Validating Higher Order Structure of Biologics using Nuclear Magnetic Resonance (NMR)

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CASSS HOS 2019, April 10, San Mateo CA

Outline

- Introduction
- Precision, Sensitivity, …
- Data Acquisition
- Availability

Christian Fischer

- Biologics HOS Software

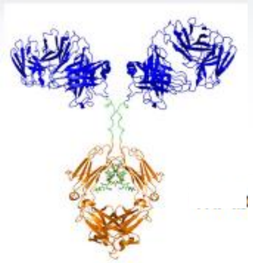
Mike Bernstein

Emerging Technology

Solution for high precision, high resolution routine assessment of HOS

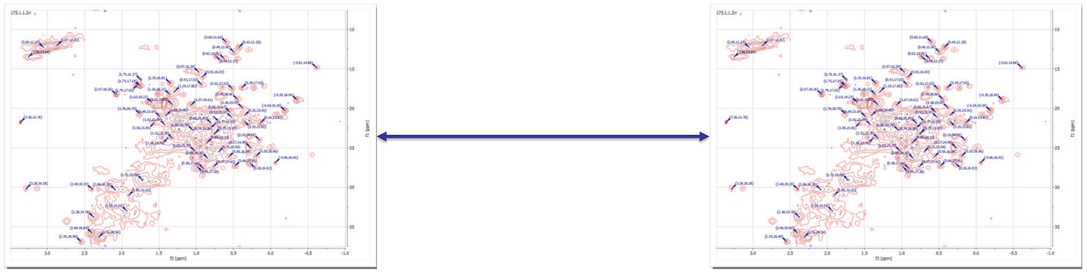
Evaluation by NMR: How

Validated Material



New Batch
- Production
- R&D, optimized conditions

Correct Structure ?



