3rd International Symposium on Higher Order Structure of Protein Therapeutics (HOS 2014)

Symposium Co-chairs:

Steven Cohen, SAC Analytical Consultants
Linda Narhi, Amgen Inc.

February 18 – 20, 2014
Crystal City Marriott at Reagan National Airport
Arlington, Virginia USA

Organized by

CASSS
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The Organizing Committee gratefully acknowledges the Corporate Program Partners for their generous support of the 3rd Symposium on Higher Order Structure of Protein Therapeutics

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Acknowledgements

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Steven Cohen, SAC Analytical Consultants
Linda Narhi, Amgen Inc.

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Norma Allewell, University of Maryland
Katherine Bowers, Fujifilm Diosynth Biotechnologies
Michael Brenowitz, Albert Einstein College of Medicine
Guodong Chen, Bristol-Myers Squibb Company
Otmar Hainzl, Sandoz Biopharmaceuticals
Damian Houde, Biogen Idec
Wim Jiskoot, LACDR, Leiden University
Aston Liu, GlaxoSmithKline Pharmaceuticals
Jamie Moore, Genentech, a Member of the Roche Group
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William Weiss, Eli Lilly and Company

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Michael Johnstone, MJ Audio-Visual Productions

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Renee Olson, Senior Program Manager
Anna Lingel, Project Coordinator
Catherine Stewart, Finance Manager
**Student Travel Grants**

**CASSS Higher Order Structure Student Travel Grants**
CASSS is pleased to provide a limited number of student travel grants for PhD students and post-docs who present applicable posters at the 3rd International Symposium on Higher Order Structure of Protein Therapeutics (HOS 2014). PhD students or post-doctoral fellows conducting research at academia throughout the world are eligible.

This year’s student travel grant recipients include:

**Evaluation of Insulin Analogues and Their Aggregates with Simultaneous Raman Spectroscopy and Dynamic Light Scattering**
Chen Zhou, *University of Colorado, Anschutz Medical Campus, Aurora, Colorado, CO USA*

**Investigating the Effects of the Metal-Binding claMP Tag on the Structure and Stability of Protein Conjugates**
Brittney Mills, *University of Kansas, Lawrence, KS USA*

**Differentiation of Protein Particles and Silicone Oil Droplets by Flow-imaging Microscopy (MFI and FlowCAM) and Resonant Mass Measurement (Archimedes)**
Daniel Weinbuch, *Leiden University, Leiden, The Netherlands*

**Epitope Mapping of EGFR Binding to an Adnectin by Fast Photochemical Oxidation of Proteins (FPOP)**
Yuetian Yan, *Washington University in St. Louis, St. Louis, MO USA*
# 3rd International Symposium on Higher Order Structure of Protein Therapeutics Scientific Program Summary

**Tuesday, February 18**

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<th>Time</th>
<th>Event</th>
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<tr>
<td>07:30 – 18:00</td>
<td><strong>Registration</strong> in the Ballroom Foyer</td>
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<tr>
<td>07:30 – 08:30</td>
<td><strong>Continental Breakfast</strong> in the Chesapeake Ballroom</td>
<td></td>
</tr>
<tr>
<td>08:30 – 08:45</td>
<td><strong>Welcome and Introductory Comments</strong> in the Potomac Ballroom</td>
<td>Steven Cohen, <em>SAC Analytical Consultants, Hopkinton, MA USA</em></td>
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**Keynote Session** in the Potomac Ballroom

**Session Chair:** Linda Narhi, *Amgen Inc., Thousand Oaks, CA USA*

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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>08:45 – 09:45</td>
<td><strong>Mechanisms of Protein Aggregation</strong></td>
<td>Thomas Laue, <em>University of New Hampshire, Durham, NH USA</em></td>
</tr>
<tr>
<td>09:45 – 10:15</td>
<td><strong>Break</strong> – Visit the Exhibits and Posters in the Chesapeake Ballroom</td>
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**Higher Order Structure in Development Session** in the Potomac Ballroom

**Session Chair:** William Weiss, *Eli Lilly and Company, Indianapolis, IN USA*

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<tbody>
<tr>
<td>10:15 – 10:35</td>
<td>TBD</td>
<td>Michael Boyne, <em>CDER, FDA, Silver Spring, MD USA</em></td>
</tr>
<tr>
<td>10:35 – 10:55</td>
<td><strong>Evaluating Higher Order Structure of Antibody Drug Conjugates</strong></td>
<td>Fred Jacobson, <em>Genentech, A Member of the Roche Group, South San Francisco, CA USA</em></td>
</tr>
<tr>
<td>10:55 – 11:15</td>
<td><strong>HOS Characterization Strategies to Support Development of Biosimilars</strong></td>
<td>Andreas Seidl, <em>Sandoz – HEXAL AG, Oberhaching, Germany</em></td>
</tr>
<tr>
<td>11:15 – 11:40</td>
<td><strong>Discussion – Questions and Answers</strong></td>
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<tr>
<td>11:40 – 11:55</td>
<td><strong>Break</strong> – Visit the Exhibits and Posters in the Chesapeake Ballroom</td>
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**11:55 – 12:55**

**Lunch and Learn - Technical Seminar**

**Biotherapeutic Structure and Dynamics: A Critical Attribute of the Highest Order**

Asish Chakraborty, *Waters Corporation, Milford, MA USA*

Sponsored by Waters Corporation *Potomac Ballroom*
Tuesday, February 18 continued…

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<td></td>
<td><strong>Higher Order Structure Fundamentals Session</strong> in the Potomac Ballroom</td>
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<tr>
<td>13:10 – 13:30</td>
<td><strong>PFAST: Protein Fluorescence and Structural Toolkit</strong></td>
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<td>Yana Reshetnyak, <em>University of Rhode Island, Kingston, RI USA</em></td>
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<td>13:30 – 13:50</td>
<td><strong>Studying Reversible Self-association of Biopharmaceuticals using AUC and Light Scattering</strong></td>
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<td>John Philo, <em>Alliance Protein Laboratories, San Diego, CA USA</em></td>
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<td>13:50 – 14:10</td>
<td><strong>Automated Chemical Denaturation as a Tool to Evaluate Protein Stability and Optimize the Formulation of Biologics</strong></td>
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<td>Ernesto Freire, <em>Johns Hopkins University, Baltimore, MD USA</em></td>
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<tr>
<td>14:10 – 14:35</td>
<td><strong>Discussion – Questions and Answers</strong></td>
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<tr>
<td>14:35 – 15:05</td>
<td>Break – Visit the Exhibits and Posters in the Chesapeake Ballroom</td>
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<td></td>
<td><strong>HOS in Protein Therapeutics Discovery and Early Candidate Selection Session</strong> in the Potomac Ballroom</td>
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<tr>
<td>15:05 – 15:25</td>
<td><strong>Dynamics of Protein-Protein, Ligand and Membrane Interactions by H/D Exchange Mass Spectrometry</strong></td>
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<td></td>
<td>Ganesh Srinivasan Anand, <em>National University, Singapore, Singapore</em></td>
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<tr>
<td>15:45 – 16:05</td>
<td><strong>All Atom Molecular Dynamics Simulations of Antibodies in Explicit Solvent: Quite a Mouthful But the Results Are Worth Gnawing On</strong></td>
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<td>Tom Patapoff, <em>Genentech, A Member of the Roche Group, South San Francisco, CA USA</em></td>
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<tr>
<td>16:05 – 16:25</td>
<td><strong>Computational Assessment of Pharmaceutical Properties for Protein Therapeutics</strong></td>
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<td>Stanley Krystek, <em>Bristol-Myers Squibb Company, Princeton, NJ USA</em></td>
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<tr>
<td>16:25 – 16:50</td>
<td><strong>Discussion – Questions and Answers</strong></td>
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<tr>
<td>16:50 – 18:00</td>
<td><strong>Exhibitor Reception</strong> in the Potomac Ballroom</td>
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<td>Continental Breakfast in the Chesapeake Ballroom</td>
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</table>
| 07:30 – 08:30 | **Technical Seminar**  
**FT-IR Spectroscopy for Structure Elucidation of BioTherapeutics: The Do’s and Do NOT’s – Because the Correct Spectrum Matters!**  
Rina Dukor, *BioTools, Inc., Jupiter, FL USA* | Potomac Ballroom |
| 07:30 – 08:30 | Sponsored by BioTools, Inc.  
**H/D Exchange Analysis Session** in the Potomac Ballroom  
**Session Chairs:** Steven Cohen, *SAC Analytical Consultants, Hopkinton, MA USA* and Wasfi Al-Azzam, *GlaxoSmithKline, King of Prussia, PA USA* |                               |
| 08:30 – 08:50 | **Hydrogen Deuterium Exchange FTIR Analysis of Protein Higher Order Structure and Dynamics**  
Yijia Jiang, *Amgen Inc., Thousand Oaks, CA USA* |                               |
| 08:50 – 09:10 | **H/D Exchange Mass Spectrometry for Probing Higher Order Structure of Protein Therapeutics: Current Status and Future Directions**  
Guodong Chen, *Bristol-Myers Squibb Company, Princeton, NJ USA* |                               |
| 09:10 – 09:30 | **Position Specific Effects of Chemical Composition on Protein Stability**  
Jennifer Laurence, *University of Kansas, Lawrence, KS USA* |                               |
| 09:30 – 09:55 | **Discussion – Questions and Answers**                                                     |                               |
| 09:55 – 10:25 | **Break** – Visit the Exhibits and Posters in the Chesapeake Ballroom                      |                               |
| 10:25 – 10:45 | **Interaction and Dynamics Session** in the Potomac Ballroom  
**Session Chairs:** Katherine Bowers, *Fujifilm Diosynth Biotechnologies, Cary, NC USA* |                               |
| 10:25 – 10:45 | **High-throughput Biophysical Methods for Improving Monoclonal Antibody Selection and Formulation**  
Peter Tessier, *Rensselaer Polytechnic Institute, Troy, NY USA* |                               |
| 10:45 – 11:05 | **High-resolution NMR as a Higher Order Structure Assessment Tool for Protein Therapeutics**  
John Marino, *NIST, Rockville, MD USA* |                               |
Wednesday, February 19 continued…

