visually, but nevertheless encode all the structural information of interest.

PCA Discrimination of Glycan Variants may be possible with NUS Sampling < 10%

NISTmAb NMR Interlaboratory Study

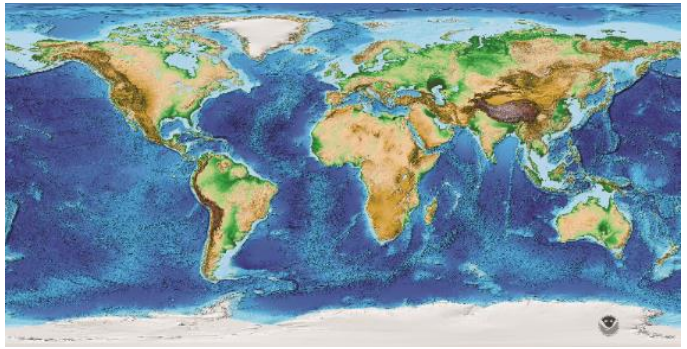
- To establish a harmonized community standard for the measurement of the higher order structure (HOS) by 2D-NMR
- To provide assurance for industrial and regulatory agencies that 2D-NMR can be applied to biopharmaceuticals with high precision and reproducibility
- To develop and benchmark chemometric tools to aid in the translation of the 2D-NMR method into the biopharmaceutical development and manufacturing workflow.

RG Brinson, et. al. (48 co-authors …) Enabling adoption of 2D-NMR for the higher order structure assessment of mAb therapeutics. Submitted to mAbs **2018**.

NISTmAb NMR Interlaboratory Study

Moving the 2D-NMR method from an emerging technology to a harmonized, routine measurement that can be generally applied with confidence to high precision assessments of biopharmaceutical HOS

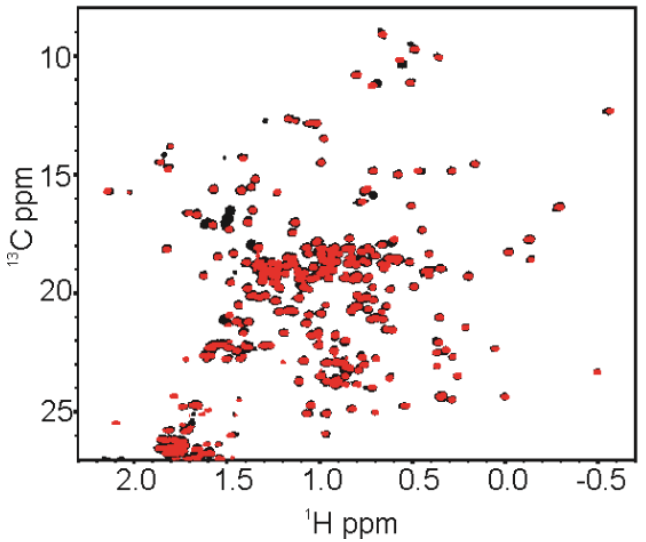
Global Collaborating Partners



8 Pharmaceutical companies

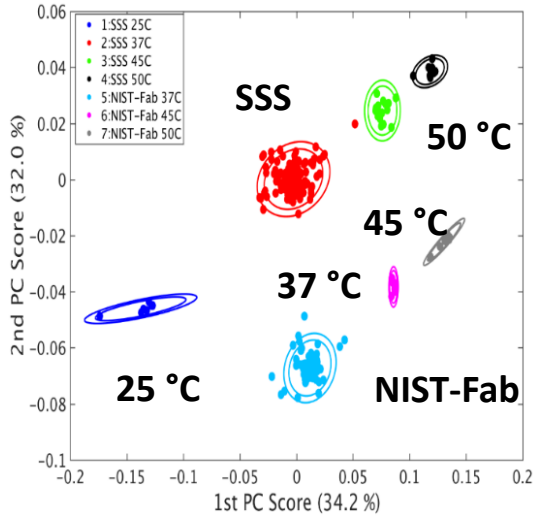
1 vendor
8 Government Laboratories
9 academic labs

