Dear all,

A Mass spectrometry based proteomics course will take place on 18-20, March 2014. The course arrangements and description are shown below.

The course is free but we will ask for a refundable bond of £200 which will be taken from your Research and Training Grant if you do not attend having had a place confirmed.

Tuesday, 18th March 1:00-5:00pm Lecture 1 by Juri Rappsilber: Basics of mass spectrometry for protein identification - CH Waddington Building Room 1.08 <http://www.ed.ac.uk/maps?building=ch-waddington>

Wednesday, 19th March 9:00-12:00 Lecture 2 by Lauri Peil: Introduction to quantitative proteomics - CH Waddington Building Room 1.08 <http://www.ed.ac.uk/maps?building=ch-waddington>

Wednesday, 19th March 1:00-5:00 Computational practice by Juan Zou: Peptide spectrum Match using xiSPEC and Protein identification by Mascot - JCMB Teaching Studio 3211 <http://www.ed.ac.uk/maps?building=james-clerk-maxwell-building>

Thursday, 20th March, 9:00-12:00 Seminar by Zhuo Angel Chen, Lutz Fisher, Georg Kustatscher, Matthias Trost (special guest speaker, Programme Leader and Head of Proteomics, College of Life Sciences, University of Dundee) Swann Building Room Room 7.20 <http://www.ed.ac.uk/maps?building=michael-swann-building>

**Lecture 1** is appropriate for individuals with little or no experience in mass spectrometry who desires to learn a general work flow for protein identification in a simple mixture. Participants will receive an introduction to proteomics, including the physical basis of mass spectrometry, available instrumentation, sample preparation methods and mass spectra interpretation.

**Lecture 2** includes an introduction to quantitative proteomics and then explains in more detail label-free and isotope-labelled protein quantification approaches. Techniques such as SILAC (stable isotope labelled amino acids in cell culture) and iTRAQ/TMT will be covered, together with common concepts of label-free quantification, such as spectral counting, peak intensity/area measurements and protein abundance index. Finally, a quick introduction to absolute and targeted quantification will be given.

**Computational Practice:** using software Qual Browser to displays and manipulates chromatograms and spectra of raw data collected by Orbitrap Mass spectrometer. Practical aspects of peptide sequence identification using xiSPEC and protein identification using MASCOT.

Target audience: Students and post-doctoral research staff who are considering incorporating proteomics tools into their own research or who wish to familiarize themselves with typical proteomics work flows, or who are interested in quantitative measurements from protein mixtures.

**Seminar:**

**1. Proteomics of a fuzzy organelle: interphase chromatin by Georg Kustatscher**

Chromatin proteins mediate replication, regulate expression and ensure integrity of the genome. So far, a comprehensive inventory of interphase chromatin has not been determined. This is largely due to its heterogeneous and dynamic composition, which makes conclusive biochemical purification difficult, if not impossible. As a fuzzy organelle it defies classical organellar proteomics and cannot be described by a single and ultimate list of protein components. Instead we propose a new approach that provides a quantitative assessment of a protein’s probability to function in chromatin. We integrate chromatin composition over a range of different biochemical and biological conditions. This resulted in interphase chromatin probabilities for 7635 human proteins, including 1840 previously uncharacterized proteins. We demonstrate the power of our large-scale data-driven annotation during the analysis of CDK regulation in chromatin. Quantitative protein ontologies may provide a general alternative to list-based investigations of organelles and complement Gene Ontology.

**2. How wrong we are – FDR by Lutz Fischer**

In mass spectrometry we basically measure mass over charge values in a scan and then try to guess what peptide could have produced these peaks. Obviously we are not perfect at doing so. But we can score these guesses and the higher the score the more likely a guess was correct. Only how big is the error we are making? That's where False Discovery Rate (FDR) comes into play. By using so called decoy proteins we can model the error we are making. Especially we can model the error we are making for a given minimum score. This is the basis of identifying proteins, characterising their post-translational modifications and determining their structures by mass spectrometry.

**3. Structural characterisation of protein and protein complexes using chemical cross-linking and mass spectrometry by Zhuo Angel Chen**

After more than a decade of method development, the combination of chemical cross-linking with mass spectrometry and bioinformatics has become a powerful tool for characterising, in solution, structural details of proteins, functional complexes and macromolecular assemblies. The structure of proteins or protein complexes is ascertained by identifying amino acid pairs that are positioned in close proximity to each other. We demonstrate this technology with the analysis of the architecture of the Pol II-TFIIF complex. Additionally, with the study on the conformational change-driven activation of the key complement component, C3, we elucidate that further integration of quantitation into cross-linking/mass spectrometry provides opportunities for studying dynamics of proteins and protein complexes.

**4. Quantitative proteomics in macrophage and phagosome biology by Matthias Trost**

Phagosomes are organelles formed by uptake of particles (>0.5 um) through immune cells such as macrophages. Proteomics has been in key in the last 13 years to help us understand how this organelle is organised, how it matures by constant fission and fusion with other organelles and how specific functions such as antigen presentation take place. Matthias will present past successes in the field and the latest results from his own lab.

**Reading:**

1. Steen H & Mann M. (2004) The ABC’S (and XYZ’S) of peptide sequencing, Nature Reviews Molecular Cell Biology. 5: 699-711.

2. Mascot (<http://www.matrixscience.com>). Probability-based, peptide-identification software, which works with most mass-spectrometry formats.

3. Mann M. (2006) Functional and quantitative proteomics using SILAC. Nature Reviews Molecular Cell Biology. 7: 952-958.

4. Ishihama Y, et al. (2005) Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. Molecular and Cellular Proteomics. 4: 1265-1272.

**Further Reading:**

1. Hu Q, et al. (2005) The Orbitrap: a new mass spectrometer. Journal of Mass Spectrometry. 40:430-443.

2. Walther TC and Mann M. (2010) Mass spectrometry-based proteomics in cell biology.  Journal of Cell Biology.  190: 491-500.

3. Schulze WX and Usadel B (2010) Quantitation in mass-spectrometry-based proteomics.  Annual Review Plant Biology. 61:491-516.

4. Bantscheff M., Lemeer S., Savitski M.M. & Kuster B. (2012) Quantitative mass spectrometry in proteomics: critical review update from 2007 to the present. Anal Bioanal Chem. 404:939–965

5. Liebler D.C. and Zimmerman L.J. (2013) Targeted Quantitation of Proteins by Mass Spectrometry. Biochemistry. 52:3797−3806