BiologicsHOS SOP’s
intact molecule at **natural abundance**

BiologicsHOS Software
evaluate differences at
atomic resolution

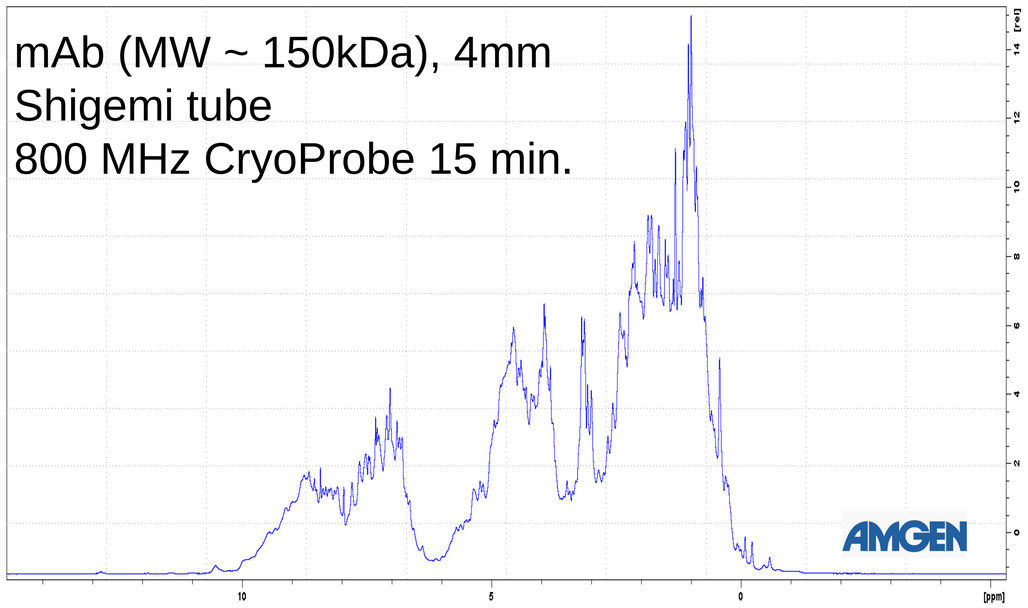
BiologicsHOS: SOP’s + Software

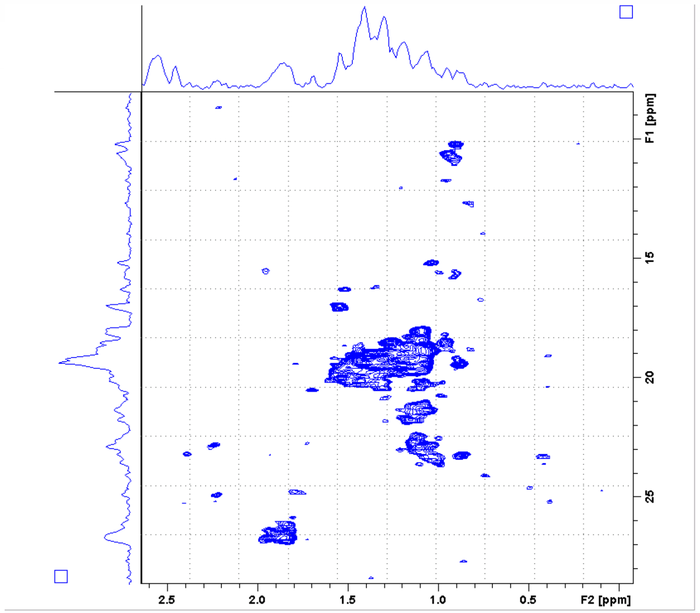
- NMR provides a solution to the need for **atomic resolution** in HOS characterization;
- NMR can be applied to **intact molecules** at **natural abundance**, with acquisition times reasonable for **routine** analysis;
- NMR is a **high precision** analytical technique that is the ideal input for **robust statistical** tools required for HOS evaluation;

1D and 2D NMR

HOS different?

mAb (MW ~ 150kDa), 4mm
Shigemi tube
800 MHz CryoProbe 15 min.



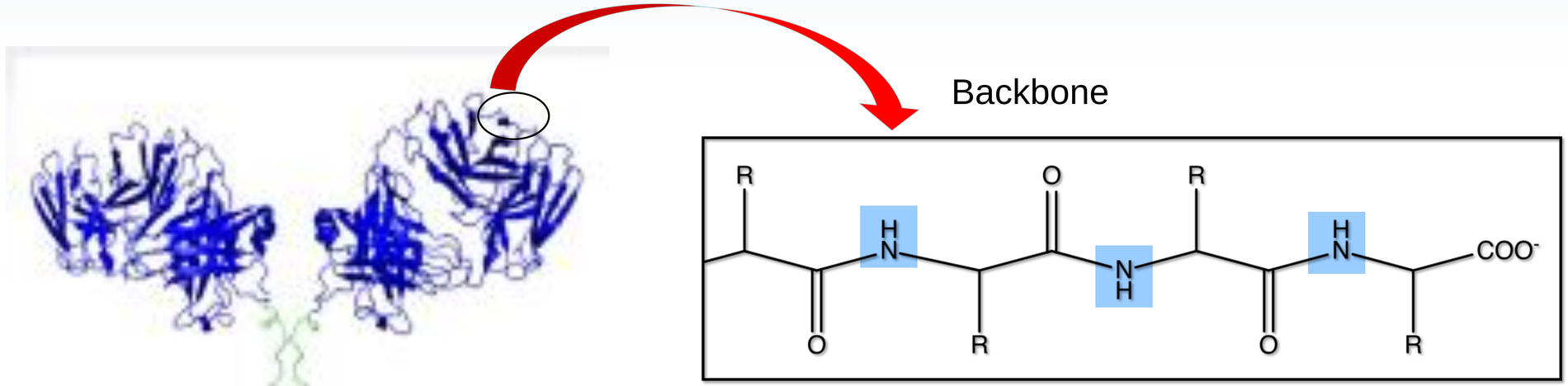


200μM IgG(MW ~ 150kDa)

600MHz CryoProbe 4.5hr

Where are they different?

2D NMR
Protein Fold Detection



^{1}H ^{15}N HSQC: “Gold Standard”
amide correlation for each amino acid

Challenges:

- ^{15}N natural abundance: 0.37 %
- Broad lines due to long rotational correlation time

2D NMR: Focus on Methyl Signals

Amino Acids containing Methyl groups:
Ala, Ile, Leu, Met, Thr, Val

Methyl groups present throughout the primary sequence and the spectral dispersion of the 2D methyl fingerprint spectrum is a sensitive monitor of HOS at atomic resolution

*John Marino, AT-Europe CASSS, 2016

Advantages ^{1}H ^{13}C HSQC of Methyl Signals

- Natural abundance
  - ^{13}C: 1.1 %
  - ^{15}N: 0.37%
- Methyl groups: sharp signals

Challenges

- Additions like Tween (Polysorbate) overlap with methyl signals

Highly Reproducible – 2D ^{1}H-^{13}C HSQC Spectra

Enabling adoption of 2D-NMR for the higher order structure assessment of mAb therapeutics: a multi-national, inter-laboratory comparison*