11:05 – 11:25  Monitoring and Analysis of Protein Pharmaceutical High Molecular Weight Species During Manufacturing: A Case Study
Stephen Raso, Biogen Idec, Cambridge, MA USA

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

11:50 – 12:05  Break – Visit the Exhibits and Posters in the Chesapeake Ballroom

12:05 – 13:05  Lunch and Learn - Technical Seminar
TBD
Sponsored by Agilent Technologies  Potomac Ballroom

13:05 – 14:30  Poster Session in the Chesapeake Ballroom

Young Scientist Session in the Potomac Ballroom
Session Chairs: Damian Houde, Biogen Idec, Cambridge, MA USA

14:30 – 14:45  Investigating the Effects of the Metal-binding claMP Tag on the Structure and Stability of Protein Conjugates
Brittney Mills, University of Kansas, Lawrence, KS USA

14:45 – 15:00  Epitope Mapping of EGFR Binding to an Adnectin by Fast Photochemical Oxidation of Proteins (FPOP)
Yuetian Yan, Washington University, St. Louis, MO USA

15:00 – 15:15  Differentiation of Protein Particles and Silicone Oil Droplets by Flow-imaging Microscopy (MFI and FlowCAM) and Resonant Mass Measurement (Archimedes)
Daniel Weinbuch, Leiden University, Leiden, The Netherlands

Formulation Development Session in the Potomac Ballroom
Session Chairs: Wim Jiskoot, LACDR, Leiden University, Leiden, The Netherlands and Radhika Nagarkar, KBI Biopharma Inc., Durham, NC USA

15:15 – 15:35  Protein Solubility as a Predictive Assay for Formulation Development
Douglas Banks, Amgen Inc., Thousand Oaks, CA USA

15:35 – 15:55  Impact of Sub-visible Particles (SVPs) on Viscosity of Monoclonal Antibody (mAb) Solutions
Prasad Sarangapani, MedImmune, Gaithersburg, MD USA
Wednesday, February 19 continued…

15:55 – 16:10  **Formulation Optimization and Aggregates-detection Methodology for a Classic Biological Product: IGIV**
                Douglas Frazier, *Retired CBER, FDA CMC Reviewer, Bethesda, MD USA*

16:10 – 16:35  **Discussion – Questions and Answers**

16:35 – 16:45  **Mini Break**

| **Workshop** in the Potomac Ballroom  
**Facilitators:** John Gabrielson, *Amgen Inc., Longmont, CO USA*  
and William Weiss, *Eli Lilly and Company, Indianapolis, IN USA* |
|---------------------------------------------------------------|

# Thursday, February 20

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<td>07:30 – 08:30</td>
<td>Continental Breakfast in the Chesapeake Ballroom</td>
</tr>
<tr>
<td><strong>Biological Consequences of HOS Session</strong> in Potomac Ballroom</td>
<td>Session Chair: Linda Narhi, <em>Amgen Inc., Thousand Oaks, CA USA</em></td>
</tr>
<tr>
<td>08:30 – 08:50</td>
<td>Regulatory Considerations for the Assessment Higher Order Structure</td>
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<td>Peter Adams, <em>CDER, FDA, Bethesda, MD USA</em></td>
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<tr>
<td>08:50 – 09:10</td>
<td>Novel Antibody Therapeutics with Engineered Features and Impact on Immunogenicity</td>
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<td>Sally Fischer, <em>Genentech, A Member of the Roche Group, South San Francisco, CA USA</em></td>
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<tr>
<td>09:10 – 09:30</td>
<td>B-cell Epitope Prediction and Cloning Monoclonal ADAs to Assess Break of Immune Tolerance Against Human Protein Therapeutics</td>
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<td>Stefan Ryser, <em>Trellis Bioscience LLC, South San Francisco, CA USA</em></td>
</tr>
<tr>
<td>09:30 – 09:55</td>
<td>Discussion – Questions and Answers</td>
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<td>09:55 – 10:25</td>
<td>Break – Visit the Exhibits and Posters in the Chesapeake Ballroom</td>
</tr>
<tr>
<td><strong>Higher Order Structure Emerging and Novel Technologies Session</strong> in the Potomac Ballroom</td>
<td>Session Chairs: Norma Allewell, <em>University of Maryland, College Park, MD USA</em> and Aston Liu, <em>GlaxoSmithKline, King of Prussia, PA USA</em></td>
</tr>
<tr>
<td>10:25 – 10:45</td>
<td>Solution Structural Biology: The Use of Small-angle X-ray Scattering in Drug Discovery for Multi-domain or Flexible Protein Targets</td>
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<td>Nino Campobasso, <em>GlaxoSmithKline, Collegeville, PA USA</em></td>
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<tr>
<td>10:45 – 11:05</td>
<td>Peptide-conjugation Induced Antibody Conformational Changes Revealed by Individual-particle Electron Tomography</td>
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<td>Gang (Gary) Ren, <em>Lawrence Berkeley National Laboratory, Berkeley, CA USA</em></td>
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<tr>
<td>11:05 – 11:25</td>
<td>Biofabrication: An Assembly Paradigm for Device Construction that Facilitates Communication</td>
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<td>William Bentley, <em>University of Maryland, College Park, MD USA</em></td>
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<tr>
<td>11:25 – 11:40</td>
<td>Discussion - Questions and Answers</td>
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<tr>
<td>11:40 – 11:50</td>
<td>Closing Remarks and Invitation to HOS 2015</td>
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<td></td>
<td>Linda Narhi, <em>Amgen Inc., Thousand Oaks, CA USA</em></td>
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13
Mechanisms of Protein Aggregation

Thomas Laue

University of New Hampshire, Durham, NH USA

Energetics provide insights into the causes and remedies for nuisances like flocculation, liquid-liquid phase separation and high viscosity solutions. Both thermodynamic and kinetic views provide valuable understanding for the processes that lead to these higher-order structures. A two-step description of hydrophobic interactions allows their incorporation into the proximity energy framework, and provides some awareness as to their cause and possible remedies.

NOTES:
TBD

Michael Boyne, CDER, FDA, Silver Spring, MD USA

Abstract not available at the time of print.

NOTES:
Evaluating Higher Order Structure of Antibody Drug Conjugates

Fred Jacobson

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

Attachment of a hydrophobic chemotherapy drug to a monoclonal antibody in producing an ADC has the potential to significantly alter the properties of the protein. This presentation will focus on the studies that were performed to evaluate the impact of conjugation on the structure and function of trastuzumab (a humanized anti-HER2 MAb) during the production of Kadcyla® (trastuzumab emtansine). Although conjugation can affect solution stability of the ADC, these results demonstrate that conjugation has little impact on the biological properties that are indicative of the higher order structure.

**NOTES:**
HOS Characterization Strategies to Support Development of Biosimilars

Andreas Seidl

Sandoz, Hexal AG, Oberhaching, Germany

Targeted technical development of follow-on biologics (biosimilars) requires up-front definition of the development target on the basis of data of the originator product and continuous comparison of the biosimilar candidate with the originator product during development. For these purposes, a thorough assessment of dozens of characterization parameters of the product is performed, with biophysical characterization and HOS methods becoming more sensitive and important and receiving recently more and more attention. In order to develop sensitive methodologies that are able to deliver meaningful and reliable results, these methods need to be both well understood and qualified. With well-established HOS methods at hand, biosimilarity can be demonstrated in detail, turning HOS analytics to a major pillar of biosimilar development. This presentation will provide an overview about the area including some case studies. Examples from comparability exercises in the case of process changes or to demonstrate biosimilarity illustrate the power and performance of HOS methods nowadays.

NOTES:
**PFAST: Protein Fluorescence and Structural Toolkit**

Oleg Andreev; Yana Reshetnyak

*University of Rhode Island, Kingston, RI USA*

Fluorescence spectroscopy is a powerful tool for the investigation of protein structure, conformations and dynamics since fluorescence properties of tryptophan residues vary widely depending on the tryptophan environment in a given protein. The major goal in the application of tryptophan fluorescence spectroscopy is to interpret the fluorescence properties in terms of structural parameters and to predict of the structural changes in the protein.

We have developed methods for the mathematical analysis of fluorescence spectra of multitryptophan proteins aimed at revealing the spectral components of individual tryptophan or clusters of tryptophan residues located close to each other. Also, we have created an algorithm for the structural analysis of the tryptophan environment in 3D atomic structures of proteins from Protein Data Bank (PDB). The successful design of the methods of spectral and structural analysis opened an opportunity for establishing a relationship between the spectral and structural properties of a protein. The developed software modules were intergrated into a web-based toolkit PFAST: Protein Fluorescence and Structural Toolkit.

PFAST contains 3 modules:

1) FCAT is a fluorescence-correlation analysis tool, which decomposes protein fluorescence spectra to reveal the spectral components of individual tryptophan residues or groups of tryptophan residues located close to each other, and assigns spectral components to one of five previously established spectral-structural classes.

