7th International Symposium on Higher Order Structure of Protein Therapeutics (HOS 2018)

Symposium Co-Chairs:
Guodong Chen, *Bristol-Myers Squibb Company*
David Keire, *CDER, FDA*

April 9-11, 2018
Omni Providence Hotel
Providence, RI USA

*Organized by*
# Table of Contents

Program Partners, Exhibitors, and Media Partners .................................................3  
Acknowledgements ........................................................................................................6  
Student Travel Grants and Make the CASSS Connection .......................................7  
Scientific Final Program Summary .............................................................................8  
Roundtable Discussion Topics ......................................................................................15  
Plenary and Session Abstracts .....................................................................................17  
Poster Abstracts ..............................................................................................................49
The organizing committee gratefully acknowledges the program partners and exhibitors for their generous support of the 7th International Symposium on Higher Order Structure of Protein Therapeutics

**Strategic Platinum Program Partner**

MedImmune, A member of the AstraZeneca Group

**Strategic Gold Program Partners**

Eli Lilly and Company
Pfizer, Inc.

**Strategic Silver Program Partner**

Biogen

**Platinum Program Partner**

Amgen Inc.

**Silver Program Partner**

Genentech, a Member of the Roche Group

**Bronze Program Partners**

FUJI Film Diosynth Biotechnologies
Sanofi
Exhibitor Partners

Beckman Coulter Life Sciences
Bruker BioSpin
Charles River Laboratories
NanoImaging Services, Inc.
Postnova Analytics Inc.
Protein Metrics Inc.
Thermo Fisher Scientific
Waters Corporation
The organizing committee gratefully acknowledges the following media partners for their promotional consideration of HOS 2018.

Media Partners

American Laboratory/labcompare
American Pharmaceutical Review
The Analytical Scientist
BioProcessing Journal
Genetic Engineering & Biotechnology News
LCGC North America
The Pathologist
Pharmaceutical Outsourcing
Technology Networks
Acknowledgements

Symposium Co-Chairs:
Guodong Chen, *Bristol-Myers Squibb Company*
David Keire, *CDER, FDA*

Scientific Program Committee:
Wasfi Al-Azzam, *GlaxoSmithKline*
Yves Aubin, *Health Canada*
Alexander Bepperling, *Novartis*
Katherine Bowers, *FUJIFILM Diosynth Biotechnologies*
Shawn Cao, *Amgen Inc.*
Natalie Ciaccio, *BioMarin Pharmaceuticals Inc.*
Galahad Deperalta, *Genentech, a Member of the Roche Group*
Tom Lerch, *Pfizer, Inc.*
Krishna Mallela, *University of Colorado Anschutz Medical Campus*
Anders Nielsen, *Novo Nordisk A/S*
Gang (Gary) Ren, *Lawrence Berkeley National Laboratory*
John Schiel, *National Institute of Standards and Technology (NIST)*
William Weiss, *Eli Lilly and Company*

Audio-Visual:
Michael Johnstone, *MJ Audio-Visual Productions*

CASSS Staff:
Karen Bertani, Director of Meetings
Amy Cano, Administrative Assistant
Stephanie Flores, Executive Director
Julie Fowle, Meeting Coordinator
Anna Lingel, Meeting Coordinator
Carisa Lubeck, Business Information Analyst
Renee Olson, Senior Program Manager
Catherine Stewart, Finance Manager
CASSS is pleased to provide a limited number of student travel grants for Ph.D. students and post-docs who present applicable posters at the 7th International Symposium on Higher Order Structure of Protein Therapeutics (HOS 2018). Ph.D. students or post-doctoral fellows conducting research at academia throughout the world are eligible.

This year’s grant winners include:

**Effect of Deamidation on the Structure and Function of a Therapeutic Protein Interferon alpha-2a**
Swati Bandi, *University of Colorado Anschutz Medical Campus, Aurora, CO USA*

**Physiologically-relevant Crowding Effects on a Protein-peptide Interaction**
Samantha Stadmiller, *University of North Carolina at Chapel Hill, Chapel Hill, NC USA*

**Biophysical Analysis of Zinc-induced Amyloidogenesis of a Biofilm Adhesion Protein**
Alexander Yarawsky, *University of Cincinnati College of Medicine, Cincinnati, OH USA*

**Characterization of IL-7/ IL-7Rα Binding Interface and Structural Dynamics through Chemical Cross-linking**
Mengru Zhang, *Washington University in St. Louis, St. Louis, MO USA*

New to this year’s symposium is a professional mentoring program designed to guide students, post-doctoral researchers, and entry-level professionals with interest in HOS of protein therapeutics towards career opportunities in industry. This program seeks to foster deeper engagement of researchers new to the study of HOS with experts in the field. The program offers guidance to the opportunities presented at the symposium, and career development discussions.

Interested in participating? Please see the registration desk for more information.
7th International Symposium on Higher Order Structure of Protein Therapeutics
Scientific Program Summary

Monday, April 9, 2018

07:30 – 17:30 Registration in the Narragansett Ballroom Foyer

07:30 – 08:30 Continental Breakfast in Narragansett B&C Ballroom

08:30 – 08:45 Welcome and Introductory Comments in Narragansett A Ballroom
David Keire, CDER, FDA, St. Louis, MO USA

Keynote I Session in Narragansett A Ballroom
Session Chair: William Weiss, Eli Lilly and Company, Indianapolis, IN USA

08:45 – 09:45 Manipulating Protein Structure for Therapeutic Utility (Engineering for Properties That Mother Nature Never Had to Consider)
John Beals, Eli Lilly and Company, Indianapolis, IN USA

09:45 – 10:15 Networking Break – Visit the Exhibits and Posters in Narragansett B&C Ballroom

Discovery and Candidate Selection Session in Narragansett A Ballroom
Session Chair: Wasfi Al-Azzam, GlaxoSmithKline, King of Prussia, PA USA

Mandi Hopkins, University of Colorado Anschutz Medical Campus, Aurora, CO USA

Stanley Krystek, Bristol-Myers Squibb Company, Princeton, NJ USA

10:55 – 11:15 Early Analytical Characterization and Selection of Novel Biotherapeutics
Dana Filoti, AbbVie Inc., Worcester, MA USA

11:15 – 11:40 Discussion – Questions and Answers

11:40 – 12:40 Hosted Lunch in Narragansett B&C Ballroom
### Monday, April 9, 2018 continued

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:40 – 13:40</td>
<td>Technical Seminar</td>
<td><strong>Narragansett A Ballroom</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Facilitating Drug Discovery and Formulation with HDX-MS</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Christopher Morgan, <em>Sanofi, Framingham, MA USA</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Harry Sterling, <em>BioMarin Pharmaceuticals, Novato, CA USA</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sponsored by Waters Corporation</td>
<td></td>
</tr>
</tbody>
</table>

### HOS in Development Session in Narragansett A

**Session Chairs:** Natalie Ciaccio, *BioMarin Pharmaceuticals Inc., Novato, CA USA* and Anders Nielsen, *Novo Nordisk A/S, Måløv, Denmark*

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:40 – 14:00</td>
<td>Application of HDX-MS to Biopharmaceutical Development Requirements: Improved Sensitivity to Detection of Conformational Changes</td>
<td><strong>Narragansett A Ballroom</strong></td>
</tr>
<tr>
<td></td>
<td>Lea Bonnington, <em>Roche Diagnostics GmbH, Penzberg, Germany</em></td>
<td></td>
</tr>
<tr>
<td>14:00 – 14:20</td>
<td>What X-Ray Footprinting Can Tell You About Protein Interactions and Conformation</td>
<td><strong>Narragansett A Ballroom</strong></td>
</tr>
<tr>
<td></td>
<td>Corie Ralston, <em>Lawrence Berkeley National Laboratory, Berkeley, CA USA</em></td>
<td></td>
</tr>
<tr>
<td>14:20 – 14:40</td>
<td>Effects of Excipients of Filgrastim Products on the Structure and Dynamics of the Drug Substance</td>
<td><strong>Narragansett A Ballroom</strong></td>
</tr>
<tr>
<td></td>
<td>Yves Aubin, <em>Health Canada, Ottawa, ON Canada</em></td>
<td></td>
</tr>
<tr>
<td>14:40 – 15:05</td>
<td>Discussion – Questions and Answers</td>
<td></td>
</tr>
<tr>
<td>15:05 – 16:05</td>
<td>Poster Session in Narragansett B&amp;C Ballroom</td>
<td></td>
</tr>
</tbody>
</table>

### Biological Consequences of HOS Session in Narragansett A Ballroom

**Session Chairs:** Galahad Deperalta, *Genentech, a Member of the Roche Group, South San Francisco, CA USA* and Krishna Mallela, *University of Colorado Anschutz Medical Campus, Aurora, CO USA*

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:05 – 16:25</td>
<td>Assessing Impact of Thiol-related Attributes on Structure and Function of mAb Products</td>
<td><strong>Narragansett A Ballroom</strong></td>
</tr>
<tr>
<td></td>
<td>Yan Chen, <em>Genentech, a Member of the Roche Group, South San Francisco, CA USA</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wim Jiskoot, <em>Leiden University, Leiden, Netherlands</em></td>
<td></td>
</tr>
<tr>
<td>16:45 – 17:10</td>
<td>Discussion – Questions and Answers</td>
<td><strong>Narragansett A Ballroom</strong></td>
</tr>
</tbody>
</table>
Monday, April 9, 2018 continued

17:10 – 18:30  **Exhibitor Reception** in Narragansett B&C Ballroom
Tuesday, April 10, 2018

07:30 – 08:30  Continental Breakfast in Narragansett B&C Ballroom
08:00 – 19:00  Registration in the Narragansett Ballroom Foyer

<table>
<thead>
<tr>
<th>Keynote II Session</th>
<th>in Narragansett A Ballroom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session Chair:</td>
<td>Krishna Mallela, University of Colorado Anschutz Medical Campus, Aurora, CO USA</td>
</tr>
</tbody>
</table>

08:30 – 09:30  Approaches and Challenges for Quantifying Multi-Body Interactions as Higher-Order Structure for Key Product Attributes
Christopher J. Roberts, University of Delaware, Newark, DE USA

09:30 – 10:00  Networking Break – Visit the Exhibits and Posters in Narragansett B&C Ballroom

<table>
<thead>
<tr>
<th>Established Methods and Fundamentals for HOS Session</th>
<th>in Narragansett A Ballroom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session Chair:</td>
<td>Yves Aubin, Health Canada, Ottawa, ON Canada and William Weiss, Eli Lilly and Company, Indianapolis, IN USA</td>
</tr>
</tbody>
</table>

