

Workshop Presiding	H/D Exchange, Covalent Labeling & Cross-Linking David Schriemer (University of Calgary) Joshua Sharp (University of Georgia)
Date of workshop	Tuesday June 17, 2014
Estimate of attendance	200-250

Theme

“The workshop will provide a forum for discussing the latest HDX, covalent labeling and crosslinking methods for protein analysis. Presentations will provide an opportunity to discuss MS-based methods, data analysis routines and applications with the attendees. The goal of these presentations will be to stimulate discussion and convey useful experimental detail you can take back to your lab.”

Workshop Synopsis

The aim of the workshop program was to introduce new members to the three broad technology classes covered in our interest group, highlight the particular strengths of each class, and promote a discussion of technical challenges and experimental considerations for each of these technologies. To promote this aim, we chose one speaker to represent each class of technology. Prior to the workshop, we presented each speaker with a biological system of interest—the human κ -opioid G-protein coupled receptor. We then invited each speaker to outline a strategy for research based on H/D exchange, covalent labeling, or cross-linking to investigate this biological system. Through these presentations and following discussions from attendees, we explored the types of problems that can be explored using each of the three core technologies of this Interest Group.

Summary of program and discussion

The workshop began with a brief outline of the program, followed by an introduction to the human κ -opioid G-protein coupled receptor. This introduction included the biological importance of the receptor, as well as the importance of the larger class of G-protein coupled receptors; the current state of knowledge of the structure of the receptor, and three sample questions that each presenter was invited to explore as appropriate.

1. Patrick Griffin (Scripps)

Patrick Griffin presented H/D exchange technology and its use in studying the structure and function of G-protein coupled receptors. Patrick presented the Workshop with a summary of recent progress in the application of H/D exchange in the field of G-protein coupled receptors. He then discussed potential applications of H/D exchange to addressing questions of structure-function relationships in proteins, technical challenges of H/D exchange in integral membrane proteins, and the application of H/D exchange to questions of receptor oligomerization.

2. Lars Konermann (Western Ontario)

Lars Konermann presented covalent labeling technology, starting with a brief overview of the diversity of covalent labeling chemistries and their use in protein structural biology. Lars then moved on to a more specific discussion of hydroxyl radical protein labeling. Lars argued that many fundamentals of hydroxyl radical protein labeling remain under-investigated, including the flow paths in flowed FPOP samples and the potential for multiple exposures, as well as questions regarding the lifetime of the hydroxyl radical in these experiments. Lars also brought up concerns regarding the use of hydroxyl radical covalent labeling for detergent- and membrane-embedded proteins due to observations of poor oxidation, potentially due to radical scavenging from the matrix. Discussion within the workshop focused on the usage of synchrotron radiation for covalent labeling of proteins by hydroxyl radicals, reports of superior amino acid coverage using the synchrotron method, and methods of improving the flow profile within flow FPOP systems.

3. James Bruce (University of Washington)

Jim Bruce presented cross-linking technology for investigating protein structure and complex formation. Jim began with an overview of the principles behind cross-linking for studying protein complexes. Jim then discussed some of the previous results of using cross-linking to study protein-protein interactions within a membrane milieu, including the quantitative aspects of the technology. Jim ended the workshop with some thoughts on the possible applications of cross-linking technology for assignment of agonist and antagonist-induced interactions, as well as the probing of structural mechanisms of receptor action.

(pictures below)

