JULY 29-31, 2015 • LOEWS HOTEL • PHILADELPHIA, PA, USA

"The Separation and Characterization of Biologically Important Molecules"

# ISPPP 2015 FINAL PROGRAM


- CO-CHAIRS
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  Advanced Materials Technology

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  University of Georgia

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**35th International Symposium and Exhibit on the Separation of Proteins, Peptides and Polynucleotides**

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ISPPP 2015
*35th International Symposium and Exhibit on the*
**Separation and Characterization of Biologically Important Molecules**

**ISPPP 2015 Scientific Program** (as of 7-24-2015)

## Wednesday, July 29, 2015

7:15 AM **Symposium Registration Open**
Location: Commonwealth Hall, 2nd floor

10:20 AM **Exhibition Open**
Location: Commonwealth Hall B/C, Exhibition/Poster Session Hall, 2nd floor

8:20 AM **Welcome and Opening Remarks**
Location: Commonwealth Hall A, 2nd floor

**Wednesday Session 1. Proteomics Analysis**
Session Chair: Robert Hodges, University of Colorado, Denver
Location: Commonwealth Hall A, 2nd floor

8:30 AM L-101 **Proteomic Studies of Pancreatic Cancer Stem Cells.** David Lubman, Jianhui Zhu, University of Michigan, Ann Arbor, MI, USA

9:00 AM L-102 **Development of a µCE-MS/MS Platform for Comprehensive Single-cell Proteomics: Identification and Relative Quantitation of Proteins from Xenopus Embryonic Cells.** Camille Lombard, Sally A. Moody, Peter Nemes, The George Washington University, Washington, DC, USA

9:25 AM L-103 **Proteomic Readouts for Advancement of Tissue Engineering Efforts**. Ryan Hill, Kirk Hansen, Department of Biochemistry & Molecular Genetics, University of Colorado Denver, Aurora, CO, USA

9:50 AM L-104 **Multi-Dimensional Separation for Deep Coverage of the Proteome.** Roland S. Annan, GlaxoSmithKline, Collegeville, PA, USA

10:20 AM **Break and Social in Exhibition/Poster Session Hall**
Location: Commonwealth Hall B/C, Exhibition/Poster Session Hall, 2nd floor

**Wednesday Session 2. Separation Methodologies (I)**
Session Chair: Milton Hearn, Monash University
Location: Commonwealth Hall A, 2nd floor

11:00 AM L-105 **Development and Application of Silica Monoliths for Bioanalysis.** Karin Cabrera, Gisela Jung, Peter Knoell, Benjamin Peters, Tom Kupfer, Merck Millipore, Darmstadt, GERMANY

11:25 AM L-106 **The Influence of Biocompatible Coating Thickness of Hybrid Polymer on Protein and Nucleic Acid Recoveries and Resolution.** Michael Lu, BioChrom Labs Inc., Terre Haute, IN, USA

11:50 AM L-107 **Separation of Monoclonal Antibody and its Fragments by a Newly Developed Hydroxyapatite Resin.** William Evans, Atis Chakrabarti, Tosoh Bioscience, LLC, King of Prussia, PA, USA

12:15 PM **PAUSE, EXHIBITION, POSTERS** (lunch on own)

12:30 - 1:30 PM **Free Vendor Seminar sponsored by Shimadzu Scientific Instruments**
**"Enhanced Detection of Hydrophobic Peptides and Proteins by MALDI-TOF MS"**
Location: Adams Room, 3rd floor *(light lunch will be provided)*
Must register at the Shimadzu exhibit by Wednesday @ 10:50 AM

**POSTER SESSION I & EXHIBITION**
**1:30 PM- 3:00 PM**
Location: Commonwealth Hall B/C, 2nd floor

P-W-108 **Effect of Larger Exclusion Limit on the Separation of HMW Species – A Study using a 3 µm Size Exclusion Chromatography Column.** Atis Chakrabarti, Tosoh Bioscience LLC, King of Prussia, PA, USA

P-W-109 **Analysis of Proteins and Water-soluble Polymers using Macroion Mobility Spectrometry.** Axel Zerrath, Patrick Hutchins, Richard Cavalere, Erik Willis, TSI Inc, Shoreview, MN, USA

