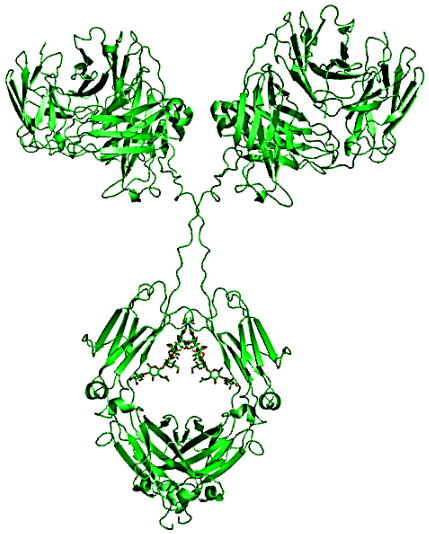
The NIST NMR Interlaboratory Study: Conference Calls #1-4



Dr. Robert G. Brinson
Dr. John P. Marino

May 20, May 26, June 2, 2016

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

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INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH

NMR Interlaboratory Study: NIST-IBBR Team

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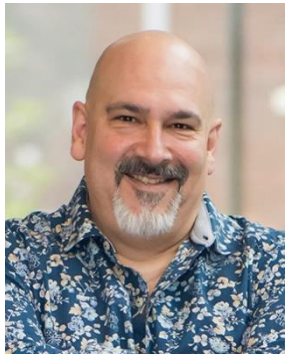
www.ibbr.umd.edu

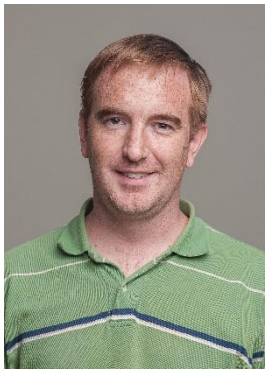
National Institute of Standards and Technology
U.S. Department of Commerce

www.nist.gov

Dr. John Marino, Leader Biomolecular Structure and Function Group



Dr. Frank Delaglio, Software Development



Dr. Robert Brinson, Research Chemist

Dr. Luke Arbogast, Research Chemist

Dr. John Schiel, Head of NISTmAb program

NMR Interlaboratory Study: System Suitability Sample Preparation

Dr. Yves Aubin Geneviève Gingras

Santé Canada Health Canada

***** A special thanks to Ms. Geneviève Gingras for her great effort preparing the system suitability sample, U-^{15}N,20%-^{13}C NIST-Fab *****

Additional Acknowledgement: Non-Uniform Sampling Experimental Protocol

Harvard Medical School

Dr. Gerhard Wagner

Dr. Sven Hyberts

Dr. Haribabu Arthanari

Call #1 Participants (May 20, 10:00 ET)

- NIST Team: Drs. John Marino, Robert Brinson, Luke Arbogast, Frank Delaglio
- Dr. Yves Aubin Health Canada

- Chuck Ellis National Association of Proficiency Testing (NAPT)
- Dr. Carlos Amezcua Baxter Healthcare
- Dr. Clemens Anklin Bruker Biospin
- Dr. Donna Baldsisseri Bruker Biosping
- Dr. Göran Widmalm Stockholm University
- Dr. Kristian Schweimer University of Bayreuth
- Dr. Maurício Sforça Brazilian Biosciences National Laboratory
- Dr. Ana Zeri Brazilian Biosciences National Laboratory
- Dr. Desiree Tsao Momenta Pharmaceuticals
- Thea Stahel ETH Zurich

****For accounting purposes, we need to log who participates on each conference call****

Call #2 Participants (May 26, 16:00 ET)

- NIST Team: Drs. Robert Brinson, Luke Arbogast, Frank Delaglio
- Dr. Yves Aubin — Health Canada

- Chuck Ellis — National Association of Proficiency Testing (NAPT)
- Dr. Brad Jordan — Amgen
- Dr. Mats Wikstroem — Amgen
- Dr. Scott Bradley — Eli Lilly and Company
- Dr. Gregory Ilc — EN-FIST Centre of Excellence
- Dr. David Keire — FDA
- Dr. Kang Chen — FDA
- Dr. Ken Skidmore — Genentech
- Dr. Stu Parnham — MUSC
- Dr. Feng Ni — NRC – Canada
- Dr. John Cort — PNNL
- Dr. Robert Kutlik — Pfizer
- Dr. Teddy Zartler — Pfizer

****For accounting purposes, we need to log who participates on each conference call****

Call #3 Participants (May 26, 20:00 ET)

- NIST Team: Drs. John Marino, Robert Brinson, Luke Arbogast, John Schiel *(tentative),* Frank Delaglio
- Dr. Yves Aubin — Health Canada

- Chuck Ellis — National Association of Proficiency Testing (NAPT)