10:00 – 10:20  IgG Cooperativity
Tom Laue, University of New Hampshire, Durham, NH USA

10:20 – 10:40  Using Dynamic Light Scattering to Detect and Quantify the Effect of Small Molecule Additives on Protein Associations
Allen Minton, National Institutes of Health (NIH), Bethesda, MD USA

10:40 – 11:00  Time-Resolved Fluorescence Spectroscopy: An Old Technique to Monitor Protein Higher Order Structure Changes
Sergey Arzhantsev, CDER, FDA, St. Louis, MO USA

11:00 – 11:25  Discussion – Questions and Answers

11:25 – 12:25  Technical Seminar

<table>
<thead>
<tr>
<th>Expanding the Biomolecular Structural Analysis Capabilities using Orbitrap Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aaron Bailey, Thermo Fisher Scientific, San Jose, CA USA</td>
</tr>
</tbody>
</table>

Sponsored by Thermo Fisher Scientific

Tuesday, April 10, 2018 continued

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Presenter</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:25</td>
<td>Seeing Is Believing: How We Use Electron Microscopy to Understand HOS Changes Observed for a Bispecific Antibody</td>
<td>Ming Lei</td>
<td>Genentech, a Member of the Roche Group, South San Francisco, CA USA</td>
</tr>
<tr>
<td>13:45</td>
<td>Identifying HOS Variation with Multivariate Analysis of 2D NMR</td>
<td>John Marino</td>
<td>NIST-IBBR, Rockville, MD USA</td>
</tr>
<tr>
<td>14:05</td>
<td>Coherent 2D IR: Introduction to a Powerful New Structural Spectroscopy and Application to Difficult Protein Systems</td>
<td>Chris T. Middleton</td>
<td>PhaseTech Spectroscopy, Inc., Madison, WI USA</td>
</tr>
<tr>
<td>14:25</td>
<td>Discussion – Questions and Answers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:50</td>
<td>Networking Break – Visit the Exhibits and Posters in Narragansett B&amp;C Ballroom</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Presenter</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:20</td>
<td>Characterization of IL-7/ IL-7Rα Binding Interface and Structural Dynamics through Chemical Cross-linking</td>
<td>Mengru Zhang</td>
<td>Washington University in St. Louis, St. Louis, MO USA</td>
</tr>
<tr>
<td>15:35</td>
<td>Biophysical Analysis of Zinc-induced Amyloidogenesis of a Biofilm Adhesion Protein</td>
<td>Alexander Yarawsky</td>
<td>University of Cincinnati College of Medicine, Cincinnati, OH USA</td>
</tr>
<tr>
<td>15:50</td>
<td>Effect of Deamidation on the Structure and Function of a Therapeutic Protein Interferon alpha-2a</td>
<td>Swati Bandi</td>
<td>University of Colorado Anschutz Medical Campus, Aurora, CO USA</td>
</tr>
<tr>
<td>16:05</td>
<td>Physiologically-Relevant Crowding Effects on a Protein-Peptide Interaction</td>
<td>Samantha Stadmiller</td>
<td>University of North Carolina at Chapel Hill, Chapel Hill, NC USA</td>
</tr>
</tbody>
</table>
**Tuesday, April 10, 2018 continued**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Chair(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:20 – 16:40</td>
<td><strong>The Role of Higher Order Structure in the Assessment of Comparability and Biosimilarity</strong>&lt;br&gt;Brad Jordan, <em>Amgen Inc., Thousand Oaks, CA USA</em></td>
<td></td>
</tr>
<tr>
<td>16:40 – 17:00</td>
<td><strong>Leveraging X-ray Crystallography for Biosimilarity Assessment</strong>&lt;br&gt;Thomas Lerch, <em>Pfizer, Inc., Chesterfield, MO USA</em></td>
<td></td>
</tr>
<tr>
<td>17:00 – 17:20</td>
<td><strong>Structural Characterization and Comparability Between a Therapeutic Protein and its Novel Fc Fusion Counterpart</strong>&lt;br&gt;George Bou-Assaf, <em>Biogen, Cambridge, MA USA</em></td>
<td></td>
</tr>
<tr>
<td>17:20 – 17:45</td>
<td><strong>Discussion – Questions and Answers</strong></td>
<td></td>
</tr>
<tr>
<td>17:45 – 18:30</td>
<td><strong>Networking Break</strong> – Refreshments in the Narragansett Foyer</td>
<td></td>
</tr>
<tr>
<td>18:30 – 19:30</td>
<td><strong>Roundtable Discussion Session</strong> in the Narragansett B&amp;C Ballroom</td>
<td></td>
</tr>
</tbody>
</table>
Wednesday, April 11, 2018

07:30 – 08:30  Continental Breakfast in Narragansett B&C Ballroom

08:00 – 12:00  Registration in the Narragansett Ballroom Foyer

| Computational Methods for HOS Session in Narragansett A Ballroom |
| Session Chair: Katherine Bowers, FUJIFILM Diosynth Biotechnologies, Cary, NC USA |

08:30 – 08:50  Prediction of Protein-Protein Binding Sites and Epitope Mapping  
Nels Thorsteinson, Chemical Computing Group (CCG), Montreal, QC Canada

08:50 – 09:10  Using Automation and Statistical Analysis to Enhance Sensitivity and Reduce Subjectivity of Biological Therapeutic Comparability using Circular Dichroism  
Leo Bowsher, UCB Celltech, branch of UCB Pharma S.A., Slough, UK

09:10 – 09:30  Structure-Based Engineering to Improve Homogeneity of Bi-specifics and ADCs  
Eric Bennett, Pfizer, Inc., Cambridge, MA USA

09:30 – 09:55  Discussion – Questions and Answers


| Late Breaking Session in Narragansett A Ballroom |
| Session Chairs: Guodong Chen, Bristol-Myers Squibb Company, Princeton, NJ USA and David Keire, CDER, FDA, St. Louis, MO USA |

10:25 – 10:45  Native Peptide Mapping – New Method to Monitor HOS Changes in a QC Laboratory  
Michel Degueldre, UCB Pharma S.A., Braine-L’alleud, Belgium

10:45 – 11:05  Consequences of Sample Age on Biotherapeutic Higher Order Structure: Insights from Native Ion Mobility-Mass Spectrometry Methods  
Richard Kerr, CDER, FDA, St. Louis, MO USA

11:05 – 11:25  Electron Microscopy Imaging Reveals Unique Higher Order Structures of Adalimumab-TNFα and Infliximab-TNFα Complexes  
Siew Leong Chan, AbbVie Inc., Worcester, MA USA

11:25 – 11:50  Discussion - Questions and Answers

11:50 – 12:00  Closing Remarks  
Guodong Chen, Bristol-Myers Squibb Company, Princeton, NJ USA
Roundtable Discussion Topics

Tuesday, April 10, 2018 – 18:30-19:30

The Roundtable session will be a truly interactive workshop to connect and discuss real issues with your peers. This session was designed to offer informal, yet structured discussions on topics of interest to participants, but were not able to be incorporated into the other sessions within the program. There are ten Roundtable topics. To create meaningful discussion, we are going to limit each table to ten attendees. **Seating will be on a first come, first serve basis.** These discussions will include a facilitator, whose role is to help assist the discussion and ensure a lively exchange, and a scribe, whose role is to make general, anonymous notes about the discussion that will be posted on the HOS 2018 website and mobile app. Listed below are the Roundtable topics, facilitators, and scribes. Please view abstracts in the mobile app or on the HOS 2018 meeting webpage (https://casss.site-ym.com/page/HOS1814b).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Biological Consequences – Function, Safety and Other Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACILITATOR: Wim Jiskoot, <em>Leiden University</em></td>
<td></td>
</tr>
<tr>
<td>SCRIBE: Yves Aubin, <em>Health Canada</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Comparability/Similarity: Past, Present, and Future</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACILITATOR: Asish Chakraborty, <em>Waters Corporation</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Characterization and Formulation of Novel Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACILITATOR: Tony Leone, <em>Bristol-Myers Squibb Company</em></td>
<td></td>
</tr>
<tr>
<td>SCRIBE: Katherine Bowers, <em>FUJIFILM Diosynth Biotechnologies</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4</th>
<th>HOS Method Qualification Strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACILITATOR: Lea Bonnington, <em>F. Hoffman – La Roche Ltd.</em></td>
<td></td>
</tr>
<tr>
<td>SCRIBE: Alexander Bepperling, <em>Novartis</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5</th>
<th>New and High-resolution Methods for HOS Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACILITATOR: Jason Wood, <em>Bruker BioSpin</em></td>
<td></td>
</tr>
<tr>
<td>SCRIBE: William Weiss, <em>Eli Lilly and Company</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 6</th>
<th>HOS in Downstream Process Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>FACILITATOR: St John Skilton, <em>Protein Metrics Inc.</em></td>
<td></td>
</tr>
<tr>
<td>SCRIBE: Galahad Deperalta, <em>Genentech, a Member of the Roche Group</em></td>
<td></td>
</tr>
</tbody>
</table>
Table 7  Characterization Requirements for Phase Appropriate Filings
FACILITATOR:  Yan Chen, Genentech, a Member of the Roche Group
SCRIBE:  Shawn Cao, Amgen Inc.

Table 8  HOS Analysis in Problem Solving
FACILITATOR:  John Gabrielson, Elion Labs
SCRIBE:  Tom Lerch, Pfizer, Inc.

Table 9  HOS in Candidate Selection – Developability and Technology Evaluation
FACILITATOR:  Brad Jordan, Amgen Inc.
SCRIBE:  Anders Nielsen, Novo Nordisk A/S

Table 10  CTD/IND: Past, Present, and Future
FACILITATOR:  Mark Schenerman, CMC Biotech-MAS Consulting
SCRIBE:  John Schiel, National Institute of Standards and Technology (NIST)
Manipulating Protein Structure for Therapeutic Utility (Engineering for Properties That Mother Nature Never Had To Consider)

John Beals

Eli Lilly and Company, Indianapolis, IN USA

Converting endogenously secreted proteins into exogenously administered therapeutics can introduce challenges with shelf-life/in-use stability, immunogenicity, adsorption, distribution, metabolism, and excretion. The discrete, exogenous administration of protein therapeutics represents a stark contrast to the “on-demand” production of some proteins. Moreover, human physiology evolved to exploit the unique properties of these proteins. Consequently, subcutaneous (SC) and intravenous (IV) administration of naturally evolved proteins may present challenges with recapitulating the pharmacokinetic (PK), pharmacodynamic (PD), and biodistribution of the endogenous protein. The evolution of insulin therapy is an example of how various protein-engineering approaches exploit different strategies to create therapies that attempt to approximate more closely the prandial and basal needs of the patient. In addition, these strategies simplify the use and administration of the drug as well as improve the safety profile of the therapy. The talk will explore the challenges of modifying self-association to recapitulate rapid exposure from the SC injection for prandial control as well as a cadre of strategies (insulin derivatization, pI alterations, PEGylation) to slow exposure for basal control.