2) SCAT is a structural-correlation analysis tool for the calculation of the structural parameters of the environment of tryptophan residues from the atomic structures of the proteins from the PDB (Protein Data Bank), and for the assignment of tryptophan residues to one of five spectral-structural classes.

3) The last module is a PFAST database that contains protein fluorescence and structural data obtained from results of the FCAT and SCAT analyses.

Applications of the PFAST approaches will be presented on the examples of protein-ligand and peptide-membrane interactions. Other spectroscopic approaches such as a circular dichroism and oriented circular dichroism will be introduced for the studies of peptide insertion into lipid bilayer of membrane of liposomes and cancer cells.

**NOTES:**
Studying Reversible Self-association of Biopharmaceuticals using AUC and Light Scattering

John Philo

Alliance Protein Laboratories, San Diego, CA USA

The distinction between reversible and irreversible association/aggregation of biopharmaceuticals can be difficult to make, and in our experience is often not well understood by our clients. Another common misunderstanding is that clients think it is possible to physically separate different oligomers in order to size them and quantify the fractions (which in general cannot be done). This talk will focus on characterizing reversible self-association using analytical ultracentrifugation (sedimentation equilibrium or sedimentation velocity) and classical light scattering (CG-MALS), and will discuss exactly what information can be obtained from these methods, using some real-world examples for small peptides and proteins.

NOTES:
Automated Chemical Denaturation as a Tool to Evaluate Protein Stability and Optimize the Formulation of Biologics

Ernesto Freire

*Johns Hopkins University, Baltimore, MD USA*

Automated chemical denaturation provides an accurate way of measuring protein stability under many different solvent or formulation conditions, yielding reliable thermodynamic stability parameters. Contrary to temperature denaturation, chemical denaturation is reversible under a majority of conditions, especially for monoclonal antibodies, fusion proteins and other proteins of therapeutic interest. The dynamic range of the technique permits stability measurements over protein concentrations spanning five orders of magnitude, allowing measurements in the important 100mg/mL concentration range. In addition, chemical denaturation experiments performed in the presence of ligands provide enough information to determine binding affinities from chemical denaturation shifts.

In this presentation, the fundamentals of automated chemical denaturation and its application to the evaluation of protein stability and optimization of formulation conditions will be discussed.

NOTES:
Dynamics of Protein-Protein, Ligand and Membrane Interactions by H/D Exchange Mass Spectrometry

Ganesh Srinivasan Anand¹,²

¹Department of Biological Sciences, National University, Singapore, Singapore; ²Mechanobiology Institute, NUS, Singapore, Singapore

Describing the dynamics of large macromolecular assemblies that constitute signaling proteins is an area of great importance for cellular signaling processes. Amide H/D exchange mass spectrometry (HDXMS) is a powerful biophysical tool for describing the effects of various perturbants such as protein-ligand, protein-protein interactions, membrane anchoring and the effects of osmolytes on protein dynamics. HDXMS offers important advantages including small sample amounts, automation, and is not limited by the sizes of the target proteins. My talk will cover applicability of HDXMS in mapping protein-protein interactions and together with computational docking provides a rapid method to model large multiprotein macromolecular assemblies. The model proteins described are the cyclic adenosine monophosphate (cAMP) phosphodiesterases and their interactions with cAMP receptor proteins. In addition to mapping protein-protein interactions in this system, we have also shown the power of HDXMS in monitoring allosteric relays and dynamics of transient complexes in this pathway involving the dynamic interactions of multiple proteins (phosphodiesterases and cAMP receptor) with the ligand cAMP. Such ternary complexes are not readily characterized by other methods. This has important implications for development of multiprotein complexes as alternate targets for small molecule drug discovery. HDXMS is also especially suited to probing conformational dynamics of membrane proteins, not easily amenable to X-ray crystallography or NMR. The last part of my talk focuses on the mechanism of osmolyte sensing by a bacterial membrane anchored kinase, EnvZ. This kinase is also membrane anchored and HDXMS has provided important insights into membrane effects on receptor dynamics. Our results reveal osmolytes promote intrahelical H-bonding that enhances helix stabilization, increasing autophosphorylation and downstream signaling. The model provides a conserved mechanism for signaling proteins that respond to diverse physical and mechanical stimuli and has important biopharmaceutical applications in identifying the effects of osmolytes in therapeutic protein formulation, storage and stability.

NOTES:
All Atom Molecular Dynamics Simulations of Antibodies in Explicit Solvent… Quite a Mouthful But the Results Are Worth Gnawing On

Tom Patapoff

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

Higher Ordered Structures… We talk about them but do we really have an understanding of what they truly are or might be? One way of investigating the nature of higher ordered structures is to perform in silico molecular dynamics simulations to "see" what could potentially occur. Knowing what may happen can give insight into what techniques might be useful in confirming the presence of the predicted structures in solution. This presentation will examine the progress being made to evaluate possible higher ordered structures of MAbs and some experimental results that corroborate those structures.

NOTES:
Computational Assessment of Pharmaceutical Properties for Protein Therapeutics

Stanley Krystek

*Bristol-Myers Squibb Company, Princeton, NJ USA*

Challenges for developing antibody-based therapies include the identification of minor components resulting from protein production such as purification, charge variants, glycoforms, disulphide bridge isoforms and other chemical modifications (oxidation and deamidation) within protein milieu. Because the protein variants often differ in their biophysical and biochemical properties it is essential to characterize protein stability along with pharmacokinetic and pharmacodynamic properties. We have developed a series of computational methods that augment experimental methodologies and are part of a strategy used to understand and optimize the structure of clinical candidates increasing protein homogeneity and providing a strategy for development.

NOTES:
Hydrogen Deuterium Exchange FTIR Analysis of Protein Higher Order Structure and Dynamics

Yijia Jiang; Cynthia Li; Hai Yue; Xichdao Nguyen

Amgen Inc, Thousand Oaks, CA USA

Protein higher order structure and dynamics play key roles in the function and stability of these macromolecules. Hydrogen deuterium exchange (HDX) has been used with spectroscopic methods, especially mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectrometry, to assess protein dynamics and flexibility. In the presentation, we are going to show that hydrogen deuterium exchange can also be used with Fourier transform infrared (FTIR) spectroscopy for the analysis of protein higher order structure and dynamics. HDX and FTIR is used to probe the dynamics of specific conformational motifs within a protein rather than the linear sequence. Method characteristics such as reproducibility, and robustness will be investigated. Application case studies will be shown to demonstrate the sensitivity and utility of this approach. Comparison with other methods such as circular dichroism (CD), differential scanning calorimetry (DSC) and hydrogen deuterium exchange with MS will also be discussed.

NOTES:
H/D Exchange Mass Spectrometry for Probing Higher Order Structure of Protein Therapeutics: Current Status and Future Directions

Guodong Chen

*Bristol-Myers Squibb Company, Princeton, NJ USA*

One of the key challenges in the characterization of protein therapeutics is how to define the higher order structures (HOS) that often dictate their biochemical functions. Classical biophysical methods do not provide detailed conformational information. Characterization at the molecular level is possible by NMR, but these experiments are often limited to smaller proteins, are time consuming and require high sample concentrations. X-ray crystallography provides detailed structural information, however, it requires the formation of appropriate crystals and the solid state structure may not reflect the protein conformational dynamics in solution. Hydrogen/deuterium exchange mass spectrometry (HDX-MS) has emerged as a powerful technology for probing HOS of protein therapeutics. This presentation will highlight current status and future directions in HOS analysis of protein therapeutics by HDX-MS technology, including epitope mapping for protein/drug interactions and biopharmaceutical comparability studies.

NOTES:
Position Specific Effects of Chemical Composition on Protein Stability

Jennifer Laurence

University of Kansas, Lawrence, KS USA

Protein stability depends on both the composition of the protein and the solution environment into which it is placed. Often approaches to examining stability are chosen because they are amenable to high-throughput screening and these tools are applied to evaluate the effects of different formulation conditions. These techniques tend to rely on low-resolution analysis tools and assessment is performed as a function of temperature or agitation to detect alteration of the structure or aggregation of the product. These fast, low-resolution techniques have enabled more rapid identification of best conditions, but little understanding has been gained about how stabilization is achieved. Compositions that stabilize one protein do not necessarily affect similarly another, even homologous, protein. This suggests stability may be determined by surface properties, local interactions and/or conformational dynamics. Solution NMR was used to detect perturbations to individual residues and specific influences on stability were extracted from cross-correlation with standard analysis approaches to assess mechanisms of inherent and induced instability in proteins.