Robert G. Brinson[1,*], John P. Marino[1], Frank Delaglio[1], Luke W. Arbogast[1], Ryan M. Evans[2], Anthony Kearsley[2], Geneviève Gingras[3], Houman Ghasriani[3], Yves Aubin[3], Gregory K. Pierens[4], Xinying Jia[4], Mehdi Mobli[4], Hamish G. Grant[5], David W. Keizer[5], Kristian Schweimer[6], Jonas Ståhle[7], Göran Widmalm[7], Edward R. Zartler[8], Chad W. Lawrence[9], Patrick N. Reardon[9,†], John R. Cort[9], Ping Xu[10], Feng Ni[10], Saeko Yanaka[11], Koichi Kato[11], Stuart R. Parnham[12], Desiree Tsao[13], Andreas Blomgren[14], Torgny Rundlöf[14], Nils Trieloff[15], Peter Schmieder[15], Alfred Ross[16], Ken Skidmore[17], Kang Chen[18], David Keire[18], Darón I. Freedberg[19], Thea Suter-Stahel[20], Gerhard Wider[20], Gregor Ilc[21,22], Janez Plavec[21,22], Scott A. Bradley[23], Donna M. Baldisseri[24], Mauricio Luis Sforça[25], Ana Carolina de Mattos Zeri[26], Julie Yu Wei[27], Christina M. Szabo[28], Carlos A. Amezcua[28], John B. Jordan[29], Mats Wikström[30]

26 industrial, government and academic laboratories worldwide, 39 spectrometers, 451 2D spectra

Identical samples

Identical acquisition parameters

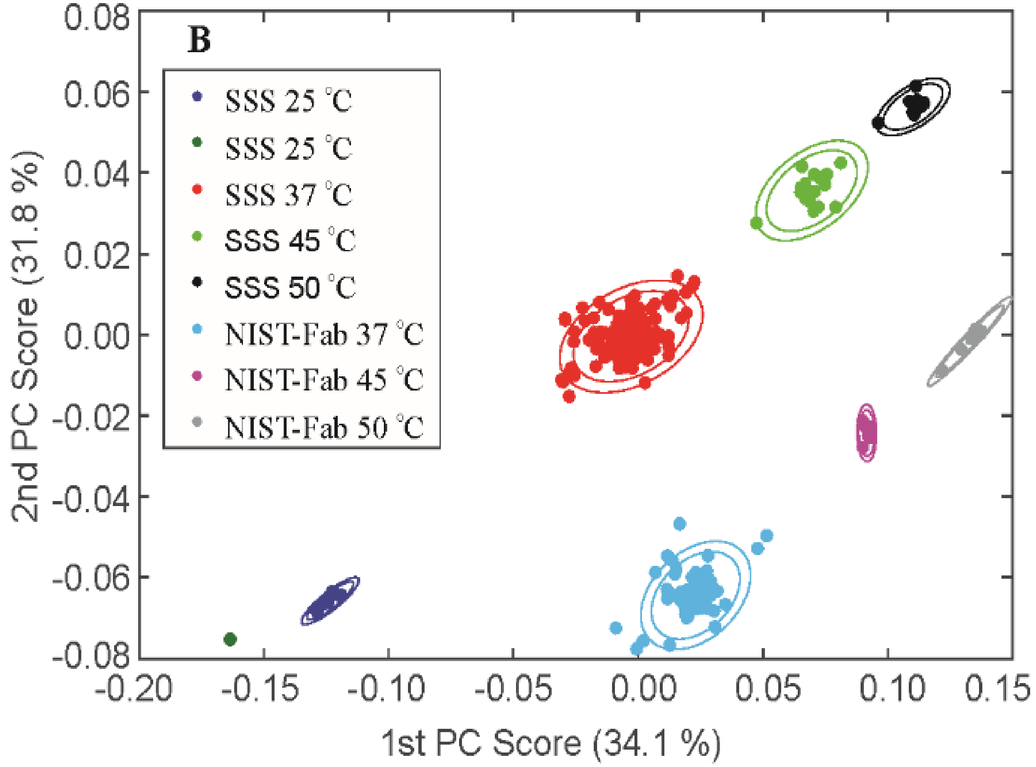
500 – 900 MHz spectrometers, virtually all with cryoprobes

