ISPPP 2013

33rd International Symposium and Exhibit on the

Separation and Characterization of Biologically Important Molecules

July 17-19, 2013 in Boston, MA, USA

www.ISPPP.org

Welcome to ISPPP 2013!

We would like to personally welcome each of you to the 33rd International Symposium for the Separation of Proteins, Peptides and Polynucleotides. It's an exciting time for the separation science of biological molecules. The field continues to grow and adapt, enjoy better materials, instruments and methods, and a seeming never-ending list of interesting molecules to work on. We continue to confront analytical, productivity and financial challenges, with ever more asked. The world of biological molecule separations and analysis is an exciting area in which to work/study/play, and we intend to continue to meet and bring inspired people together in this forum, to ensure ISPPP remains relevant and timely for the science and technology discussions that we all enjoy.

Over the course of the conference, we would encourage you to take advantage of our assembled experts, practitioners, and exhibitors, and to engage our presenters and attendees in the various presentation formats at the Symposium, whether this is during the Lectures, Posters Sessions, Exhibitor Meetings, or during breaks. We are of the mind that there are no bad questions, just bad answers!

This 33rd ISPPP Symposium is testing some changes in the way things have been done, with our joint assembly with the Prep 2013 Symposium, and by shifting from Fall to Summer. Our feelings were that there was a great opportunity for synergy by meeting on a joint day (Wednesday), and placing ISPPP in series with this important Symposium on Preparative Methods. Our congratulations go to the Program Chair, Prof. Giorgio Carta, and his excellent Committees, on a great Program for that Symposium. We were always confident that the weather in Boston would be fully cooperative, and it appears we are pretty lucky (fingers crossed). It is a pleasure to return to this vibrant city, which we last visited as ISPPP in 1995. As always, the Organizing Committee is extremely interested in feedback on the ISPPP Symposium, so do not hesitate to be forward with suggestions for future meetings.

We would like to thank you again for attending this Symposium and bringing your expertise to our gathering. As a personal note, we would like to particularly thank Dr. Joseph DeStefano, for his help in organizing this Symposium, and for his decades of service as a Committee Member and as Chair of several ISPPP Symposia. We fervently hope that Joe will stay involved, and continue to support ISPPP with his excellent drive, judgment and scientific interest. Throughout this conference, we ask you to stay engaged, keep us proactive, and help us shape the future of ISPPP.

Barry Boyes, Ph.D
Ron Orlando, Ph.D.
Co-Chairmen, 33rd ISPPP

TABLE OF CONTENTS

	Page
Welcome Message	1
General Information	3-4
Scientific Program	5-13
Free Vendor Seminars	14
Exhibitors	15-16
Abstracts	17-43
Author Index	44-46

GENERAL INFORMATION

Venue

Westin Boston Waterfront, 425 Summer Street, Boston, MA 02210, USA
Phone 617-532-4600 ◊ www.westinbostonwaterfront.com

Name Badges

A name badge must be worn to all conference functions.

Registration

Symposium Registration is located near the Harbor Ballroom

Exhibits

The exhibition is an important component of the meeting, so please take the time to thank all the exhibitors for their support of the program by visiting the booths located in Harbor Ballroom I & II.

Exhibit Hours

Wednesday	10:00 AM – 7:00 PM
Thursday	10:00 AM – 5:10 PM

Social Functions

Welcome Mixer on Wednesday at 5:05-7:00 PM in Harbor Ballroom II & III
Symposium Banquet on Thursday at 6:30-8:30 PM in Burroughs room (ticket required)

Free Vendor Seminars

Wednesday 12:00 – 1:30 pm

Free Vendor Seminar Sponsored by Waters Corporation
"Looking at Innovator and Biosimilar Biotherapeutics through a Kaleidoscope"
Location: Carlton Room *(light lunch will be provided)*
Must register at the Waters exhibit by Wednesday @10:30 AM

Thursday 12:00-1:30 PM

Free Vendor Seminar Sponsored by Shimadzu
"Turning Mountains into Molehills: The New Landscape of Protein Analysis"
Location: Griffin Room *(light lunch will be provided)*
Must register at the Shimadzu exhibit by Wednesday @ 3:45 PM

