Protein PTM workshop –Forest White

Cellular regulatory networks are typically regulated, at the protein level, by a combination of altered protein expression and protein post-translational modifications (PTM). Protein expression changes tend to take much longer and often can represent a state change in the cell, while protein PTMs are much more dynamic and reversible, providing rapid response to environmental perturbations. While there are well over 100 different protein PTMs that have been identified, phosphorylation, glycosylation, ubiquitination, and lysine acylation (e.g., methylation, acetylation, etc…) are among the most well-characterized PTMs to date. Over the past 15 years the truly proteome-wide nature of protein PTMs has begun to be appreciated, with thousands of sites identified for many of the more common PTMs. Moreover, the response speed of protein PTM signaling networks is now beginning to be revealed, with network-wide changes occurring within seconds of cell stimulation. Quantifying protein PTM networks in different disease states has revealed novel therapeutic targets and combinatorial therapeutic strategies. Despite the promising potential of protein PTM analysis, data acquisition from limited amounts of sample remains challenging and the extraction of biological insight from highly complex data sets can also be difficult. In this workshop we will discuss a range of techniques to enrich, identify, and quantify protein PTMs, by mass spectrometry and other methods. We will highlight the advantages and disadvantages of selected techniques and discuss critical data quality considerations. Data analysis techniques will also be discussed. Technical (e.g., how do I do this?), experimental design (e.g., will the data from this experiment answer my question?), and philosophical (e.g., why am I even doing this experiment?) questions will be addressed.

Bio statistics workshop: Detecting differentially abundant proteins with R and MSstats - Meena Choi and Olga Vitek

This workshop will introduce practical aspects of basic data visualization and data analysis with R, statistical experimental design (such random sampling of subjects and randomization of mass spectrometry runs), and calculation of simple summary statistics. It will then discuss the use of MSstats in R for differential analysis of mass spectrometry-based proteomic experiments, to detect proteins that systematically change in abundance between conditions.

The instructors will make an on-screen demonstration. Interested participants will also be able to follow the exercises on their own computers. If you are interested in a hands-on session, please bring your own computer, and follow the installation instructions below before arriving to the workshop.

1. Download and install **R**, currently version 3.3.1 (https://www.r-project.org/)
2. Download and install the latest version of **Rstudio** (https://www.rstudio.com/). Accept all default options during installation.
3. Launch Rstudio. During the course we'll be using additional packages that are not pre-installed.
4. Make sure you're online and within Rstudio while completing the installations. Copy the three lines below and paste them in **Console** pane in the top-left corner and press enter-key. Then all required packages will be installed.

```
install.packages(c("ggplot2", "reshape2", "psych"))
source("http://bioconductor.org/biocLite.R")
biocLite('MSstats')
```

Protein Quantification workshop – Shabaz Mohammed

Often the very first question after identifying anything interesting is 'how much?'. Quantification has become a routine aspect of most proteomics experiments. The wide range of biological questions and sample origin has led to a plethora of quantification strategies and within each strategy a multitude of methodologies. This workshop will cover the three main areas; label free, isotope labelling (at the MS labelling) and isobaric labelling (MSMS level). We will also discuss the strengths and weakness of introducing labels (either metabolically or isotopically) at different stages of the work flow and what issues may arise. We will also discuss the requirements for statistical significance with particular emphasis on replicates. Often many scenarios will have multiple solutions each with their strengths and weaknesses. A common experimental design will involve a cell line, 2 conditions and 2-3 biological replicates. Such an experiment can be addressed with metabolic labelling which has best precision/accuracy but is most problematic to set up and requires most mass spec time. You have intermediary methods based on chemical isotope labelling, easier to set up but lower precision and significant mass spec time. One can also use isobaric labelling which will allow all samples to be run as one mass spec experiment but it is costly and (depending on instrumentation) has poor accuracy. In this workshop we will discuss a number of situations based on the desires of the participants.

Manual Spectra Interpretation workshop – Kai Scheffler

The focus of this workshop is to teach the basics skills in spectra interpretation every "proteomicist" should hold. Even though protein identification is nowadays routinely performed using computer-aided database searches, the user is often required to verify the quality of identification results. This requires very often quite some experience but also detailed knowledge on peptide fragmentation basics. This knowledge is also essential for de-novo sequencing, a technique that can be assisted with software but also often verified in a manual fashion.