***mAbs Vol. 11, 2019, issue 1, pp. 94-105**

High Precision: 2D methyl HSQC

NIST inter-laboratory results

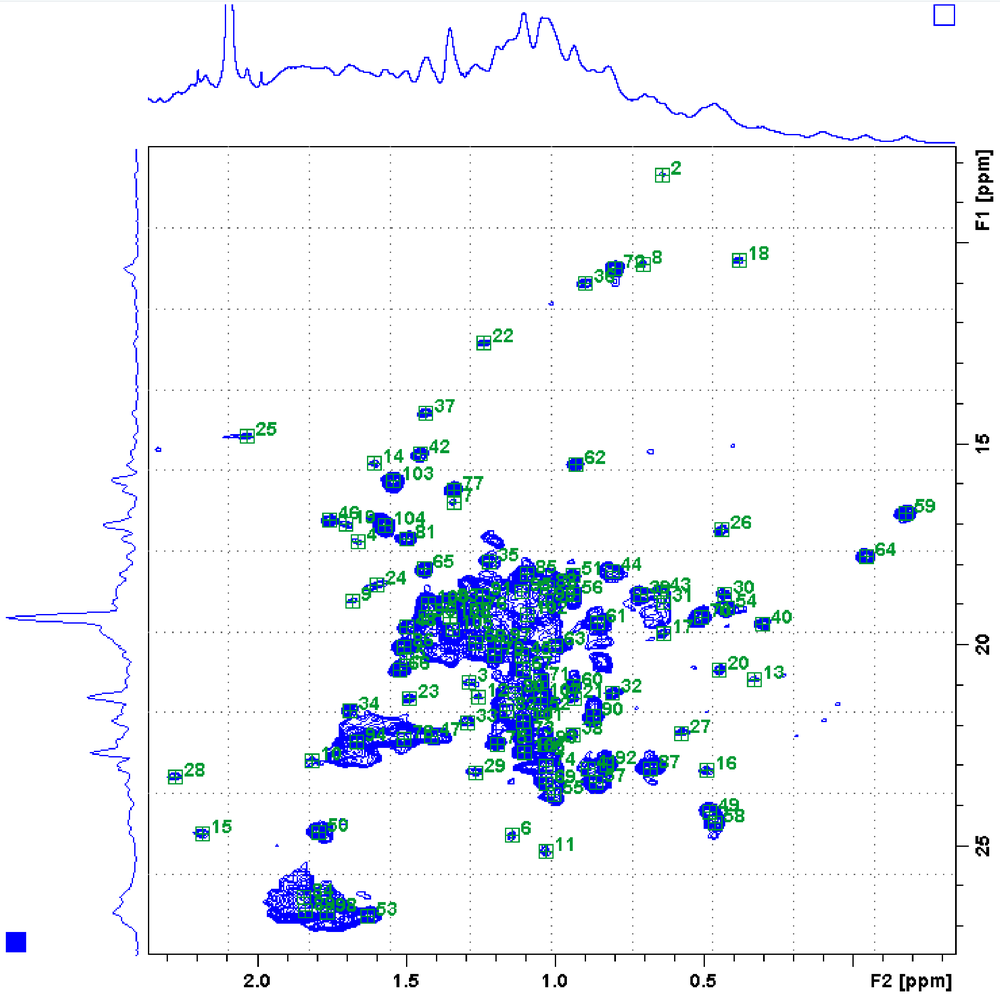
PCA on peak positions



Clustered PCA scatter plots of all peak lists from 354 ^{13}C-HSQC

Outliers from T deviations, inadequate S/N or resolution (sfHMQC)

2D NMR methyl fingerprint intact mAb



~300µM ~ 150kDa mAb at 600MHz
TCI-F CP
15hr US ALSOFAST-HMQC
108 peaks

Excipient signal at 2.1, 23.1 ppm

removal by SIERRA filter*

*

Journal of Biomolecular NMR (2018) 72:149–161
https://doi.org/10.1007/s10858-018-0214-1

ARTICLE

Selective suppression of excipient signals in 2D ^{1}H–^{13}C methyl spectra of biopharmaceutical products

Luke W. Arbogast[1] · Frank Delaglio[1] · Joel R. Tolman[2] · John P. Marino[1]

Further Improvements in Acquisition

~300μM ~ 150kDa	15 h	600 MHz
	7.5 h	600 MHz + 50% NUS
	5.15 h	800 MHz + 50% NUS

First version of a script for automated setup of SIERRA filtered experiment available

~400μM ~60 kDa Protein; no excipient removal; **1.5h / 1h**

NMR Sensitivity Compared to Other Methods

Talk by Fabio Baroni, EMD Serono

“Application of 1D and 2D NMR to HOS Characterization Studies: How to Make NMR a Routine Technique”

Software: Our Vision for the Future

Arxpan

Automated and intelligent acquisition

Data analysis Vendor independent

Common Library

Development Teams

Acquisition
- Donna Baldisseri (US)
- Francesca Benevelli (IT)
- Daniel Mathieu (DE)
- Matteo Pennestri (UK)
- Martial Piotto(FR)

Analysis
Mike Bernstein (UK)
Ian Clegg (UK)
Christian Fischer (DE)
Michael Fey (US)

