27th International Symposium & Exhibit on the

Separation of Proteins, Peptides & Polynucleotides

ISPPP 2007
— 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 21, 2007

Short Course # 1: Monolithic Columns: How to Make and Use Them
9:00 AM - noon

Presented by Prof. Frantisek Svec, University of California, Berkeley, CA, USA

This workshop will give an introduction to the basics of monolithic stationary phases, their preparation, and selected applications. First, a brief history of monolithic stationary phases, their rebirth at the end of the 1980's and their fast development ever since will be presented. The various approaches to the stationary phases with reduced discontinuity including aligned fibers, rolled textiles, as well as monolithic discs, and columns based on both silica and synthetic polymers will also be introduced. Then, several specific examples of the preparation of monolithic columns based on synthetic polymers will be shown with emphasis on simplicity of both thermally and UV initiated processes and variety of chemistries easily available. Approaches to larger scale monoliths for preparative separations will be described. Also, monolithic materials placed in the currently very popular capillary and microfluidic formats will be presented in more detail. In addition, methods leading to desired chemistries by grafting of pores with selected functional monomers and combinations of various chemistries and functions within the same monolith will also be described. Due to the specifics of monolithic columns enabling high flow rates without compromising the efficiency, high speed/high throughput separations of a variety of compounds including proteins, peptides, nucleic acids, synthetic polymers, and small molecules in HPLC mode can be achieved. Several examples of these separations using monoliths of very diverse shapes and sizes from very large radial flow columns, over analytical scale units, to capillary columns will be shown. Since monolithic columns also represent quite a significant share of all columns used in capillary electrochromatography, their preparation and use in CEC will also be presented. This workshop will be wrapped up by discussing current trends and future developments of this promising new format of stationary phases.

Short Course # 2: Mass Spectrometry in Glycomics and Glycoproteomcs
9:00 AM - noon

Presented by Prof. Ron Orlando, The 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 # 3: 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 # 4: Particle Packed Columns and Monolithic Columns in HPLC:A Comparison and Critical Appraisal
1:30-4:30 PM

Presented by Prof. Klaus K. Unger and Romas Skudas, Department of Inorganic Chemistry and Analytical Chemistry, Duesbergweg 10 – 14, Johannes Gutenberg-University, 55099 Mainz , Germany

The course is divided into four parts and is thought for beginners in this field as well as for advanced chromatographers. The first part surveys the most important process steps in the manufacture of particle packed columns : synthesis and particle formation, sizing and size analysis, packing procedure and evaluation of column performance.

The second part highlights the most attractive developments in the field of particle packed columns: the ultimate minimum particle size in HPLC – fiction and facts, totally vs. core/shell particles in view of the currently introduced Halo particles, column miniaturization: from meso to micro to nano – where is the end ? and how to gain higher resolution in HPLC ?

The third part discusses the most relevant features of monolithic columns with regard to their structure and chromatographic properties and is divided in three paragraphs: the basic idea and the pioneers, monolithic silica columns, polymer-based monolithic columns. The latter subject will be treated in depth by F. Svec at the short course Number.1 at the beginning.

The fourth part is entitled : comparison of the structure and performance of particle packed and monolithic columns. It summarizes all aspects and will answer the question: Where are we now and where are we going.

