

# 3<sup>rd</sup> European Summer School in Proteomic Basics – “Quantification and Post-Translational Modifications of Proteins”

August 2<sup>nd</sup>-8<sup>th</sup> 2009 at Kloster Neustift, Brixen, Italy

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## Introduction

The European Summer School in Proteomics Basics was initiated in 2007 as a series of three courses, with a different focus each year. The topic of the first Summer School (in 2007) was ‘Sample Preparation and Separation’ and the 2008’s course was on ‘Protein Identification by Mass Spectrometry’. The topic of this year was ‘Quantification and Post-Translational Modifications of Proteins’. As the two previous years, the course was organised at the monastery Neustift in South Tyrol, by Henning Urlaub (Max Planck Institute for Biophysical Chemistry, Germany) and Katrin Marcus (Medizinisches Proteom-Center, Germany). 64 motivated students from about 20 of the European countries attended the course. Lectures, workshops and poster sessions were mixed with social activities such as climbing, rafting and hiking in the surrounding mountains. The atmosphere was informal and communicative, with most of the speakers being around throughout the week of the course.

## Scientific lectures

The introduction talk was given by **Ole N. Jensen** (University of Southern Denmark, Denmark) about state-of-the-art proteomics. Jensen gave examples of current research topics in his group, such as multi-site modifications and multi-variance of post-translational modification (e.g. histone analysis) and the use of “supercharge” solvent to increase the charge state of tryptic peptides for MS/MS analysis (using ETD). Jensen encouraged the participants by pointing out the enormous available space for improvement and discovery within proteomics and mass spectrometry.

## Post-translational modifications

Different enrichment methods for phosphoproteomics were summarised by **Karl Mechtler** (IMP, Austria). Mechtler reviewed the main approaches for IMAC, TiO<sub>2</sub> and ERLIC. **Carolin Huhn** and **Renee Ruhaak** (Leiden University Medical Center, Netherlands) gave a survey of the analysis of protein glycosylation, divided into three levels: intact glycoproteins, glycopeptides and released glycans. For each level, Huhn and Ruhaak presented the most important analytical techniques currently applied (for separation and detection).

A less well-known field of analysis is that of the change in the redox state of Cysteine residues, which was presented in detail in a lecture by **Angela Bachi** (San Raffaele del Monte Tabor Foundation, Italy). Recent advances in the field were presented as well as a description of a methodology for identification and the characterisation of nitric oxide targets and of the S-nitrosylated residues.

A presentation on array based methods for phosphorylation analysis was held by **Oliver Pötz** (Natural and Medical Science Institute Tübingen, Germany). Different assay systems and set-ups for detection and quantification of phosphorylated proteins were presented. **Pier Giorgio**

**Righetti** (Politecnico di Milano, Italy) presented the technology behind the *ProteoMiner* beads, which are utilised to decrease the dynamic range of the protein concentration and make low abundant proteins detectable.

## Quantification

Strategies for 2-DE-based quantification were presented by **Thierry Rabilloud** (CEA-Grenoble, France). He presented a critical comparison of the most widely used types of protein detection methods when it comes to sensitivity, linearity of detection, homogeneity of detection between different proteins and compatibility with the digestion and mass spectrometry process.

**Bernd Thiede** (University of Oslo, Norway) gave an introductory lecture to labelling and quantification by mass spectrometry. Thiede encouraged discussions during the talk, with the experience from the participants as basis. The current strategies of metabolic labelling, especially SILAC and  $^{15}\text{N}$  labelling, were further presented by **Jeroen Krijgsveld** (EMBL, Germany). Many of the speakers of the summer school pointed out the high accuracy of metabolic labelling compared to other techniques (thanks to the early incorporation of the label). Krijgsveld discussed the issues of arginine-to-proline conversion in SILAC, and sub-optimal labelling when using  $^{15}\text{N}$ , and presented ways to increase the accuracy of the quantification. Further on, an overview of the software MaxQuant, which may be used for SILAC-based quantification, was presented by **Matthias Selbach** (Max-Delbrueck-Center for Molecular Medicine, Germany).

A well structured lecture was given by **Bernhard Küster** (Technical University Munich, Germany) about the different chemical labelling strategies for MS based quantification. Küster discussed the advantages and disadvantages of protein versus peptide labelling, as well as quantification in MS1 versus MS2 spectra. Küster explained the use of two-sided regression analysis to calculate the protein fold change instead of averaging the peptide fold change.

**Bettina Warscheid** (Ruhr-University, Germany) gave an overview of label-free quantification, which is becoming more and more used, even though it is still suffering from low quantitative accuracy. Warscheid introduced the two common concepts of label-free quantification - spectral counting and peak intensity/area measurements - and gave examples of different methods used for each of the two strategies. Warscheid further discussed the importance of biological and technical replicates for accurate label-free quantification measurements.

## Company representatives

In addition to the above intensive scientific lecture programme, company representatives gave in depth lectures on the latest developments in their respective fields and presented a wide variety of technical applications. **Marcus Macht** (Bruker Daltonics, Germany) described how the two fragmentation technologies collision induced dissociation (CID) and the more recently developed electron transfer dissociation (ETD) can be applied for the analysis of posttranslational modifications of peptides and proteins. Macht discussed the complementarities of the two methods and how CID and ETD can be combined in experiments to achieve optimal results.

**Kai Scheffler** (Thermo Fisher, Germany) gave a summary of the history of the introduction of the LTQ Orbitrap followed by a detailed introduction to its functionalities. Scheffler further presented recent hardware advances and new developments in dissociation techniques for the Orbitrap (currently five possible fragmentation technologies: CID, ETD, HCD, PQD and in-source). Scheffler also gave a brief summary of the improvements in the recently introduced LTQ Orbitrap Velos.

From Applied Biosystems (Germany), **Christof Lenz** gave an overview of how Multiple Reaction Monitoring (MRM) is applied to the quantification of peptides and proteins in proteomics studies. Lenz presented the design of peptide MRM experiments as well as strategies for optimisation and standardisation. **Mark A. McDowall** (Waters Corporation, United Kingdom) also discussed MRM and presented how ion mobility adds another dimension for sample separation (based on ion size and shape).

### Other scientific activities

Apart from the scientific lectures, the participants also benefited from workshops on the various scientific topics that were chaired by the speakers and from intense discussions during two long poster evenings. Short presentations of 1 minute were held of each participant prior to the poster sessions to trigger interest and give a first overview of each poster. Poster prizes were awarded to four participants, who later on held 15 minutes talks about their current research project. The awards were given to Violette Gautier (IPBS-CNRS Université de Toulouse, France) for her poster on 'Quantitative mass spectrometry for the identification of specific interaction partners of THAP transcription factors', to Rieuwert Hoppes (Netherlands Cancer Institute) for his poster on 'Epitope identification upon proteasomal protein degradation', to Marit Terweij (Netherlands Cancer Institute – NPC, Netherlands) for her poster on 'Dynamics of histones and their post-translational modifications' and to Sonja Volk (NMI Reutlingen, Germany) for her poster on 'Plasma proteome analysis by immunoaffinity Triple X Proteomics'.

### Final remark

The organizers plan a Summer School also for 2010, with the preliminary topics softwares and treatment of proteomics data.