29th International Symposium & Exhibit on the Separation of Proteins, Peptides & Polynucleotides

ISPPP 2009
— SCIENTIFIC PROGRAM —

The ISPPP Symposium reserves the right, without notice, to modify or amend the roster of Sunday short courses and/or presenters. Any changes will be updated on the web site at www.isppp.org.

Sunday, October 25, 2009

Short Course # 1: The role of Mass Spectrometry in Glycomics and Glycoproteomcs
1:00 PM – 4:30 PM

Presented by Prof. Ron Orlando, University of Georgia, Athens, GA, USA

Glycosylation is one of the two most common post-translational modifications found on proteins. Glycan structures and sites of glycosylation have been shown to change with the state/condition of the cell in which the proteins are produced. For example, it has been known for over 40 years that cancerous cells attach different glycans than those of corresponding "normal" cells from the same tissue/organ. Since many glycoproteins are excreted, altered glycosylation has the potential to be used as a biomarker for cancer. Numerous other disease states, ranging from arthritis to alcoholism, are also characterized by altered glycoprotein glycans, as is normal cell growth, differentiation, and development. Identifying glycan structures and how these structures change as cells differentiate or as tumor cells progress, for example, is the focus of an emerging field called glycomics. This workshop will focus on the role of mass spectrometry in the emerging field of glycomics and glycoproteomics. An overview will be presented on the biosynthetic pathway that leads to protein glycosylation and how this, in turn, leads to diverse structures of glycoprotein glycans. Other topics that will be discussed include: the analytical challenges of characterizing glycoproteins and their glycans; the methods used to determine glycan structure, sites of glycosylation, and identify glycans present at individual glycosylation sites. Approaches used for comparative glycomic studies will also be covered. Many of the techniques discussed are applicable to both whole cell glycoprotein extracts (i.e., glycomics) as well as the characterization of purified glycoproteins. Although the emphasis of this workshop will be on N-linked glycosylation, the methodology discussed can be extrapolated to other types of glycosylation

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Short Course # 2: Preparative-scale Separation of Biomolecules
1:30-4:30 PM

Presented by Prof. Alois Jungbauer, University of Natural Resources and Applied Life Sciences, Vienna, Austria

Chromatographic methods play a pivotal role in biotechnology and biopharmaceutical technology, particularly for high molecular mass compounds such as proteins and plasmids. The high level of purity can be only achieved by chromatographic methods. Beside bulk contaminants traces of bioactive compounds such as endotoxins, DNA and other adventitious agents must be efficiently removed from the process solution. In the workshop special emphasis will be put on the description of the characteristics of chromatography media used in bioseparation and how they differ from analytical media and media used for separation of small molecules. Process optimization, scale up and important design criteria will be discussed. The influence of mobile phase composition on resolution , and guidelines for the optimization of selectivity will be presented. An overview on novel stationary phases for protein and polynucelotide separation and examples for novel bioseparation processes using these phases will be given. The difference and applicability of monoliths, beads with a porous shell and polymer grafted beads will be elaborated. In the second part of the workshop the progress on biorecognition for affinity chromatography will be discussed.

Short Course # 3: Affinity Chromatography
1:30-4:30 PM

Presented by Prof. David S. Hage, University of Nebraska, USA

Affinity chromatography is a type of liquid chromatography that relies on the use of a biologically-related ligand as a stationary phase for the selective retention of sample components. This method has been popular for decades as a means for the rapid and selective isolation of proteins and other biological agents. This technique has also seen increased use in recent years as a tool for the selective analysis of chemicals, sample preparation in multidimensional analytical techniques, and the study of biological interactions.

In this course we will discuss the basic principles of affinity chromatography and key factors to consider in the development of an affinity-based separation. A survey of popular types of affinity chromatography and their applications will also be presented, including bioaffinity chromatography, immunoaffinity chromatography, immobilized metal-ion affinity chromatography, lectin affinity chromatography, dye ligand/biomimetic affinity chromatography, and the use of affinity chromatography in chiral separations. Several examples illustrating the use of affinity chromatography in preparative or analytical work and multidimensional methods will also be described during this discussion.

Short Course # 4: 2D Liquid chromatography: Basic operating principles, advantages, key applications, instrumentation, methods development, and implementation.
1:00 PM – 4:30 PM

Presented by Dr. Mark R., Schure, The Dow Chemical Company, USA

Multidimensional Liquid Chromatography is a technique that keeps growing and developing. The two-dimensional comprehensive version of this technique, which we will call 2DLC, is a very interesting technique where sample zones are fractionated over two different types of

retention mechanisms, from two independent columns. For many bioseparations, one needs to have at least two different retention mechanisms to spread the separation over an area (two-dimensional), rather than along a one-dimensional separation axis, as is the normal way with HPLC. Often people talk about how 2DLC can increase peak capacity so that more peaks can be resolved. This is often true but not always.

In this course we will review the basic operation of 2DLC and discuss the key biochemistry applications where one needs to use this type of technology. The present state of instrumentation will be surveyed in separate groups:

1) Commercially available as complete turn-key systems
2) How to add 2DLC to an existing HPLC with an add-on kit

We will not discuss how to build a system from the ground up. The various methods development processes will be taught. A survey of the present state-of-the art work in 2DLC will be given with emphasis on the application. Students will be expected to be familiar with HPLC.