NOTES:
Determination of Interaction Parameters for Reversibly Self-Associating Antibodies: A Comparative Analysis

David Bain, Mandi Hopkins

University of Colorado Anschutz Medical Campus, Aurora, CO USA

Monoclonal antibodies (mAbs) represent a major class of biotherapeutics and are the fastest growing category of biologic drugs on the market. However, mAb development and formulation are often impeded by reversible self-association (RSA), defined here as the dynamic exchange of monomers with native-state oligomers. Here we present a comparative analysis of the self-association properties for five IgG mAbs, under matched conditions and using orthogonal methods. Concentration-dependent dynamic light scattering, and sedimentation velocity studies revealed that the majority of mAbs examined exhibited weak to moderate RSA. However, because these studies were carried out at mAb concentrations in the mg/mL range, we also observed significant nonideality. Noting that nonideality frequently masks RSA and vice-versa, we conducted direct boundary fitting of the sedimentation velocity data to determine stoichiometric binding models, interaction affinities, and nonideality terms for each mAb. These analyses revealed equilibrium constants ranging from micromolar to millimolar, and stoichiometric models ranging from monomer-dimer to isodesmic. Moreover, even for those mAbs described by identical models (e.g. monomer-dimer), we observed distinct kinetics of self-association. The accuracy of the models and their corresponding equilibrium constants were addressed using sedimentation equilibrium and simulations. Overall, these results serve as the starting point for the comparative dissection of RSA mechanisms in therapeutic mAbs.

NOTES:
Sequence and Structure Inspire Predictive Tools for Developability Assessment of Antibody Molecules

Stanley Krystek

Bristol-Myers Squibb Company, Princeton, NJ USA

Protein therapeutics is the fastest-growing class of pharmaceutical agents. Driven by the need to provide therapeutic solutions to unmet medical needs advances in protein engineering requires the expanded use of in silico methods to meet drug discovery timelines and reduce overall development costs. Industry, academic, and government researchers face a common challenge: How do we take advantage of increasing amounts of experimental data and computational power to develop approaches that have the opportunity to impact treatment of patients afflicted with severe disease? This presentation will focus on exploring the application of computational tools for the optimization and development of biologics.

Identification of manufacturability hot-spots and mitigation via protein engineering solutions that enhance the protein’s properties, such as its activity, affinity, specificity, and stability will be discussed. Computational approaches that examine protein aggregation and estimate physical stability of proteins, and identify intrinsic liabilities with regard to safety, efficacy, and manufacturability will be presented.

NOTES:
Early Analytical Characterization and Selection of Novel Biotherapeutics

Dana Filoti

*Abbvie Inc., Worcester, MA USA*

IgG based novel biologics pose a challenging developability profile due to their structure-function symbiosis. We will discuss approaches towards implementation of a highly desirable platform methodology for early biologics development to ensure a systematic screening funnel selection as early as candidate selection.

NOTES:
Application of HDX-MS to Biopharmaceutical Development Requirements: Improved Sensitivity to Detection of Conformational Changes

Lea Bonnington

Roche Diagnostics GmbH, Penzberg, Germany

The usefulness of the higher order structure information provided by hydrogen/deuterium exchange mass spectrometry (HDX-MS) in the protein therapeutic field is undisputed, however its applicability as a method for critical quality and comparability assessment has until now not been demonstrated. Results from a modified method workflow demonstrate the applicability of the HDX-MS technique for monitoring structural changes due to i.e. oxidation, deamidation and glycosylation at increased sensitivity levels and throughput, realistic to the requirements of biopharmaceutical research and development. Significant deuterium uptake differences for low level oxidations and glycosylation variants could be verified, down to the lowest increments tested, and the methodology could hence be applied to structurally differentiate other relevant chemical modifications.

NOTES:
What X-Ray Footprinting Can Tell You About Protein Interactions and Conformation

Corie Ralston, Sayan Gupta

Lawrence Berkeley National Laboratory, Berkeley, CA USA

The use of X-ray footprinting mass spectrometry (XFMS) to investigate structural features and conformational changes of macromolecules in the solution state has grown substantially in the past decade and has been successfully applied to systems ranging from single domain proteins to in vivo ribonucleoprotein assemblies. The method is highly complementary to the more widely used structural elucidation techniques for biological macromolecules such as x-ray diffraction, HDX, and cryo-electron microscopy. XFMS is an in situ hydroxyl radical (•OH) labeling method; X-ray irradiation dissociates solvent water to produce hydroxyl radicals, which covalently modify side chains which are solvent accessible. More specifically, residues which are in proximity to water molecules (either bulk or bound) are modified to a greater extent than residues which are not in proximity to water. Because liquid chromatography-mass spectrometry is then used to analyze the stable covalent modifications produced, the data provide a “water map” at the single residue level, which is then used to determine sample conformation. In this talk, I will describe the XFMS method, its advantages and disadvantages relative to other methods, and some recent exciting examples of structural information obtained on protein systems using the method.

NOTES:
Effects of Excipients of Filgrastim Products on the Structure and Dynamics of the Drug Substance

Houman Ghasriani, Derek Hodgson, Sara Ahmadi, Grant Frahm, Michael Johnston, Yves Aubin

Health Canada, Ottawa, ON Canada

Filgrastim is the generic name for recombinant methionyl human granulocyte colony-stimulating factor. Filgrastim products are formulated as a low ionic strength solution containing cryoprotectant such as sorbitol, non-denaturing detergents, usually polysorbate-80, in a buffer at an unusually low pH of 4.0, or 5.0 in some case. It is well documented that the low pH stabilizes the protein fold and we have proposed the presence of a cation-p (pi) interaction as a major stabilizing interaction. In addition, there is a significant body of literature suggesting that interactions of excipient molecules with the API may provide benefits from a conformational stability aspect. In order to shed some light in this area, we have used NMR spectroscopy to probe the effects of the structure, via chemical shifts, and dynamics using relaxation measurements of filgrastim when subjected to various excipient conditions. The NMR results are analyzed in parallel with thermal unfolding studies using circular dichroism.

NOTES:
Assessing Impact of Thiol-related Attributes on Structure and Function of mAb Products

Yan Chen

Genentech, a Member of the Roche Group, South San Francisco, CA USA

Disulfide bonds formed between the thiol groups of cysteine residues are critical for maintaining protein tertiary and quaternary structure. Free thiols and trisulfides are two common thiol-related variants observed in all subclasses of IgG antibodies. Various tools were developed to study the structural impact of free thiols and trisulfides in therapeutic mAb products. A systemic approach for assessing the potential quality impact of these variants will be presented.

NOTES:
Immunogenicity of Protein Aggregates: What’s New?

Wim Jiskoot

Leiden University, Leiden, Netherlands

For several decades protein aggregates have been among the usual suspects with regard to risk factors for therapeutic protein immunogenicity. Nevertheless, despite a huge amount of research it is still not clear which exact aggregate attributes (such as size, conformation, reversibility, chemical modifications, etc.) contribute most to the risk of aggregates as immune-stimulating impurities. In this presentation I will discuss some of the work that has been performed during the past few years on this topic. In particular, I will present and discuss recent data on the effect of aggregate size on protein immunogenicity.

NOTES:
Approaches and Challenges for Quantifying Multi-Body Interactions as Higher-order Structure for Key Product Attributes

Christopher J. Roberts

University of Delaware, Newark, DE USA

Protein higher-order-structure can manifest as well-defined or loosely defined structures (spatial ordering of proteins or their domains), depending on the system of interest. For example, higher-order structures such as transient "clusters" have been invoked to explain anomalously high values for the viscosity of protein solutions, although the amount of data to support this is limited. Low-concentration measures of reversible protein-protein interactions have been offered as a means to predict interactions at high-concentration ("crowded") conditions. In some cases, this works well, while in others it is of questionable utility. This has implications for how one designs candidate selection processes, as well as formulation development efforts. A combination of experimental biophysical characterization and coarse-grained molecular models offers a means to resolve discrepancies in some of these challenges, while recent results also highlight outstanding challenges when one considers the dynamics of higher-order structures formation. This presentation will review examples where experimentally detectable protein-protein interactions and spatial correlations at high concentrations are predictive, or not predictive, of key product properties such as physical stability and solution viscosity.

NOTES:
IgG Cooperativity

Tom Laue

University of New Hampshire, Durham, NH USA

IgG molecules consist of two separable regions with distinct functions: a variable (V) region responsible for specific antigen binding, and a constant (C) region whose binding sites determine which effector functions will occur, such as complement activation or specific Fc-receptor (FcR) binding. The various forms of cooperativity potentially occurring in IgGs will be identified, focusing on the structure-function relationship in which the antigen-binding fragment (Fab) and crystallizable fragment (Fc) may exert cooperative influence on each other’s function or within its own functions. While available research does not support the existence of antigen-induced conformational allosteric cooperativity in IgGs, there is substantial evidence for configurational allostery due to glycosylation and sequence variations. The possibility of allosteric signals propagating through the IgG domains complicates our understanding of the antibody structure-function relationship and challenges the current subclass selection process in therapeutic antibody design. The importance of sample purity on detecting cooperativity will be shown.

NOTES:
Using Dynamic Light Scattering to Detect and Quantify the Effect of Small Molecule Additives on Protein Associations

Di Wu, Allen Minton

National Institutes of Health (NIH), Bethesda, MD USA

We have developed a high throughput system for quantifying the effect of an individual small molecule additive or combination of small molecule additives upon the tendency of a protein to self-associate in solution. The system consists of a Hamilton NIMBUS pipetting robot in conjunction with a Wyatt Dynapro DLS plate reader and custom software. Using this system, we have measured the effect of Guanidine hydrochloride (GuHCl), trimethylamine-N-oxide (TMAO), and mixtures of the two additives upon the dimer-tetramer equilibrium of cyanmet-hemoglobin. The system permits a large number of additive compositions and hemoglobin concentrations to be rapidly explored. An extensive data set is obtained that is amenable to global modeling, providing a quantitative picture of the effect of the two additives, individually and jointly, on the free energy of dimer-tetramer conversion.