- Dr. Hiroaki Sasakawa — JEOL, Inc
- Dr. Pavlos Stampoulis — JEOL, Inc
- Dr. Naoyuki Fujii — JEOL, Inc

- Dr. Koichi Kato — Okazaki Institute for Integrative Bioscience
- Dr. Saeko Yanaka — Okazaki Institute for Integrative Bioscience
- (1 additional staff) — Okazaki Institute for Integrative Bioscience

- Dr. Medhi Mobli — The University of Queensland

****For accounting purposes, we need to log who participates on each conference call****

Call #4 Participants (June 2, 10:00 ET)

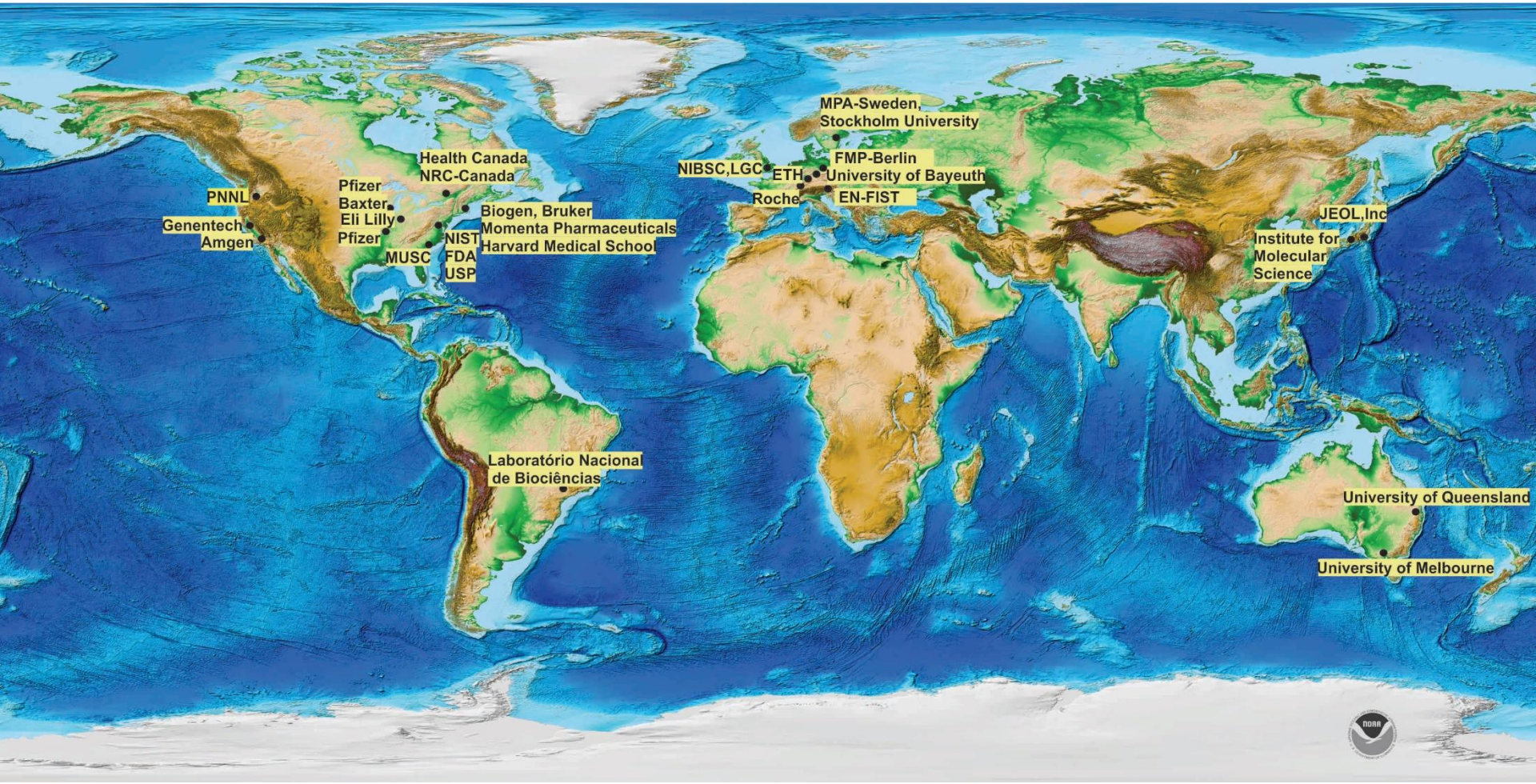
- NIST Team: Drs. John Marino, Robert Brinson, Luke Arbogast, Frank Delaglio
- Dr. Yves Aubin — Health Canada

- Chuck Ellis — National Association of Proficiency Testing (NAPT)