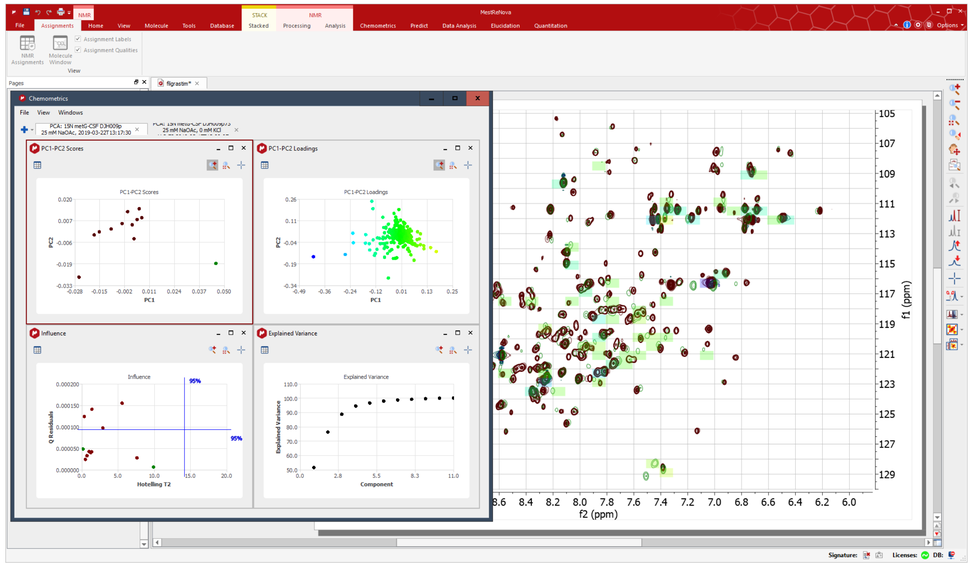
Software (Mestrelab, Spain)
- Agustin Barba
- Noa Campos
- Isaac Iglesias
- Nikolay Larin
- Joaquín Ossorio

BiologicsHOS Software

Mestrelab Research
chemistry software solutions

Three published and accepted methods are available (2D NMR)

- **ECHOS** – simple representation of spectral differences
- **CCSD** – represents shifts in peak positions
- **PCA** – unsupervised chemometrics



1D PROFILE method
Amgen

Profiling Formulated Monoclonal Antibodies by ^{1}H NMR Spectroscopy

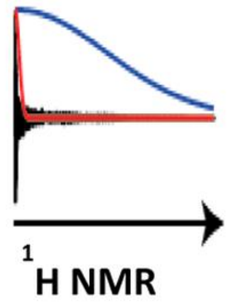
Leszek Poppe,[†,*] John B. Jordan,[†] Ken Lawson,[‡] Matthew Jerums,[‡] Izydor Apostol,[‡] and Paul D. Schnier[†]

[†]Molecular Structure and Characterization and [‡]Process and Pr... lopment, Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320, United States