P-W-110 **Development of a Therapeutic Protein Separation Method on a Superficially-porous 120Å Reversed-phase Column through Design of Experiments.** Nicholas Woon, Cynthia Quan, Genentech, South San Francisco, CA, USA

P-W-111 **Impact of Mobile Phase Composition on the Separation of Peptides by Reversed Phase and Hydrophilic Interaction Chromatography.** Milton Hearn, Reinhard Boysen, Monash University, Clayton, AUSTRALIA

P-W-112 **Charge-based Separation Profile Analysis of Proteins for the Clinical Immunodiagnostics Industry: Generation of Quantitative Results from Classic Qualitative Data.** Tracey Rae, Ryan Bonn, Sam Diep, Jeffrey Fishpaugh, Abbott Diagnostics, Abbott Park, IL, USA

P-W-113 **2D-HPLC: An Automated Method for Detecting Monoclonal Antibody Product and Aggregate.** Michael Bruce, Rong-Rong Zhu, Robert Smith, Kristina Cunningham, EMD Millipore, Bedford, MA, USA

P-W-114 **Hydrophobic Interaction Process Scale Separation of Protein, mAb and Antibody Drug Conjugate.** Sixi Wang, Haiying Chen, Xueying Huang, Ke Yang, Sepax Technologies, Newark, DE, USA

P-W-115 **Chiral Purity Analysis of Synthetic Therapeutic Cyclic Peptides with Unnatural Components by Achiral Reversed Phase HPLC-UV-MS.** Yuefei Shao, Atsu Apedo, Oliver Mcconnell, Bristol-Myers Squibb, Pennington, NJ, USA

P-W-116 **Separation of Oligonucleotides using Reversed-Phase Columns- A High pH Stable, Superficially Porous Particle Columns For LC/UV and LC/MS Analysis.** Phu Duong[1], Luke Stephen[2], Alex Zhu[1], [1]Agilent Technologies, Inc., Wilmington, DE, USA; [2]Agilent Technologies, Inc., Church Stretton, Shropshire, UK

P-W-117 **"Snapshot" RP-UHPLC Method to Monitor Post-Translational Modifications in Monoclonal Antibody Therapeutics.** Justin Jeong, Bing Zhang, Braydon Burgess, Taylor Zhang, Genentech, South San Francisco, CA, USA

**POSTER SESSION I & EXHIBITION** *(continued)*
**1:30 PM- 3:00 PM**
Location: Commonwealth Hall B/C, 2nd floor

P-W-118 **Development of a Stochastic Approach for the Prediction of the Probability of Successful Separation in HPLC and Sequential Elution LC.** Erin Ennis, Joe Foley, Drexel University, Philadelphia, PA, USA

P-W-119 **Rapid and Specific HILIC Method to Assay Guanine Deaminase and Application to Characterize the Brain Enzyme.** Barry Boyes, William Miles, Benjamin Libert, Advanced Materials Technology, Inc., Wilmington, DE, USA

P-W-120 **Protein Profiling (HDMSE) for Monitoring of Chondrocyte Differentiation of Mesenchymal Stem Cells in 3D Pellet Culture in a Multi-omic Approach.** Shujuan Tao, Andrea Tan, David Chen, Clark Hung, Lewis Brown, Columbia University, New York, NY, USA

P-W-121 **Prediction of Peptide Retention Times in Hydrophilic Interaction Liquid Chromatography (HILIC) Based on Amino Acid Composition.** Majors Badgett[1], Ron Orlando[1], Barry Boyes[2], [1]University of Georgia, Athens, GA, USA; [2]Advanced Materials Technology, Wilmington, DE, USA

P-W-121-B **Advancing Untargeted Proteomics to Single Cells for the 16-cell Xenopus Embryo using µCE-ESI-MS/MS.** Sally Moody, Peter Nemes, Camille Lombard, The George Washington University, Washington, DC, USA

**Wednesday Session 3. Separation Methodologies (II)**
Session Chair: Yehia Mechref, Texas Tech University
Location: Commonwealth Hall A, 2nd floor

