Biotherapeutics Interest Group Workshop
63rd ASMS Conference and Allied Topics, May 31 - June 4, 2015, St. Louis, MO

Jason Hogan, Ph.D. and Damian Houde, Ph.D.

The Biotherapeutics Interest Group (formerly the Protein Therapeutics Interest Group) workshop, entitled "Characterization of Protein Therapeutics by Mass Spectrometry", was held from 5:45 PM to 7:00 PM on Wednesday, Jun. 3, 2015. Approximately 125 people attended the workshop. This year the Biotherapeutics workshop coincided with both the Hydrogen-Deuterium Exchange and the Regulated Bioanalysis interest groups, potentially splitting the attendance. The attendees were also encouraged to update their ASMS member profile to the Biotherapeutics interest group, as their profile may not have been migrated over from the Protein Therapeutics interest group.

The primary goal of the workshop was to allow the audience to learn from one another. A pre-conference survey was created using SurveyMonkey to provide potential discussion topics for conversation. The workshop started with a brief presentation by the organizers of a few slides from the survey results, included below, on various topics related to protein therapeutics characterization and served as a starting point for discussion.

The discussion started with a focus on the industry term 'Developability' for protein therapeutics and the common sources of protein heterogeneity. This focused on the identification of multiple types of post-translational modifications, such as deamidation and oxidation, which are commonly observed in proteins. Mass spectrometry plays an important role in the characterization of these modifications. A main discussion topic involved different protocols to limit the amount of analysis-induced modifications to protein samples which interferes with accurate quantitation. One observation was that it is important to provide adequate and rapid denaturation of the protein therapeutic in order to limit the amount of artifact induced modification. Glycosylation was also discussed which also incorporated the use of electron transfer dissociation (ETD) for site identification. While there are still challenges to using ETD in routine protein characterization experiments, the audience agreed that it provided unique capabilities for the analysis of many post-translational modifications such as glycosylation or phosphorylation.

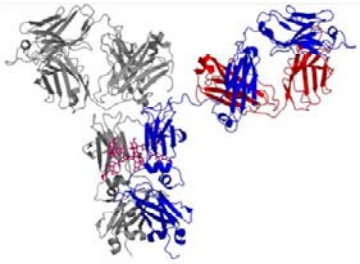
The topic of hydrogen-deuterium exchange (HDX) was also discussed despite the presence of a separate interest group workshop. HDX is becoming a useful component of protein characterization workflows due to its ability to probe protein structure and dynamics. It has utility in the determination of epitope binding, verification of product manufacturing consistency, probing protein aggregation propensity, and biosimilarity analysis. An example of the use of HDX for the analysis protein structure was shown. Two denaturation and reduction protocols were shown resulting in obtaining different amounts of protein sequence coverage highlighting the importance of rapid disulfide bond reduction for HDX experiments. This led to a quick presentation about the potential utility of incorporating electrochemical reduction into HDX workflows. The discussion was directed to two representatives of Antech, a supplier of electrochemical instrumentation. They provided a brief overview of the technology and how it can be interfaced with existing mass spectrometers and workflows. This led to many follow-up questions by the audience regarding potential reaction conditions and types of proteins that would benefit from this technology.

The final discussion topic involved the use of software for data analysis and quantitation. The survey results were quite surprising as the respondents were evenly split between analyzing data manually and allowing software to perform the analysis task. The discussion was directed towards how well the various vendor and third-party software packages perform the analysis tasks. While many software users utilize the software provided by their particular instrument vendor, there is interest in software that can handle multiple instrument vendor data files. An important discussion point was focused on the need to verify the results automated software packages provide. While many attendees use software packages to analyze their data, they still end up verifying the results manually. This may help direct future software development towards providing validation metrics for peak quantitation to limit the amount of user intervention.

Overall, the discussion was informative and some quality feedback was obtained afterwards from the attendees. The workshop was adjourned around 7pm. Next year, Damian Houde will be joined by Ashley Gucinski as the Biotherapeutics Interest Group workshop organizers.