NOTES:
Time-Resolved Fluorescence Spectroscopy: An Old Technique to Monitor Protein Higher Order Structure Changes

Sergey Arzhantsev

CDER, FDA, St. Louis, MO USA

Time-resolved fluorescence spectroscopy is a powerful tool in biochemical research due to its high sensitivity and the simplicity of the method. The intrinsic fluorescence of proteins originates from the fluorescence of the aromatic amino acid residues: tryptophan, tyrosine, and phenylalanine. The strongest contribution to the fluorescence of proteins comes from the tryptophan emission. The tryptophan emission properties (fluorescence lifetime, quantum yield, emission maximum) are very sensitive to the local microenvironment, such as solvent accessibility, polarity of the solvent, solvent pH, concentration of salt, energy transfer and quenching. The changes in the tryptophan microenvironment due to conformational changes of proteins can be detected by the intrinsic fluorescence. In addition, time-resolved fluorescence anisotropy measurements can be used to monitor aggregation and fragment rotation. This presentation will discuss the application of time-resolved fluorescence spectroscopy to monitor changes of protein higher order structure.

NOTES:
Seeing is Believing: How We Use Electron Microscopy to Understand HOS Changes Observed for a Bispecific Antibody

Ming Lei

Genentech, a Member of the Roche Group, South San Francisco, CA USA

Bispecific antibody has opened up new opportunities to treat diseases that require simultaneous binding to multiple targets. Bispecific antibodies can be achieved by a “knob-into-hole” system where the Fc domains in the two half antibodies are engineered with a “knob” and a “hole”, respectively. One of the impurities generated from this process is the “hole-hole” homodimer that can behave differently from a normal antibody possibly due to the hole mutation. For another impurity composed of the dimer form of the “knob-into-hole” bispecific antibody, increased potency was observed. In both cases, we used multiple chromatography and MS-based analytical methods to demonstrate that these changes are related to the HOS differences rather than chemical modifications. One of the key technologies that help to understand these HOS differences is electron microscopy (EM). For the “hole-hole” homodimer, we first used negatively staining EM (NT-EM) to generate high contrast images where a new X-shaped conformation was identified. This conformation as well as increased Fc flexibility were further demonstrated by reconstructing the 3D structure of each individual molecule using the individual-particle electron tomography (IPET) technology. This increased Fc flexibility can explain the many unusual behaviors of this homodimer. NT-EM was also used to understand the structural basis for the increased potency of the HMWF of “knob-into-hole” bispecific antibody. Instead of random interactions between molecules, EM images revealed a preferred arrangement between the two bispecific antibody molecules in this dimer form. This preferred arrangement may lead to increased valency of the dimer and lead to increased potency. These case studies show that together with other techniques such as native MS and HDX-MS, EM can lead a better holistic understanding of protein HOS-related issues encountered during the development of protein therapeutics.

NOTES:
Identifying HOS Variation with Multivariate Analysis of 2D NMR

John Marino¹, Luke Arbogast¹, Robert Brinson¹, Frank Delaglio¹, John Schiel²

¹NIST-IBBR, Rockville, MD USA, ²NIST-IBBR, Gaithersburg, MD USA

The development of advanced techniques for the characterization of the higher order structure (HOS) of protein therapeutics, including monoclonal antibodies (mAbs), is emerging as a major priority in the pharmaceutical industry. To this end, two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy has been shown to provide a robust approach for producing spectral 'fingerprints' of the HOS of protein biologics in solution at atomic resolution. The individual peak positions from NH and CH correlation spectra are sensitive to HOS and can serve as spectral indicators for establishing consistency in drug manufacturing, assessing stability of drug formulations and for establishing biosimilarity to an innovator reference product. Using a standard monoclonal antibody developed by NIST (NISTmAb), 2D-NMR methods were used to acquire ¹H-¹³C correlation spectra of the NISTmAb and glycan remodeled NISTmAb at natural isotopic abundance. Statistical and chemometric tools were then applied to the 2D spectral 'fingerprints' to establish HOS comparability between mAb samples. With principal component analysis (PCA) applied directly to the spectral data matrix, spectra from highly similar species could be successfully discriminated, with low limits of detection, which could not be distinguished by visual inspection or simple intensity based statistical approaches. This approach enabled discrimination to be automated without any requirement for interactive analysis of spectral features. Further, using PCA loading plots the sources of spectral variation between species could be identified and provisionally ascribed to structure. Taken together, the results demonstrate the feasibility of applying 2D NMR techniques to mAbs, the precision with which these methods can be used to assess the HOS of mAb therapeutics, and the extent to which structural differences can be detected.

NOTES:
Coherent 2D IR: Introduction to a Powerful New Structural Spectroscopy and Application to Difficult Protein Systems

Chris T. Middleton

*PhaseTech Spectroscopy, Inc., Madison, WI USA*

Coherent 2D IR has moved from a cutting-edge experimental technique to a versatile and powerful research tool with broadening applications in biosciences including membrane proteins, ion channels, amyloids, tissues and more. PhaseTech Spectroscopy has commercialized the latest technological advances in order to bring 2D IR to laboratories worldwide. We will introduce the technique and its advantages for protein systems as well as some exciting example applications from PhaseTech co-founder Prof. Martin Zanni’s laboratory at the University of Wisconsin-Madison: detailed structural studies of membrane proteins, structural dynamics of amyloid protein aggregation and HOS characterization of cataract tissues. These examples will highlight ways that 2D IR is distinctly capable of providing structural details on difficult protein systems.

NOTES:
Characterization of IL-7/IL-7Rα Binding Interface and Structural Dynamics through Chemical Cross-linking

Mengru Zhang\textsuperscript{1}, Guodong Chen\textsuperscript{2}, Richard Huang\textsuperscript{2}, Jagat Adhikari\textsuperscript{1}, Ekaterina Deyanova\textsuperscript{2}, Jing Li\textsuperscript{2}, Brett Beno\textsuperscript{2}, Adrienne Tymiak\textsuperscript{2}, Michael Gross\textsuperscript{1}

\textsuperscript{1}Washington University, St. Louis, MO USA, \textsuperscript{2}Bristol-Myers Squibb Company, Princeton, NJ USA

Here, we test whether chemical cross-linking of reactive groups coupled with mass spectrometry (MS) identification can be a useful tool in structural proteomics especially for defining a protein/protein binding interface. To test this possibility, we selected IL-7 and IL-7Rα as a model.

In the extracellular matrix, the common \(\gamma_c\) family and its interleukin receptors play important roles in the development, proliferation and homeostasis of B and T cells. The interaction between \(\gamma_c\), including interleukin-2 (IL-2), IL-7, IL-9, IL-15 & IL-21, and its corresponding receptors initiates a signal cascade. Among these interleukin complexes, a structural study of IL-7 and IL-7Rα would serve as a model to afford understanding of binding events for all the different protein complexes in the \(\gamma_c\) family.

We chose \textit{bis}(sulfosuccinimidyl)suberate (BS3) as the cross-linking reagent to test whether we can locate the binding interface and evaluate the interaction dynamics between IL-7 and IL-7Rα. We found cross-linked peptides between the loop region of IL-7Rα and helix-A and C of IL-7, which delineates their binding interface according to the x-ray structure. In addition, we found many other cross-linked peptides spanning distances much larger than expected from the x-ray crystal structure. Observation of these long-range cross-linked peptides suggests that the IL7/IL7Rα complex is highly dynamic and flexible. The study of the IL-7 and IL-7Rα interaction serves as a model for other interleukin complex interactions and an opportunity to provide more targets in the design of novel therapeutics.

NOTES:
Biophysical Analysis of Zinc-induced Amyloidogenesis of a Biofilm Adhesion Protein

Alexander Yarawsky¹, Andrew Herr²

¹University of Cincinnati College of Medicine, Cincinnati, OH USA, ²Cincinnati Children's Hospital Medical Center, Cincinnati, OH USA

Staphylococcus epidermidis is a primary organism responsible for hospital-acquired infections. The ability of this gram-positive, human commensal to cause infection can be attributed to its incredible capacity to form biofilms on indwelling medical devices, such as catheters, pacemakers, and joint replacements. Biofilms are organized bacterial communities that offer strong mechanical and chemical resistance to the bacteria, resulting in infections that often require prolonged antibiotic treatment and removal of the device. The Accumulation Associated Protein (Aap) is critical for S. epidermidis infection. Aap is a multifunctional, cell wall-anchored protein containing a superdomain composed of 5-17 B-repeats. Our lab has previously characterized a minimal B-repeat construct containing one full B-repeat and a C-terminal half repeat important for stability (Brpt1.5). This construct formed an anti-parallel dimer in the presence of Zn²⁺ and led us to hypothesize a “zinc-zipper” model of biofilm accumulation. We have recently shown that Aap is capable of forming functional amyloid, which could offer strength and stability to S. epidermidis biofilms. Using a new construct containing a biologically relevant number of B-repeats (Brpt5.5), we have begun to understand the initial assembly events of Aap amyloidogenesis. Utilizing analytical ultracentrifugation (AUC), we have been able to characterize early, reversible assembly states, along with the number of Zn²⁺ ions involved in each assembly. Temperature-dependent experiments performed using circular dichroism (CD) and dynamic light scattering (DLS) have provided complementary information regarding secondary structure changes along the pathway of amyloidogenesis, and to follow the formation of insoluble amyloid aggregates.

NOTES:
Effect of Deamidation on the Structure and Function of a Therapeutic Protein Interferon alpha-2a

Swati Bandi, Surinder Singh, Dinen Shah, Krishna Mallela

University of Colorado Anschutz Medical Campus, Aurora, CO USA

The two most common chemical modifications that compromises therapeutic protein quality are the deamidation and oxidation. These modifications can occur during protein purification, formulation, and storage depending on the solution conditions such as pH, temperature, and buffer composition. Interferon alpha-2a (IFNA2a) drug products have been shown earlier to undergo chemical modifications during their shelf-life. Here, we probed how deamidation affects the structure, stability, aggregation, and function of IFNA2a. It is often impossible to differentiate the specific effect of deamidation from that of oxidation, since both chemical modifications occur in parallel during the product’s shelf-life. Therefore, to study the specific effect of deamidation, we mutated the four Asn (N45, N65, N93 and N156) residues in IFNA2a to Asp using genetic engineering. Significant changes were observed in the local structure of the protein upon deamidation, despite no significant changes in its global secondary structure and minor changes in its tertiary structure. In addition, deamidation destabilized the protein, and increased its propensity to aggregate under stress conditions like freeze-thaw, agitation and isothermal incubation. In addition, IFNA2a showed a significant loss of anti-proliferative activity compared to its antiviral activity. 2D NMR experiments indicated that deamidation did not perturb the overall global structure of IFNA2a, but rather affected the structure of certain local regions in the protein. Identifying these local regions in the three-dimensional protein structure provided a mechanistic understanding of how deamidation induced protein instability, caused protein aggregation, and resulted in the loss of protein function.