- Dr. Julie Wei — Biogen
- Dr. Kim Colson — Bruker Biospin
- Dr. Alfred Ross — Hoffman LaRoche
- Dr. Peter Schmieder — Leibniz-Institut Fur Molekulare Pharmakologie
- Dr. John Warren — LGC Group
- Dr. Torgny Rundlöf — Medical Products Agency of Sweden
- Dr. Andreas Blomgren — Medical Products Agency of Sweden
- Dr. Tim Rudd — NIBSC
- Dr. David Keizer — The University of Melbourne

****For accounting purposes, we need to log who participates on each conference call****

Participating Institutions



Interlaboratory Study: Goals

- **To harmonize** and **to validate** NMR structural fingerprinting measurements for the assessment of higher order structure of large protein biologics and/or domains from these proteins. The validation of NMR methods for the characterization of the higher order structure of mAbs is specifically targeted due to the large interest of the pharmaceutical industry in using mAbs as platforms for therapeutic development.

Current Timetable

- **2016 May 27** Test Shipments were Successful!
- **2016 June 1** Soft Launch of Online Forum
- **2016 June 2** Conference Call #4,10:00 ET

  June 3 Release of Final Documentation

  June 3, 6, 7 Shipping of NMR kits
- **2016 September 15** – reporting of NMR results to National Association of Proficiency Testing (NAPT)***
- **2016 November** – release of initial data analysis by NIST and 2nd round of conference calls
- **2017 January** – Draft of manuscript

*All data and completed forms must be submitted to NAPT *

(more details in later slides)

Sample #1

100% Methanol-d4, in a sealed tube. This sample is to be used for temperature calibration.

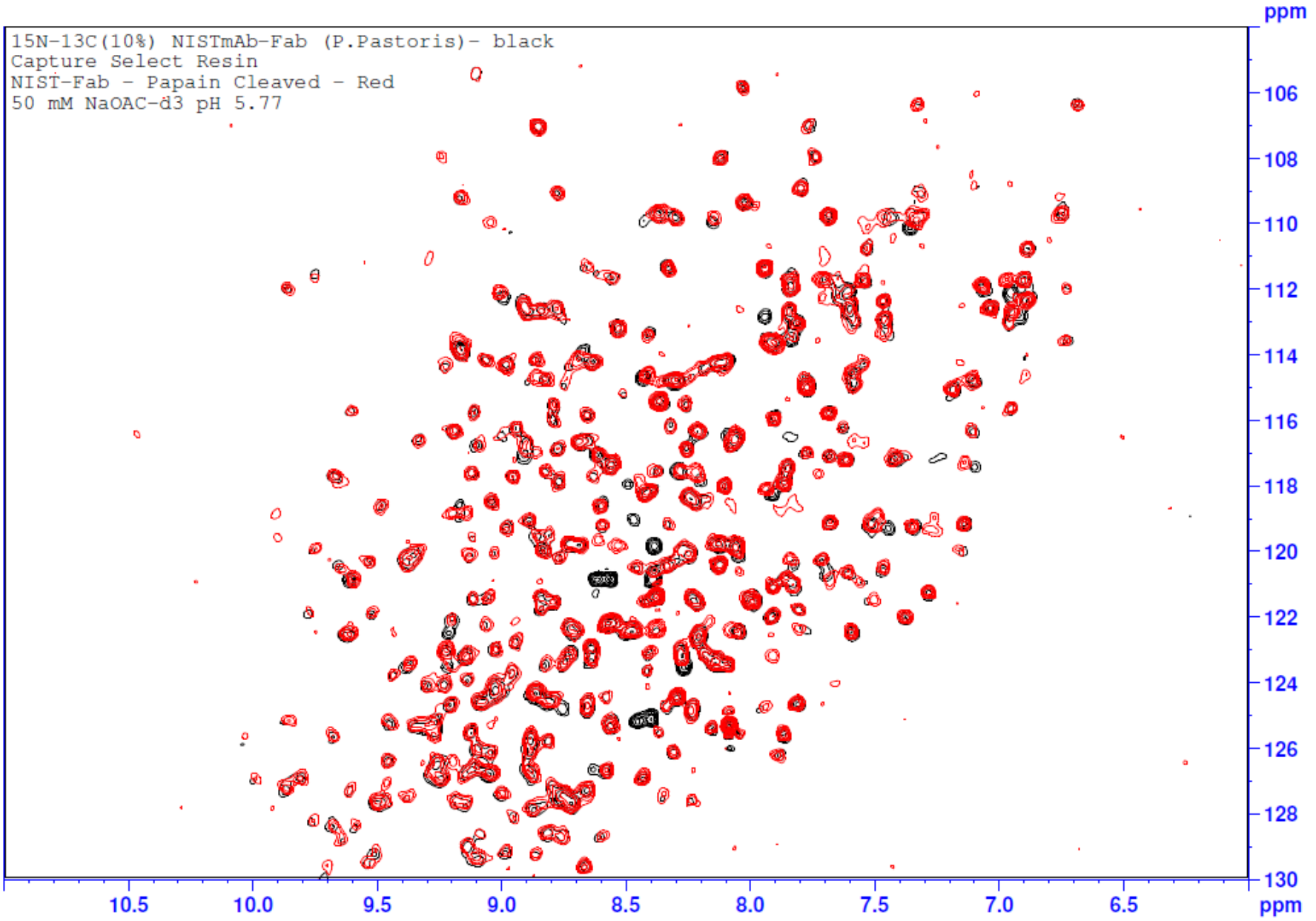
Sample #2: 53 µM U-^{15}N, 20%-^{13}C NIST-Fab, System Suitability Sample (SSS)