6:00 – 8:00 PM WELCOME RECEPTION

Monday, October 22, 2007

7:45 am	**Registration Opens**
8:20 am	**Opening Remarks –** Mark Schure, Rohm & Haas, Springhouse, PA, USA
Session I:	**Column Technology I. Frantisek Svec, chair**
8:30 am	**L1. Ultratrace LC/MS Analysis Using 10 µm i.d. PLOT Columns.** **Barry Karger,** Quanzhou Luo; Tomas Rejtar, Shiaw-Lin Wu Barnett Institute, Northeastern University, Boston MA.USA.
9:00 am	**L2. UPLC Separation of Oligonucleotides: Method Development.** **Martin Gilar,** Uwe Neue, Waters Corp. Milford, MA USA
9:20 am	**L3. New Packing Materials for Applications in Analysis of Cell Lysates.** **Xueying Huang,** Sepax Technologies, Inc., Newark, Delaware USA
9:40 am	**L4. Theoretical and Practical Considerations in the Application of UPLC to the Separation of Peptides.** **Thomas E. Wheat**, Jo-Ann M. Jablonski, Beth L. Gillece-Castro, Diane M. Diehl and Uwe Neue, Waters Corporation**,** Milford, MA USA
10:10 am	**break, exhibition and posters**
Session II:	**Affinity Separation Methods. Milton T. W. Hearn, chair**
10:50 am	**L5. Novel capturing method for currently spread influenza viruses from cell cultures by affinity separation.** **Lars Opitz,** Anke Zimmermann, Sylvia Lehmann, Yvonne Genzel, Holger Lübben, Udo Reichl and Michael W. Wolff Bioprocess Engineering Max-Planck-Institute for Dynamics of Complex Technical Systems, Sandtorstrasse 1 Magdeburg, Sachsen-Anhalt Germany
11:10 am	**L6. Biothermodynamic studies of adsorption of monoclonal antibodies.** **Michael Dieterle,** Hans Hasse and Dieter Hoehn, Institute of Thermodynamics and Thermal Process Engineering, University of Stuttgart, Pfaffenwaldring 9 Stuttgart, Germany
11:30 am	**L7. Developments in membrane affinity chromatography for monoclonal antibody recovery.** **Giulio C. Sarti,** Cristiana Boi, Simone Dimartino, Ingegneria Chimica Mineraria e delle Tecnologie Ambientali Università di Bologna viale Risorgimento 2 Bologna, Italy
Session III:	**Glycomics I. Milton T. W. Hearn, chair**
11:50 am	**L8. New Glycomic and Glycoproteomic Tools and Methods for a Better Understanding of Human Diseases.** **Yehia Mechref**, Pilsoo Kang, Zuzana Kyselova,, John Goetz, Milan Madera, Benjamin Mann and Milos Novotny, Chemistry Dept. Indiana University Bloomington, Indiana USA
12:20 pm	Break: (**Lunch on own)**
1:20 pm	**Posters and exhibit**

Session IV: **Proteomics I. David M. Lubman chair**

3:20 pm **L9**. **Analytical and biological implications of dynamics of protein abundances, molecular isoforms and localizations.**
André Schrattenholz, ProteoSys AG, Mainz, Germany

3:50 pm **L10. Automated Metal-free Nanoscale HPLC System for Phosphoproteomic Analysis.**
Rui Zhao, Shi-Jian Ding, Yufeng Shen, Feng Yang, Robert A Maxwell, Harold Udseth, and Richard Smith**,** Environmental Molecular Sciences Laboratory and Biological Sciences Division**,** Pacific Northwest National Laboratory**,** Richland, WA USA

4:10 pm **L11. Innovative Mass Spectrometry Technology for the Study of Cell Signaling.**
Donald F. Hunt, Chemistry Department, University of Virginia, Charlottesville, VA USA

4:40 pm **L12. Rapid identification of protein variants.**
Reinhard I. Boysen, Asif Alam, Donald K. Bowden and Milton T. W. Hearn
ARC Special Research Centre for Green Chemistry, Monash University,Clayton Campus, Wellington Road Building 75, Melbourne, Victoria Australia

5:00 pm **Adjourn**

Tuesday, October 23, 2007

Session V: Monoliths. Abraham M. Lenhoff chair

8:20 am **L13. Monolithic columns for bioseparations: Present state-of-the-art and future trends.**
Frantisek Svec, The Molecular Foundry**,** E.O. Lawrence Berkeley National Laboratory Berkeley, CA USA

9:00 am **L14. Macroporous Polymeric Monoliths by Reactive Gelation for Protein Purification.**
Alessandro Butté, N. Marti, M. Küthe, and M. Morbidelli
Institute for Chemical and Bioengineering, ETH Zurich, Zurich, Switzerland

9:20 am **L15. Purification of large plasmids on ion-exchange monolithic columns.**
Nika Lendero, F. Smrekar, M. Ciringer, A. Štrancar, A. Podgornik
Research and Development, BIA Separations**,** Ljubljana, Slovenia