Slides presented:

Characterization of Protein Therapeutics by Mass Spectrometry



ASMS 2015

Damian Houde Biogen
Jason Hogan Bristol-Myers Squibb

Biotherapeutics Interest Group

To receive updates for future ASMS Biothereaputics Interest Group workshops, please update your ASMS Member Profile

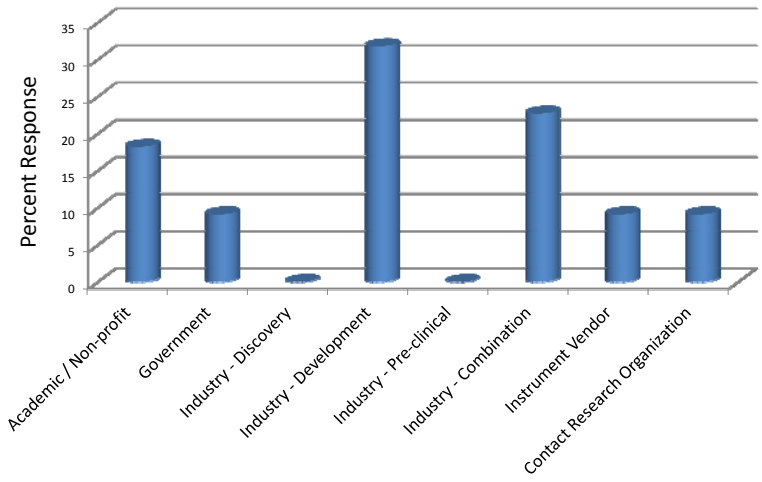
Interest Group Selections

Interest Groups:

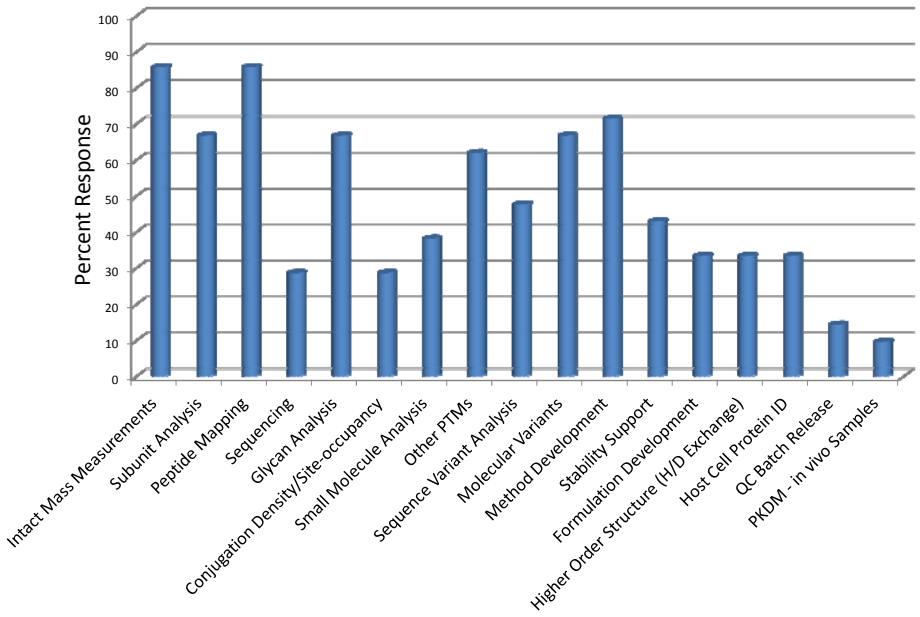
- [ ] Analytical Lab Managers
- [ ] Bioinformatics for MS
- [x] Biotherapeutics
- [ ] Clinical Chemistry
- [ ] Data Independent Acquisition
- [ ] DNA/RNA
- [ ] Drug Metabolism & Pharmacokinetics
- [ ] Energy, Petroleum & Biofuels
- [ ] Environmental Applications
- [ ] Exposomics
- [ ] Flavor, Fragrance and Foodstuff
- [ ] Forensics and Homeland Security
- [x] FTMS
- [ ] Fundamentals
- [ ] H/D Exchange, Covalent Labeling & Cross Linking
- [ ] Imaging MS
- [ ] Ion Mobility MS
- [x] Ion Trap MS
- [ ] LC-MS & Related Topics
- [ ] Lipids & Lipidomics
- [ ] Metabolomics
- [ ] Metal Ion Coordination Chemistry
- [ ] Peptide Fragmentation
- [x] Pharmaceuticals
- [ ] Photoionization MS
- [ ] Polymeric Materials
- [ ] Quantitative Intact Proteomics
- [ ] Regulated Bioanalysis
- [ ] Undergraduate Research in MS
- [ ] Young Mass Spectrometrists
- [x] Protein Therapeutics

The Protein Therapeutics group was renamed the Biotherapeutics group last year so your profile may not have been migrated over.