- Expressed in *Pichia pastoris* in the laboratory of Health Canada in Ottawa, Ontario, Canada
- This sample will serve as the benchmark for all measurements.
- Contains an extra 4 amino acid signal peptide (EAEA) on both the light and heavy chains that did not get cleaved as expected.
  - This afforded a few extra peaks on the spectrum of the system suitability sample
- Health Canada shipped two batches of the SSS to NIST-IBBR. NIST mixed these batches and will distribute to all partners.

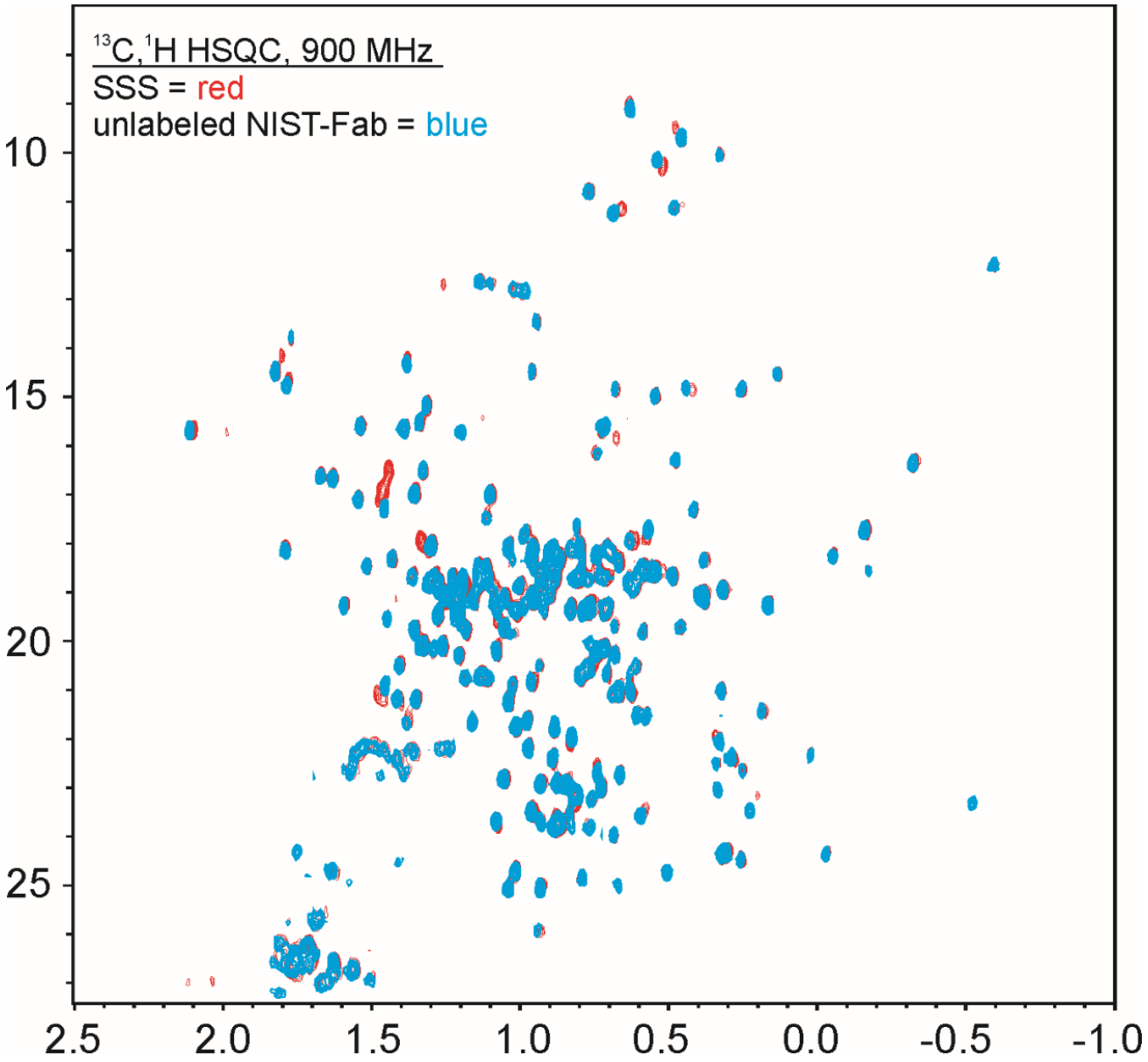
Do these extra residues compromise the purpose of the samples? NO!

