Title **H/D Exchange and Covalent labeling**
Presiding **Lars Konermann (University of Western Ontario)**
David Schriemer (University of Calgary)

Theme
This workshop provided a forum for discussing methods and experimental parameters for protein structural studies by hydrogen/deuterium exchange and covalent labeling. There were a number of brief talks to highlight new advances within the field. The goal of these talks (5 min. maximum) was to stimulate discussion. The workshop also included a question and answer session, with questions being submitted in advance.

Date of workshop **Tuesday May 22, 2012**
Estimate of attendance **300**

Summary of program and discussion
The workshop was a lively discussion of current issues and opportunities in the area of structural mass spectrometry, and in particular the application of H/D exchange, covalent labeling and crosslinking to the wider objectives of this important and emergent application area. The workshop was organized around a selection of short topical summaries by leaders in the area.

1. **Michael Chalmers (Scripps Florida) HDX: Automation, Acquisition, Analysis**

Michael Chalmers provided an overview of the Scripps platform in support of an automated HDX workflow, from data acquisition to data analysis. Considered by many to be the largest and most streamlined of pipelines for HDX measurements, Michael provided advice on implementing various aspects of the pipeline.

2. **Keith Fadgen (Waters Corp) Ion Alignment Considerations for HDX Analysis**

Waters has asserted itself in the area of HDX-MS for the last few years, and Keith Fadgen had the opportunity to describe their recent efforts using MS^E for the analysis of MS/MS-domain data. This has become particularly interesting of late, given requirements for higher structural resolution using electron-mediated dissociation, and the need for higher tolerance of sample complexity.

3. **Derek Wilson (York University) Microfluidics and System Integration for HDX Workflows**

Microfluidics has made an impact on many areas of bioanalysis, and structural mass spectrometry is no exception. Derek Wilson gave an overview of how this has evolved over the last few years, and highlighted a recent contribution from his lab. A lively discussion followed on just what aspects of the HDX method could (or should) be installed in microfluidics.

4. **Joshua Sharp (University of Georgia) New Developments in Hydroxyl Radical Labeling**

Hydroxyl radical labeling is a powerful complement to HDX methods. Side chains are usually labeled rather than the peptide backbone and the label does not back-exchange. Joshua Sharp described the degree of correlation one can expect between label incorporation and certain structural parameters. There is growing appreciation and enthusiasm for the role of mass spectrometry in structural biology, which was evidenced by a spirited discussion on the possibilities.

5. **Christoph Borchers (University of Victoria) Protein Structural Studies by Crosslinking**

Crosslinking is already used for refining structural models, particularly of multiprotein complexes. Christoph Borchers provided the audience with insight on just how successful the effort can be, and the role of isotopic coding within crosslinker design.

6. **Kaspar Rand (University of Copenhagen) Characterizing the Structure of Gas-Phase Proteins by HDX**

The field of structural mass spectrometry continues to offer up new approaches. Kaspar Rand described his efforts in the labeling of intact proteins with deuterium, but in the gas phase. The reagent matters, and the nature of the labeling event is quite unique from solution phase, and Kaspar described the potential of the strategy using examples from his lab.

As organizers, what struck us most was the level of engagement by the audience. We consider this to be one of the most participatory groups in recent years. There is a clear range of interest – from the expert to the novice. One question that was discussed at length, was the perceived need for standardizing the HDX-MS method. It was recognized that, to establish confidence in the data obtained from a range of commercial and lab-built systems, a standard protein set should be characterized for such purposes. This is no trivial task, as there are many endpoints in HDX-MS experiments (e.g. kinetics, thermodynamics, binding site definition). However, there was considerable interest in such a venture. HDX-MS is finding application in biopharmaceuticals as a means of monitoring product conformational stability and even uniqueness (in the case of biosimilars). We sensed that the standardization discussion was driven in no small part by this application area.

In general, the success of the workshop points to a healthy interest group, and the undercurrent of excitement in structural applications speaks well for future growth and the emergence of structural mass spectrometry as an important new venture.

A view of the audience, deep in thought

The speakers and the organizers. From left to right: Derek Wilson, Christoph Borchers, Lars Konermann, Joshua Sharp, Michael Chalmers, David Schriemer, Keith Fadgen, Kaspar Rand.