NOTES:
Physiologically-Relevant Crowding Effects on a Protein-Peptide Interaction

Samantha Stadmiller, Jhoan Sebastian Aguilar, Gary Pielak

University of North Carolina at Chapel Hill, Chapel Hill, NC USA

Two-thirds of disease associated missense mutations disrupt protein-protein interactions, making them a significant target for pharmaceutical development. The majority of these interactions occur within crowded and dynamic living cells, an environment organized by protein quinary structure. Most biophysical characterizations of protein complexes, however, are conducted in dilute buffered-solutions where cosolute conditions rarely exceed 10 g/L. Here, we examine the effects of crowded environments on protein-peptide binding with a model system comprising the N-terminal SH3 domain from the Drosophila signal transduction protein drk and a 12-amino acid, proline-rich peptide derived from Son of Sevenless (Sos). The SH3-Sos interaction mediates eukaryotic mitogen activated protein kinase signaling, a pathway that is often disrupted in cancer, because it controls cell growth, differentiation, and apoptosis. Labeling SH3 with fluorine on its sole tryptophan enables one-dimensional $^{19}$F NMR detection of the complex. Subsequent lineshape analysis is then used to determine a dissociation constant as well as rate constants for the SH3-peptide interaction. Our use of biologically relevant small molecules, protein cosolutes, and traditional crowding molecules demonstrate differing effects on the thermodynamics and kinetics of the interaction. These results have immediate implications for understanding the effects of the cellular environment on this essential eukaryotic protein-peptide interaction. More broadly, these data enable the development of more accurate computational models for cell signaling, can aid the design of drugs to target protein-protein interactions, and provide insight into cellular effects on peptide therapeutics.

NOTES:
The Role of Higher Order Structure in the Assessment of Comparability and Biosimilarity

Brad Jordan

Amgen Inc., Thousand Oaks, CA USA

Improvements in production methods and process and control test methods along with the development of highly sensitive analytical techniques for product characterization have helped to evolve the regulation of biological products by allowing manufacturers to readily identify and assess the impact of manufacturing changes on the product. Key to these methods are cutting edge and highly sensitive techniques to assess differences in higher order structure (HOS). In many cases, sensitive analytical and functional characterization can be paired with prior knowledge of the process and product in order to demonstrate product comparability between a pre- and post-change biologic through a comparability exercise, many times without the need for additional clinical data. When developing a biosimilar, no prior knowledge of the reference product manufacturing process is available, and thus analytical/functional characterization alone cannot be relied upon to establish the clinical equivalence of the prospective biosimilar to the reference product. While techniques to probe higher order structure have advanced significantly, without prior knowledge of the production process, the relationship between small, but detectable, structural differences and the safety, efficacy, and immunogenicity of a biological product cannot yet be readily established and thus requires additional clinical data. Therefore, while the assessment of higher order structure is highly important in the establishment of comparability and biosimilarity, the degree to which HOS data obtained by currently available methods can be relied upon in these exercises is distinctly different.

NOTES:
Leveraging X-ray Crystallography for Biosimilarity Assessment

Thomas Lerch

*Pfizer, Inc., Chesterfield, MO USA*

Higher order structure (HOS) assessment is an important component of biosimilarity evaluations. For example, noninvasive spectroscopy methods are routinely used to evaluate comparability and similarity. The addition of X-ray crystallographic analysis to these biophysical methods enables further orthogonal evaluation of higher order structure. In this presentation, crystal structures for a proposed biosimilar and the reference product will be discussed. These high-resolution structures provide orthogonality to circular dichroism, Fourier-transform infrared, and fluorescence emission spectroscopic methods typically used to evaluate HOS. In contrast to spectroscopic methods, crystal structures enable three-dimensional assessment of complementarity-determining regions (CDRs) and other local regions at near-atomic resolution. Lastly, the structures provide new insights into the physicochemical properties of the proposed biosimilar and the reference product, further strengthening “totality of evidence” in the evaluation of similarity.

NOTES:
Structural Characterization and Comparability Between a Therapeutic Protein and Its Novel Fc Fusion Counterpart

George Bou-Assaf

*Biogen, Cambridge, MA USA*

Fusion of the Fc domain of monoclonal antibodies to therapeutic proteins enables half-life extension of these biopharmaceuticals. Extensive characterization of fusion proteins is required to ensure that the conjugation of the Fc domain does not perturb the structural and functional properties of the unmodified therapeutic protein. Here, we employ several biophysical and structural characterization tools to demonstrate that the higher-order structure and dynamics of a therapeutic protein are comparable to those of its Fc-fusion counterpart. Among these techniques, some such as X-ray crystallography and electron microscopy (EM), elucidated the protein structure with very high resolution but lacked information on the flexible domains of the protein. Other techniques, such as hydrogen/deuterium exchange monitored by mass spectrometry (HDX-MS) and small angle X-ray scattering, capture the dynamic properties of this protein because they are performed in solution. The results indicate that the higher-order structure of the fusion protein is highly similar to that of its individual elements (therapeutic protein alone and Fc alone). Finally, a series of other biophysical tests including surface plasmon resonance, HDX-MS, and EM confirmed that the binding of the fusion protein to its functional counterparts is not hindered by the conjugation of the Fc domain to the therapeutic protein.

NOTES:
Prediction of Protein-Protein Binding Sites and Epitope Mapping

Nels Thorsteinson

Chemical Computing Group (CCG), Montreal, QC Canada

We present a method for identifying important interaction sites in protein interfaces and carrying out epitope mapping. An analysis is carried out of molecular properties mapped onto the protein surface to determine patches which play a role in determining properties and binding interactions. Docking calculations generate an ensemble of protein-protein poses, sampling orientational space. An interaction fingerprint encoding patch contacts is used to generate pose clusters ranked by ensemble free energy and used to extract consensus interactions comprising the predicted epitopes.

NOTES:
Using Automation and Statistical Analysis to Enhance Sensitivity and Reduce Subjectivity of Biological Therapeutic Comparability using Circular Dichroism

Leo Bowsher¹, John O’Hara¹, Lindsay Cole², Paul McColgan³, Clare Trippett³, Oliver Durrant¹

¹UCB Celltech, branch of UCB Pharma S.A., Slough, UK, ²Applied Photophysics Limited, Leatherhead, UK, ³CPI, Darlington, UK

Circular Dichroism (CD) is a spectroscopic technique commonly used to provide structural information on proteins. The inherent chirality of most amino acids means that proteins exhibit a difference in their absorbance of left and right circularly polarised light resulting in the circular dichroism effect. Common secondary structure elements (alpha helix, beta sheet etc.) show distinct spectral features in the far ultraviolet (UV) region, and aromatic residues and disulphide bonds influence the near UV region which gives insight into tertiary structure. Due to the sensitivity to changes in both protein secondary and tertiary structure, CD spectroscopy is routinely used to monitor structural comparability and stability throughout the product life cycle in the biopharmaceutical industry.

There is a trend to introduce a more statistics driven approach to the analysis of biophysical spectroscopic techniques, such as CD, to remove the inherent subjectivity and potential source of error of a single user evaluating comparability by visual inspection of overlaid spectra. Statistical analysis also shows potential in fully understanding and exploiting the sensitivity of these techniques.

Here, we demonstrate the potential of automation coupled with statistical comparison to discern small structural differences between a control and degraded samples of three antibody therapeutic formats (IgG1, IgG4 and Fab). These molecules were degraded under a range of conditions and characterised by orthogonal testing in order to relate observed spectral differences to distinct structural modifications. Far and near UV CD were able to detect statistically significant structural differences that only a combination of orthogonal tests was able to.

NOTES:
Structure-Based Engineering to Improve Homogeneity of Bispecifics and ADCs

Eric Bennett

*Pfizer, Inc., Cambridge, MA USA*

Engineered biologics platforms such as site-specific antibody-drug conjugates (ADCs) and bispecific antibodies allow exploring additional mechanisms of action relative to standard IgG formats. However, both platforms present unique manufacturing challenges. ADCs may exhibit chemical instability at the conjugation site, and bispecific production often yields unwanted species of mis-paired protein chains that can be difficult to remove. We used protein structure analysis to further investigate a previously reported reaction affecting ADC stability, leading to proposed mutations near the conjugation site which proved able to alter the reactivity of the linker. We also used structural analysis to reengineer the VL/VH interface to improve bispecific chain pairing fidelity, finding several designs which reduced or prevented formation of mispaired species. Our results show that application of structural analysis early in lead development can reduce technical challenges that may occur later in manufacturing.

NOTES:
Native Peptide Mapping – New Method to Monitor HOS Changes in a QC Laboratory

Michel Degueldre, Carl Jone, Annemie Wielant, Sandrine Van Leugenhaeghe, Erica Bortolotto, Eglantine Girot, Gaël Debauve, Annick Gervais

UCB Pharma S.A., Braine L'alleud, Belgium

In biopharmaceutical environments, defining and controlling the Critical Quality Attributes (CQA) of products are imperative to prevent changes which may impact their function and consequently generate safety concerns for the patient. These attributes are monitored for batch-to-batch consistency and in stability studies. Nowadays, a plethora of physico-chemical techniques and bioassays screen and monitor these CQAs.

Higher order structure (HOS), which may be a CQA, is studied by techniques such as circular dichroism, X-Ray structure, NMR, epitope detection and FTIR. These techniques are not commonly considered amenable to QC laboratories.

Therefore, a new peptide mapping-based method focused on HOS analysis will be presented. Compared to current HOS techniques, this method presents several advantages such as its speed, simplicity and automation to operate in a QC laboratory.

The utility of this method to discriminate material resulting from different stress factors and its correlation with bioassay results will be presented.