26 total laboratories
25 total Institutions



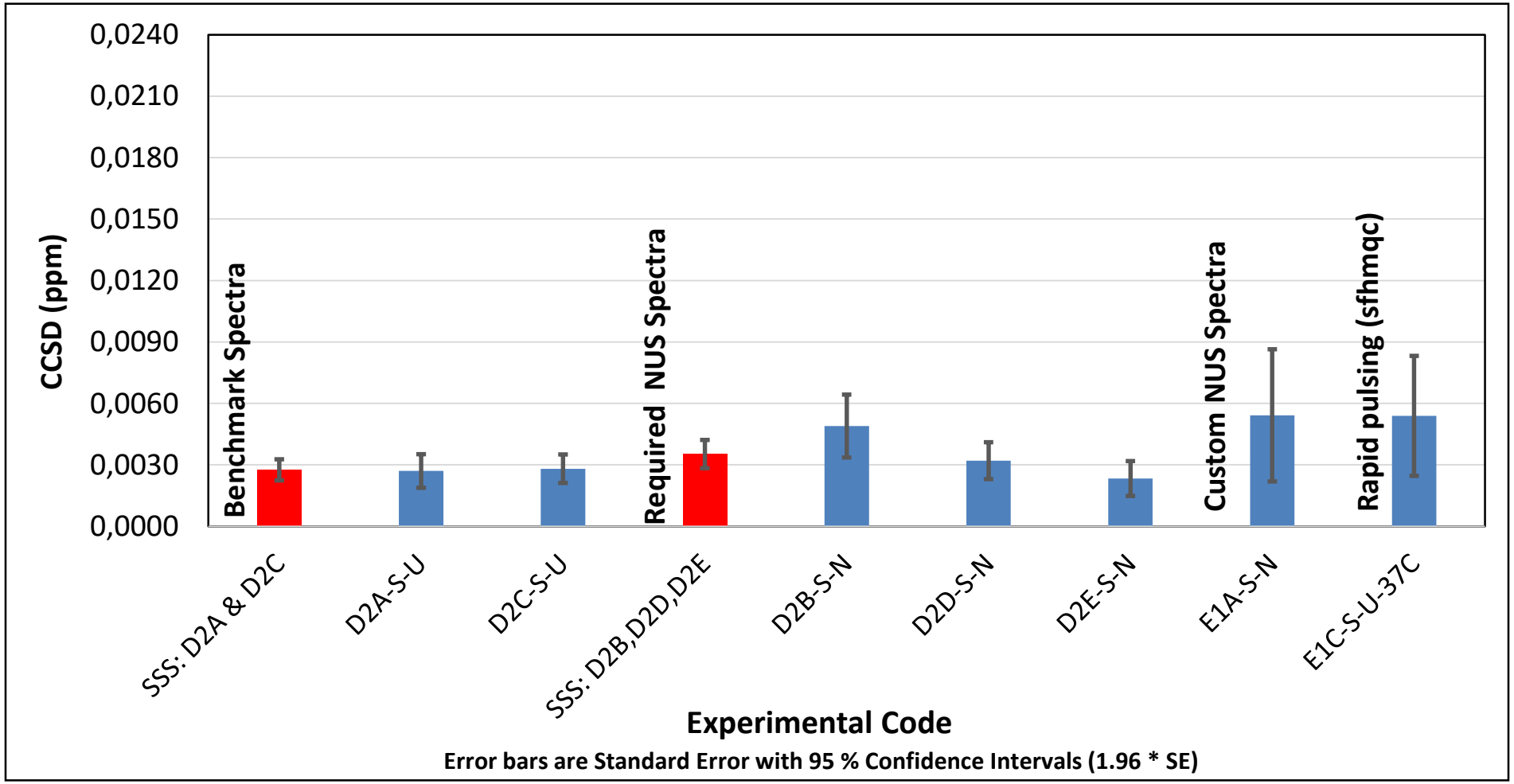
375 ^{1}H,^{13}C spectra
From 7 Magnetic fields
And 39 magnets

Model Therapeutic: NISTmAb
Primary Sample #8670



Robust Chemometric Analyses

Average ^{1}H-^{13}C Methyl CCSD of SSS Spectra: Evaluating the Precision of the 2D-NMR Method



Data are benchmarked against D2A and D2C spectra

Summary

2D NMR Fingerprinting of Biopharmaceutical (including mAbs) is precise, practical and robust

- Natural Abundance (label-free technique)
- Applicable at **600 MHz**, the 'workhorse' NMR spectrometer of most labs
- ^{13}C Methyl maps can take < 1 hr using NUS/SOFAST
- Excipient Signals can be filtered/suppressed
- Interferograms can be analyzed directly, eliminating need for NUS reconstruction

Data Analysis (Beyond visual inspection by the expert operator)

- Combined Chemical Shift Deviation
- Point-by-point comparison
- Multivariate analysis (PCA) – other Chemometric Approaches

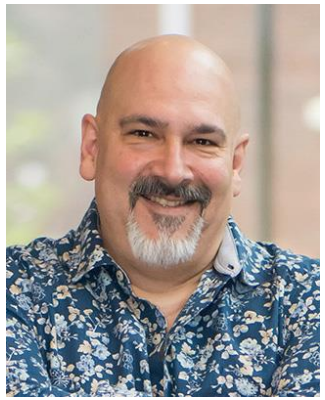
Multivariate analysis of 2D spectra is sensitive to subtle difference in HOS

- Methods can be automated without interactive analysis of spectral features
- Potential for application of machine learning

Acknowledgements


Luke Arbogast


Robert Brinson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