6:00 – 8:00 PM WELCOME RECEPTION

Monday, October 26, 2009

7:45 am	**Registration Opens**
8:45	**Opening Remarks –** Mark Schure, Dow Chemical Company, Springhouse, PA, USA
Session I:	**LC/MS. Rainer Bischoff, chair**
9:00	**L1. Development of high-resolution gas-phase separations for analysis of complex biological mixtures.** David E. Clemmer Dept. of Chemistry, Indiana University, .Bloomington, Indiana USA.
9:45	**L2. Using Chromatographic Intelligence to simplify LC/MS Peptide Map Analysis.** Scott Berger**,** Waters Corp. Milford, MA USA
10:15	**break, exhibition and posters**
Session II:	**Affinity Separation Methods. Rainer Bischoff, chair**
11:00	**L3. Ultrafast immunoextraction and microaffinity columns: Recent developments in the use of affinity-based separations for pharmaceutical and biomedical applications.** David Hage Dept. of Chemistry, University of Nebraska, Lincoln, Nebraska USA
Session III:	**Column Technology I: Hydrophilic Interaction Chromatography. Rainer Bischoff, chair**
11:30	**L4. Solubility Studies and Binding Capacity and Resolution Evaluation on HIC Resins for PEG Lysozyme.** Egbert Müller, Tosoh Bioscience, Shuttgart, Germany
11:50	Break: (**Lunch on own)**
1:00 pm	**Posters and exhibit**
Session IV:	**Proteomics I. David Lubman, chair**
2:30	**L5. Chemical Proteomics: Beyond the Profiling of Proteins** Rainer Bischoff, Dept. of Analytical Biochemistry, University of Groningen, The Netherlands
3:00	**L6. On-column Refolding of Autoprotease Fusion Proteins Using N^{pro} Fusion Technology** Elisabeth Schmoeger, Alex Trefilov, Petra Gerster, Alois Jungbauer, Rainer Hahn, Department of Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria
3:30	**L7. An Alternative to 2DGE: PEC-TLC for Peptidomics and Proteomics** Ira Krull, Dept. of Chemistry and Chemical Biology, Northeastern University, Boston, MA USA
3:50	**L8. Shotgun Proteomics has some holes** Mark Duncan, Department of Pediatrics/Div Endocrinology, University of Colorado Denver, Aurora, Colorado 80045

4:20 **Break, Posters and exhibit**

Session V: **Column Technology II.: Ion Exchange Chromatography, Scott Berger, chair**

4:40 **L9. Antibodix Packings**
Xueying Huang, Sepax Technologies, Inc., Newark, Delaware USA

5:00 **L10. Temperature responsive ion-exchange chromatography resins**
Pankaj Maharjan, Milton T.W. Hearn and William R. Jackson, ARC Special Research Centre for Green Chemistry, Monash University, Clayton, Victoria, Australia

5:20pm **Adjourn**

Tuesday, October 27, 2009

Session VI: **Peptides, Barry Boyes, chair**

9:00 am **L11. The use of de novo designed synthetic peptide mixtures to develop effective reversed-phase materials for separation and purification of peptides**
Robert Hodges, Dziuleta Cepeniene, Tivadar Farkas, Colin Mant
Biochemistry and Molecular Genetics, University of Colorado Denver, Aurora, Colorado USA

9:30 **L12. Use of peptide retention prediction model for investigation of RP-LC peptide separation selectivity**
Martin Gilar, Hongwei Xie, Aleksander Jaworski, Bipharmaceutical Sciences, Waters Milford, MA USA

9:50 **L13. An alternative method for peptide mapping using mixed-mode stationary phases**
Romain Dabre, Achim Schwämmle, Michael Lämmerhofer, Wolfgang Lindner, Renato Froidevaux, Didier Guillochon, Dominique Vercaigne-Marko,
PC-R Merck KGaA, Darmstadt, Germany

10:10 **L14. High-resolution Separations of Polypeptides Using Fused-Core™ Particles**
Barry Boyes, S. A Schuster, B. M. Wagner and J. J. Kirkland
Advanced Materials Technology, Wilmington, DE USA

10:30 **break, exhibition and posters**

Session VII: **Column Technology III: Chromatofocusing, Mark Schure, chair**

11:10 **L15 pISep, a multi-dimensional LC system for separating proteins using fully controllable pH gradients**
Allen Hirsh, Latchezar Tsonev Cryobiophysica, Inc. Rockville, MD USA

11:40 **L16. Development of a Chromatofocusing Method for Charge Variant Determination of a Monoclonal Antibody**
Gary Console, John Briggs
Protein Analytical Chemistry, Genentech, South San Francisco, CA USA

12:00 Break: (**Lunch on own)**

1:00 pm **Posters and exhibit**

Session VIII: **Proteomics II. Robert Hodges, chair**

2:30 **L17. A Glycoprotein Approach to Biomarker Discovery**
David Lubman, Yashu Liu, Chen Li, Anna Yang, Mack T Ruffin, Diane M Simeone, Dean E. Brenner
Surgery, University of Michigan, Ann Arbor, MI USA