NOTES:
Consequences of Sample Age on Biotherapeutic Higher Order Structure: Insights from Native Ion Mobility-Mass Spectrometry Methods

Richard Kerr, Hongping Ye

CDER, FDA, St. Louis, MO USA

Given their complex higher order structure (HOS), biotherapeutic products such as monoclonal antibodies (mAb) exhibit comparatively short shelf-lives. Combining data from several analytical sources with appropriate statistical modelling, these shelf-lives accurately estimate the duration over which a product is expected to retain its stated therapeutic efficacy, purity and safety.

To assure the quality of the product, over its stated lifetime, the effect of sample age on the HOS stability of biotherapeutics needs to be evaluated. Based on quality guidelines outlined by the International Conference on Harmonisation (ICH), we have initiated accelerated aging studies to assess the capability of native Ion Mobility Mass Spectrometry (IM-MS) to identify age-dependent degradations that may compromise the safety and/or efficacy of a chosen biotherapeutic.

Using native IM-MS approaches, assuming several instrumental and sample handling considerations are met, data support that solution phase structures can be maintained within the gas phase. The conformational state of these analytes can then be assessed to identify differences in structure based on changes in analyte collision cross section (CCS). The same instrumental considerations may be further leveraged to perform gas phase Collision Induced Unfolding (CIU) experiments, akin to differential scanning calorimetry methods, to assess the gas phase structural stability of the analyte.

Our data support that despite no change in the measured mAb CCS, significant differences in analyte structural stability can be observed using CIU. These data highlight the impact of age to induce subtle changes in mAb HOS, that may compromise the in situ safety and/or efficacy of the product.

In this presentation, we will discuss in greater detail the mAbs and experimental methods used, in addition to the significance of our data pertaining to the impact of sample age. Reference to additional supporting analytical methods, including hydrogen-deuterium exchange mass spectrometry, will be made to support these conclusions.

NOTES:
Electron Microscopy Imaging Reveals Unique Higher Order Structures of Adalimumab-TNFα and Infliximab-TNFα Complexes

Siew Leong Chan

AbbVie Inc., Worcester, MA USA

Therapeutic monoclonal antibodies form complexes with target antigens, through interactions between the Fab arms and epitope surfaces on antigens. Adalimumab and Infliximab are recombinant IgG1 monoclonal antibodies (mAbs) that bind and neutralize human tumor necrosis factor alpha (TNFa). TNFa forms a stable homotrimer with unique surface-exposed sites for Adalimumab, Infliximab and TNF receptor binding. Since Adalimumab, Infliximab and trimeric TNFa are multivalent, the interactions between these antibodies and TNFa may form higher order complexes. Here, we report the complex structures of Adalimumab-TNFα and Infliximab-TNFα, obtained from negative stain EM and cryo-EM imaging. EM images reveal complex structures consisting of 1:1, 1:2, 2:2 and 3:2 complexes of Adalimumab-TNFα and Infliximab-TNFα. The 2:2 complex structures of Adalimumab-TNFα and Infliximab-TNFα show distinct orientations of the Fab domains, indicating different binding modes by Adalimumab and Infliximab to TNFα. Cryo-EM analysis of 3:2 Adalimumab-TNFα complex generated a low-resolution structure model with a TNFα trimer bound with 3 Fab domains from 3 individual antibody molecules, while each antibody molecule binds to 2 molecules of TNFα trimer. However, the highly flexible Fc domains are not visible in the model reconstruction. These results show that the two mAbs form structurally distinct complexes with TNFα.

NOTES:
Facilitating Drug Discovery and Formulation with HDX-MS

Christopher Morgan\textsuperscript{1}, Harry Sterling\textsuperscript{2}

\textsuperscript{1}Sanofi, Framingham, MA USA, \textsuperscript{2}BioMarin Pharmaceuticals, Novato, CA USA

Hydrogen deuterium exchange mass spectrometry (HDX-MS) is a key technology that provides critical information on both protein conformation and dynamics. The automation of sample handling, data collection, and data analysis has catalyzed the expansion of HDX-MS from academia into the world of biopharmaceuticals. Ease of use, sensitivity, speed, and resolution in the study of the higher order structure of proteins and protein complexes make this technique a uniquely valuable tool in the biophysical toolbox.

Waters is the innovation leader in HDX-MS and remains the only vendor to offer an end-to-end integrated system solution for HDX-MS studies. Our speakers for this seminar will highlight the benefits of using HDX-MS in drug discovery, development and formulation.
Expanding the Biomolecular Structural Analysis Capabilities using Orbitrap Technology

Terry Zhang, Rosa Viner, Aaron Bailey, Stephane Houel, Jonathan Josephs

Thermo Fisher Scientific, San Jose, CA USA

To understand protein function in the cellular environment, it is essential to characterize the protein complex assembly and its spatial structure. Equipped with this knowledge, structural biologists can find innovative ways to intervene in disease processes and find new preventive measures, treatments, and pharmaceutical agents. Various MS methods have been developed for specific structural and functional studies. While no single MS method is suitable for all analyses, each can be applied for certain experiments. In this presentation, the latest advanced Orbitrap technology-based characterization tools: including native mass spectrometry, chemical cross-linking, hydrogen deuterium exchange and the complementary technique, cryo-electron microscopy will be presented.

NOTES:
Emerging Technology for HOS Evaluation

P-102

Characterisation of Peptide-receptive Major Histocompatibility Complex Class I Molecules by HDX-MS

Asish Chakraborty1, Malcolm Anderson2, Laetitia Denbigh2

1Waters Corporation, Milford, MA USA, 2Waters Corporation, Wilmslow, UK

Hydrogen-deuterium exchange (HDX) analysis of MHC I proteins has previously been limited to peptide-loaded complexes. Here, we have prepared peptide-receptive MHC I protein by refolding MHC I molecules with UV photolabile peptide and subjecting to UV irradiation, enabling comparison of both peptide-loaded and peptide-receptive states.

The study included non-irradiated and UV-irradiated samples of two MHC I allotypes, together with a further sample of UV-irradiated complex which had been incubated with excess peptide ligand to demonstrate that UV exposed MHC I was receptive to binding peptide. These five combinations of MHC I allotype and condition were subjected to HDX mass spectrometry analysis: After dilution into deuterium oxide buffer for specific incubation periods, quenched samples were subjected to online pressurized pepsin digestion. After chromatographic separation at 0 degrees C, eluted peptides were detected via an MSe method using a Synapt G2-Si mass spectrometer. Selected peptides were subjected to ETD fragmentation. Data processing was performed using ProteinLynx Global Server and DynamX.

We found allotype-specific differences between the hydrogen-deuterium exchange profiles of peptide-loaded and peptide-receptive MHC I molecules. Most of the differences involved the peptide-binding domain and were most noticeable in the alpha2 and alpha1 helices.

NOTES:
Electro-flow Asymmetric Field Flow Fractionation Characterization of the NIST Monoclonal Antibody Standard RM 8671

Robert Reed¹, Soheyl Tadjiki¹, Thorsten Klein²

¹Postnova Analytics Inc., Salt Lake City, UT USA, ²Postnova Analytics GmbH, Landsberg, Germany

The NIST monoclonal antibody (mAb) standard reference material provides a platform for evaluating methods used to characterize physicochemical and biophysical attributes of other mAbs and large biomolecules. In this study, the NIST mAb was used to evaluate separation parameters for asymmetric flow field flow fractionation – multi angle light scattering (AF4-MALS) analysis of mAbs, especially in comparison to size exclusion chromatography (SEC-MALS). The amount of aggregates present in the NIST mAb was measured to be 10% by FFF with refractive index detection, whereas SEC-RI did not detect any aggregates. This non-detect by SEC-RI is possibly due to loss of aggregate material on the SEC column during separation. A new module for AF4 using an auxiliary electric field for separation by macromolecule surface charge, electro-flow AF4 (EAF4) was employed to measure the electrophoretic mobility of the NIST mAb during size separation. The EAF4-measured value for electrophoretic mobility of the NIST mAb in 1x phosphate buffered saline was -1.68 +/- 0.05 μm cm s⁻¹ V⁻¹.

NOTES:
High-Resolution Hydroxyl Radical Protein Footprinting Introduction and Workflow

John Schenkel

NeoProteomics, Inc, Cleveland, OH USA

Hydroxyl radical protein footprinting (HRF) with mass spectrometry (MS) is a recognized approach for assessing structure of proteins. Hydroxyl radicals react with a wide variety of side-chains resulting in their oxidation. Advances in liquid chromatography and MS approaches permit an examination of the labeling of individual residues, transforming the approach to high-resolution. Overall, the technology can provide an accurate measures of side-chain solvent accessibility in a wide range of interesting and useful contexts for the study of protein structure and dynamics in both academia and industry. Here we demonstrate the extension of the HRF workflow to include single residue examination, showcasing the importance of individual labels as significant telltale of local conformational change to a complex.

The typical workflow for high-resolution HRF is illustrated here by examination of a free antigen and an antigen-Fab complex. All samples are exposed to hydroxyl radicals for 0-800 microseconds of the x-ray beam. The amount of exposure for both samples is assessed using fluorophore Alexa-488. After exposure, the samples are subjected to deglycosylation using PNGase F. Deglycosylated samples are precipitated with 10% trichloroacetic acid (TCA)/acetone, reduced and alkylated with 10 mM iodoacetamide and 25 mM of DTT respectively, and digested with trypsin. The MS data are acquired using high-resolution LC-MS and analyzed, resulting in dose response plots for each peptide. Peptides from the antigen that show changes in modification against the complex are further analyzed for labeling at each residue.