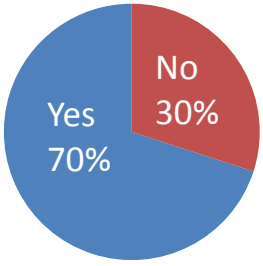
What is Your Primary Work Focus?

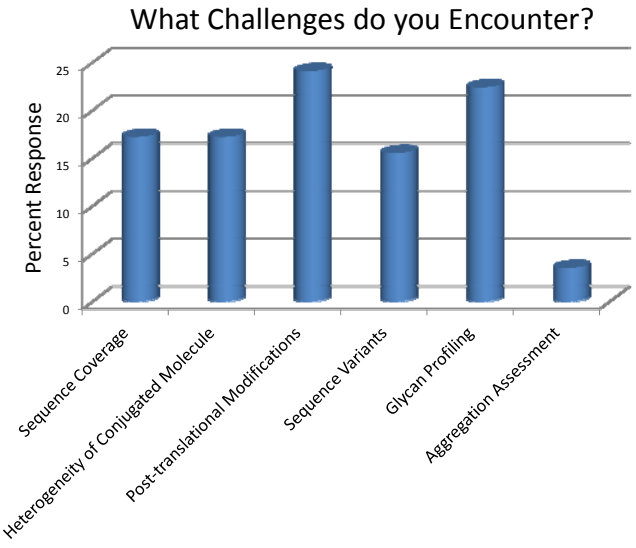


What Do You Use Mass Spectrometry For?



Are you Developing Mass Spectrometry Methods for Complex/Heterogeneous Biopharmaceuticals?





'Developability' of a Protein Therapeutic: Common Sources of Protein Heterogeneity

- Covalent
  - Primary sequence
  - Deamidation
  - Iso-asp formation
  - Methionine oxidation
  - Unpaired cysteine
  - Asp hydrolysis
  - Disulfide Scrambling
  - Glycation
- Non-covalent
  - Stability
    - Thermal
    - Chemical
    - Conformational
  - Aggregation
  - Precipitation/particle formation
  - Viscosity

Mass Spectrometry is an important component for these analyses

Other biophysical techniques used. Mass spectrometry used in specific cases.

Peptide-based Issues for Discussion

- Sample Preparation:
  - Techniques to limit method induced artifacts
  - Ways to analyze samples either from neat and complex matrices.

- Post-translational modifications:
  - How to handle quantification? Can we adjust for ionization issues?

- Glycan / Glycopeptide Analysis:
  - Do we have to remove the glycans?
  - Is enrichment required?
  - Can we be confident in MS/MS identifications or structures?

- Bottom-up vs. Middle Down vs. Top Down:
  - Can intact protein techniques be used to steer or replace peptide based approaches for rapid analysis?

Some areas where to use H/DX-MS…

- Research
  - Epitope mapping, structural dynamics, mutation analysis, etc..
- General characterization/investigations
  - PTMs, variants, stability, etc..
- Comparability
  - Manufacturing consistency, effect of process changes, etc..
- Aggregation (self-association)
  - Better understand aggregation hot spots?
  - Insight into concentration-dependent self-association?
  - Study high concentration viscosity issues?
- Biosimilarity

How do we reduce S-S bonds in < 1minute?

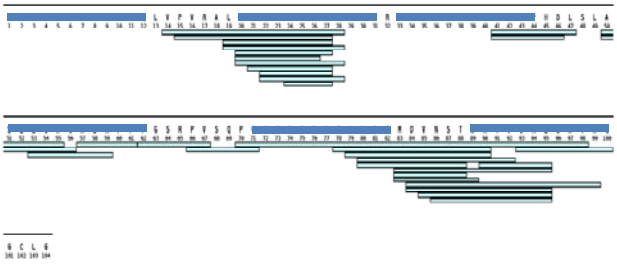
- 22 kDa Protein
- 7 S-S bonds

Quench procedure:

