

2nd EU-Summer school in Proteomic Basics 'Protein Identification- Mass Spectrometry'

13-19.07.2008

**Kloster Neustift
(Brixen, South Tyrol, Italy)**



By

Varaprasad Kolla
Prenatal Medicine Lab
Dept of Biomedicine (DBM)
University Hospital Basel
Basel, Switzerland
Kollap@uhbs.ch

Introduction:

The 2nd European- Summer School in Proteomic Basics took place in Kloster Neustift, Brixen, South Tyrol, Italy during 13th and 19th July 2008. The location was perfect for the summer school, in a monastery with very serene and quiet surroundings. The awesome mountains, the roaring Eisack River and tempting wine yards in the backdrop.

This year the main focus of the summer school was on **Protein Identification – Mass Spectrometry**. Proteomics has become an important tool in molecular and cellular biology. As this field of proteomics has evolved over a period of time, from traditional two dimensional gel electrophoresis to the sophisticated mass spectroscopy magnanimously, to work in this area, one must have both a solid background of the traditional method as well as a good knowledge of the new sensitive technique used for the protein identification. This summer school gave me an opportunity to explore the new horizon of proteomics.

All the speakers were par from excellence; they touched every single aspect of Mass Spectrometry and there was enough opportunity for extensive exchange and discussion. The lectures were well articulated covering a theoretical basics, perspectives and general understanding of the basic proteome technologies and their potential fields of application. In addition to lectures there were two poster sessions giving the opportunity for everyone to present her/his own scientific data and with this to extend the discussions among each other. Combined with this scientific program there were also planned recreations which has encourage communication and feeling of togetherness.

Program

Sunday, July 13

School started with the welcome address from organizer **Prof. Henning Urlaub** from Max Planck Institute for Biophysical Chemistry, Gottingen, Germany. He spoke about the idea behind the organizing the school and also gave an overview about the week long proceedings of the school.

This was followed by plenary lecture from Prof. Matthias Wilm, Conway Institute, University Collage Dublin, Ireland. He spoke about Nano electrospray based peptide sequencing: from the last millennium to today. He spoke about how the nano electrospray based peptide sequencing has revolutionised the field and the how important is this tool for a high quality of biological research.

Monday, July 14

Prof. Thierry Rabilloud Lecture

On the first day of the school Prof. Thierry Rabilloud, CEA, DSV, ITRSV; LBBSI & CNRS URM 5092, Grenoble, France, gave the lecture on sample preparation for mass spectrometry, he described for several reasons and mainly precision reasons, most proteomics analyses are carried out on peptides and not on whole protein. He also elaborately discussed about the influence of various experimental parameters with special emphasis on

- Chemical vs. enzymatic cleavage
- In Solution vs. In gel digestion
- Which enzyme to select
- Detection of contaminants

Prof. Martina Schnölzer Lecture

This was followed by the talk of Prof. Martina Schnölzer, Functional Proteome Analysis, German Cancer Research Center, Heidelberg, Germany. She spoke about Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) and Electrospray ionization mass spectrometry (ESI-MS). The lecture was mainly focused on

- Basics of MALDI-MS including instrumentation and principles of the ionization
- Sample preparation for MALDI-MS in-gel digestion of protein
- Protein identification by peptide mass fingerprinting (PMF)
- Analysis of posttranslational modification
- Amino acid sequencing by MALDI-MS/MS
- Strategies for quantitation of proteins with MALDI-MS

Dr. Marcus Macht Lecture

This was followed by the talk of Dr. Marcus Macht, Bruker Daltonics, Bremen, Germany. He spoke about the approaches to protein characterization in bottom up versus top down analysis by MALDI-TOF and ESI trap mass spectrometry. Why to take a top down approach and relevance, as well as how these can be combined for the mutual benefit with bottom-up approaches. He also discussed when and how these technologies can be applied for achieving more comprehensive information about the proteins present in a sample

Prof. Daniele Fabris Lecture

This was followed by the lecture of Prof. Daniele Fabris, University of Maryland Baltimore County (UMBC), U.S.A. He covered the fundamentals of ESI-MS and highlighted the specific properties enabling the functional study of biomolecules and their assemblies. He also gave a rapid overview about the application of ESI-MS to obtain valuable information about enzymes, substrate, transition state and product.. The future direction of this type of ESI-MS application was discussed in the context of the challenges presented by the analysis of biological systems of ever increasing complexity.