Thursday 12:00-1:30 PM

Free Vendor Seminar Sponsored by Knauer
"Contichrom®: A Versatile Purification Platform for Batch/CaptureSMB/MCSGP Biochromatography"
Location: Carlton Room *(light lunch will be provided)*
Must register at the Knauer exhibit by Wednesday @ 3:45 PM

Author Index

The Author Index is located on pages 44-46 of the Final Program book.
"L" preceding the abstract number = Lecture
"P" preceding the abstract number = Poster
"P-W" is Wednesday poster presentation day
"P-Th" is Thursday poster presentation day

GENERAL INFORMATION

Oral Presenters

Prior to the start of each session, please arrive at your session at least 20 minutes before the start of the session to introduce yourself to the session chair and to submit your presentation on a flash drive labeled with the presenter's name. Important to note that if there is no time to submit your presentation between sessions, please submit the presentation during the break that immediately precedes your session.

Please come to the podium and get your presentation set up during the question period for the previous talk.

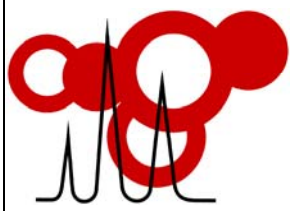
Kindly note that session chairs are under very strict instructions to keep their sessions on schedule.

Poster Presenters

Details regarding set-up, presentation, and tear-down days and times:

- ALL posters in Poster Sessions I and II should be put up on Wednesday, July 17, between 8:00-10:40 AM and remain on the poster boards for participants to view both days (do not remove until Thursday at 3:30-5:30 PM).
- Poster presentations are numbered in the scientific program to correspond with the poster board number and its abstract located in the Final Program book.
- At the back of the Final Program book, please refer to the Author Index to assist in locating a poster number.
- Presenters must be in attendance at their posters on the day and time of their poster presentation.
- Authors of posters in the P-W series should stand at their posters and be available to discuss the research during Poster Session-I on Wednesday, July 17, at 1:30-3:00 PM.
- Authors of posters in the P-Th series should stand at their posters and be available to discuss the research during Poster Session-II on Thursday, July 18, at 1:30-3:00 PM.
- Remove all posters from the poster boards only on Thursday, July 18, between 3:30-5:30 PM. Anything remaining on the poster boards after 6:00 PM will be discarded.

Use of still or video cameras and cell phones is prohibited during oral sessions and in poster areas.
Opinions expressed by presenters are not necessarily the opinions of the PREP Symposium.



ISPPP 2013

33rd International Symposium and Exhibit on the

Separation and Characterization of Biologically Important Molecules

ISPPP 2013 Scientific Program

Wednesday, July 17, 2013

7:30 AM **Symposium Registration Open**
Location: Harbor Ballroom

10:00 AM **Exhibition Opens in Harbor Ballroom II & III**

8:10 AM **Welcome and Opening Remarks in Burroughs Room**

Wednesday Oral Session 1. Analytical Separations
Session Chair: Milton Hearn, Monash University
Location: Burroughs Room

8:20 AM L-101 **Orthogonal Bioseparations.** Mark Schure, Kroungold Analytical, Blue Bell, PA, USA

8:40 AM L-102 **New Advances in Hydrophilic Interaction Chromatography of Peptides: Comparison of HILIC/SALT to HILIC and RPC.** Colin Mant, Robert Hodges, Department of Biochemistry and Molecular Genetics, University of Colorado Denver, Aurora, CO, USA

9:00 AM L-103 **Highly Efficient LC-MS of Intact Proteins using Sub-0.5µm Particles with Slip Flow.** Zhen Wu[1], Bingchuan Wei[2], Ximo Zhang[1], Mary Wirth[1], [1]Purdue University, West Lafayette, IN, USA; [2]University of Washington, Seattle, WA, USA

9:20 AM L-104 **Capillary-channeled Polymer (C-CP) Fibers for High Throughput Analytical Separations and Desalting of Proteins Prior to MALDI and ESI-MS.** R. Kenneth Marcus[1], Abby Schadock-Hewitt[1], Benjamin T. Manard[1], Carolyn Q. Burdette[2], Marissa Pierson[1], [1]Clemson University, Clemson, SC, USA; [2]National Institute of Standards and Technology, Gaithersburg, MD, USA