3:00 PM L-122 **Recombinant Antibodies Purified by Affinity Chromatography or Precipitation, are They Different?** Nikolaus Hammerschmidt[1], Beate Hintersteiner[2], Nico Lingg[2], Peter Satzer[2], Alois Jungbauer[2], [1]ACIB, Vienna, AUSTRIA; [2]BOKU, Vienna, AUSTRIA

3:25 PM L-123 **Application of a New Class of Immobilized Metal Ion Affinity Chromatographic Adsorbents for the Purification of Specifically Tagged Recombinant Proteins.** Milton T. W. Hearn, Dale Fredericks, Chunfang Zhang, Campbell Coghlan, Martin Petzold, Yuangzhong Yang, Eva Campi, Reinhard Boysen, Monash University, Clayton, AUSTRALIA

3:50 PM L-124 **Protein and Other Bio-active Large Molecule Separation by Centrifugal Precipitation Chromatography.** Martha Knight[1], Yoichiro Ito[2], Moon-Jun Brian Kim[1], [1]CC Biotech, Rockville, MD, USA; [2]NIH, Bethesda, MD, USA

4:15 PM L-125 **Prediction of Peptide Retention Times in Hydrophilic Interaction Liquid Chromatography (HILIC) Based on Amino Acid Composition.** Majors Badgett[1], Barry Boyes[2], Ron Orlando[1], [1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA, [2]Advanced Materials Technology, Inc., Wilmington, DE, USA

4:40 PM L-126 **Optimization and Evaluation of Peptide and Protein LC-MS using Well-designed Test Mixes.** Ben Cutak, Kevin Ray, Sigma Chemical Company, St. Louis, MO, USA

5:10 PM L-127 **TUTORIAL: Overview of Protein and Peptide Separations via Various Modes of Chromatography.** Andrew Alpert, PolyLC Inc. Columbia, MD, USA

6:00 PM **Pause**

6:30-7:30 PM **WELCOME MIXER in Exhibition/Poster Session Hall**
Location: Commonwealth Hall B/C, Exhibition/Poster Session Hall, 2nd floor

7:45 AM **Symposium Registration Open**
Location: Commonwealth Hall, 2nd floor

10:15 AM **Exhibition Open**
Location: Commonwealth Hall B/C, Exhibition/Poster Session Hall, 2nd floor

**Thursday Session 4. Therapeutics**
Session Chair: Mark Lowenthal, National Institute of Standards and Technology
Location: Commonwealth Hall A, 2nd floor

8:30 AM L-128 **Challenges in the Analysis of Biotherapeutics and Biosimilars.** Joseph Glajch, Momenta Pharmaceuticals, Cambridge, MA, USA

9:00 AM L-129 **Process Characteristics of Chromatographic Separation of Mono-PEGylated Exenatide.** Nguyen Thi Ngoc Than, E. K. Lee, Hanyang University, Ansan, KOREA

9:25 AM L-130 **Online UPLC Applications for Biotherapeutic Development.** Zhi Chen, Douglas Richardson, Maria Khouzam, Xiaodun Mou, Daisy Richardson, John Higgins, David Pollard, Merck Research Laboratories, Kenilworth, NJ, USA

9:50 AM L-131 **Characterization of Low Level Impurities During the Development of Biotherapeutics.** Richard Ludwig, Bristol-Myers Squibb, Pennington, NJ, USA

10:15 AM **Break/Social in Exhibition/Poster Session Hall**
Location: Commonwealth Hall B/C, Exhibition/Poster Session Hall, 2nd floor

**Thursday Session 5. Glycobiology**
Session Chair: Mark Schure, Kroungold Analytical
Location: Commonwealth Hall A, 2nd floor

10:50 AM L-132 **Isomeric Separation of Free Oligosaccharides and N-linked Glycans in Human Milk.** Yehia Mechref, Texas Tech University, Lubbock, TX, USA

11:15 AM L-133 **Using Evolution to Predict N-glycosylation on Novel Non-canonical Protein Motifs; Using LC-MS/MS to Validate.** Mark Lowenthal, Kiersta Davis, Catherine Formolo, Lisa Kilpatrick, National Institute of Standards and Technology, Gaithersburg, MD, USA