The orbitrap is the only original type of mass analyzer to be developed in the past 25 years and has resulted therefore in a great deal of interest. It is a detector implemented in hybrid type and benchtop instruments. During the course of the workshop an introduction into the Orbitrap instrument platform is presented focusing on the different fragmentation techniques, namely CID (collision induced dissociation), PQD (Pulsed q Dissociation), HCD (higher energy collision induced dissociation) and ETD (Electron Transfer Dissociation). Further on basic rules in peptide fragmentation will be discussed in detail as well as differences in spectra obtained from CID, HCD and ETD as the most widely used fragmentation types.

This first part focusing on the theory of peptide fragmentation is the essential basis for the second part, the hands-on spectra interpretation (with printed spectra, pen and calculator – no computers!) on selected examples which will be performed to inherit and practice the skills. What seems tough on the first example becomes easier and much clearer with every further example during practicing. This workshop will sharpen your view and understanding of fragment ion spectra and will enable you to judge database search results with much higher confidence

The workshop will consist of three parts:

1) Basic introduction into the Orbitrap technology with focus on different fragmentation techniques
2) Discussion of peptide fragmentation rules
3) Hands-on spectra interpretation

All materials needed for the workshop will be provided.

Skyline workshop – Brendan McLean

Setting up targeted proteomics experiments in Skyline:

This workshop will cover the basics of targeted method editing with Skyline, defining target lists and exporting transition (SRM) or isolation (PRM) lists for targeted acquisition on mass spectrometers. Spectral library and background proteome building will be covered along with their uses in targeted proteomics. Retention time prediction and scheduling with iRT libraries will also be covered. All in an interactive tutorial setting that will get you actually using Skyline. Laptop required. In preparation, please install Skyline v3.5 from the Skyline web site (http://skyline.maccosslab.org)

Analyzing targeted proteomics data with Skyline

This workshop provides an introduction to targeted mass spectrometry data analysis with Skyline, by working through an interactive tutorial on a real targeted case-control data set exploring heart failure in a salt-sensitive rat model. Participants will use Skyline to analyze this data and learn powerful methods of directed interrogation of targeted data sets. Statistical comparison of cases and controls will be performed with issues in data quality and bias discussed. Laptop required. In preparation, please install Skyline v3.5 from the Skyline web site (http://skyline.maccosslab.org).

Intact protein analysis workshop –Julia Chamot-Rooke

In a top-down or native mass spectrometry experiment the first step is to ionize the intact protein or protein complex and measure its molecular mass. With electrospray ionization, an envelope of multiply charged ions is obtained, which has to be deconvoluted.
In the first part of this workshop, several spectra obtained for the analysis of intact proteins in both positive and negative mode will be analyzed and manually deconvoluted. The concept of monoisotopic and average mass will be discussed. We will then move to the analysis of mass spectra obtained for multimeric protein complexes and the goal of the exercise will be to determine the composition and stoichiometry of the complex.
In a second part, we will review the different fragmentation methods that can be used to obtain either primary structure information on intact proteins (HCD, CID, ETD, ECD, EThCD) or structural information on the topology of intact protein complexes. The benefits and limitations of the different activation methods will be discussed (in particular for the analysis of posttranslationally modified proteins) and several MS/MS spectra will be analyzed.

Clinical Proteomics workshop – Michael Gillette

“Clinical Proteomics” encompasses a spectrum of activity, from pre-clinical discovery (proteomics applied to clinically relevant materials and/or addressing a clinical question or need) to applied diagnostics intended to inform clinical decision-making. This translational potential is alluring, and many investigators aspire to include clinical proteomics in their research program. Unfortunately, while technological advances have addressed some historical limitations and increased the promise of effective clinical proteomics, many projects continue to be compromised by inattention to pre- and post-analytical factors including inadequate definition of the clinical problem being addressed, insufficient quality or suitability of clinical samples, and inappropriate statistical methods. This talk will provide a précis for improving the likelihood of success in clinical proteomics, illustrated by ongoing projects in our and collaborator’s laboratories.