1:1 dilution

200 mM phosphate/citrate
4 M GuHCl
0.2 M TCEP
pH ~2.3

Incubate 0.5 to1 min
inject



<70% sequence coverage not reproducible

This is OK but not great…

One Attempt to Rapidly Reduce the S-S bonds

- MW ~22 kDa
- 7 SS bonds

Aggressive quench procedure:

Two step quench

Step 1:
dilute sample 1:1

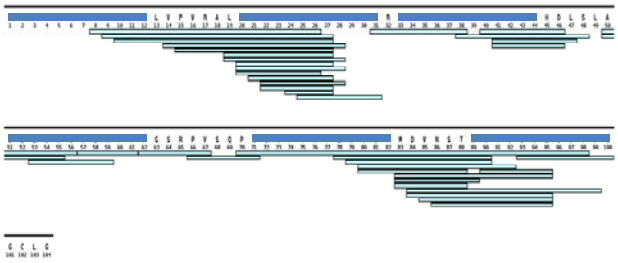
200 mM Phosphate/citrate
~8 M GuHCl
0.5 M TCEP
pH ~2.3

Incubate 0.5 to1 min

Step 2:
dilute quenched sample 1:1

0.1% formic acid (maintains pH ~2.6)

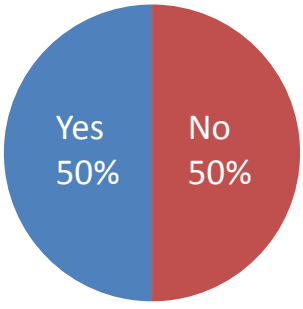
Inject sample



88% sequence coverage
A gain of ~18%

Do You Rely Heavily on Software Packages to Aid in the Characterization of Biomolecules?

- Yes, The software does most of the work.
- No, I do the work manually.



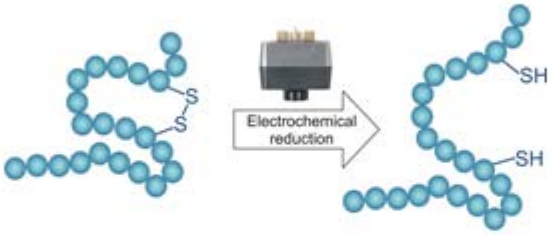
What are the Challenges?

- Price
- Vendor Availability
- Usability
- Validity of the Software
- Inability to compare multiple samples
- Difficulties in analyzing the output
- Reliance on MS1 or MS/MS

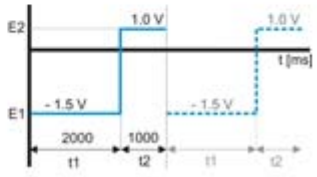
Controlled Reduction of Disulfide Bonds in Protein Therapeutics using an Electrochemical Reactor Cell online with LC/MS

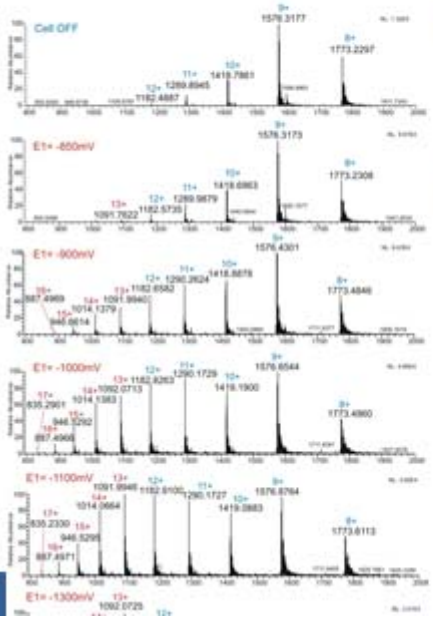
Martin Eysberg
Antec LLC, USA
ASMS, June 3th, 2015

Electrochemical Reduction of D-Bonds



1. ROXY EC system (syringe pump, Potentiostat, Antec)
2. µ-PrepCell (Antec)
3. Titanium based WE[1)]
4. Square wave potential pulse
5. Mobile phase containing formic acid: 0.5 – 1.0 % FA