Anal. Chem. 2013, 85, 9623−9629

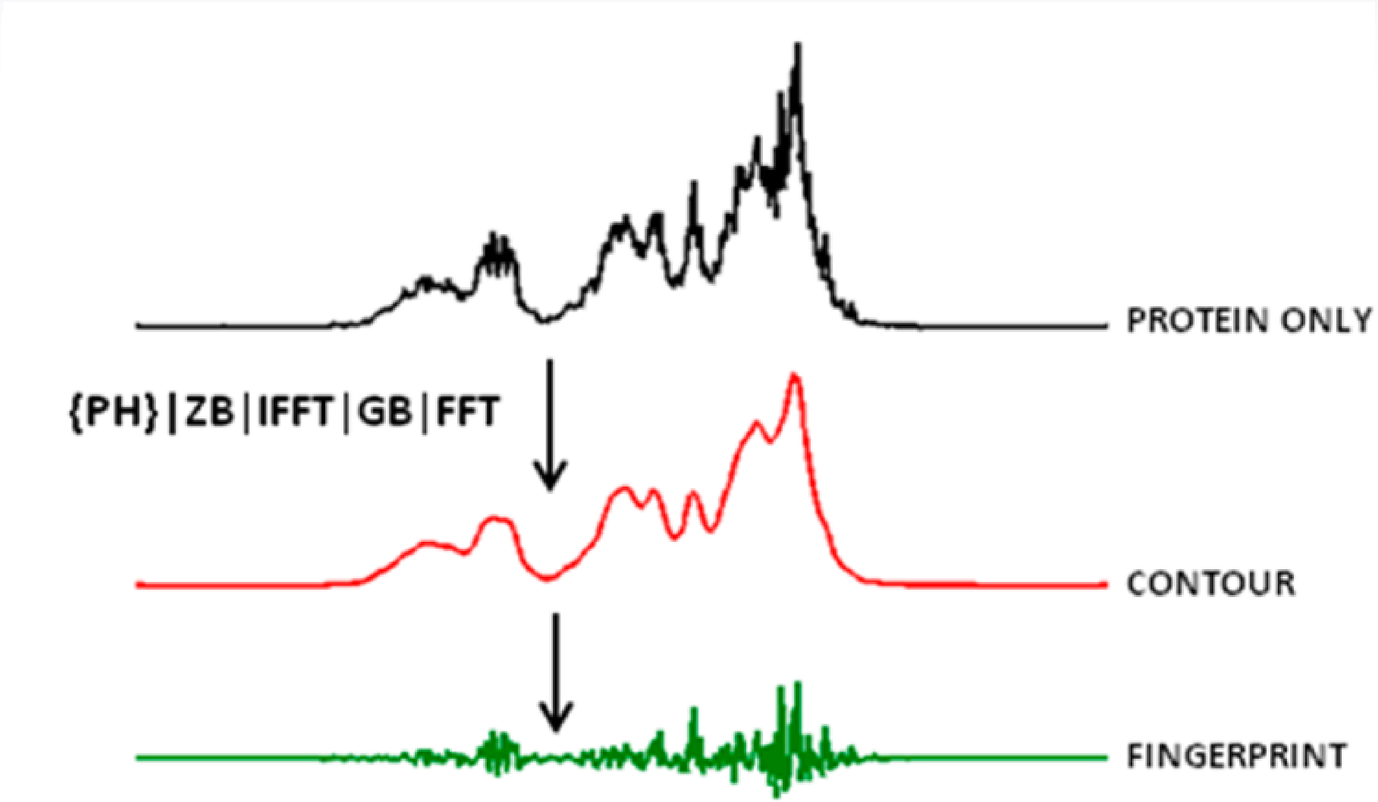
Already Available

AssureNMR



PROtein FIngerprint by Line shape Enhancement method (PROFILE)