NOTES:
A key challenge in developing therapeutic monoclonal antibodies (mAbs) is their complex self-association behavior at high concentrations (>50 mg/mL). Unfortunately, the low purities, small quantities and large numbers of mAb candidates available during antibody discovery are incompatible with conventional methods of measuring mAb self-association. We report a method (affinity-capture self-interaction nanoparticle spectroscopy, AC-SINS) for identifying mAbs with low self-association propensity that is robust at extremely low antibody concentrations (<0.05 mg/mL) and in the presence of contaminants. Gold nanoparticles are conjugated with polyclonal antibodies specific for human mAbs, and these conjugates are used to capture mAbs from dilute solutions. We find that the interparticle distance-dependent plasmon wavelength of the gold conjugates redshifts in a manner that is well correlated with light scattering measurements conducted at three orders of magnitude higher antibody concentrations. We are currently using AC-SINS for identifying mAb candidates with high solubility during antibody discovery as well as for improving mAb formulation during antibody development.
High-resolution NMR as a Higher Order Structure Assessment Tool for Protein Therapeutics

John Marino

NIST, Rockville, MD USA

Protein biologics vary greatly in size from a few thousand daltons to hundreds of kilodaltons and require higher-order folding of the primary sequence (i.e. tertiary structure) for therapeutic function. High-resolution NMR provides a simple, robust spectroscopic approach for obtaining higher-order structural 'finger-prints' of the bioactive form(s) of protein therapeutics at atomic resolution in solution. Such 'finger-prints' of the structure(s) of protein therapeutics can be used as a tool for establishing consistency in drug manufacturing, for detecting drug product variations inherent to or resulting from modifications in the manufacturing process, and for comparing a biosimilar to an innovator reference product. In this presentation, I will describe an NMR spectral 'finger-printing' assessment of various formulations of granulocyte colony stimulating factor (G-CSF a.k.a. Filgrastim) carried out through an inter-laboratory comparability study, involving groups from the FDA, NIST, Health Canada and MPA-Sweden. Results from the study demonstrate the precision with which NMR spectral 'finger-prints' can be used as a structure assessment and comparability tool for protein therapeutics.

NOTES:
Monitoring and Analysis of Protein Pharmaceutical High Molecular Weight Species During Manufacturing: A Case Study

Stephen Raso

*Biogen Idec, Cambridge, MA USA*

This presentation centers on the characterization and analysis of protein-based pharmaceuticals during development. Of particular focus is the in-process monitoring of high-molecular weight (HMW) protein species during manufacturing at an industrial scale. A case study will be provided wherein biophysical characterization of a protein drug substance batch was used to investigate out-of-trend HMW levels observed in some of the process intermediates. These out-of-trend HMW results correlated with an atypical process step incurred specifically during the manufacturing of this batch.

NOTES:
Investigating the Effects of the Metal-binding *cla*MP Tag on the Structure and Stability of Protein Conjugates

Brittney J. Mills; Jennifer S. Laurence

*University of Kansas, Lawrence, KS USA*

Incorporation of peptide tags into proteins is desirable because addition of a short peptide sequence can greatly expand the capabilities of the protein. The development of peptide-based metal-binding tags has gained interest because addition of the tag to a protein allows for targeted metal delivery for imaging and therapeutic applications. Incorporation of a metal-binding tag into a protein is typically achieved through chemical conjugation; inline incorporation options are limited. Chemical conjugation often results in a heterogeneous product, poor yield, and decreased stability. Our lab discovered a novel tripeptide (Asn-Cys-Cys) known as the *cla*MP tag, which can be incorporated inline and potentially used as a tag in imaging and therapeutic applications. In this work, epidermal growth factor (EGF), which contains three disulfide bonds, was selected as a model to determine the effects of the *cla*MP tag on the structure and stability of a disulfide containing protein. The *cla*MP tag was genetically engineered into the plasmid and *E. coli* was used to generate *cla*MP-tagged EGF. Various analytical methods were used to characterize metal-bound *cla*MP-tagged EGF and to assess the stability of both the protein and the *cla*MP tag. NMR spectroscopy confirms the overall fold of the protein is maintained in the presence of the *cla*MP tag with only minor perturbations of residues near the tag. Because addition of the metal-bound *cla*MP tag causes a change in charge of 2’, anion exchange chromatography was also used to evaluate the stability of the conjugate through monitoring of the overall surface charge of the molecule. The effects of extrinsic variables, such as buffer conditions, on the stability of the conjugate were also investigated. Through this evaluation, it was determined that *cla*MP-tagged EGF is stable under the conditions studied, which allows for great potential in the application of the *cla*MP tag for therapeutic or imaging purposes.

**NOTES:**
Epitope Mapping of EGFR Binding to an Adnectin by Fast Photochemical Oxidation of Proteins (FPOP)

Yuetian Yan¹; Guodong Chen²; Hui Wei²; Richard Huang²; Jingjie Mo²; Don Rempel¹; Adrienne Tymiak²; Michael Gross¹

¹Washington University, St. Louis, MO USA; ²Bristol-Myers Squibb Company, Princeton, NJ USA

Epitope mapping has gained more importance during the past 25 years, accompanying the development of monoclonal antibodies (mAbs) as protein therapeutics. Recently, a class of therapeutic mAb alternatives, which are termed adnectins and are derived from the tenth type III domain of human fibronectin (10Fn3), has been developed as targeted biologics. To understand their binding to targets, a suite of appropriate tools is needed. One approach is X-ray crystallography, and it was used to determine the structure of a highly specific Adnectin that binds to human EGFR. We are interested in implementing a relatively new approach, namely protein footprinting coupled with mass spectrometry as readout, as a structural biology tool that complements X-ray crystallography but is more sensitive and has faster turnaround. We report here the use of fast photochemical oxidation of proteins (FPOP), a footprinting method that uses OH radical reaction with protein side chains, coupled with MS, to map the epitope of EGFR/Adnectin at both the peptide and residue levels. The epitope binding site, as determined by footprinting, is located near the N-terminus of the EGFR, consistent with the previously determined epitope from the X-ray crystal structure. The outcome adds credibility to oxidative labeling in epitope mapping and suggests more applications as a stand-alone method or in conjunction with X-ray crystallography or possibly NMR.

NOTES:
Differentiation of Protein Particles and Silicone Oil Droplets by Flow-imaging Microscopy (MFI and FlowCAM) and Resonant Mass Measurement (Archimedes)

Daniel Weinbuch¹,²

¹Leiden University, Leiden, The Netherlands; ²Coriolis Pharma, Martinsried-Munich, Germany

We comparatively evaluated flow-imaging microscopy and the recently introduced technique of resonant mass measurement (Archimedes, RMM) as orthogonal methods for the quantitative differentiation of silicone oil droplets and protein particles in the submicron and micron size range.

Representative samples of artificially generated silicone oil droplets and protein particles were prepared and analyzed both separately and in defined mixtures by the different instruments (MFI4100 and MFI5200, Protein Simple; FlowCAM VS1 and FlowCAM PV, Fluid Imaging; Archimedes, Affinity Biosensors).

An accurate differentiation of silicone oil droplets and protein particles could be achieved by all flow-imaging microscopy systems in the micron size-range. Clearly lower standard deviations (higher precision), were obtained by MFI5200 and FlowCAM PV due to the higher sampling efficiency, as compared to MFI4100 and FlowCAM VS1. The customized filter (MFI4100) was more reproducible and enabled reliable discrimination for more extreme mixing ratios. RMM showed highly accurate discrimination form about 0.5 to 2 µm independent of the ratio between silicone oil droplets and protein particles, provided that sufficient particles were counted.

We recommend combining flow imaging microscopy and resonant mass measurement for a comprehensive analysis of biotherapeutics potentially containing silicone oil droplets and protein particles in the submicron and micron size range.

NOTES:
Protein Solubility as a Predictive Assay for Formulation Development

Douglas Banks

Amgen Inc., Seattle, WA USA

Two formulation strategies, based on different aggregation models, were compared for their ability to predict which co-solutes would minimize the aggregation rate of an IgG1 monoclonal antibody stored long term at refrigerated and room temperatures. These studies indicate that the stabilizing effect of co-solutes formulated at isotonic concentrations is derived from their ability to solubilize the native state ensemble, not by the increase of protein conformational stability induced by their presence. The degree the co-solutes solvate the native state was estimated from the apparent transfer free energy of the native state from water into each of the co-solutes.

NOTES:
Impact of Sub-visible Particles (SVPs) on Viscosity of Monoclonal Antibody (mAb) Solutions

Prasad Sarangapani\textsuperscript{1}; Maria Miller\textsuperscript{1}; Maria Monica Castellanos\textsuperscript{2}; Ralph H. Colby\textsuperscript{2}; Jai A. Pathak\textsuperscript{1}

\textsuperscript{1}MedImmune, Gaithersburg, MD USA; \textsuperscript{2}Penn State, University Park, PA USA

The impact of SVPs on common unit operations and drug delivery is of significant interest to the biopharmaceutical industry due to the deleterious consequences during processing and possible immunogenicity concerns. During fill/finish and purification, the interactions of mAbs with solid-liquid and liquid-air interfaces in pumps may result in the formation of SVPs, leading to higher solution viscosities ($\eta$) and pressure drops required to transport material. In this two part talk, we first assess the impact of titanium and steel surfaces on SVP formation and $\eta$ in situ using a combination of rheology and small-angle neutron scattering (rheo-SANS). Stainless steel surfaces are found to increase $\eta$ with a simultaneous increase in SVPs. In the second part of the talk, we address the impact of prolonged aggregation on $\eta$ of a mAb under accelerated stability conditions, where we find an increase in solution viscosity. A transition from Newtonian behavior ($t=0$) to non-Newtonian behavior occurs and was proven to be related to SVPs, where upon filtration, a purely Newtonian response was recovered. SANS measurements of the aggregated mAbs also showed an increase in scattered intensity at low-angles, whereas the control sample showed no such feature. We discuss a possible assembly mechanism for SVPs in mAb solutions and its impact on $\eta$.