Protein Identification by Database Searching workshop- John S. Cottrell

Protein identification and characterisation by database searching is a well-established technique, generating in excess of 1000 peer reviewed publications each year.
The procedure is non-trivial because real mass spectra are not ideal transformations of molecular sequences. A real mass spectrum has finite resolution and finite signal to noise ratio, mass spectrometers have limited mass accuracy, enzymes do not cleave proteins with perfect fidelity, and peptide ions rarely dissociate into complete fragment ion series.
This talk is intended to provide an introduction to the subject. It will include an overview of the most commonly used methods, the software packages that are available, a discussion of the statistical nature of the technique, and some practical tips.
Suggested reading:
J. S. Cottrell, *Protein identification using MS/MS data*, J. Proteomics 74(10) 1842-51 (2011), http://www.sciencedirect.com/science/article/pii/S1874391911002053
Eidhammer, Ingvar; Flikka, Kristian; Mikalsen, Svein-Ole; Martens, Lennart. 2008. *Computational Methods for Mass Spectrometry Proteomics*. John Wiley & Sons. 284 pp. ISBN: 978-0-470-51297-5.

Sample preparation workshop - Thierry Rabilloud

As exemplified in the lecture on sample preparation, the motto "garbage in, garbage out" applies to sample preparation to make it a key issue in every proteomic experiment.
The purpose of the workshop is thus to complement the lecture, i.e. to go deeper into the details of sample preparation and to explore the very practical nuts and bolts for this critical step for different sample types (e.g. mammalian cell cultures, bacteria and plants). The workshop is also designed to be interactive so that the precise questions of the students for their own samples of interest will be dealt with collectively.

Protein-Protein Cross-Linking workshop – Henning Urlaub

The workshop introduces techniques for, and applications on the mass spectrometry-based identification of protein-protein cross-links (CX-MS). CX-MS has become a powerful approach in structural biology. It allows for the identification of cross-linked peptide pairs after chemical cross-linking of isolated protein complexes, proteins co-isolated by affinity purification approaches, cellular organelles, and even entire cells. The workshop will describe the use of various cross-linking reagents (chemical cross-linking, photo reactive cross-linkers, cleavable cross-linkers, isotopically labelled cross-linkers for quantification), purification strategies for cross-linked peptides, mass spectrometric instrument settings for their analysis, and finally database search strategies and softwares. Applications in structural and cell biology will be discussed. Participants are welcome to report their own case studies.

Selected literature:
Belsom A, Schneider M, Fischer L, Brock O, Rappsilber J. Serum Albumin Domain Structures in Human Blood Serum by Mass Spectrometry and Computational Biology. Mol Cell Proteomics. 2016 Mar;15(3):1105-16. doi: 10.1074/mcp.M115.048504.
Leitner A, Faini M, Stengel F, Aebersold R.Crosslinking and Mass Spectrometry: An Integrated Technology to Understand the Structure and Function of Molecular Machines. Trends Biochem Sci. 2016 Jan;41(1):20-32. doi: 10.1016/j.tibs.2015.10.008.
Liu F, Heck AJ. Interrogating the architecture of protein assemblies and protein interaction networks by cross-linking mass spectrometry. Curr Opin Struct Biol. 2015 Dec;35:100-8. doi: 10.1016/j.sbi.2015.10.006.
Politis A, Stengel F, Hall Z, Hernández H, Leitner A, Walzthoeni T, Robinson CV, Aebersold R. A mass spectrometry-based hybrid method for structural modeling of protein complexes. Nat Methods. 2014 Apr;11(4):403-6. doi: 10.1038/nmeth.2841.

Sinz A. The advancement of chemical cross-linking and mass spectrometry for structural proteomics: from single proteins to protein interaction networks. Expert Rev Proteomics. 2014 Dec;11(6):733-43. doi: 10.1586/14789450.2014.960852. Epub 2014 Sep 16. Review.
Walzthoeni T, Leitner A, Stengel F, Aebersold R. Mass spectrometry supported determination of protein complex structure. Curr Opin Struct Biol. 2013 Apr;23(2):252-60. doi: 10.1016/j.sbi.2013.02.008. Epub 2013 Mar 20. Review.
Rappsilber J. Cross-linking/mass spectrometry as a new field and the proteomics information mountain of tomorrow. Expert Rev Proteomics. 2012 Oct;9(5):485-7. doi: 10.1586/epr.12.44.
Leitner A, Walzthoeni T, Kahraman A, Herzog F, Rinner O, Beck M, Aebersold R. Probing native protein structures by chemical cross-linking, mass spectrometry, and bioinformatics. Mol Cell Proteomics. 2010 Aug;9(8):1634-49. doi: 10.1074/mcp.R000001-MCP201.