HOS of Biologics – 1D PROFILE method

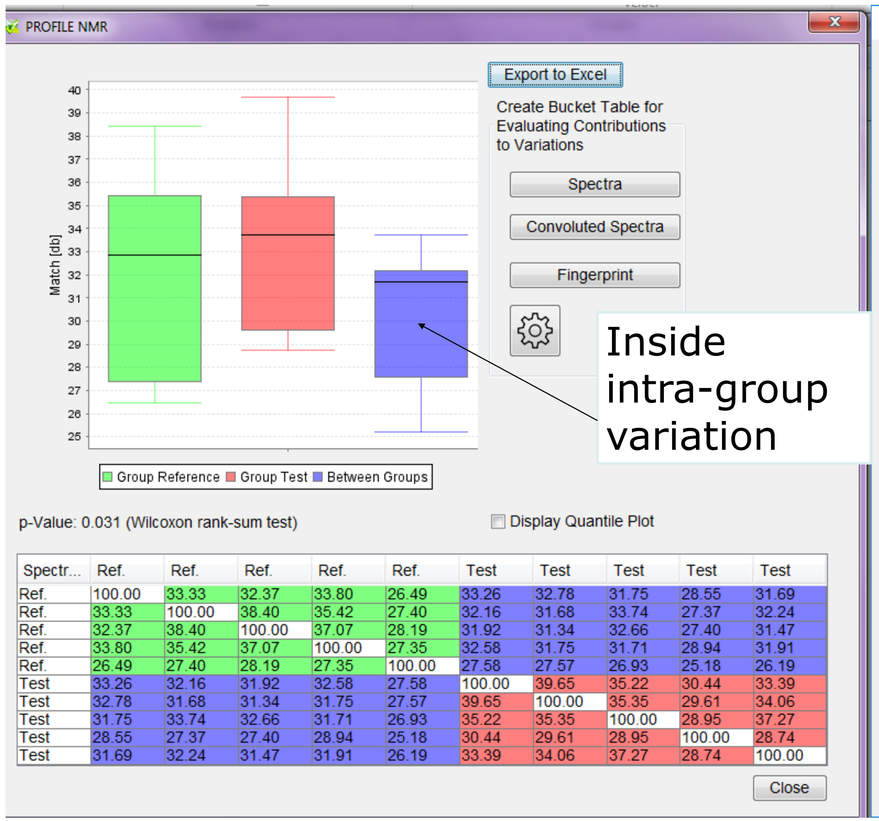


HOS of Biologics – 1D PROFILE method

Compare “Pass” and “Fail” Samples

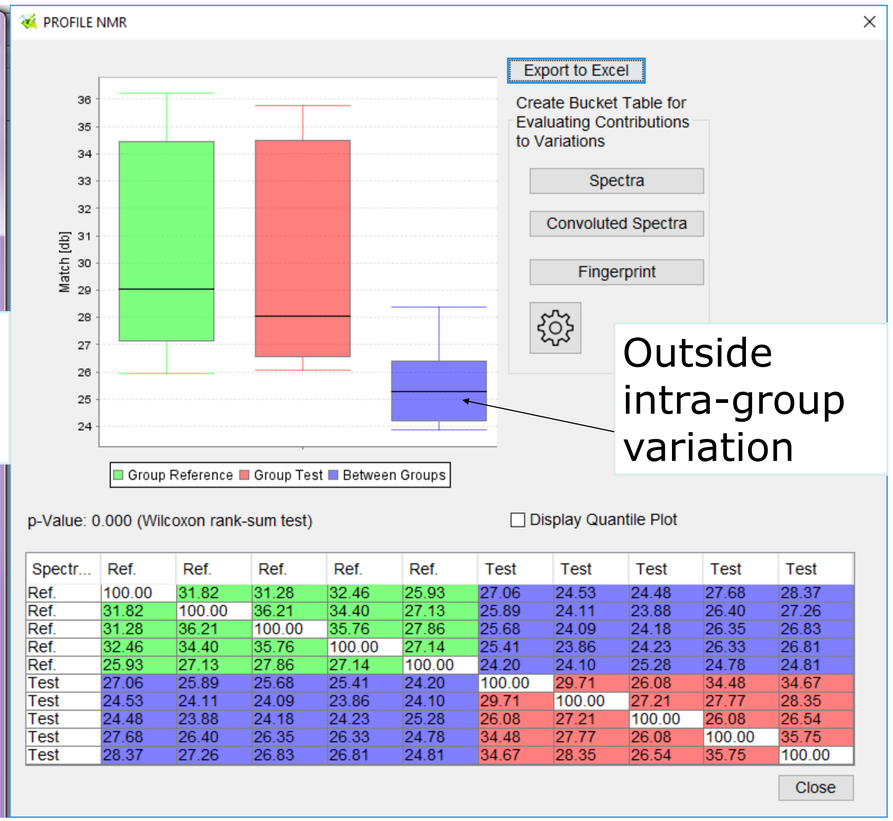
5 reference spectra and 5 test spectra

‘Pass’ Spectra



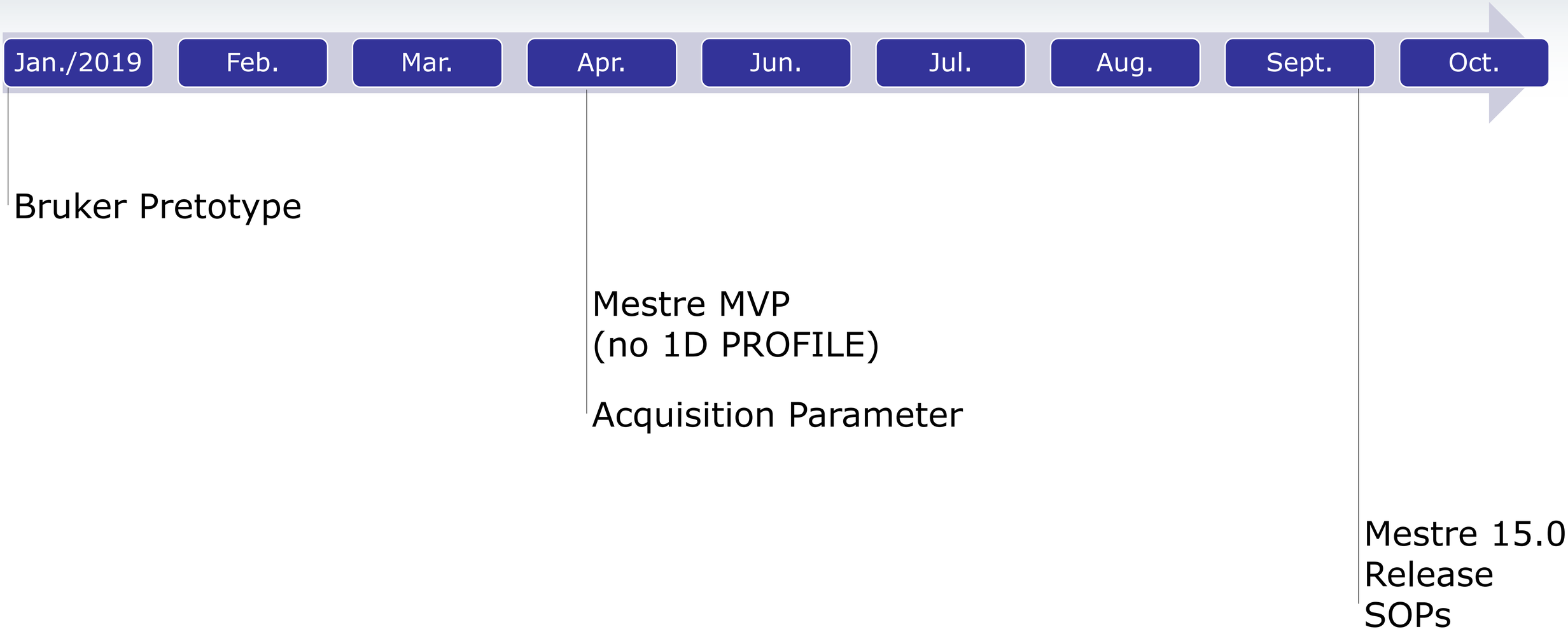
Spectr...	Ref.	Ref.	Ref.	Ref.	Ref.	Test	Test	Test	Test	Test
Ref.	100.00	33.33	32.37	33.80	26.49	33.26	32.78	31.75	28.55	31.69
Ref.	33.33	100.00	38.40	35.42	27.40	32.16	31.68	33.74	27.37	32.24
Ref.	32.37	38.40	100.00	37.07	28.19	31.92	31.34	32.66	27.40	31.47
Ref.	33.80	35.42	37.07	100.00	27.35	32.58	31.75	31.71	28.94	31.91
Ref.	26.49	27.40	28.19	27.35	100.00	27.58	27.57	26.93	25.18	26.19
Test	33.26	32.16	31.92	32.58	27.58	100.00	39.65	35.22	30.44	33.39
Test	32.78	31.68	31.34	31.75	27.57	39.65	100.00	35.35	29.61	34.06
Test	31.75	33.74	32.66	31.71	26.93	35.22	35.35	100.00	28.95	37.27
Test	28.55	27.37	27.40	28.94	25.18	30.44	29.61	28.95	100.00	28.74
Test	31.69	32.24	31.47	31.91	26.19	33.39	34.06	37.27	28.74	100.00

