

Workshop H/D Exchange and Covalent labeling
Presiding Lars Konermann (Western University)
David Schriemer (University of Calgary)
Date of workshop Wednesday June 12, 2013
Estimate of attendance 150-200

Theme

“This workshop will provide a forum for discussing HDX and covalent labeling approaches for protein analysis (structure, function, folding, dynamics). There will be a number of brief presentations introducing new advances in MS-based methods, experiments, data analysis and applications to the attendees. The goal of these presentations (5 min. maximum) will be to stimulate discussion. The workshop will also contain a question and answer session, with questions submitted in advance. “

Workshop Synopsis

The workshop clearly showcased the need for integrating multiple methods in order to achieve higher-order protein characterization. Real insights in protein folding, function and assembly can be addressed by mass spectrometry, but no single method can fully represent all the protein properties we might want to probe. The presentations and conversation captured this. But they also captured a need to build confidence in the use of an increasingly complex toolkit. A representative from NIST (Dr. Jeffrey Hudgens) announced a study to probe inter-laboratory “stress test” on the application of H/DX-MS for protein conformational analysis. It is good to see NIST pick up the challenge we laid down in last year’s workshop!

Not surprisingly, we also voted to change the name of the interest group, to capture the growing complexity in our methodological basis. **H/D Exchange, Covalent Labeling & Cross-Linking Interest Group** will be our new name. We will not attempt an acronym! The workshop was another success. Although the numbers were a bit lower from last year, we believe this reflects the time slot (Wednesday night vs. Tuesday night in 2012).

Summary of program and discussion

The workshop began with a brief tribute to two pioneers who passed away since the last ASMS meeting: Dr. Virgil L. Woods Jr. and Dr. Max L. Deinzer. Both were early advocates for H/DX and labeling methods in protein analysis. A sombre beginning to the evening perhaps, but at least we could celebrate their work and vision with some great science. We heard five brief presentations from existing and emerging leaders in a relaxed atmosphere, and then finished up with some thought-provoking topics and questions.

1. David Russell (Texas A&M)

David Russell started us off with reminding us just how complex protein characterization can be, when you are interested in more than just sequencing! Metalloproteins were the chosen vehicle for this demonstration. He focused on determining where metal coordination occurs and the effect differential coordination can have on structure. Characterizing such things requires a full-court press: different labeling chemistries, multiple fragmentation methods, IMS and the informatics to tie it all together.

2. Scott McLuckey (Purdue)

There is a healthy debate between mass spectrometrists who investigate gas-phase protein structure vs. those who chemically code for structure in the solution-phase. Is there a right way to use mass spec for characterizing higher order properties of proteins? Scott McLuckey blurred the edges of this debate by highlighting his research on gas-phase chemical labeling. Whether you call it “building bridges between neighbors” or adding new capabilities, his presentation highlighted the possibilities of crosslinking without the added complication of solvent.

3. George Bou-Assaf (Biogen Idec)

Are these methods only of academic interest? No way! George Bou-Assaf provided an industry perspective on the use of H/D exchange for characterizing biopharmaceutical products. Whether for

profiling the conformational equivalence of competing products, determining the effect of glycosylation on conformation, or measuring the effect of excipients on product stability, H/DX-MS is here to stay. He convinced us that ongoing method development will be of great practical benefit to the industry. Still work to be done.

4. Jessica Bereszczak (University of Utrecht)

Why work on problems that other biophysical methods can handle? Jessica Bereszczak from Albert Heck's lab in Utrecht gave us the details on an H/DX-MS analysis of antibodies bound to whole viruses. This is a large molecular system! Jessica provided some advice on how to conduct such studies, and what can be gleaned (or not) from the data.

5. Petr Man (Academy of Sciences, Czech Republic)

Does crosslinking – or any chemical labeling for that matter – distort the proteins under study? Petr Man from the Czech Republic asked this important but uncomfortable question! If crosslinking forces relationships between proteins that don't exist in solution, are the data any good? To illustrate, Petr monitored the effect of crosslinking on enzyme activity, and monitored structural transitions by NMR. There are definitely conditions under which crosslinking can alter activity, and structure too. But happily, Petr showed we can tolerate a wide range of reaction conditions using conventional reagents. It is a good question to pursue further, so we can counsel each other on the right controls to use.

(pictures below)