NOTES:
Optimization of the formulation and storage of protein biologics products must be done empirically, and may be difficult to achieve. Aggregation and loss of activity over time may occur together or individually; both process conditions and formulation choice can affect stability. For polyclonal IgG preparations, stability is dependent on stabilizer choice, pH, and salt content of the formulation medium, as well as ionic strength, protein concentration, and shear stress during processing. Routine characterization of IGIV products for molecular size distribution can be done using off-the-shelf technologies, e.g., SE-HPLC, dynamic light scattering (DLS), light obscuration per USP <788>, particle counting, and differential scanning calorimetry (DSC), although development of better methods and standards is underway. Developing reference standards to allow absolute measurement of protein aggregates size is problematic, since the process of aggregation is dynamic; currently, clinical efficacy and safety of parenteral protein products must be linked to consistent analytical methodology and controls, and well-controlled process conditions. Primary sources of official guidance include the USP, other harmonized compendia, FDA guidances, and individual Agency feedback for specific products.

NOTES:
Regulatory Considerations for the Assessment Higher Order Structure

Peter Adams

CDER, FDA, Bethesda, MD USA

The higher order structure of a protein is fundamentally linked to its biological activity. Assessment of higher order structure is an important component of the development of protein therapeutics and the manufacturing process should yield a product with the appropriate higher order structure. The biological activity and clinical profile of a protein therapeutic can be significantly affected by multiple factors between the time of its manufacture and subsequent delivery to a patient. Similarly, modifications introduced into a manufacturing process can have effects on protein structure and function, that can in turn alter the clinical profile of the protein therapeutic. This presentation will provide an overview of the regulatory requirements and guidances that are associated with the evaluation of higher order structure of protein products during product development and comparability.

NOTES:
Novel Antibody Therapeutics with Engineered Features and Impact on Immunogenicity

Sally Fischer

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

To improve the effectiveness of antibody therapeutics, a variety of antibodies with engineered features have been generated. These engineered features are designed to improve various characteristics of the molecules. This presentation will focus on a case study where a mutation intended to increase FcRn binding affinity caused unforeseen challenges in the immunogenicity evaluation of the molecule.

NOTES:
B-cell Epitope Prediction and Recovery of Monoclonal ADAs to Assess Frequency and Significance of Break of Immune Tolerance Against Protein Therapeutics

Stefan Ryser

*Trellis Bioscience LLC, South San Francisco, CA USA*

Although most Anti-Drug Antibodies (ADAs) are innocuous, some low abundance or low frequency ADAs may pose a significant clinical development risk which is difficult to evaluate at the serum level. Trellis’ established technology platform CellSpot® for cloning rare memory B-cells enables deconvolution of serum into its component monoclonal ADAs, thereby providing a cost effective approach to managing the ADA risk. Our computational B-cell epitope prediction technology accelerates cloning of a comprehensive suite of ADAs against all epitopes on the protein. As CDRs are the only areas in mAbs that are not necessarily immune tolerated, we compared the pattern of predicted B-cell epitopes in the CDRs of commercially available mAbs and found a higher density of such potentially immunogenic epitopes for adalimumab, which is known to induce a high frequency of ADAs. The prediction technology further enables efficient immunological silencing of problematic epitopes, a task that has been pioneered by Ira Pastan at the NCI for PE-38. Interestingly, we have found in some healthy donors polyspecific IgG antibodies that bind multiple antigens at nanomolar affinity. Unlike the more common IgM mAbs that bind more weakly, such polyspecific IgG antibodies may represent pre-existing antibodies against protein based therapeutics with potential to mature into clinically significant ADAs. The finding may offer a solution to pre-screen for patients with a higher risk to develop ADAs.

NOTES:
Solution Structural Biology: The Use of Small-angle X-ray Scattering in Drug Discovery for Flexible Protein Targets

Nino Campobasso

*GlaxoSmithKline, Collegeville, PA USA*

Protein crystal structures are routinely used for drug discovery and the targets being investigated with crystallography are increasing in complexity. Multi-domain proteins and multi-component systems are complex and can be difficult to crystallize. Even though a typical protein crystal may be 50% solvent, component domains may be different in solution to what is seen in the crystal due to crystal packing interactions. Crystal growth requires a protein to maintain a rigid pose to crystallize. Therefore, one must determine whether the pose seen from a crystal structure is an artifact of crystallization by investigating solution state properties. Various technologies can be used to characterize the solution state of proteins and to develop more detailed structure-function conclusions. This talk will discuss Small-Angle X-ray Scattering (SAXS) as a tool for drug discovery. We show that a multi-domain protein can crystallize but the behavior of the protein in solution shows that the relative domains are not the same as what is seen in the crystal. In addition, the solution scattering properties of proteins have also guided efforts in gaining a crystal structure of complex systems. Crystallography provides a static model of a protein structure. With additional solution scattering data, one can begin to generate more detailed understanding of the dynamic nature of complex systems.

**NOTES:**
Peptide-conjugation Induced Antibody Conformational Changes Revealed by Individual-particle Electron Tomography

Gang (Gary) Ren

Lawrence Berkeley National Laboratory, Berkeley, CA USA

Antibody-based therapies are currently undergoing a renaissance, in which, more than two dozens of antibody-based therapeutics have been approved to date by the FDA, and numerous others are currently undergoing development. Given the need for therapies, a high-throughput tool for antibody structural characterization is urgently required.

Antibodies in solution are naturally flexible and dynamic. Dynamic personalities and structural heterogeneities of antibodies are essential to understanding their proper function. However, structural determination of dynamic/heterogeneous antibodies is limited by current technology such as: X-ray crystallography, nuclear magnetic resonance spectrum, small angle scattering, and electron microscopy single-particle reconstruction. A common weakness of all current techniques is requiring an averaged signal from thousands to millions of different macromolecules.

A fundamental solution in the structural determination of dynamic antibodies should rely on the structure determination of each individual antibody instead of averaging thousands of different antibodies. We recently report our novel approach, individual-particle electron tomography (IPET) for 3D reconstruction of each individual antibody structure via imaging a series of tilted viewing images of a single antibody by electron tomography and computing these images into a 3D density map. By comparing the “snap-shot” 3D structures from different antibodies, IPET provide an experimentally approach to study antibody fluctuation and dynamics, even to examine the conformational change of domain structure of antibody after conjugation of peptides. For example, we studied the conformational change of antibody drug conjugates (ADCs). The monoclonal antibodies (mAbs) attached to peptides by chemical linkers with labile bonds. Peptides show much promise as potent and selective drug candidates. Fusing peptides to a scaffold monoclonal antibody produces a conjugated antibody which has the advantages of peptide activity yet also has the pharmacokinetics determined by the scaffold antibody. However, the conjugated antibody often has poor binding affinity to antigens that may be related to unknown structural changes. The study of the conformational change is difficult by conventional techniques because structural fluctuation under equilibrium results in multiple structures co-existing. By our individual-particle electron tomography (IPET) technique, we the domains of antibodies present an elongated peptide-conjugated conformational change based on two-dimensional (2D) image analyses and three-dimensional (3D) maps. The example suggests that our technique may be a novel tool to monitor the structural conformation changes in heterogeneous and dynamic antibodies before and after syntheses.

NOTES:
Biofabrication: An Assembly Paradigm for Device Construction that Facilitates Communication

William Bentley

University of Maryland, College Park, MD USA

Definition: Biofabrication is the use of biological or biomimetic materials and mechanisms for fabrication. Biofabrication complements traditional microfabrication methods by enabling both the hierarchical assembly of nanocomponents into devices and the interfacing of biology with electronics.

Vision: The five human senses are complex networked systems that enable exquisitely sensitive, rapid and reliable interrogation of the surroundings. They involve the chemo-, photo-, mechano- and thermo-reception of cues. They rely on biological transducer molecules (e.g. transmembrane glycoproteins, porins) whose function is often mediated by small changes in structure. When stimulated, they transduce information by the generation and transport of molecules. They are information processing systems that filter out noise, scale stimuli, and reformulate these cues into focused signals for actuation. Actuation again is mediated by molecular signals; the precise outcomes are typically context dependent. For example, the same signal, the same transduction cascade in the context of the identical cell can lead to a completely different transcriptional response when cells are cultured at different temperatures. Conversely, microelectronic devices communicate via electrons and photons and we expect identical outputs for given inputs irrespective of context. Microfabricated devices are typically not adaptive and are constructed using completely orthogonal design principles from those of biology. Yet, we expect devices to communicate with and modulate biological function.

Many have predicted that the effective interfacing of biology and microfabrication will enable remarkable advances in medicine, industry and national security. We will discuss the engineering of a device/bio interface to facilitate transduction of chemical cues to microelectronic devices and back. Fundamental to our approach is the desire to assemble proteins, DNA, and polysaccharides onto devices that preserves function. The goal of our research is to enlist biomolecular, biofabrication, and microsystems engineering to “translate” messages between biology and microfabricated devices. That is, we are attempting to create a “thesaurus” for understanding the language of biology and to transform the way in which nanoscale components of biological origin are assembled and actuated to sense and modulate biological signaling. In this way, it may be reasonable to expect that biomedical devices of the future will be comprised of biological materials and assembled via biological means.

NOTES:
Workshop Abstract

Wednesday, February 19
16:45 – 18:15
Potomac Ballroom

Facilitators:
John Gabrielson, Amgen Inc., Longmont, CO USA
William Weiss, Eli Lilly and Company, Indianapolis, IN USA

This workshop will explore the evolving role of higher order structure data in technical decision-making in biopharmaceutical development. The discussion will be structured around two hypothetical case studies with practical relevance to the industry. Risk-tolerance and opportunity cost will be central themes. Open and transparent participation is highly encouraged and will largely determine the overall success of the discussion. The workshop will conclude by reviewing the results of an anonymous pre-meeting survey that will give participants additional insight into how peers and colleagues understand the use of higher order structure tools now and in the future.