11:40 AM L-134 **The use of Isotopically Labeled IgG for the Relative and Absolute Quantitation of N-linked Glycans.** Shujuan Tao[1], Yining Hung[1], Emily Betchy[1], Alex Harvey[2], Ron Orlando[2], [1]University of Georgia, Athens, GA, USA; [2]Glyco Scientific, Athens, GA, USA

12:05 PM **PAUSE, EXHIBITION, POSTERS** (lunch on own)

12:30 - 1:30 PM **Free Vendor Seminar sponsored by TSI Incorporated**

**"Macroion Mobility Spectrometry for Particle Size Analysis of Large Proteins, Protein Aggregates and Aqueous Polymers"**
Location: Adams Room, 3rd floor *(light lunch will be provided)*
Must register at the TSI exhibit by Wednesday @ 3:00 PM

12:30 - 1:30 PM **Free Vendor Seminar sponsored by Supelco**

**Analyze Glycoproteins with Exceptional Reproducibility!**
**"Analysis of Glycoproteins using Size Exclusion and Hydrophilic Interaction Chromatography Technologies"**
Location: Anthony Room, 3rd floor *(light lunch will be provided)*
Must register at the Supelco exhibit by Wednesday @ 3:00 PM

**POSTER SESSION II & EXHIBITION**
**1:30 PM- 3:00 PM**
Location: Commonwealth Hall B/C, 2nd floor

P-TH-135 **Aggregation Analysis of Proteins using Size Exclusion Chromatography.** Larissa Harwick, Jeffrey Fishpaugh, Kevin Rupprecht, Abbott Laboratories, Abbott Park, IL, USA

P-TH-136 **Recent Results on the Immobilization of Ligands to Silica Monoliths and their Application in Bioanalysis.** Tom Kupfer, Gisela Jung, Peter Knoell, Benjamin Peters, Karin Cabrera, EMD Millipore, Darmstadt, GERMANY

P-TH-137 **Application of Fused-Core® Reversed-phase HPLC Columns for Biotherapeutic Analyses.** Hillel Brandes, Lauren Swiger, Stacy Squillario, Sigma Aldrich / Supelco, Bellefonte, PA, USA

P-TH-138 **A Study to Optimize the Design of Superficially Porous Particles for the Separation of Large Biomolecules.** Wu Chen, Anne Mack, Kunqiang Jiang, Bo Sachok, Xiaoli Wang, Agilent Technologies, Wilmington, DE, USA

P-TH-139 **Using Evolution to Predict N-glycosylation on Novel Non-canonical Protein Motifs; Using LC-MS/MS to Validate.** Mark Lowenthal, Kiersta Davis, Catherine Formolo, Lisa Kilpatrick, National Institute of Standards and Technology, Gaithersburg, MD, USA

P-TH-140 **Estimation of Partition Coefficients in Aqueous Two-phase Systems Based on Osmotic Virial Coefficients.** Christian Kress, Christoph Brandenbusch, Technical University Dortmund, Dortmund, GERMANY

P-TH-141 **Multiple Reaction Monitoring (MRM)-based Quantitation of Oxidation during Hydroxyl Radical Protein Footprinting for Protein Biopharmaceutical Conformation Analysis.** Franklin Leach, Peter Todd, Ron Orlando, Joshua Sharp, PhotoChem Technologies, Athens, GA, USA

P-TH-142 **Kinetic Model of Column Re-equilibration after Gradient Elution.** Michael Fletcher, Joe Foley, Drexel University, Philadelphia, PA, USA

P-TH-143 **Sequential Elution Liquid Chromatography using a Wide-range, Mass Spectrometry Compatible pH Gradient.** Catherine Kita, Joe Foley, Drexel University, Philadelphia, PA, USA

P-TH-144 **Optimization of High Performance Separations of Labeled N-glycans on Three Ultra High Performance HILIC Columns.** Oscar Potter[1], Phu Duong[2], Linda Lloyd[3], [1]Agilent Technologies, Santa Clara, CA, USA; [2]Agilent Technologies, Wilmington, DE, USA; [3]Agilent Technologies, Church Stretton, UK