1) The purpose SSS is to assess measurement performance on a high sensitivity sample
2) This is also fortuitous. The difference from the unlabeled NIST-Fab will allow for robust testing of chemometric analyses

Amide Fingerprint Overlay



Methyl Fingerprint Overlay



All experimental parameters as given in documentation (Experiment 2A), 37 °C.

Sample #3: 429 µM Unlabeled NIST-Fab

This NIST-Fab sample was derived from the NISTmAb candidate reference material #8670 by papain cleavage. The unlabeled fragment was generated at NIST-IBBR in Rockville, MD, USA.

Buffer Conditions

- 275 µL in 25 mM Bis-Tris-d19, pH 6.0.

- All samples will be provided in vendor-specific 5mm Shigemi Tubes
  - If your lab has both Agilent and Bruker systems, you will receive a Bruker kit. These tubes can be used successfully in Agilent systems.

Shipping and Storage Conditions

- **Shipping** – The NMR kit is designed to keep the samples cold for 3 – 4 days. If the samples arrive at ≤20 °C, immediately refrigerate. If warmer, contact NIST. We may recommend sending you a second kit.

- **Storage** – in a Refrigerator (approximately 5 °C)

- From NMR measurements, the sample has shown good stability at elevated temperatures for several month. However, it is good practice to keep it refrigerated reduce the chance of degradation

- Pre-warm sample to 37 °C for at least 30 minutes to degas samples.

Inquiry regarding shipping and storage conditions courtesy of Carlos Amezcua of Baxter Healthcare

Sample Stability

- Every month during data collection phase NIST will collect ^{15}N and ^{13}C HSQC data at 900 MHz to track stability of NIST-Fab samples.

Criteria for Field Strength Selection

1) No fields lower than 500 MHz
2) At least 3 magnets at each field strength to help ensure data anonymity

*****We will now accept 900 MHz data*****

*****You may collect data on as many magnets as desired. Please do not tell us if you add or subtract a field from what you have already told NIST. This will help ensure data anonymity.*****

Fields higher than 900 MHz *not* accepted in study.

Fields represented in the Study

Field Strength	Number of Magnets
500 MHz	8
600 MHz	18
700 MHz	7
750 MHz	3
800 MHz	6
850 MHz	3
900 MHz	4

- **Total: 49 magnets**

- Both room temperature and cold probes will be used by participants

Measurement Plan: Required Experiments

All required data collected at 37 °C

D. Experimental List– All Experimental Parameters are given in Tables 2 – 5

1. $^{1}H,^{15}N$-gsHSQC: U-^{15}N, 20%-^{13}C-NIST-Fab (Tables 2 – 3)

a. Uniform sampling (US), acquisition time in F1 = 20 ms

2. $^{1}H,^{13}C$-gsHSQC: U-^{15}N, 20%-^{13}C-NIST-Fab (Tables 4 – 5)

a. US 128 total points in F1 (64 complex points)

b. 50% NUS Schedule 1, 128 total reconstructed pts in F1

c. US acquisition time in F1 = 25 ms

d. 50% NUS Schedule 2 Field Dependent, acquisition time in F1 = 25 ms

e. 50% Time Equivalent (TE) NUS, Schedule 2

3. $^{1}H,^{13}C$-gsHSQC: unlabeled NIST Fab Fragment, derived from the NISTmAb (Tables 4 – 5)

a. US acquisition time in F1 = 25 ms

b. 50% NUS Schedule 2, acquisition time in F1 = 25 ms

*****Reminder: calibrate the temperature of your spectrometer*****

*****We are double/triple checking all parameters in the tables.** Please use the Final documentation to run your experiments. **Throw out Version 4.2 after this call*****

*****Submit all data to NAPT*****

Measurement Plan: Optional Experiments

E. Optional ^{1}H ,^{13}C and ^{1}H,^{15}N Experiments

Use the the system suitability sample or the unlabeled NIST-Fab.