NOTES:
Electron Microscopy: A Powerful Tool for Characterization of Biotherapeutics

Anette Schneemann, Jeffrey A. Speir, Travis Nieuema, Maria Janssen, Anchi Cheng, Sarah Dunn, Bridget Carragher, Clinton S. Potter

NanoImaging Services, Inc., San Diego, CA USA

We present the 2.4Å resolution structure of apoferritin determined with our Titan Krios microscope as an example of the cryo transmission electron microscopy (cryo-TEM) services available at NIS. Cryo-TEM is an established and powerful imaging technique applicable to many specimens, including the three-dimensional (3D) reconstruction of macromolecules and their associated complexes to high resolution. The technique is parsimonious in its material requirements and captures the specimens in their fully hydrated state, close to their native environment. The resolution of cryo-TEM reconstructions was limited to the subnanometer range until the recent development of direct electron detectors and improvements in image processing software, which has led to a so-called “resolution revolution” in the cryo-TEM field. Several protein structures have now been solved at near atomic resolution, establishing the technique as a viable alternative to X-ray analysis for high resolution structure determination. We have now determined several structures to better than 4Å resolution for our clients, which are being integrated into drug discovery and development workflows. While high resolution 3D structure determination by cryo-TEM is at the forefront of structural biology, averages of 2D projection images at moderate resolution can also provide a wealth of information that may be difficult to obtain using other methods. This is illustrated in an example study of IgM that uses imaging and analysis techniques to address common and important structure-function relationships of protein-protein complexes. Alignment and 2D projections of mammalian IgM, combined with selective masking and particle sub-classification, revealed that the five antibody monomers are arranged asymmetrically around a central hub. This is in contrast to prevailing models, which show the monomers to be symmetrically arranged. Thus, both the moderate resolution TEM and high resolution cryo-TEM methods are well suited to extensively characterize protein structure-function relationships that may be refractory to other

NOTES:
Sedimentation Coefficient Distributions From Nonideal Solutions At High Concentrations

Peter Schuck, Sumit Chaturvedi, Huaying Zhao

National Institutes of Health (NIH), Bethesda, MD USA

Sedimentation velocity analytical ultracentrifugation has emerged as a powerful solution-based approach to characterize the homogeneity and oligomeric aggregate content of protein therapeutics solutions. The most sensitive and high-resolution analysis can be carried out with the computation of the sedimentation coefficient distribution \( c(s) \), which is based on the direct fitting of the experimentally measured evolution of sedimentation boundaries by combinations of Lamm equation solutions, each separately describing the sedimentation and diffusion of an ideally sedimenting particle with different sedimentation velocity. Their best-fit combination describes the populations of differently sized particles in solution. A current limitation of this approach occurs when the sedimentation process is nonideal and dominated by hydrodynamic interactions at high protein concentrations. Under these conditions, the theoretical description of sedimentation underlying the \( c(s) \) method fails to describe the experimentally observed process, which can exhibit sedimentation boundaries distorted in height and shape, for example, through the Johnston-Ogston effect and boundary sharpening. Here we report progress on the design of an extension for \( c(s) \) suitable for highly concentrated solutions under formulation conditions using a mean-field approach for the hydrodynamic interactions, implemented in the software SEDFIT.

NOTES:
Evaluating Empty and Fully-Packaged Adeno-Associated Virus (AAV) Capsid Species using Analytical Ultracentrifugation (AUC) and Cryo-Electron Microscopy (Cryo-EM)

Caitlin Wappelhorst¹, Thomas Lerch¹, Tatiana Shapkina², Herb Runnels¹, Alex Berrill¹, William Wellborn¹, Qin Zou¹

¹Pfizer, Inc., Chesterfield, MO USA, ²Pfizer, Inc., Morrisville, NC USA

AAV is a non-enveloped, single-stranded DNA virus with an icosahedrally symmetric protein capsid. The capsid structure dictates essential vector functions such as tissue tropism, cell entry, and delivery of therapeutic cargo to the nucleus. The growth in gene therapy development for clinical use has highlighted the need to reliably monitor AAV-specific attributes such as capsid size and the relative abundance of empty and fully packed capsids, the latter of which is generally considered a critical quality attribute (CQA). Whereas AAV size is evaluated using dynamic light scattering (DLS) and negative stain transmission electron microscopy (TEM), the empty to fully packaged capsid ratios are determined using both analytical ultracentrifugation- sedimentation velocity (AUC-SV) and cryo electron microscopy (cryo-EM). As the relative quantities of empty and full capsids can affect transduction efficiency, AUC-SV and cryo-EM are powerful tools for AAV process development and higher order structure characterization.

NOTES:
NMR Represents a Superior Method for the Assessment of Higher Order Structure of Biopharmaceuticals

Mats Wikström, Xingxiang Cao

Amgen Inc., Thousand Oaks, CA USA

The higher order structure (HOS) of protein therapeutics is a critical quality attribute directly related to the function of these molecules. Recently, high-resolution nuclear magnetic resonance (NMR) techniques have emerged as a powerful tool for HOS characterization utilizing both one-dimensional (1D) and two-dimensional (2D) NMR methods. NMR is a true multi attribute method with unique capabilities to provide structural information of proteins including primary, secondary, tertiary, quaternary, and quinary structure.

In this study we have compared Profile NMR (1D) with the current platform methods for higher order structure (HOS); FTIR and far UV CD for secondary structure, and intrinsic fluorescence and near UV CD for tertiary structure, using a set of blended samples of IgG1 and IgG2 monoclonal antibodies. The study shows that Profile NMR can distinguish between most sample combinations (~93%), whereas no distinction could be made between any samples using the current platform methods. Our data therefore shows that NMR has superior ability to address subtle differences in HOS, a feature that could be directly applicable in comparability and similarity assessments of biopharmaceuticals.

NOTES:
Development and Proof-of-Concept of a New Flow-through System for High Resolution NMR to Evaluate Biologics-producing Cell Lines and Their Growth Environments

Jason Wood

Bruker BioSpin, Billerica, MA USA

We have developed a new flow-through system for NMR spectrometers that allows detection and evaluation of cells, their metabolites and higher-order structure of their products in one convenient, fully bio-compatible, system that sits within the magnet field of any standard bore NMR system from 400 MHz to 1 GHz. Two modalities have been evaluated; one in which cells are held externally in a temperature-controlled way and their products are sent directly (online) to the spectrometer and secondly, cells themselves are trapped within a temperature-controlled zone (tube) within the magnet field. In this way, cellular products and/or metabolites, or the cells themselves, can be evaluated under various conditions either supporting or deterring growth and the effects evaluated in terms of changes or processes of the cellular mechanism(s). Case studies and potential (additional) usages will be highlighted and discussed.

NOTES:
Electron Transfer Dissociation for Hydrogen/Deuterium Exchange Mass Spectrometry and Its Application to the Study of Protein Conformation

Terry Zhang, Stephane Houel, Jonathan Josephs

*Thermo Fisher Scientific, San Jose, CA USA*

Proteins possess primary, secondary, ternary and quaternary structures. Hydrogen/deuterium exchange mass spectrometry (HDX) has emerged as a powerful tool to study protein conformation, conformation dynamics and protein-protein interactions. When HDX is analyzed at peptide or protein level, only the overall deuterium uptake of peptides or proteins is obtained. Determination of deuterium incorporation at single amino acid resolution by CID and related techniques is perturbed by “hydrogen scrambling”. Electron transfer dissociation (ETD) is known in the literature as a potential technique that would yield very low level of hydrogen scrambling and therefore allows for localization of incorporated deuterium with single residue resolution. A model peptide and proteins were used to evaluate the feasibility of the technique at single residue resolution.

Model peptide, HHHHHHIKIIK was used to optimize source and capillary temperatures measure hydrogen scrambling and deuterium back exchange. Cytochrome C was studied by both top-down and bottom-up HDX-ETD experiments on Thermo’s fully automatic HDX workflow station with an Orbitrap Fusion.

With the model peptide, very low level of hydrogen scrambling was observed at normal HDX bottom-up experimental conditions. Based on this observation, 50 °C source and 220 °C capillary temperatures were selected as experimental conditions. For cytochrome C HDX-ETD top-down experiment. Around 90% sequence coverage was obtained. Single amino acid deuterium incorporation results were obtained from the consecutive N and C terminal fragments. Bottom-up experiment also was performed using the same experimental conditions for Cytochrom C. Both MS full scan and ETD MS2 scan were conducted at the same time. The MS full spectra were analyzed with HDExaminer first to probe the region of significant change in deuterium incorporation. The ETD MS2 spectra were then used to pinpoint at the single amino acid level deuterium incorporation for the region of significant change.

NOTES:
Enhanced Protein Structural Characterization using Microfluidic Modulation Spectroscopy (MMS)

Jeff Zonderman, Eugene Ma

RedShift BioAnalytics, Burlington, MA USA

Measurement and characterization of the secondary structure of proteins are critical in many research applications. Traditional analytical tools are not optimized for the demanding requirements of this application, which include high sensitivity, wide dynamic range, simplified workflow, and high repeatability. We demonstrate a new technique, Microfluidic Modulation Spectroscopy (MMS), that is designed to address these needs.

The platform utilizes a tunable mid-infrared quantum cascade laser to generate an optical signal 1000x brighter than conventional sources used in FTIR spectroscopy. This also allows the use of simpler detectors without the need for liquid nitrogen. Additionally, the sample (protein) solution and a matched buffer reference stream are automatically introduced into a microfluidic flow cell, and the two fluids are rapidly modulated (e.g. 5 Hz) across the laser beam path to produce nearly drift-free background subtracted measurements.

NOTES:
HOS Biosimilarity and Comparability

P-112

More Than A-helix and -Sheet: Expanding the Role of Circular Dichroism

Deborah Litman

*Applied Photophysics Inc., U.S.A., Beverly, MA USA*

This poster demonstrates how state-of-the-art CD spectrometers go beyond the traditional use of CD. High-quality data now provides unique insights into the higher order structure (HOS) of complex biomolecules. Two applications are presented. 1. A HOS comparison of NIST mAb variants revealed minor differences in secondary and tertiary structure. The statistical significance of these differences was determined using the Tier 2 quality range approach as recommended by FDA guidelines. 2. A multiwavelength, thermal denaturation (temperature ramp) experiment revealed changes to both secondary structure and protein stability in a globular protein after transient exposure to nanoparticles.

NOTES:
Use of Surface Plasmon Resonance to Study C1q/Antibody/Antigen Complex Assembly

Alvaro Amor, Christopher Sucato, Mario DiPaola

Charles River Laboratories, Woburn, MA USA

Human protein complement component 1q (C1q) is an important recognition factor for the innate immune system. C1q binds to antibodies along with their target antigen to allow for complement binding recognition to initiate an immune response. Thus C1q’s affinity for antibodies can be a hallmark of immunogenically preferable immunoglobulins. This interaction is becoming increasingly important in the antibody-driven therapeutic market to select Ig molecules with favorable immunogenic properties. The C1q/Ab interaction is often studied by in vivo methodologies, however in vitro methodologies can allow for more precise measurements using biochemical and biophysical instrumentation. One such technique is Surface Plasmon Resonance (SPR) using Biacore instrumentation. Despite the important role of C1q interactions with immunoglobulins, the large size of this protein, its unusual shape, and distribution of charged groups makes it prone to non-specific binding and other challenges in the setting of a SPR sensorchip surface. The Biophysical Characterization Group of Charles River Laboratories has developed a Biacore methodology to study the kinetic aspects of the C1q/Ab/antigen binding reaction. We describe here experimental set-ups that have optimized C1q binding to surface-immobilized antibody, generating kinetic association and dissociation parameters, as well as the thermodynamic dissociation constant (KD). We demonstrate that the method is rapid and sensitive and can fully assess binding of a drug product against C1q with a quick turnaround time.