α-Lactalbumin

EQLTKCEVFR ELKDLKGYGG VSLPEWVCTT

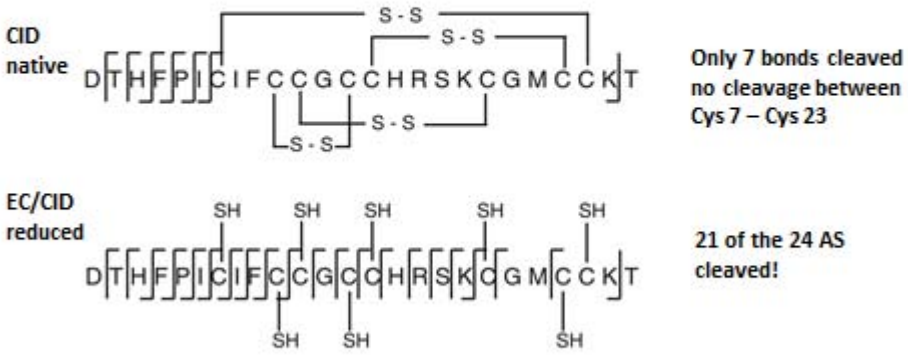
FHTSGYDTQA IVQNNDSTEY GLFQINNKIW

CKDDQNPHSS NICNISCDKF LDDDLTDDIM

CVKKILDKVGI NYWLAHKAL CSEKLDQWLC EKL

- Controlled disulfide bond cleavage → via applied ΔV
- More negative potentials increased cleavage and unfolding.
- Partial opening important in top-down proteomics → location of disulfide bonds

Backbone Cleavage of Hepcidin after EC Reduction

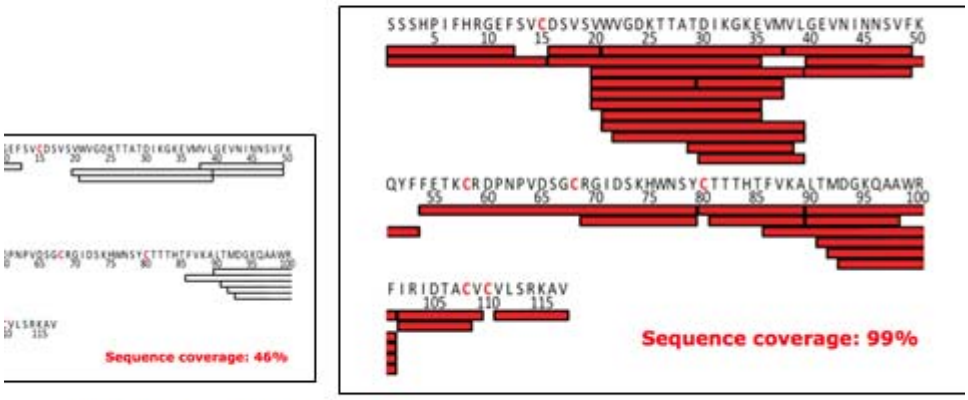


Only 7 bonds cleaved
no cleavage between
Cys 7 – Cys 23

21 of the 24 AS
cleaved!

S. Nicolardi et al., JASMS, 2013, 24 (12) 1980

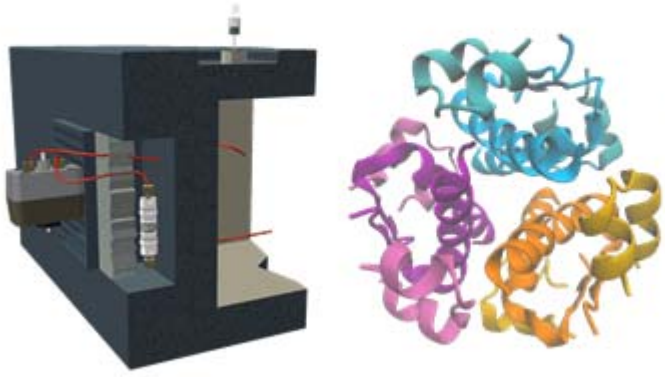
Chemical vs Electrochemical Reduction



Courtesy: Esben Trabjerg et al., Department of Pharmacy, Univ. Copenhagen, Denmark

Superior HDX/MS of TECP Resistant Proteins

Insulin hexamers



T6 hexamers
Stable assemblies

R6 hexamers
Very stable assemblies

Simon Mysling et al., Anal.Chem. 2014, 86, 340

Conclusion

- Fast, Clean & Green on-line reduction
- Controlled reduction by applied voltage
- Applicable for automated HDX workflows (top-down)

See us at Booth 61