1. Suggested ^{1}H,^{13}C Experiments

a. Generate your own NUS sampling schedule and re-run some of the experiments listed in Section D.

b. Run the same experiment at different temperatures. Please use any of the following: 15 °C, 25 °C, or 45 °C. *Remember to calibrate the temperature using the methanol-d4 sample!*

c. SOFAST-HMQC – set the acquisition time to 50 ms in the ^{1}H dimension. (Figure 5)

2. Suggested ^{1}H,^{15}N Experiments

a. Run the same experiment at different temperatures. Please use any of the following: 15 °C, 25 °C, or 45 °C. *Remember to calibrate the temperature using the methanol-d4 sample!*

b. Phase sensitive HSQC: hsqcfpf3gpphwg (Figure 6)

c. SOFAST-HMQC: sfhmqcf3gpph (Figure 5)

*****Reminder: calibrate the temperature of your spectrometer*****

*****Submit all data to NAPT*****

Total Experimental Time for Unlabeled NIST-Fab

Goal: Establish guidance for attaining an average S/N of 10:1 in the final 2D spectrum

Table 2. Guidance on Experimental Time from S/N of 1st Fid of 2D Experiment, measured from NIST 600 MHz spectrometer with cold probe[1]

	$^{1}H,^{15}N$ gsHSQC			$^{1}H,^{13}C$ gsHSQC		
	Experimental Time	1st Fid S/N	Experiment Number	Experimental Time	1st Fid S/N	Experiment Number
SSS	2 h 0 min	61:1	1A	2 h 16 min	51:1	2C
Unlabeled NIST-Fab	N/A	N/A	N/A	18 h 13 min	40:1	3A

[1]All experiments performed with a recycling delay = 1.0 s

1. Collect and Fourier Transform 1st FID of gsHSQC experiment and calculate S/N using the regions defined in Table 8 (*see guidance document*). The target for the ^{1}H-^{15}N gsHSQC is 60:1 and for the ^{1}H-^{13}C gsHSQC is 40:1.
2. Collect full 2D spectrum and calculate S/N from the average of peaks heights in the signal region (set threshold just above noise) by the RMS amplitude of noise peak heights in the noise region (set threshold so noise peaks cover most of noise region) to ensure a minimum S/N of 10:1.

Remember: This is a guide only. You are not required to follow this precise protocol

Query from Scott Bradley of Eli Lilly & Company and Carlos Amezcua of Baxter Healthcare

Blinded Data

- Why blinded data?

  (1) The reason is simple. If a participating company provides very poor quality data in the public domain, that company then would potentially develop a very poor analytical reputation. The blinded data is a protection for the industrial partners in this study.

  (2) Freedom of Information Act (USA law)
    - To assure anonymity of data, we need multiple magnets represented from each field.
- All raw data
  - Converted to NMRPipe format by third party vendor, National Association of Proficiency Testing (NAPT)
  - Given a randomized code
- NIST receives anonymized data from NAPT for spectral processing and analysis

Anonymization Process

- Master V2.xlsm

datafile_name	sample_info	gradient_enhanced	x_car_ppm	y_nucleus	y_sw_hz	y_obs_mhz	y_car_ppm	temperature_k	scans	nus_name
NIST/CH_gHSQC_US/5/ser	15N,13C NIST-Fab	yes	4.721	13C	7692.308	226.351	25.000	323.0	128	
NIST/CH_gHSQC_n50/7/ser	15N,13C NIST-Fab	yes	4.721	13C	7692.308	226.351	25.000	323.0	128	nuslist

- Submit data and all forms to OneDrive, set up by NAPT (*Forms A and B described later*)

- Do not submit data directly to NIST. This will compromise the anonymity of your data.

Administrative Forms: Form A

Form A: The Instrumentation Survey

Please provide the following information about your NMR system. The information provided here will not be used for discussions of head-to-head performance comparisons (*e.g.*, brand-name comparisons).

Please submit this form directly to NAPT, who will compile the information and send NIST a complete list of magnets, consoles, and probes used in the study.

Here are is a NIST example:

Magnet #1

Frequency of ^{1}H	900 MHz
Manufacturer	Bruker BioSpin
Console	AVANCE III
Probe (e.g., cold or RT probe; double or triple resonance; pulsed gradient capability)	5mm TCI ^{1}H-^{13}C/^{15}N/D Cryoprobe™, Z-gradients

Please add as many magnets as you used for the study.

Magnet #1

Frequency of ^{1}H	
Manufacturer	
Console	
Probe (e.g., cold or RT probe; double or triple resonance; pulsed gradient capability)	

Available at NMR Study Online Forum: See slide 28

Administrative Forms: Form B

Form B: Authorship and Acknowledgement Form

Please provide a list of authors in the form below. Add rows, as necessary. The personal address will be used only when a work address fails, and we need to obtain the scientist's signature on a copyright agreement.

Please add your acknowledgements. Please understand that these acknowledgements will be edited to conform to the policies of the journal. NAPT will release the author list to NIST after all anonymized data has been given to NIST.