**POSTER SESSION II & EXHIBITION** *(continued)*
**1:30 PM- 3:00 PM**
Location: Commonwealth Hall B/C, 2nd floor

P-TH-145 **Single-cell CE-MS Combined with Ventralization Studies Reveal Blastomere-specific Metabolic Composition in the 16-Cell Xenopus Embryo.** Rosemary M. Onjiko, Sydney Morris, Sally A. Moody, Peter Nemes, The George Washington University, Washington, DC, USA

P-TH-146 **Integrated Bioprocess Development Based on Micro-Scale Cultivations and Feed Stock Characterization by Chromatography Modeling.** Pascal Baumann, Tobias Hahn, Juergen Hubbuch, Karlsruhe Institute of Technology (KIT), Karlsruhe, GERMANY

P-TH-147 **New Nano-diamond Core-shell Stationary Phase for Peptides and Proteins Separations.** Andrew E. Dadson, David S. Jensen, Janusz Zukowski, Diamond Analytics, Orem, UT, USA

P-TH-148 **Studying the Kinetics of N-glycan Release by PNGase F with MRM Quantitation of the Glycopeptides from Human Serum Glycoproteins.** Yining Huang, Ron Orlando, University of Georgia, Athens, GA, USA

P-TH-148-B **Characterization of Charge Variant Profiles and Glycation Level of Monoclonal Antibodies In Vivo.** Yu Huang, Wei Chen, Yinyin Li, Shashi Prajapati, Yelena Lyubarskaya, Biogen, Cambridge, MA, USA

## Thursday, July 30, 2015

**Thursday Session 6. Predicting Separation Behavior**
Session Chair: Richard Ludwig, Bristol-Myers Squibb
Location: Commonwealth Hall A, 2nd floor

3:00 PM L-149 **Prediction of Column Performance from Small Scale Pre-packed Disposable Columns.** Susanne Schweiger[1], Christian Jungreutmayer[1], Rupert Tscheliessnig[1], Natalie Wehrwein[2], Jasmin Haas[2], Tim Schröder[2], Astrid Dürauer[3], Alois Jungbauer[3], [1]ACIB, Vienna, AUSTRIA; [2]Atoll, Weingarten, GERMANY; [3]BOKU, Vienna, AUSTRIA

3:25 PM L-150 **Integrated Biopharmaceutical Process Design: High-throughput Technologies and Modeling Supported by Protein Tags.** Pascal Baumann, Juergen Hubbuch, Karlsruhe Institute of Technology (KIT) Institute for Biomolecular Separation Engineering, Karlsruhe, GERMANY

3:50 PM L-151 **Estimation of Partition Coefficients in Aqueous Two-phase Systems Based on Osmotic Virial Coefficients.** Christian Kress, Christoph Brandenbusch, Technical University Dortmund, Dortmund, GERMANY

4:15 PM L-152 **A Study to Optimize the Design of Superficially Porous Particles for the Separation of Large Biomolecules.** Wu Chen, Anne Mack, Kunqiang Jiang, Bo Sachok, Xiaoli Wang, Agilent Technologies, Wilmington, DE, USA

4:40 PM L-153 **Development of Multi-product Salt Gradient Ion Exchange Chromatography Method from a Novel Understanding of the Charge Characteristics of Therapeutic Proteins.** Daniel McDonald, Thomas Patapoff, Benny Freistadt, Jennifer Wang, Pat Rancatore, Genentech, South San Francisco, CA, USA

5:10 PM L-154 **TUTORIAL: Stationary Phases, Particle Size and Pore Size and How These Effect Separations.** Mark Schure, Kroungold Analytical, Bluebell, PA, USA

6:00 PM **Pause**

6:30-8:30 PM **Conference Banquet** (Ticket Required)
Location: Loews Lescaze Room, 33rd floor

8:00 AM **Symposium Registration Open**
Location: Commonwealth Hall, 2nd floor

**Friday Session 7. Metabolomics and Nucleic Acids**
Session Chair: Alois Jungbauer, BOKU, Vienna
Location: Commonwealth Hall A, 2nd floor

8:30 AM L-155 **Single-cell CE-MS Combined with Ventralization Studies Reveal Blastomere-specific Metabolic Composition in the 16-Cell Xenopus Embryo.** Rosemary M. Onjiko[1], Sydney Morris[1], Sally A. Moody[2], Peter Nemes[1], [1]George Washington University Department of Chemistry, Washington, DC, USA; [2]George Washington University Department of Anatomy & Regenerative Biology, Washington, DC, USA