‘Fail’ Spectra



Spectr...	Ref.	Ref.	Ref.	Ref.	Ref.	Test	Test	Test	Test	Test
Ref.	100.00	31.82	31.28	32.46	25.93	27.06	24.53	24.48	27.68	28.37
Ref.	31.82	100.00	36.21	34.40	27.13	25.89	24.11	23.88	26.40	27.26
Ref.	31.28	36.21	100.00	35.76	27.86	25.68	24.09	24.18	26.35	26.83
Ref.	32.46	34.40	35.76	100.00	27.14	25.41	23.86	24.23	26.33	26.81
Ref.	25.93	27.13	27.86	27.14	100.00	24.20	24.10	25.28	24.78	24.81
Test	27.06	25.89	25.68	25.41	24.20	100.00	29.71	26.08	34.48	34.67
Test	24.53	24.11	24.09	23.86	24.10	29.71	100.00	27.21	27.77	28.35
Test	24.48	23.88	24.18	24.23	25.28	26.08	27.21	100.00	26.08	26.54
Test	27.68	26.40	26.35	26.33	24.78	34.48	27.77	26.08	100.00	35.75
Test	28.37	27.26	26.83	26.81	24.81	34.67	28.35	26.54	35.75	100.00

Availability



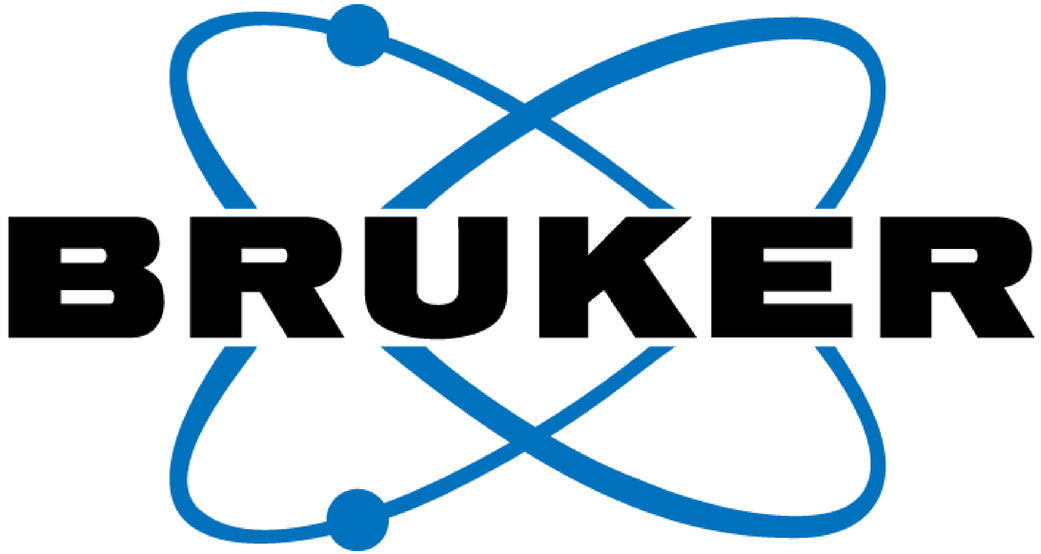
Do you want more information on BiologicsHOS ?

Do you want to test it now?

biologicsHOS@bruker.com

Mestrelab: http://mestrelab.com/software/biologicals-hos/

Bruker: https://www.bruker.com/products/mr/mr-in-pharma/quality-assessment-of-biologics-drugs.html



Innovation with Integrity