Here is a NIST example:

Name	Institution, Department , Address	Institutional E-Mail/ Personal email	Contribution to Study (Succinct description please)
John P. Marino	Institute of Bioscience and Biotechnology Research, National Institute of Standards and Technology, 9600 Gudelsky Drive, Rockville, MD 20850 USA	john.marino@nist.gov, jmarino2@umd.edu	Study organizer, analyzed data, edited the manuscript
Robert G. Brinson	Same as above	robert.brinson@nist.gov, robert_brinson@hotmail.com	Study organizer, analyzed data, edited the manuscript
Acknowledgements: 1) We acknowledge the support by NIST Biomanufacturing Initiative and NIST and W.M. Keck for support of Biomolecular NMR instrumentation.			

Name	Institution, Department , Address	Institutional E-Mail/ Personal email	Contribution to Study (Succinct description please)
Aknowledgements: 1)			

Available at NMR Study Online Forum: See slide 28

Procedures for Communication: NMR Interlaboratory Study Online Forum

- Hosted by the Institute for Bioscience and Biotechnology Research (IBBR) in Rockville, MD, USA
- Participation in Forum by Invitation only
- Password protected – you will receive a guest IBBR account
- Purpose:
  - Repository of study documentation, administrative forms (Form A and Form B), NUS schedules, AV1 NUS pulse sequence, pulse sequence Code
  - Technical Discussion. Once you establish your account, begin and comment on discussion threads from your email
- Online forum was launched June 1. An invitation should have come from webmaster@ibbr.umd.edu
- Do not share your data code, or your confidentiality will be compromised

Submit to NAPT via OneDrive

1) All Data and acquisition files, zipped
2) Master_V2.xlsm
3) Form A
4) Form B

Procedures for Communication: Questions about Your Data

- **Specific questions or problems after analysis of your data**: In order to maintain anonymity of data, specific questions regarding the analysis of your data should be done through NAPT.
- NAPT will remove identifiable information from your inquiry and send it anonymously to NIST.
- NIST will send its answer to NAPT who will forward it to the appropriate institution.

Data Processing and Analysis

- All blinded data will be processed by nmrPipe at NIST-IBBR
- Data Analysis
  - Combined Chemical Shift Deviation (CCSD)
  - Principal Components Analysis (PCA)
  - Other multivariate analyses in development with NIST mathematicians using other datasets. The best algorithms will be applied to this study.
  - Other suggestions welcome

Emailed Questions

??Chemical Shift Referencing??

- There is a descrepancy in frequency referencing depending on the spectrometer calibration
  - ^{1}H: 0.07 ppm difference (4.70 versus 4.77 ppm)
  - ^{13}C: 2.7 ppm difference

- This discrepancy will be rectified during spectral processing with nmrPipe.

- This information will be added to the study documentation

Question courtesy of Dr. Medhi Mobli of the University of Queensland

?? E-mail Question ??

- From Kang Chen of the FDA:

Among [our questions] the salient one is regarding the Fab at 50mg/mL. As we all know, a realistic IgG drug product is at 10 mg/mL, roughly 6.7 mg/mL for Fab. So is there any way to harmonize?

- Response: Many IgG drugs are formulated at higher concentrations
- Purpose of study: harmonization of the NMR method using a drug-like molecule
- Other comments?

??? Questions ???

- A compilation of slides from the conference calls will be posted on the Online Forum after *all* conference calls are completed

- Please confirm your shipping address via email.