Tuesday, July 15

Prof. Tine Thingholm Lecture

Prof. Tine Thingholm, Department of Endocrinology and metabolism, University Hospital of Odense, Denmark gave the lecture about the overall principle of protein identification by MS and MS/MS analysis was discussed. Tricks and troubleshooting for the sample preparation, data interpretation, and database searches was presented. We were guided through the manual interpretation of a MS/MS spectrum and subsequent database search, looking into the sequence information found from two ion series and diagnostic immonium ions.

Parallel Educational Seminars

This lecture was followed by the educational workshop. I took the spectral interpretation and data analysis, which was very interesting and informative. We were given an assignment to work on and we learn to use **Mascot** a powerful search engine that uses mass spectrometry data to identify proteins from primary sequence databases.

Wednesday, July 16

Prof. Hanno Steen Lecture

Prof. Hanno Steen, Department of pathology, Harvard Medical School and Children's Hospital Boston, Boston, MA. In his lecture, he gave more emphasis on LC-Coupled MS; combining mass spectrometry with reverse-phase liquid chromatography is the method of choice for the analysis of protein mixture of high complexity. In order to provide more analytical depth and to identify proteins of lower abundance, which normally comprise the house-keeping and structural proteins. In order to provide more analytical depth and to identify proteins of lower abundance, such as signalling molecules and transcription factors, additional dimension of separation have to be added prior to the LC/MS step. Several liquid chromatography methods and different

strategies for the fractionation and analysis of highly complex protein mixture were explained

Prof. Martin R. Larsen Lecture

Prof. Martin R. Larsen, Department of Biochemistry and molecular Biology, University of Southern Denmark, Denmark gave the next lecture. This lecture was mainly designed to introduce the relatively new field of **Phosphoproteomics**. It's very useful in identifying and mapping the phosphorylation sites in the numerous proteins inside a cell or tissue. It can be used to compare phosphorylation profile before and after a key cellular process or disease state, to better understand the biological regulation. It is mostly achieved by mass spectrometry (MS) techniques. In the lecture latest method for phosphoproteomics studies including phosphopeptide enrichment techniques and mass spectrometric fragmentation of phosphopeptides were majorly discussed.

Dr. Antonio Serna Sanz Lecture

Dr. Antonio Serna Sanz, Applied Biosystem, Madrid, Spain spoke about **Targeted Quantitative Proteomics**. In this talk he covered the basics of MRM analyses, design of experiment, different software and new technologies for MRM application

Thursday, July 17

Prof. Bernhard Küster Lecture

Prof. Bernhard Küster, TMU, Freising, Germany. In his lecture he spoke about the Quantitative Mass Spectrometry, isotopic labelling, label free quantification, its merits and challenges

Friday, July 18

Prof. Michael Washburn Lecture

Prof. Michael Washburn, Stowers Institute for Medical Research, Kansas City, MO. He mainly spoke about the different multidimensional separation coupled to tandem mass spectrometry and the application of these techniques for quantitative proteomics analysis using spectral count.

Dr. Kai Stühler Lecture

Dr. Kai Stühler, spoke about application of label-free proteomics and presented the results of this approach regarding reproducibility of the instrumental set-up and compare it with the 2D-DIGE study.

Dr. Kai Scheffler Lecture

Dr. Kai Scheffler, spoke about the advances in the development of mass spectrometry and selected application of protein identification, protein quantitation, top-down analysis and post-translational modification

Saturday, July 19

Prof. Juri Rappsilberg Lecture

Prof. Juri Rappsilberg, University of Edinburgh, UK. He spoke about the need of fragmentation in mass spectrometric process to obtain structural information of molecules.

Prof. Friedrich Lottspeich Lecture

Prof. Friedrich Lottspeich, MPI for Biochemistry, Martinsried, Germany. He spoke about the MS vision and presented the gist of whole MS based research from past to the current and its application in different walk of life sciences

Closing summer school

Dr. Katrin Marcus, Ruhr.Universitat Bochum, Germany gave the closing remarks on the summer school

Special activities

Apart from the busy sessions of school, special activities were organised like hiking, rafting, football match, wine tasting, guided tour to the monastery

My contribution

I presented a poster on the current proteomics work which I am pursuing, under the title "Biomarker Discovery strategy for Trisomy 21 using iTRAQ and LC-MALDI". I must admit this summer school has added a lot to my knowledge.

Acknowledgement

My registration for the school and travel expenses were covered by the travel grant given by the **Swiss Proteomics Society (SPS)**, I am really grateful to the Chair SPS for showing the confidence in me by accepting my grant application.