Notes:
Biotherapeutic Structure and Dynamics: A Critical Attribute of the Highest Order

Asish Chakraborty

Waters Corporation, Milford, MA USA

The drive to produce safer, more powerful and market-differentiated biotherapeutics has accelerated the need for tools that efficiently demonstrate the interactions, dynamics, and structural comparability of ever more complicated biotherapeutic proteins. Waters has commercialized innovative technologies such as UPLC, High Resolution Mass Spectrometry, Ion Mobility, and HDX along with associated chemistries, standards, and informatics that are enabling researchers to address a variety of difficult questions encountered during the development of their innovator or biosimilar biotherapeutics:

- Is this mutation likely to produce a biobetter or a bio-headache?
- Does this new manufacturing process produce a protein with the same structure as the old one?
- Why is my protein aggregating?
- Does PEGylation or drug conjugate addition at site X likely cause protein functional changes?
- Why does the latest production batch peptide map profile look fine, but exhibit altered bioassay results?
- Does my refolded recombinant protein look like the native protein?
- Can I protect my new mAb with IP by showing unique interactions with its target?
- It has the same sequence but is it structurally biosimilar?
- Do I have a formulation that maintains the structural stability of my molecule?
- What happens when this small molecule drug binds my protein?

Waters is continuing our investments to advance these innovative technologies and make them routinely accessible across biopharmaceutical organizations. Please join us for lunch, and a discussion of how new approaches to biotherapeutic structural analysis are expanding the range of scientific questions, proteins, and biological systems that can be addressed using the combination of these powerful technologies.

NOTES:
BioTools, Inc. Technical Seminar
Wednesday, February 19
07:30 – 08:30
Potomac Ballroom

FT-IR Spectroscopy for Structure Elucidation of BioTherapeutics: The Do’s and Do NOT’s – Because the Correct Spectrum Matters!

Rina Dukor

BioTools, Inc., Jupiter, FL USA

During the past decade, FT-IR spectroscopy has gained significant prominence as a tool for structure elucidation in all facets of bio-therapeutic development – from formulations in R&D to Quality Control in Manufacturing. FT-IR has many advantages including measuring: 1) at dosage concentrations – both low and high; 2) in different states such as liquid and solids; and 3) with variety of excipients. The measurements are fairly easy and equipment very affordable. In the last two meetings in this series, speakers from FDA have listed FT-IR as one of the techniques recommended for proof of folding and structure comparability. And although FT-IR is a well-established technology for small molecules, there appears to be much confusion in the bio industry. Specifically the following questions arise:

- is there a difference between transmission and ATR? Why do the spectra look different?
- What region of the spectra to consider? And to present to regulatory agencies?
- What is considered ‘the same’ in spectral comparability?
- How to deal with excipients especially those that have different polymorphic forms?
- ‘unknown’ peaks in the spectra (due to vapor? Noise? Or real differences in structure?)
- and most important of all – ‘what does the CORRECT FT-IR spectrum looks like’?

It will be difficult to address all the questions in this short workshop, but we will cover the few fundamentals and how to judge spectral quality.

NOTES:
Agilent Lunch and Learn Seminar
Wednesday, February 19
12:05 – 13:05
Potomac Ballroom

TBD

TBD

TBD

Abstract not available at the time of print.

NOTES:
High-throughput Tools for Molecular Assessment and Formulation Screening

Arun Alphonse Ignatius; Anthony Young; Sandeep Kumar; Wei Wang; Donna Luisi

Pfizer, Inc., Chesterfield, MO USA

Purpose: To demonstrate the potential of high-throughput biophysical tools in predicting protein aggregation, viscosity behavior and storage stability.

Methods: Colloidal stability ($K_D$) and conformational stability ($T_m$) have been determined for a set of mAbs and fusion proteins using dynamic light scattering and extrinsic-fluorescence techniques, respectively. The physical parameters were qualitatively correlated to known protein behavior.

Case Study 1: A database of $K_D$ and $T_m$ was determined for a set of 11 candidate molecules, selected based upon their known aggregation and/or viscosity behavior. $T_m$ did not show significant correlation with aggregation. $K_D$ showed a clear qualitative correlation for the candidates with known aggregation and viscosity issues.

Case Study 2: Fc1, a bispecific fusion protein, exhibited negative $K_D$ values between pH 5.5-6, suggesting attractive protein-protein interactions and a positive value around pH 5, implicating repulsive forces in solution. The conformational stability ($T_m$) showed an increasing trend from pH 4 to 6. The $K_D$ data qualitatively correlated with the aggregation rate under accelerated storage conditions; however the relative $T_m$ values did not show any significant correlation.

Conclusion: Results from this study demonstrate the potential of biophysical tests in predicting aggregation and viscosity behavior of candidates. Due to the high-throughput nature and low material requirement of these methods, it can be used as an effective screening tool in molecular assessment as well as in formulation development.

NOTES:
Characterization of Concentration-dependent Self-Association in a Monoclonal Antibody by Hydrogen/Deuterium Exchange Mass Spectrometry

George Bou-Assaf; Julie Wei

Biogen Idec, Cambridge, MA USA

Concentration-dependent self-association is observed in many biological molecules including monoclonal antibodies (mAb). This phenomenon could be reversible or irreversible. It is indispensable to assess whether increasing the concentration of a therapeutic biomolecule induces self-association and how the higher-order structure of the biomolecule is affected. In the current work, we demonstrate that a mAb undergoes concentration-dependent self-association as revealed by analytical ultracentrifugation (AUC). More specifically we show how hydrogen/deuterium exchange (HDX) monitored by mass spectrometry (MS) was used to characterize self-association in this mAb at the peptide level. We explain the experimental consideration needed to carry the experiment at high concentration. More importantly, we identify the sites affected by self-association and conclude that those sites relate to residues that are either located at the interface of the interaction or were modified by allosteric effects.

The Importance of Thresholding in Imaging Analysis of Protein Aggregates

Lew Brown

Fluid Imaging Technologies, Inc., Scarborough, ME USA

Dynamic imaging particle analysis (DIPA) shows much greater sensitivity to transparent particles, such as protein aggregates, than light obscuration can. While not yet fully accepted in industry compendia, DIPA is being used increasingly in the formulation process for characterization of sub-visible particulates in biologics. DIPA measures particle size and shape by first creating a binary image based upon a defined threshold from the background value for each pixel in the image. The resulting binary image is used for all particle measurements, so the choice of thresholding technique is critical to the validity of the measurements.

This poster will demonstrate how different thresholding techniques can produce dramatically different characterization of protein aggregates. To do this, a base set of camera images are acquired for a therapeutic protein containing aggregates. These “raw images” can then be run through image processing software using different thresholding techniques to quantify and measure the aggregates contained. By using the same set of images for each thresholding process, we insure that any variation in results can only be caused by the thresholding process. The results will show striking differences caused by the thresholding variation, thereby supporting the importance of proper thresholding when using DIPA.
NOTES:
An Improved HDX MS System for On-line Digestion, Separation and Data Analysis

Asish Chakraborty; Ying Qing Yu; Michael Eggertso; Keith Fadgen; Weibin Chen

Waters Corporation, Milford, MA USA

This study reports improvements in hydrogen deuterium exchange mass spectrometry (HDX MS) for protein higher order structure analysis. The improvements are gained from 1) on-line digestion using a novel immobilized pepsin column that can sustain high pressure digestion and 2) H/D uptake data analysis using automated software. The high pressure digestion was accomplished using a micro-scale UPLC system that can operate at 15,000 PSI, an immobilized pepsin column packed with mechanically-strong particles (Ethylene-Bridge-Hybrid BEH). Data generated from this study demonstrated that the higher operating pressure rendered by the UPLC improves chromatographic resolution; the digestion efficiency of the pepsin BEH column is enhanced during higher pressure digestion.

Pepsin was immobilized onto high-pressure resistant BEH-particles. The particles were packed into a 2.1x30 mm UPLC column. This column was placed on a LC system specifically designed for HDX-MS analysis. Phosphorylase b, cytochrome C, and IgG were digested online under a pressure range above 8000 psi at 0, 10, or 25 °C, respectively. Peptides were chromatographically separated on a 1x50 mm column, and the eluent was directed into a high resolution Q-Tof mass spectrometer for mass analysis. Deuterated LC-MS data were automatically processed using DynamX software.

Data from on-line digest using pepsin immobilized BEH-particle confirmed that the chemical immobilization process was properly carried out and enzymatic activity of pepsin was not affected by the immobilization chemistry. The online digestion of phosphorylase b protein was robust and reproducible, with and without pressurization. An increased number of overlapping peptic peptides were generated from pressurized digestion resulting in improved coverage and redundancy score. Increasing the digestion temperature of the online pepsin column resulted in higher sequence coverage of cytochrome C from 63% at 0 °C to 100% at 25 °C. The rate of back-exchange from deuterated proteins was measured for both the high-pressure tolerant BEH pepsin column and commercially available pepsin columns. Comparable back-exchange rates were obtained with the BEH pepsin column compared to the commercially available pepsin columns. An enhanced chromatographic resolution was achieved for protein digests separated in less than 10 min at 0 °C. The data processing software improves the speed and consistency of deuterium uptake measurement for the peptides generated using the HDX platform. A set of data interpretation/display tools such as coverage map and a heat map options were provided in the informatics tool, facilitating efficient data comparison.