Extra Slides

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

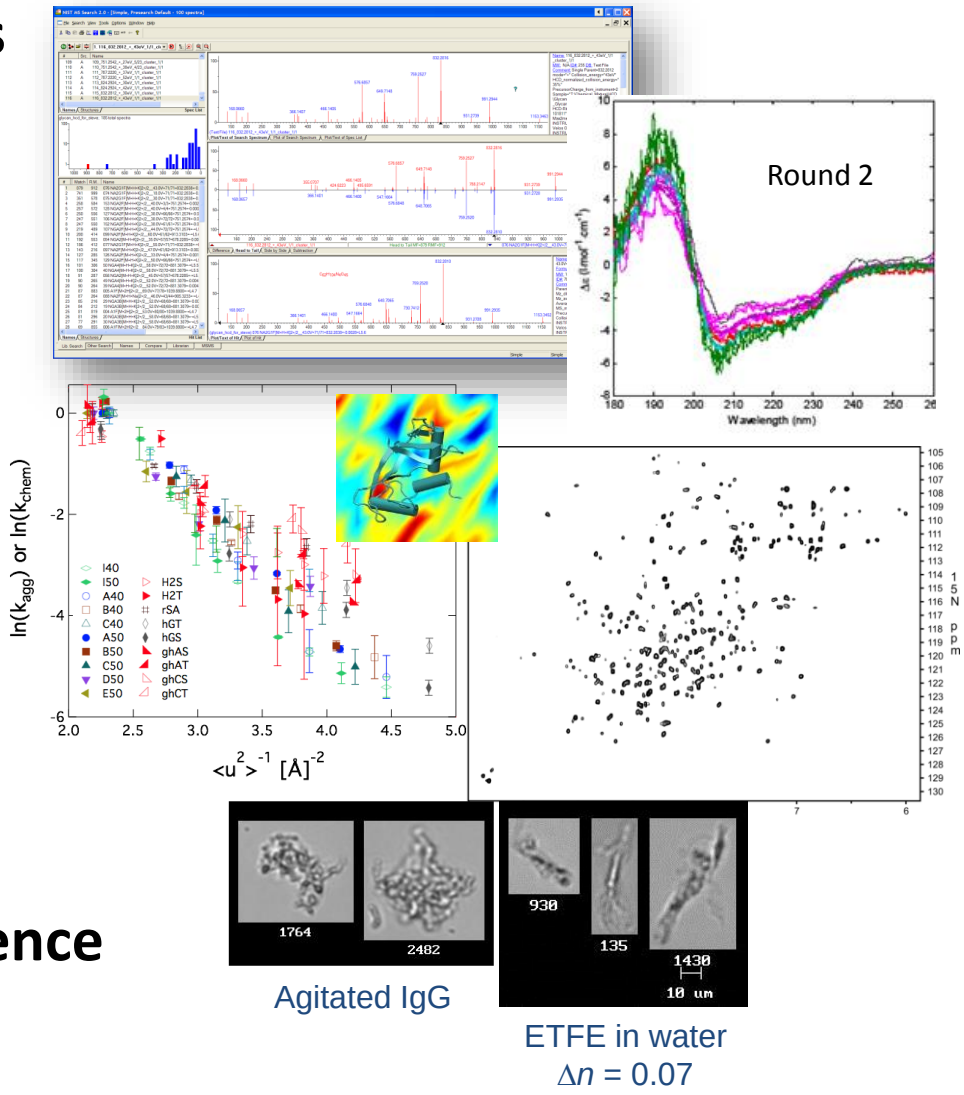
to promote U.S. innovation and industrial competitiveness by advancing measurement science, standards, and technology in ways that enhance economic security and improve our quality of life

NIST Program in Biomanufacturing Metrology

Measurement Science, Standards and Technology for:

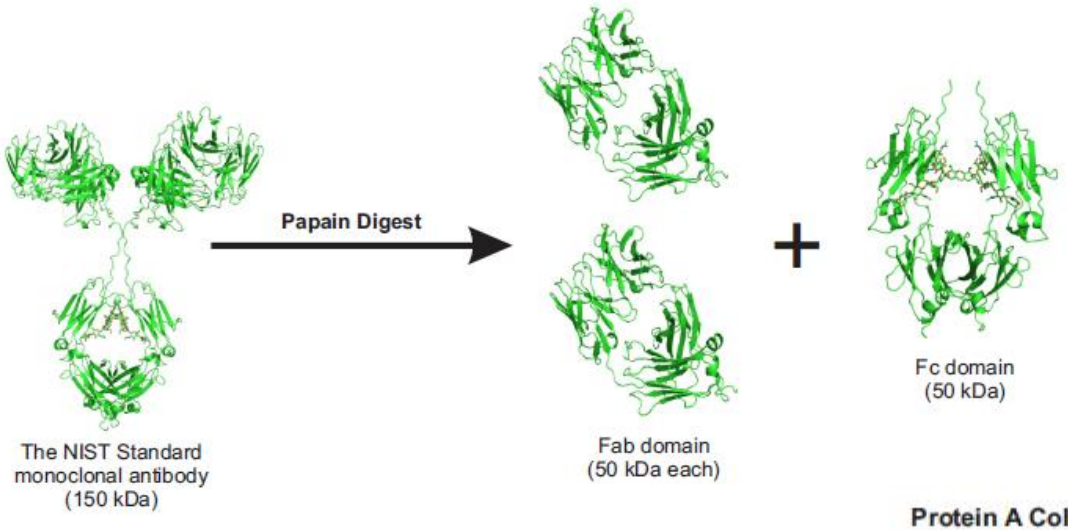
- **Protein Structure**
  - **Primary, Secondary and Higher-order structure**
  - **PTMs (glycosylation)**
- **Protein Stability, Aggregation & particulates**
- **Measurement tools & science for bioprocess development**

Future: NISTmAb Standard Reference Material (SRM)



Program Leader: Dr. Mike Tarlov michael.tarlov@nist.gov

Fab/Fc fragmentation can be accomplished by facile papain digest



- digest NISTmAb on immobilized papain resin
- Protein A affinity chromatography
- centrifugation over 30 kDa and 100 kDa filters.
- Confirmed by mass spec