9:40 AM L-105 **Increasing the Peak Capacity of Peptide Separations using Long Microcapillary Columns and Sub 2 µm Particles at 30,000+ psi.** Kaitlin Fague, Justin Godhino, Edward Franklin, Jordan Stobaugh, University of North Carolina, Chapel Hill, NC, USA

10:00 AM **Break, Exhibits and Posters**
(Location: Harbor Ballroom II & III)

Wednesday Oral Session 2. Proteomics and Protein Measurements
Session Chair: Joshua Sharp, University of Georgia
Location: Burroughs Room

10:40 AM L-106 **Application of a Robust and Modular Cartridge based NanoLC System for Proteomics.** Michael Bereman[1], Tom Corso[2], Colleen Van Pelt[2], Michael MacCoss[1], [1]University of Washington, Seattle, WA, USA; [2]CorSolutions, Ithaca, NY, USA

11:00 AM L-107 **Protein Markers for Cancer Stem Cells in Pancreatic Cancer.** David Lubman, Jianhui Zhu, University of Michigan, Ann Arbor, MI, USA

11:20 AM L-108 **Target-based Multiplex MRM Assays.** Christopher M. Colangelo, Lisa Chung, Shifman Mark, Abbott Thomas, Fumika Sakaue, Angus Nairn, Willaims Kenneth, Yale University, New Haven, CT, USA

11:40 AM L-109 **Pretreatment of Human Fluid Samples for Trace Protein Analysis.** Frank Jahnke, Sonata Biosciences, Inc., Auburn, CA, USA

12:00 PM Lunch on own

12:00-1:30 PM **Free Vendor Seminar Sponsored by Waters Corporation**
"Looking at Innovator and Biosimilar Biotherapeutics through a Kaleidoscope"
Location: Carlton Room
Must register at the Waters exhibit by Wednesday @10:30 AM

1:30 - 3:00 PM **Poster Session - I and Exhibits**
(Location: Harbor Ballroom II & III)

Wednesday Poster Session - I Presentations

P-W-110 **Epitope Mapping and the Selection of MAbs used in the Diagnostic Immunoassays.** Cheng Zhao, Bryan Tieman, Bailin Tu, Robert Ziemann, Jeffrey Fishpaugh, Carol Ramsay, Abbott Laboratories, Abbott Park, IL, USA

P-W-111 **Aggregate Removal with Cation Exchange Chromatography (NuviaTM HR-S).** Paul K. Ng, Mark A. Snyder, Bio-Rad Laboratories, Hercules, CA, USA

P-W-112 **Optimized One-day Assay for Quantitation of Monosaccharide Content in Proteins by 2-Picoline-Borane Reductive Amination.** Nicholas Woon, Genentech, South San Francisco, CA, USA

P-W-113 **Methodology of Monoclonal Antibody Charge-variant Analysis by Ion-exchange Chromatography.** Hillel Brandes, Roy Eksteen, Sigma Aldrich / Supelco, Bellefonte, PA, USA

P-W-114 **2D Separation and Immunoreactive Coverage of CHO and E.coli Host Cell Proteins (HCP) by Polyclonal Antibodies – Use of Fluorescent Labeling (Cyanine Dyes) in 2D Western Blotting.** Harbhajan Dhillon, Mark Abbott, Jonathan Basch, Mark Panek, Bristol-Myers Squibb, East Syracuse, NY, USA

P-W-115 **N-linked Glycan Profile Comparison Between the Innovator and a Biosimilar Etanercept.** Ying Qing Yu, Weibin Chen, Waters Corporation, Milford, MA, USA

P-W-116 **Charge Surface Modified C18 Columns for Increasing Peak Capacity in LC-MS Peptide Separations with Formic Acid Mobile Phases.** Matthew Lauber, Stephan Koza, Kenneth Fountain, Waters Corporation, Milford, MA, USA

P-W-117 **A Quality by Design Approach: Systematic Optimization of Malaria Vaccine Purification with IMAC.** Jessica Paul, Arthur Dukart, Jasmin Zuehlke, Gesine Cornelissen, Hamburg University of Applied Sciences, Hamburg, GERMANY

P-W-118 **Automating Workflows for Developing Separation and Reporting Methods for Size Exclusion Chromatography.** Thomas E. Wheat, Aparna Chavali, Patricia McConville, Waters Corporation, Milford, MA, USA