NOTES:
P-105

An Integrated Workflow for Automatic Mapping of Disulfide Linkages of Therapeutic Proteins using High-resolution LCMS, ETD Fragmentation and Targeted Informatics

Stephane Houel; Henry Shion; Asish Chakraborty; Weibin Chen

Waters Corporation, Milford, MA USA

Disulfide bond formation is critical for establishing three-dimensional folding and maintaining proper function of therapeutic proteins such as monoclonal antibodies (mAbs). Localization and assignment of disulfide bonds are therefore an important aspect in protein structural analysis. In this study, an advanced peptide mapping workflow was demonstrated for detection and identification of disulfide linkages in recombinant therapeutic proteins. The integrated workflow includes on-line reversed-phase ultra-performance liquid chromatographic (RP-UPLC) separation of trptic digests, enhanced mass resolution quadruple time-of-flight (Q-TOF) mass spectrometric (MS) detection, electron-transfer dissociation (ETD) fragmentation and automatic data interpretation and annotation with a dedicated peptide mapping software. Peptides and disulfide linkages were identified by accurate masses of precursors and further confirmed by peptide fragment spectra, collected by an alternating low and elevated collision energy MS acquisition (MS²E) mode. The enhanced mass resolution and mass accuracy were proven to be particularly useful for the assignment of disulfide bond-linked peptides with high molecular masses. ETD technique was used to induce both disulfide bond cleavage and backbone fragmentation, thus allowing the sequence information to be obtained from the intrachain disulfide loop region of the peptide. Both expected and unexpected (scrambled) disulfide linkages in several therapeutic proteins were rapidly assigned/characterized by the informatics tool using an automatic data processing routine. This integrated approach, combining high performance LC-MS²E, ETD and targeted software, should be applicable for fast mapping and monitoring of disulfide linkages in the development of therapeutic proteins.

P-106

Epitope Mapping of Bexsero® Vaccine Antigens using HDX-MS

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The identification of protective epitopes is an important step to understand the mechanism of an effective immune response and designing improved vaccines, therapeutics, and diagnostics. Mapping of B-cell epitopes corresponding to effective neutralizing antibodies allows the development of epitope-based vaccine designed to overcome pathogen diversity and capable to elicit potent response in vaccinees. Linear epitopes identified by conventional mapping techniques only partially reflect the immunogenic properties of the epitope in its natural conformation, thus limiting the success of these approaches. At the moment, the gold standard for epitope definition is X-ray analysis of antigen-
antibody complex crystals. This method provides atomic resolution of the epitope; however, it is not readily applicable to many antigens and antibodies, and requires a very high degree of sophistication and expertise. Among the other techniques able to map both linear and conformational epitopes, the Hydrogen/Deuterium Exchange coupled to Mass Spectrometry (HDX-MS), is one of the most effective and is able to rapidly supply nearly complete information about epitope structure at medium-high resolution. The HDX-MS approach relies on the differential solvent accessibility of the free and bound antigen to a deuterated buffer. Thus, the antigen epitopes in interaction with the antibodies are protected from the solvent and incorporate less deuterium compared to the free antigen. We report here the mapping of the epitopes recognized by 11 different monoclonal antibodies generated against the three main antigens of Bexsero®, the Novartis newly licensed multicomponent vaccine against *Neisseria meningitidis* serogroup B (MenB).

**NOTES:**
Determining Site-specific Interaction of Epidermal Growth Factor Receptor Binding to an Adnectin by HDX MS and ETD Technique

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Epidermal growth factor receptor (EGFR) is a key cellular signaling protein and blocking EGFR signaling is a validated strategy for cancer therapy. Adnectins are targeted therapeutic proteins derived from the tenth type III domain of human fibronectin, and their protein constructs can be optimized for high affinity binding to targets. The anti-EGFR adnectin was identified that specifically bound to EGFR and inhibited its intracellular signaling. In this work, we applied hydrogen/deuterium exchange mass spectrometry (HDX-MS) coupled with electron transfer dissociation (ETD) to probe the binding contacts at residue level in the EGFR/adnectin complex. Utilizing ETD on a targeted peptide of interest allowed us to obtain site-specific HDX information for the binding epitope in EGFR/adnectin.

Prior to deuterium labeling, EGFR was incubated with the adnectin for 30 minutes at room temperature. 1 hour labeling experiments were carried out on EGFR only and on the EGFR/adnectin complex. Aliquots were quenched at pH 2.5 and the samples were digested online by pepsin prior to UHPLC separation at 0 °C. MS analyses were performed using an ESI-QTOF SYNAPT G2-S with ETD coupled to a nanoACQUITY HDX system (Waters).

Peptide analysis confirmed that binding of EGFR to this adnectin revealed three different discontinuous interaction interfaces located in domain I of EGFR. The overlapping peptides from those regions showed significant differences in deuterium uptake up to 4 Da upon binding. The ETD fragments of the selected peptide, 1-19, pinpointed the protected exchange at TQL (15-17) upon binding, which reflected the same interaction contacts determined by previously published crystallography data (TQLG, 15-18). In addition, protection was observed at neighboring residues (K13, L14), which were not directly involved in the binding contacts as determined by crystallography. This study demonstrates the utility of combining HDX-MS with ETD to achieve high spatial-resolution and site-specific structural analysis of binding interfaces.

NOTES:
P-108

Size, Shape and Chemical Identification of Sub-visible Particulates via Microscopy and Raman Spectroscopy in a Single Automated Platform

Linda H. Kidder; Kevin E. Dahl; Stacy M. Kenyon; Wei Qi; E. Neil Lewis

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The enumeration of Sub-Visible Particulates (SVP) in finished biotherapeutic products is of great interest to patients, manufacturers, and regulatory agencies alike. Such particles are known to promote aggregation in bioformulations, causing reduced efficacy and possible unwanted immunogenicity. Although addressing a different concern, namely preventing the blockage of human capillaries upon injection of a parenteral formulation, manufacturers and regulators have largely followed methods for determination of SVPs as laid out in USP <788>. In this method, the number of particles greater than 10 μm and greater than 25 μm per unit volume are recorded, typically using light obscuration (LO), or through microscopy characterization of particles on a membrane filter or entrained in a flow. None of these existing techniques however, provide chemical information that is required to unequivocally differentiate between bio-aggregates and adulterant materials, which is potentially important in assessing possible immunogenic risk, for example. To this end, an automated Raman microscope (Morphologi G3-ID) has been developed to provide particle count, morphology, and chemical identification of SVPs in bioformulations. The G3-ID collects images of particulates in suspension, classifying their shape and size, as well as Raman spectra that identify bio-aggregates (intrinsic contaminants), glass and cellulose particles (extrinsic contaminants), and silicone oil droplets (inherent contaminants). The results presented will demonstrate that the G3-ID is capable of providing both enumeration and forensic-style identification of SVPs in suspension.

P-109

Colloidal and Conformation Stability Characterization via a Combined DLS/Raman Analytical Platform

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With the growing requirement to formulate protein therapeutics at high concentration, especially monoclonal antibodies, the analytical tools needed for their characterization are strongly desired, but not readily available. In this presentation, we introduce a novel technique for protein therapeutics characterization, specifically the combination of dynamic light scattering (DLS) and Raman spectroscopy in a single platform. This system simultaneously characterizes protein secondary and tertiary structure, as well as hydrodynamic size, even at high concentrations (> 50 mg/mL), enabling the link between colloidal and conformational stability to be explored by exposing samples to a variety of external stressors.
Raman spectroscopy simultaneously derives protein secondary structure (Amide I and III) and tertiary structure markers (aromatic side chains, disulfide bond, hydrogen bonding, local hydrophobicity). These higher order structural determinations are performed at actual formulation concentrations, 50 mg/mL or greater for mAbs, rather than at the diluted concentrations required by conventional methods, i.e. circular dichroism (CD) or fluorescence. DLS using a backscatter detector is capable of measuring the hydrodynamic radius of proteins at high concentrations (over 50 mg/mL). As the technique is based on light scattering, and scattering intensity scales with $r^6$, it is exquisitely sensitive to the formation of aggregates. As a result, both size and structure information is obtained as a function of a variety of perturbations (temperature, pH, salt concentration, excipients, chemical degradation, etc.) in real time, enabling the derivation of protein melting, aggregation onset, and van’t Hoff enthalpy values for specific transitions. The new insights and observations we have learned by applying the above technique to the characterization of real bio-pharmaceutical samples will be shown, including, the stability of model proteins with thermal and chemical denaturation, determination of thermal reversibility of unfolding and aggregation, the oxidization of model proteins, and the distinct difference in behavior between mAb and modified mAb samples under thermal and formulation stressors.

NOTES:
Limitations in the Use of Circular Dichroism and Fourier Transform Infrared Spectroscopy for Comparability of Monoclonal Antibodies

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Spectroscopic techniques such as circular dichroism (CD) and Fourier transform infrared spectroscopies (FTIR) are widely used to characterize the higher order structure (HOS) of proteins. Though the characterization of the HOS is important, the sensitivity of these spectroscopic techniques may not be sufficient for comparability testing especially for large proteins, e.g. monoclonal antibodies.

We assessed the relative sensitivity of techniques such as CD and FTIR spectroscopies to other conventional analytical methods such as size-exclusion and ion-exchange chromatographic methods using a degraded panel of samples of a monoclonal antibody. For FTIR, we found that samples with dramatic changes in the SEC or IEC chromatograms did not show appreciable shifts in the Amide I band of the FTIR spectra.