9:40 am **L16. Separation of viruses by monolithic columns.**
Alois Jungbauer, Biotechnology**,** University of Natural Resources and Applied Life Sciences Vienna, Austria

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

Session VI: Column Technology II. Rainer Bischoff, chair

10:50 am **L17. High Speed Separation of Peptides Using Columns of "Fused-Core" Particles.**
Joseph Kirkland, Joseph DeStefano and Timothy Langlois
Advanced Materials Technology, Inc., Wilmington, Delaware USA

11:10 am **L18. Comparison of the Performance of some Modern HPLC Columns in the Gradient Elution of a few Protein Digests.**
Georges Guiochon and Nicolla Marchetti**,** Chemistry, University of Tennessee**,** Knoxville,TN USA

11:40 am **L19. Efficient Purification and Trace Impurity Analysis of Crude Synthetic Peptides Using Reversed-Phase Displacement Chromatography and New RP Displacers.**
Barry Haymore and Hemant K. Joshi Bioprocess Technology**,** SACHEM, Inc., Austin, TX USA

Session VII: Glycomics II. Rainer Bischoff, chair

12:00 pm **L20. Novel Separation and Quantification Strategies for the Characterization of Glycopeptides from Complex Biological Mixtures.**
Ron Orlando James A. Atwood III[1] Zuzheng Lou[1] Lei Cheng[1] Brent Weatherly[2] and Barry Boyes[3]
[1]Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, GA 30602-4712 USA [2]BioInquire.LLC, Athens, GA, 30602 USA
[3]Smiths Detection, Edgewood, MD 21040 USA

12:30 pm Break: (**Lunch on own)**

1:20 pm **Posters and exhibit**

Session VIII: Advances in Multidimensional Chromatography. Mark R. Schure, chair

3:20 pm **L21. 2-D Liquid Separations, Microarrays and Microproteomics for Mapping Changes in Disease States.**
David Lubman, Diane M. Simeone, Tasneem Patwa, Hye-yeung Kim, Nancy Dai, Jia Zhao, Manoj Pal, Yanfei Wang, and Yinghua Qiu
Department of Surgery, The University of Michigan, Ann Arbor, MI USA

3:50 pm **L22. A Critical Comparison of the Peak Capacity and Information Content of One-Dimensional and Two-Dimensional Liquid Chromatography.**
Dwight Stoll, Peter W. Carr**,** Department of **C**hemistry**,** University of Minnesota Minneapolis, MN USA

4:10 pm **L23. Process Analytical Technology: Two-Dimensional Chromatography on-Line with Mass Spectrometry (2D-LC/MS) for in-Process Analysis of a Recombinant Protein Concentration and Glycosylation.**
Yelena Lyubarskaya, Zoran Sosic, Damian Houde, Steve Berkowitz and Rohin Mhatre
Analytical Development, Biogen Idec, Cambridge, MA USA

4:30 **L24. Fractionation and Characterization of Crude Protein Mixtures for Accelerating Bioseparation Process Design.**
Marcel Ottens, T. Ahamed B. K. Nfor P. E. D. M. Verhaert, G. W. K. van Dedem, L. A. M. van der Wielen, E. J. A. X. van de Sandt, and M. H. W. Eppink
Biotechnology, Delft University of Technology, Delft, The Netherlands

4:50 **short break**

Session IX: Award address.

4:55 Presentation of the ISPPP Lifetime Achievement Award to Professor Klaus K. Unger by Dr. Reinhard Ditz, Merck KGaA, Joe DeStefano, co-chairman and Mark Schure, co-chairman.

L25. Award Address: **In Search of Separation Excellence: The Future Role of HPLC in Modern Life Sciences, Klaus K. Unger**, Reinhard Ditz

5:30 pm **Adjourn**

7:00 **Conference dinner**

Wednesday, October 24, 2007

Session X: Process/Column Technology III. Alois Jungbauer, chair

9:00 am **L26. Towards Prediction of the Dynamic Binding Capacity of Proteins.**
Abraham M. Lenhoff, X. Xu, B. D. Bowes and H. Koku
Department of Chemical Engineering, University of Delaware
Newark, DE USA

9:30 am **L27. Investigation of protein – salt – interactions: Impact on dynamic binding capacity in chromatography with human monoclonal antibodies and their stability.**
Alexander Faude Heiner Böttinger, Institute of Cell Biology and Immunology**,** University of Stuttgart, Stuttgart, Germany

9:50 am **L28**. **Mixed-matrix membrane adsorber technology for the separation of therapeutic proteins.**
Michel Eppink, R. Rhemrev, and M. Snippert
Downstream Processing, NV Organon, The Netherlands

10:10 am **break**

Session XI: Proteomics III. Steven M. Cramer, chair

10:30 am **L29. Biomarker Discovery in Body Fluids by LC-MS.**
Rainer Bischoff, Peter Horvatovich, Natalia Govorukhina, Ramses Kemperman, Christin Christin, Therese Rosenling, Iwona Sobczak-Elbourne, Theo Reijmers, Frank Suits, Frits Muskiet, Ate van der Zee, Analytical Biochemistry**,** University of Groningen**,** Groningen, 9713 AV The Netherlands

11:00 am **L30. Quantitative proteomic analysis of influenza A virus infected mammalian cells: Elucidation of virus / host cell interactions with respect to the vaccine production process.**
Erdmann Rapp, Diana Vester[2], Yvonne Genzel[1], Doerte Gade[1], Udo Reichl [1,2] [1] Max Planck Institute for Dynamics of Complex Technical Systems, Bioprocess Engineering, Magdeburg, Germany [2] Otto-von-Guericke-University Magdeburg, Bioprocess Engineering, Magdeburg, Germany

11:20 am **L31.Proteomic investigation of some plasma-derived therapeutic proteins: How well characterized are "well characterized biologicals?"**
Djuro Josic and James J. Clifton**,** Proteomics Core, COBRE CCRD
Brown University, Rhode Island Hospital, Providence, Rhode Island USA

11:40 am Break: (**Lunch on own)**

Session XII: Column Technology IV. Ron Orlando, chair

1:00 pm **L32. Investigation of chemical selective displacers using robotic high throughput screening, SPR, NMR and MD simulations.**
Steven Cramer, C. Morrison, S. McCallum, R. Godawat, J. Moore and S. Garde
Chemical and biological engineering, RPI, Troy, NY US

1:30 pm **L33. Investigation of the Stability of Human Serum Albumin and its Aggregates Using High Performance Size Exclusion Chromatography.**
Jin Qian[1] Q. Tang[1], B. Cronin[2], R. Markovich[1], A. Rustum[1] [1] Global Quality Services-Analytical Sciences, Schering-Plough, 1011 Morris Avenue, Union, NJ 07083
[2] Analytical Support, Brinny, Schering-Plough, Ireland

1:50 pm **L34. Lysozyme binding orientation on different adsorber materials with varying pH and ionic strength.**
Florian Dismer F. Dismer, M. Petzold, and J. Hubbuch Institute for Biotechnology 2 Research Centre, Juelich, Germany

2:10 pm **L35. High throughput chromatographic separations at small scale in a liquid handling workstation**
Lothar Britsch, Tim Schroeder and Jürgen Friedle**,** Product Development**,** Atoll GmbH Weingarten, Germany

2:30 pm **L36.Utilization of Macrocyclic Glycopeptide Stationary Phases for the Separation of Peptides**
Hillel Brandes, William Campbell and David S. Bell, Supelco HPLC R&D, Bellefonte, PA USA

2:50 **short break**

Column Technology continued. Joeseph DeStefano, chair

3:00 pm **L37. How High Resolution Analytical Separation Methods can be Integrated into Recombinant Protein Production: From PAT to Process Intensification**
Milton T W Hearn. ARC Special Research Centre for Green Chemistry Monash University, Clayton Victoria Australia 3800

3:30 pm **L38. An Integrated Field Portable Biochemical and Instrument Platform for the Detection of Biological Threats**
Barry Boyes, John Link, Doug Green, Greg Williams, James Hazel and Jason Betley Research and Development, Smiths Detection, Edgewood, Maryland USA

3:50 **Invitation to ISPPP 2008**, Klaus Unger

4:00 **Conference closing,** Mark Schure, ISPPP 2007 co-chairman