8:55 AM L-156 **UPLC-MS Metabolomics: Applications to Red Blood Cell Biology and Biopreservation**. Angelo D'Alessandro, Travis Nemkov, Kirk Hansen, Department of Biochemistry & Molecular Genetics, University of Colorado Denver, Aurora, CO, USA

9:20 AM L-157 **Large Volume CIM® Chromatographic Monoliths for Gene Therapy Vector Purification – Scalability and Uniformity.** Lev Matoh, Viktor Zalokar, Dusan Jakopin, Mario Simic, Bostjan Kosir, Urh Cernigoj, Jana Vidic, Nika Lendero Krajnc, Ales Strancar, BIA Separations, Ajdovsona, SLOVENIA

9:45 AM L-158 **Examining the Epitranscriptome of tRNAs by a Bottom Up LC-MS/MS Approach.** Collin Wetzel, Patrick Limbach, University of Cincinnati, Cincinnati, OH, USA

10:10 AM **Break**
Location: Commonwealth Hall A Foyer, 2nd floor

**Friday Session 8. Antibody Characterization and Aggregation of Proteins**
Session Chair: Joseph Glajch, Momenta Pharmaceuticals
Location: Commonwealth Hall A, 2nd floor

10:35 AM L-159 **Analytical Separation Strategies for Efficient Purity Profiling of Bispecific Monoclonal Antibodies.** James Martosella, Geetha Goparaju, Jasna Maksimoska, Shelley, Ji, Janssen, Malvern, PA, USA

11:00 AM L-160 **High Sensitivity Intact Antibody Drug Conjugate Analysis using an Integrated Microfluidic Device Coupled to High Resolution Mass Spectrometry.** Gregory Roman, Henry Shion, Weibin Chen, James Murphy, Waters Corporation, Milford, MA, USA

11:25 AM L-161 **Experimental Investigation, Modeling and Prevention of Aggregate Formation in Downstream Processing.** Olubukayo Oyetayo, Hans Kiefer, Institute of Applied Biotechnology Biberach University of Applied Sciences, Biberach Riss, GERMANY

11:50 AM L-162 **Mobile Phase Acid Modifier Effects on Antibody Analysis by Reversed-phase LC and LC/MS.** Barry E. Boyes, Benjamin Libert, Stephanie Schuster, Joseph DeStefano, Advanced Materials Technology, Inc., Wilmington, DE, USA

12:15 PM **Closing Remarks**

# Free Vendor Seminars

**Free Vendor Seminar sponsored by Shimadzu Scientific Instruments**
**Wednesday, July 29 at 12:30-1:30pm**

**"Enhanced Detection of Hydrophobic Peptides and Proteins by MALDI-TOF MS"**
*Presented by: Brian Feild, Biotech Product Manager, Shimadzu Scientific Instruments*
Membrane protein analysis by mass spectrometry continues to be an important but challenging area of study for biomarker discovery and therapeutic drug development. In most cases, the hydrophobicity of the transmembrane domain enables minimal detection of peptides residing in these regions using traditional mass spectrometry techniques. A novel matrix for use with MALDI-TOF MS, alkylated tryhydroxyacetophenone (ATHAP), was developed to overcome these challenges and selectively provide sensitive detection of hydrophobic peptides. In this presentation, we will show the ability for this matrix to improve detection of several intact membrane proteins and their digest products in comparison to traditional matrices. Please direct inquiries to bjfeild@shimadzu.com
*Location: Adams Room on the 3rd floor (light lunch will be provided)*
*Must register at the Shimadzu exhibit by Wednesday @11:00 AM*