The same degraded samples resulted in CD signal changes that were largely within the assay variability of the Jasco CD instrument used. In addition, we used a second monoclonal antibody to experimentally quantify the sensitivity of CD in terms of a change in signal from a folded β sheet to an unfolded random coil using an inline 2-cell cuvette experiment design (Cuvette #1: native condition, Cuvette #2: 6M guanidinium denaturing condition) and compared the change to the assay variability. The protein sample analyzed on this CD instrument requires at least a 5% random coil character (i.e. 95% folded) to define a change in the secondary structure-indicating Far-UV signal, and similarly more than 15% random coil character is required when measured in the Near-UV wavelengths, which can indicate tertiary structure changes. The change in signal may originate from a partial folding of the whole ensemble, complete unfolding of a fraction of the ensemble, or most likely both. Regardless of the underlying cause of the signal change, a broad panel of chromatographic assays may be more appropriate than relatively insensitive assays such as CD or FTIR to determine comparability of mAb samples.

NOTES:
P-111

Aggregates and Particle Characterization to Support Biomanufacturing Process Development.

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Aggregates and particles can form through the life cycle of biotherapeutics from upstream and downstream processes to drug storage. Aggregates and particles should be closely monitored and eliminated during manufacturing process.

SEC-HPLC has been the most common method used for aggregate quantification because of its robustness and high-throughput. Unfortunately, there are limitations in SEC-HPLC for aggregate detection such as it cannot detect insoluble aggregates, sub-visible and visible particles. Orthogonal analytical methods (SEC-HPLC-UV and MALS, DLS and Dynamic Imaging Analysis) have been implemented in aggregates and particle characterization through mAb and vaccine manufacturing processes. By implementing DLS and Dynamic Imaging Analysis as complementary methods to SEC-HPLC for aggregates and particle characterization, we are able to improve for biotherapeutic and vaccine manufacturing processes.

P-112

Monitoring Structural Integrity in Biotherapeutic Development using SPR, DSC and 2D-DIGE

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The three dimensional conformation of biotherapeutic drugs is an important factor for the biological function and has an impact on safety and efficacy. To ensure 3D structural similarity and comparability of these complex molecules, analysis of important quality attributes throughout the entire development process as well as of the final product is essential. Here we report on an approach to antibody testing, in which the structural integrity of humanized monoclonal antibody of IgG1 class was examined by analyzing the binding characteristics of the interaction with structure-sensitive reagents. We selected a panel of molecules covering the constant domains of IgG1 and analyzed their binding to the wild-type and several stressed variants of an antibody, using a Surface Plasmon Resonance (SPR) assay. We were able to detect significant differences in binding patterns between the wild-type and the stressed variants, which could be connected to the stress-induced modifications at molecular level. A correlation was found between the results from the binding assay and Differential Scanning Calorimetry (DSC) data. In addition, the properties of biotherapeutic variant, such as molecular weight and isoelectric point as well as any impurity, can be revealed by Two-Dimensional Difference Gel Electrophoresis (2D-DIGE). The presented method might be an interesting tool for monitoring structural integrity of therapeutic antibodies during up and downstream processing.
P-113

Impact of Reversible Self-association in a Monoclonal Antibody on Viscosity, Stability, and Integrity in Structure

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Reversible self-association is observed in many biological molecules including monoclonal antibodies (mAb). While irreversible aggregation is known to cause immungenicity, as well as problems in function and stability, the reversible aggregation is largely overlooked due to its reversibility back to functional monomer state. In the current work, we demonstrate that a mAb undergoes concentration dependent reversible self-association as revealed by analytical ultracentrifugation (AUC). We show that by IR spectroscopy that the conformation state has been altered by aggregation at higher concentrations. The altered conformations are characterized by faster production of irreversible aggregate at low concentrations, and high viscosity. Work done by other researchers has demonstrated that upon change in formulation conditions the extent of reversible aggregation is limited. Here we demonstrate that this change in formulation also produces a more stable and less viscose protein, thereby linking reversible aggregation to high viscosity and instability.

P-114

Differentiation of Protein Particles and Silicone Oil Droplets by Flow-imaging Microscopy (MFI and FlowCAM) and Resonant Mass Measurement (Archimedes)

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We comparatively evaluated flow-imaging microscopy and the recently introduced technique of resonant mass measurement (Archimedes, RMM) as orthogonal methods for the quantitative differentiation of silicone oil droplets and protein particles in the submicron and micron size range.

Representative samples of artificially generated silicone oil droplets and protein particles were prepared and analyzed both separately and in defined mixtures by the different instruments (MFI4100 and MFI5200, Protein Simple; FlowCAM VS1 and FlowCAM PV, Fluid Imaging; Archimedes, Affinity Biosensors).

An accurate differentiation of silicone oil droplets and protein particles could be achieved by all flow-imaging microscopy systems in the micron size-range. Clearly lower standard deviations (higher precision), were obtained by MFI5200 and FlowCAM PV due to the higher sampling efficiency, as compared to MFI4100 and FlowCAM VS1. The customized filter (MFI4100) was more reproducible and enabled reliable discrimination for more extreme mixing ratios. RMM showed highly accurate
discrimination form about 0.5 to 2 µm independent of the ratio between silicone oil droplets and protein particles, provided that sufficient particles were counted.

We recommend combining flow imaging microscopy and resonant mass measurement for a comprehensive analysis of biotherapeutics potentially containing silicone oil droplets and protein particles in the submicron and micron size range.

NOTES:
Mapping Molecular Interfaces with Hydroxyl Radical Made by ‘Pyrite – Shrink’

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Molecular interfaces mediate cellular interactions ranging from simple dimerization to the assembly of large protein and nucleo-protein complexes. Tertiary and higher order structures are distinguishing features of protein therapeutics that underlie their function and efficacy. While the determination of atomic resolution structures is the gold standard for molecular interface maps, the structures of many biomedically important complexes are resistant to facile determination. Oxidation by the hydroxyl radical is a valuable probe of the solvent accessibly of protein side chains. Radiolytic, photolytic and chemical methods are used to generate hydroxyl radical for protein and nucleic acid ‘footprinting’. We have developed a novel method of hydroxyl radical generation for ‘footprinting’ in which the Fenton reaction is catalyzed by ‘pyrite – shrink’: pyrite nanocrystals deposited on polyolefin that is shrunk to produce a textured pyrite surface. The material is then dimpled into a multiwall format to allow for multiple sample drops to be separately and simultaneously processed. We describe a straightforward ‘pyrite – shrink’ protein ‘footprinting’ protocol demonstrating its efficacy to map the solvent accessible surface of proteins.

Evaluation of Insulin Analogues and Their Aggregates with Simultaneous Raman Spectroscopy and Dynamic Light Scattering

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Purpose
As a function of time of incubation at 37°C, insulin analogues and their aggregates were characterized with simultaneous Raman spectroscopy and dynamic light scattering (DLS).

Methods
Insulin analogues Lispro (Humalog®-Eli Lilly and Company), Aspart (Novolog®-Novo Nordisk) and Glulisine (Apridra®-Sanofi-Aventis) were purchased from the pharmacy. Preservatives (m-cresol or phenol) in the formulations were removed with a desalting column. The protein was then concentrated to 20 mg/mL with a Centricon cartridge. Insulin analogues were incubated quiescently at 37°C, and analyses were performed at day 0, 4, 10, 20 and 30 by Raman spectroscopy, DLS and UPLC-SEC.
Results
For Lispro, DLS detected a size increase from ~6 nm to ~18 nm; consistent with the conversion of dimer into higher order oligomers observed by UPLC-SEC. Raman spectra of Lispro showed a progressive loss of alpha helix during incubation; there was about a 33% reduction in alpha helix after 30 days. Disulfide bonds were also observed to be distorted progressively during the incubation. For Glulisine, with UPLC-SEC no significant amount of higher order oligomers was observed until day 30. However, because of their pronounced light scattering intensity DLS detected large aggregates as early as day 4. Raman spectra of Glulisine started to show substantial conformational perturbation only at day 30. For Aspart, the amounts of higher order oligomers detected with UPLC-SEC, large particles observed by DLS and conformational perturbation seen with Raman spectroscopy of were intermediate between results for Lispro and Glulisine.

Conclusions
The results demonstrated that the stability of insulin analogues in formulations depleted of preservatives follow Glulisine>Aspart>Lispro. The new approach combining Raman spectroscopy with DLS allows simultaneous study of structural transitions and size changes, and thus provides valuable new insights into structural perturbations arising during protein aggregation.

NOTES:
Characterization of the NIST Standard Monoclonal Antibody by 2D NMR Fingerprinting Methodologies

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The development of advanced techniques for the characterization of tertiary and higher order structure (HOS) in protein therapeutics, including monoclonal antibodies (mABs), is emerging as a major priority in the pharmaceutical industry. While Nuclear Magnetic Resonance (NMR) spectroscopy provides information on HOS in proteins at atomic resolution, limits on sensitivity, molecular size and sample volumes, as well as the need for stable-isotope enrichment, have traditionally made the technique too time and resource intensive for widespread use in the biopharmaceutical industry. However, recent hardware advances in ultra-high field NMR field strengths, cryogenically cooled probes, and reduction in sample volume requirements have allowed the development and greater widespread application of high resolution 2D heteronuclear NMR 'fingerprinting' methodologies of protein therapeutics at natural isotopic abundance. The individual peak positions from NH and CH correlation spectra are sensitive to HOS and can serve as a spectral benchmark for establishing consistency in drug manufacturing, assessing stability of drug formulations and for establishing biosimilarity to an innovator reference product. To demonstrate the viability and applicability of NMR fingerprinting techniques to the biopharmaceutical industry, we have examined the IgG-based NIST standard monoclonal antibody and present its NMR fingerprint characterization.

NOTES: