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

Dr. Christian Fischer, Senior Staff Scientist, Bruker BioSpin GmbH
CASSS HOS 2019, April 10, San Mateo CA
Outline

• Introduction
• Precision, Sensitivity, ...
• Data Acquisition
• Availability

• Biologics HOS Software

Christian Fischer

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
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

$^1H\ ^{15}N$ 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

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

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

Robert G. Brinson¹, John P. Marino¹, Frank Delaglio¹, Luke W. Arbogast¹, Ryan M. Evans², Anthony Kearsley², Geneviève Gingras³, Houman Ghasriani³, Yves Aubin³, Gregory K. Pierens⁴, Xinying Jia⁴, Mehdi Mobli⁴, Hamish G. Grant⁵, David W. Keizer⁵, Kristian Schweimer⁶, Jonas Ståhle⁷, Göran Widmalm⁷, Edward R. Zartler⁸, Chad W. Lawrence⁹, Patrick N. Reardon⁹, John R. Cort⁹, Ping Xu¹⁰, Feng Ni¹⁰, Saeko Yanaka¹¹, Koichi Kato¹¹, Stuart R. Parnham¹², Desiree Tsao¹³, Andreas Blomgren¹⁴, Torgny Rundlöf¹⁴, Nils Trieloff¹⁵, Peter Schmieder¹⁵, Alfred Ross¹⁶, Ken Skidmore¹⁷, Kang Chen¹⁸, David Keire¹⁸, Darón I. Freedberg¹⁹, Thea Suter-Stahel²⁰, Gerhard Wider²⁰, Gregor Ilc²¹, Janez Plavec²¹, Scott A. Bradley²², Donna M. Baldisseri²⁴, Mauricio Luis Sforça²⁵, Ana Carolina de Mattos Zeri²⁶, Julie Yu Wei²⁷, Christina M. Szabo²⁸, Carlos A. Amezcua²⁸, John B. Jordan²⁹, Mats Wikström³₀

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

Selective suppression of excipient signals in 2D \(^1\)H–\(^13\)C methyl spectra of biopharmaceutical products

Luke W. Arbogast\textsuperscript{1}  -  Frank Deleggio\textsuperscript{1}  -  Joel R. Tolman\textsuperscript{2}  -  John P. Marino\textsuperscript{1}
### Further Improvements in Acquisition

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Time</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\sim 300\mu M \sim 150\text{kDa})</td>
<td>15 h</td>
<td>600 MHz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(600 MHz + 50% \text{NUS})</td>
</tr>
<tr>
<td>(\sim 400\mu M \sim 60 \text{kDa}) Protein; no excipient removal;</td>
<td>1.5h / 1h</td>
<td>800 MHz + 50% NUS</td>
</tr>
</tbody>
</table>

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

Automated and intelligent acquisition

Data analysis Vendor independent

Common Library

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

- **ECHOS** – simple representation of spectral differences
- **CCSD** – represents shifts in peak positions
- **PCA** – unsupervised chemometrics
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 Product Development, Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320, United States

Anal. Chem. 2013, 85, 9623–9629

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

‘Fail’ Spectra

Inside intra-group variation

Outside intra-group variation
Availability

Bruker Prototype

- Mestre MVP (no 1D PROFILE)
- Acquisition Parameter

Mestre 15.0 Release SOPs
Do you want more information on BiologicsHOS? Do you want to test it now?

biologicsHOS@bruker.com

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