**Free Vendor Seminar sponsored by TSI Inc. - Thursday, July 30 at 12:30-1:30pm**

**"Macroion Mobility Spectrometry for Particle Size Analysis of Large Proteins, Protein Aggregates and Aqueous Polymers"**
*Presented by Axel Zerrath, Applications, TSI Inc.*
In the past 15 years, MacroIMS has established itself as a valuable tool for analyzing antibody aggregation, macromolecular protein complexes, synthetic polymers, intact viruses and virus-like particles, and lipoparticles; analytes with sizes that are too large for SEC and MS analysis, while providing better resolution than that by light scattering techniques. Advances in technology with regards to sensitivity and speed have led to a new commercial system for routine analysis of high mass macromolecules (>10 kDa). Data will be presented showing the separation of sub-micromolar protein mixtures, antibody fragments and oligomers, and analysis of aqueous synthetic polymers. Please direct inquiries to Axel.zerrath@tsi.com
*Location: Adams Room on the 3rd floor (light lunch will be provided)*
*Must register at the TSI exhibit by Wednesday @ 3:00 PM*

**Free Vendor Seminar sponsored by Supelco - Thursday, July 30 at 12:30-1:30pm**
**Analyze Glycoproteins with Exceptional Reproducibility!**
**"Analysis of Glycoproteins using Size Exclusion and Hydrophilic Interaction Chromatography Technologies"**
*Presented by Stacy Squillario, Supelco/Sigma-Aldrich*
Protein glycosylation is one of the most common and structurally diverse types of protein post translational modifications (PTM). The pattern of glycosylation as well as the location of the PTM has been shown to play a major role in numerous physiological and pathological reactions. Thus, understanding protein glycosylation is essential in elucidating a glycoprotein's cellular function. Glycoprotein analysis can be challenging, however, because it involves the identification of complex N-linked and O-linked sugar molecules that are often quite structurally similar. BIOshell™ Glycan HPLC Columns are specifically engineered to deliver fast, high resolution, reproducible glycan identification. Please direct inquiries to bobbiejo.seyler@sial.com
*Location: Anthony Room on the 3rd floor (light lunch will be provided)*
*Must register at the Supelco exhibit by Wednesday @ 3:00 PM*

**OVERVIEW OF PROTEIN AND PEPTIDE SEPARATIONS VIA VARIOUS MODES OF CHROMATOGRAPHY**

Andrew Alpert, PolyLC Inc., Columbia, MD

No single mode of chromatography suffices for every separation involving proteins and peptides. Reversed-phase chromatography (RPC) works well for most peptide applications but needs to be supplemented by other modes for complex samples or for identification of peptides with post-translational modifications (PTM's). RPC doesn't work at all for many protein applications. The following approaches will be discussed:

1) Ion-exchange chromatography (IEX): This works well with proteins. SCX (strong cation-exchange) is a general-purpose alternative to RPC for peptides. Within limitations, this method can be implemented with volatile solvents.

2) Hydrophilic interaction chromatography (HILIC): This is the inverse of RPC in many respects, separating analytes in order of increasing polarity. It can be implemented with volatile solvents, making HILIC-MS practical. HILIC works well with peptides and with proteins that don't normally occur free in aqueous media, such as membrane proteins and histones. HILIC can be superimposed upon electrostatic effects with ion-exchange columns, resulting in combinations such as IEX-HILIC and ERLIC (electrostatic repulsion-hydrophilic interaction chromatography).

3) Hydrophobic interaction chromatography (HIC): The selectivity is similar to that of RPC but is a nondenaturing mode, making it compatible with proteins. Selectivity can be remarkably high. The main drawback is the use of high concentrations of nonvolatile salts. However, a new approach to the mode suggests that even that obstacle can be overcome.

4) Size exclusion chromatography (SEC): This is useful for such applications as high-throughput drug screening and quality control analysis of proteins such as antibody-drug conjugates (ADC's).

5) Affinity chromatography.

Applications to be discussed include fraction of complex tryptic digests for bottom-up proteomics, fractionation of intact proteins for top-down proteomics, isolation of peptides with specific PTM's, and quality control analysis of commercial proteins and polypeptides.

# Thursday Tutorial 5:10-6:00 pm

**STATIONARY PHASES, PARTICLE SIZE AND PORE SIZE AND HOW THESE EFFECT SEPARATIONS**

Mark R. Schure, Kroungold Analytical, Inc., Blue Bell, PA 19422

High Performance Liquid Chromatography (HPLC) is the most commonly sought out technique for analyzing mixtures in solution. HPLC has matured to the extent that it is often a turnkey system for applications which span the range from routine environmental applications to investigations in the biomedical sciences.

Still, it is often left up to an expert to develop a robust method which tends to perform the separation (and analysis) with the required resolution, speed and reliability. These criteria are somewhat at odds and represent a compromise situation.

In this tutorial we will examine a number of optimization criteria with regards to speed of analysis, selectivity of the method (and how to measure this) and how the sample affects these. These optimization criteria are tied intimately to the effects that the stationary phase, particle and pore size have on the chromatographic process.

The views discussed here will be both practical as to how they impact the analysis and fundamental in discussing the molecular physics which takes place inside the chromatographic process. Examples from the literature and from the author's own research will be used.

# List of Exhibitors

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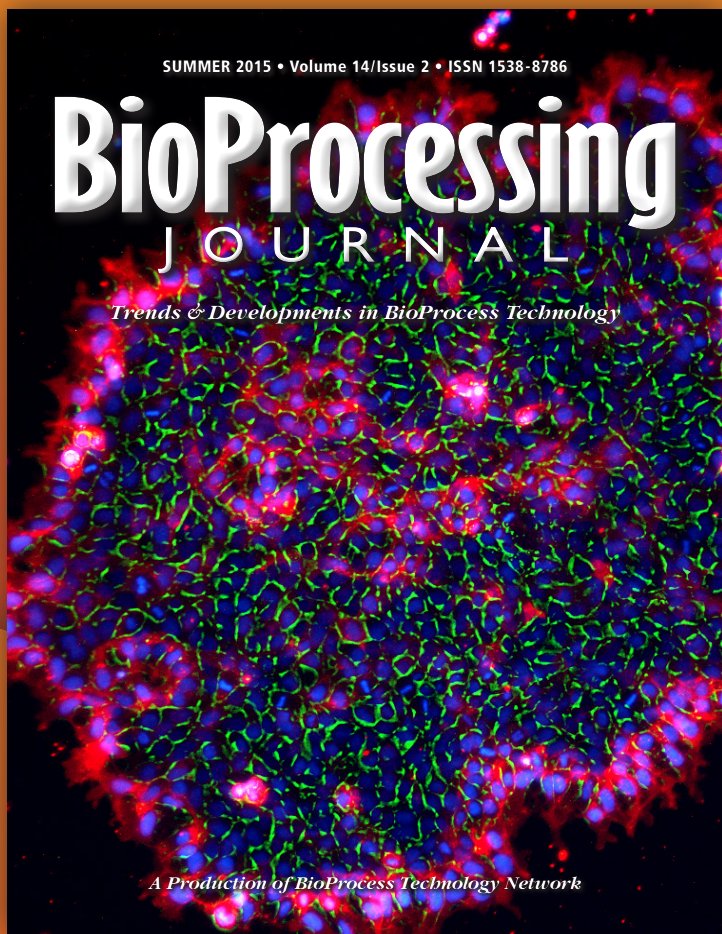

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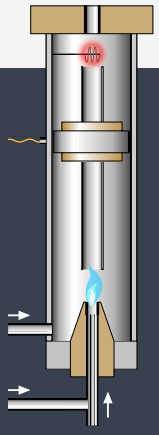

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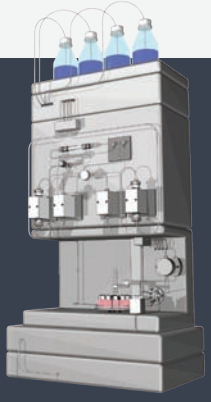

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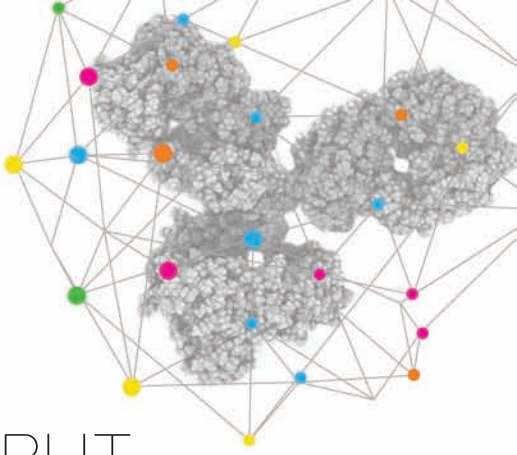

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