3:00 **L18. Beyond Protein Identification, Making Sense of Proteomic Data**
Ron Orlando, Complex Carbohydrate Research Center, University of Georgia, Athens, GA USA

Session IX: Process, Process Analytical Technology, and Simulated Moving Bed Chromatography, Abraham Lenhoff, Chair

3:30 **L19. New Proteomic Tools in Protein Characterization and in Optimization of Process Production.**
Milton Hearn, Reinhard Boysen and Yaunzhong Yang , ARC Special Research Centre for Green Chemistry, Monash University, Clayton, Victoria Australia

4:00 **short break, exhibit**

4:20 **L20. On-line HPLC as a PAT for Controlling Product Collection from Process Scale Chromatography Columns**
Rick Cooley, Dionex Corporation, Martinsville, IN USA

4:40 **L21. Continuous affinity purification of proteins by simulated moving bed (SMB) chromatography**
Anthony Grabski, Alla Zilberman, Robert Mierendorf, Semba Biosciences, Inc. Madison, WI USA

5:00 **Panel discussion**

5:30 pm **Adjourn**

7:00 **Conference dinner**

Wednesday, October 28, 2009

Session X: Monoliths, Membranes and Virus. Alois Jungbauer, chair

9:00 am **L22. Structure-Based Modeling of Transport in Monoliths**
Abraham M. Lenhoff[1], Harun Koku[1], Egor I. Trilisky[1], Kirk J. Czymmek[2], Mark R. Schure[3], Robert S. Maier[4]
[1] Department of Chemical Engineering, University of Delaware, Newark, DE USA
[2] Department of Biological Sciences, University of Delaware, Newark, DE USA
[3] Theoretical Separation Science Laboratory, Dow Chemical Co., Springhouse, PA USA
[4] Information Technology Laboratory, U.S. Army Engineer Research and DevelopmentCenter, Vicksburg, MS 39180

9:45 **L23. Membrane adsorbers to capture cell culture derived virus particles**
Michael Wolff, L. Opitz, C. Siewert, N. Petermann, M. Meininger, S. Lehmann, S. Post Hansen, R. Faber, U. Reichl, Bioprocess Technology, Max-Planck-Institute Magdeburg, Sachsony-Anhalt Germany

10:05 **L24. Chromatographic Purification of Replication-Defective Influenza Virus Vaccine: Enabling Fast and Efficient Vaccine Production**
Matjaz Peterka, Elisabeth Maurer, Manuela Gassner, H Seper, Franz Gelhart, Marko Banjac, Marko Jarc, Barbara Lah, Petra Kramberger, Ales Strancar, Thomas Muster, MBL, BIA Separations, Ljubljana, Slovenia

10:25 **L25. Polymeric Monolithic Ion-exchange Stationary Phases for the Separation and Purity Profiling of Biopharmaceuticals**
Anna Nordborg[a], Mohammad Talebi[a], Bo Zhang[b], Jian Wang[b], Emily F. Hilder[a], Paul R. Haddad[a],
[a]Pfizer Analytical Research Centre (PARC), Australian Centre for Research on Separation Science (ACROSS), School of Chemistry, University of Tasmania, Hobart, Tasmania AUSTRALIA
[b]Pfizer Research & Development, Pfizer, St. Louis, USA

10:45 **break**

Session XI: General Ron Orlando, chair

11:00 **L26. Innovative high throughput protein purification in 96-array format**
Juergen Friedle, Tim Schroeder, Atoll GmbH, Weingarten, B-W Germany

11:20 **L27. Evaluation of the Impact of the Variables Related to Thermal Melt Temperature Determination by UV Spectroscopy**
Judy Carmody, Brian. J. Rowe, Avatar Pharmaceutical Services, Inc. Marlborough, MA USA

Session XII: Metabolomics Ron Orlando, chair

11:20 **L28. Metabolic profiling of urine and tissues of laboratory animals after treatment by traditional by traditional herbal medicine**
Sam Li, Dept. of Chemistry, National University of Singapore, Singapore

11:40 break (**lunch on own)**

Session XIII: Proteomics III. Milton Hearn, chair

1:00 pm **L29. Adsorption properties of proteins on hydrophobic surfaces**
Alois Jungbauer, Department of Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria

1:30 **L30. Purification of native proteins simultaneously satisfying with high resolution, high speed, high sample loading**
Xindu Geng, Institute of Modern Separation Science, Northwest University, Xi'an, Shaanxi Province China

Session XIV: Column Technology IV: Displacement Chromatography Milton Hearn, chair

1:50 **L31. Displacement Chromatography of Peptides Comes of Age**
Barry Haymore, John Paul Woods, Sachem, Inc., Austin, Texas USA

2:10 **Panel discussion**

2:40 Conference wrap-up, **,** Mark Schure and Joe DeStefano

Invitation to ISPPP 2010, Rainer Bischoff

3:00 pm **Conference close**