NOTES:
P-114

HOS Fidelity of Biosimilars by 1D and 2D NMR

Donna Baldisseri

*Bruker BioSpin, Billerica, MA USA*

The ongoing development of biotherapeutics has led to a multi-billion dollar a year market for many pharmaceutical companies. Many of these biologics though are beginning to lose patent protection globally and thus, new biosimilars of these drugs are starting to enter the market. As such, biosimilar drug products must have a demonstrated similarity with respect to the reference products’ Higher Order Structure (HOS) in order to ensure both the effectiveness of the drug and the patients’ safety.

In this poster we discuss the latest advancements in NMR applications to the analytical challenges facing Development and Discovery laboratories in Pharma in assessing the HOS of biosimilars to targeted biologics. Innovations in hardware, software and application specific workflows will be highlighted including several biotech-relevant case studies. In particular, we will be highlighting: 1) the latest in NMR workflows for simplified HOS determination by 1D NMR and, 2) rapid determination of ideal growth conditions during manufacturing by 2D NMR.

NOTES:
HDX-MS Higher Order Structure Profiling Correlated with Crystal Structure Determination and Statistical Analysis for Development of a Highly Glycosylated Therapeutic Protein

Siyang (Peter) Li\textsuperscript{1}, Shiaw-Lin Wu\textsuperscript{1}, Qiaozhen (Cheryl) Lu\textsuperscript{2}, Chris Barton\textsuperscript{2}, Bernice Yeung\textsuperscript{2}

\textsuperscript{1}BioAnalytix Inc., Cambridge, MA USA, \textsuperscript{2}Shire Inc, Lexington, MA USA

Iduronate-2-sulfatase is a highly glycosylated therapeutic protein, consisting of complex sialylated and mannose-6-phosphate types of glycans at several Asn sites, which are crucial for cell uptake and targeting to the lysosomal degradation pathway.

In color-coded HDX-MS Heat Map analysis of the molecule, a majority of the regions of the molecule were seen as well-structured (blue, indicating low H/D exchange), with several glycosylated regions seen as more solvent accessible (yellow or light green, high H/D exchange). The tertiary structure of the molecule was also elucidated in a recent related crystal structure analysis (Demydchuk et al., Nature Communications, 2017), revealing a globular alpha/beta sandwich fold. This is consistent with HDX data, wherein the structured blue regions (low H/D exchange) indeed correspond to packed helical or beta sheet regions of the crystal structure.

In further HDX-MS analysis by a Difference Plot approach comparing the molecule with or without modified glycans (e.g., de-sialylation and/or de-phosphorylation), local conformation at each related Asn site (e.g., Asn 6, Asn 221 and Asn 255) was seen to be sensitive to changes in glycosylation. In addition, although the Asn 6 site could not be assigned in the related crystal structure analysis, the pair of 2 glycosylation sites (Asn 221 and Asn 255) in separate loop regions were revealed to be close to each other in the 3D structure, indicating that a change of local conformation at one site could affect the other. As such, the HDX-MS analysis was further used to compare different lots, particularly at these sensitive regions to assure conformation comparability. Further, a statistical “Similarity Scoring” method derived from the HDX-MS analysis was used for comparison between old and new process lots. Overall, the HDX-MS method was applied not only for characterizing and correlating Higher Order Structure but also as a powerful tool to compare structural similarities between lots.

NOTES:
Objective, Quantifiable HOS Comparisons: A Biosimilar Case Study Utilizing Circular Dichroism

Darek Sliwa

*Applied Photophysics Inc., U.S.A., Beverly, MA USA*

During biotherapeutic development a wide range of biophysical characterization techniques are required to support informed decision-making and contribute to the totality of evidence in regulatory submissions. Regulatory authorities are increasing their demand for ‘state-of-the-art’ techniques that can provide statistically-validatable data. To date, obtaining such results for higher order structure (HOS) comparisons has presented challenges in terms of data acquisition and suitability of statistical methods. This poster presents a case study in which an integrated approach to HOS analysis generates an objective, quantifiable comparison of a commercially-available biotherapeutic (Fab fragment) with a biosimilar currently under development.

NOTES:
Sedimentation Velocity-Analytical Ultracentrifugation (SV-AUC) as an Orthogonal Tool to Measure the Purity of mAbs, ADCs, and Fusion Proteins

Ziad Eletr

Takeda Pharmaceuticals Intl Co, Cambridge, MA USA

Sedimentation Velocity-Analytical Ultracentrifugation (SV-AUC) is a biophysical technique commonly used in biopharmaceutical development to quantify protein size variants, including protein aggregates. SV-AUC is often employed in circumstances when method development using traditional high throughput purity techniques (SEC and CE-SDS) is challenging. The focus of this work is to highlight instances where SV-AUC proved to be an invaluable orthogonal tool to aid in SEC method development.

NOTES:
P-119

Quantitative Evaluation Between Color and Higher Order Structure Quality Attributes of Protein Therapeutics

Conor Lake

GlaxoSmithKline, King of Prussia, PA USA

Color is one of several quality attributes of protein therapeutics under general appearance testing. Various chemical events during product and process batch delivery can result in color change, requiring thorough examination. In addition, color has been associated with increased levels of impurities, degradation, tryptophan oxidation and contamination of the product. The objective of this study was to correlate higher order structure quality attributes to color change quantitatively using the Hunter Ultrascan Vis Colorimeter. During this study, photo and thermal forced degradation conditions were employed to examine color changes in model proteins. This examination was followed with assessment of protein HOS such as SEC-MALS, near UV-(CD), and MS to systematically assess the question if HOS may be responsible or can be associated with color change.

Our findings suggest a strong correlation among the five proteins studied (4 IgG mAbs and 1 non-mAb) for the relationship between total color change (quantified by $\Delta E$) and aggregation species by SEC-MALS following photo stress. Similarly, there was aggregation for thermally force degraded samples, however at lower magnitude.

It is known that oxidation of the aromatic residues – tryptophan (Trp), tyrosine (Tyr), and histidine (His) can cause color change. In attempt to control color change, the headspace of select proteins were purged with gaseous N$_2$ to minimize oxidation of aromatics. As expected, the purged N$_2$ headspace samples following photo stress demonstrated significantly less aggregation and total color change.

These results demonstrate a novel finding that not only oxidation of aromatic residues result in color change, but also changes in protein HOS can results in color change. Additional studies are ongoing to determine if aggregation alone without aromatic residue oxidation can contribute significant color change.

NOTES:
Biophysical Characterization Of Candidates At Research Phase For Therapeutic mAbs

Helen Wu

*Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT USA*

Recombinant monoclonal antibody (mAb) therapeutics has been broadly investigated throughout pharma industries and over 300 mAb product candidates are currently in development with many for multiple indications. Significant amounts of biophysical characterization technologies/methods have been developed at early research phase to ensure delivery of optimal candidates with great developability and manufacturability as well as high potency and efficacy to the market. In this presentation, a broad spectrum of biophysical characterization methods applied in our candidate selection/optimization process will be introduced. In addition, the expanded roles of a statistically verifiable CD will be elaborated with three case studies. Incorporating this technology in our current process will make it more efficient and informative at early phase.

**NOTES:**
Enabling Robust Hydroxyl Radical Footprinting for Biotherapeutic Structural Characterization via an Internal Standard Peptide

Natalie Garcia, Galahad Deperalta, Aaron Wecksler

Genentech, a Member of the Roche Group, South San Francisco, CA USA

Biotherapeutic function is predicated on the three-dimensional arrangement of each amino acid within a macromolecule, and the physiochemical characterization of higher order structure (HOS) can support efficacy studies ensuring optimal drug quality for patients. Current analytical tools such as liquid chromatography (LC) and capillary electrophoresis are the gold standard for product quality assessment but cannot probe protein HOS directly. The limitations of size, flexibility, resolution, and analysis time render other methods (e.g. crystallography, NMR) impractical for the rapid demands of manufacturing. At Genentech, we are developing the application of the covalent-labeling structural mass spectrometry (MS) technique, hydroxyl radical footprinting (HRF-MS) via the fast-photochemical oxidation of proteins (FPOP) method. During FPOP a high-powered UV-laser is used to photolyse hydrogen peroxide into highly reactive hydroxyl radicals, which rapidly oxidize solvent accessible amino acid side-chains in a protein/complex of interest. Following FPOP, standard protein digestion and LC-MS analysis is used to quantify peptide-level side chain oxidation, which is proportional to solvent-accessibility. Common FPOP protocols require dilute protein samples (e.g. 1 mg/mL) in inert buffers and free reactive amino acids, thereby controlling the amount of hydroxyl radical available for protein oxidation. However, manufactured biotherapeutics are formulated at high concentrations, some up to hundreds of mg/mL, and in complex buffer matrices often containing surfactants capable of scavenging hydroxyl radicals. To date, it is still unclear how common biotherapeutic concentrations, common manufacturing surfactants, and different free amino acids impact the radical scavenging potential and the extent of oxidation observed during FPOP experiments. Here we will highlight the development of the small linear pentapeptide internal standard, leu-enkephalin (YGGFL) for tracking the radical scavenging potential of industry-relevant FPOP conditions and optimizing experimental conditions to study complex buffer matrices for reliable data interpretation.

NOTES:
Surface plasmon resonance (SPR) optical biosensing has been widely applied to quantitatively measure the binding affinity and kinetics of soluble macromolecules such as antibody-antigen interactions. Compared to other biophysical methods, this technique offers distinctive advantages such as high sensitivity and reproducibility. However, the involvement of the surface often introduces complex effect on the binding behavior of the molecules either immobilized or in the bulk flow, such as nonuniform density distribution of polymeric linkers, intrinsic surface roughness and nonuniform chemical microenvironment and nonuniform attachment generating various protein conformations. Fundamentally, the question arises whether the resulting surface binding sites are homogeneous. In order to reveal the heterogeneity of the binding sites with different affinities, we have developed a computational tool (EVILFIT) to determine the distribution of affinity and kinetic rate constants from the analysis of SPR binding data. Applications of this model have revealed that in most cases the sensor surface is composed of binding sites with a spectrum of binding properties. With the detailed information on the different binding events from the modeling in EVILFIT, the distinctive pattern of thermodynamic and kinetic properties of different molecules can be distinguished. We illustrate the approach with a panel of related antibodies binding their cognate antigen.