P-W-119 **Enthalpic and Entropic Contributions in Lysozyme Adsorption onto a Cation-exchange Support.** Francisco Marques[1], Goncalo Silva[1], Marvin Thrash, Jr.[2], Cristina Dias-Cabral[1], [1]CICS-UBI- Health Sciences Research Centre, University of Beira Interior, Covilha, PORTUGAL; [2]Department of Water Resources and Environmental Engineering, College of Science and Engineering, Central State University, Wilberforce, OH, USA

P-W-120 **Malaria Vaccine Purification via Expanded Bed Adsorption Chromatography Combined with an Ultrafiltration.** Sarah Schreiber, Sven Oliver Borchert, Jessica Paul, Gesine Cornelissen, University of Applied Sciences, Hamburg, GERMANY

P-W-121 **Continuous-batch Protein Chromatography – Continuous Capture Coupled with Two-step Automated Batch Polish.** Peter Tiainen, Jais Rose Bjelke, Ditte Skibstrup, Haleh Ahmadian, Novo Nordisk A/S, Malov, DENMARK

P-W-122 **Bioseparations with 3.5- and 5-Micron Wide-Pore Superficially Porous Particles.** Joseph DeStefano, Joseph Kirkland, Stephanie Schuster, Bill Johnson, Advanced Materials Technology, Inc., Wilmington, DE, USA

P-W-123 **A Novel Automated Enrichment Process for the Isolation of Product-related Impurities from Active Pharmaceutical Ingredients.** Thomas Muller-Spath, Nicole Ulmer, Lars Aumann, Guido Strohlein, Michael Bavand, ChromaCon AG, Zurich, SWITZERLAND

P-W-124 **Peptide Mapping of a Therapeutic Monoclonal Antibody (mAb): Optimizations for Increasing Speed and Peptide Identifications.** James Martosella[1], Ning Tang[2], Alex Zhu[1], [1]Agilent Technologies, Wilmington, DE, USA; [2]Agilent Technologies, Santa Clara, CA, USA

P-W-125 **Countercurrent Tangential Chromatography for Purification of Monoclonal Antibodies.** Oleg Shinkazh, Chromatan, State College, PA, USA

P-W-126 **Amphoteric Ion-Exchange Separation of Biomolecules with Porous or Non-Porous Polymer-Based Resins.** Ken Tseng[1], Toshi Ono[1], Tsunehisa Hirose[2], Kazuhiro Kimata[2], [1]Nacalai USA Inc., San Diego, CA, USA; [2]Nacalai Tesque, Kyoto, JAPAN

P-W-127 **Isomeric Separation of Procainamide Labeled N-glycans by Using Novel Superficially Porous Particle HILIC Column.** Shujuan Tao[1], Yining Huang[1], Barry Boyes[2], Ron Orlando[1], [1]CCRC, University of Georgia, Athens, GA, USA; [2]AMT, Wilmington, DE, USA

P-W-128 **Two Dimensional Separations: Which Dimension Plays the Most Important Role in Protein Identification Efficiency?** Darryl Johnson[1], Barry Boyes[2], Ron Orlando[1], [1]CCRC, University of Georgia, Athens, GA, USA; [2]AMT, Wilmington, DE, USA

P-W-129 **Microfluidic CE-MS Applied to Protein, Peptide, and Small Molecule Characterization.** Gregory Roman[1], Scott Mellors[2], Martin Gilar[1], James Murphy[1], J. Michael Ramsey[2], [1]Waters Corporation, Milford, MA, USA; [2]University of North Carolina, Chapel Hill, NC, USA

3:00 - 3:45 PM **Break/Social, Exhibits and Posters**
(Location: Harbor Ballroom II & III)

Wednesday Oral Session 3. Protein Therapeutics
Session Chair: David Lubman, University of Michigan
Location: Burroughs Room

3:45 PM L-130 **IgG Asparagine-linked Oligosaccharide Profiling by High-performance Anion-exchange Chromatography with Pulsed Amperometric Detection.** Jeffrey Rohrer[1], Deanna Hurum[2], Lipika Basumallick[1], [1]Thermo Fisher Scientific, Sunnyvale, CA, USA; [2]Palo Alto, CA, USA

4:05 PM L-131 **Size-exclusion Chromatography using Multi-angle Light Scattering (SEC-MALS) for the Characterization of Polypeptide Mixtures.** Joseph Glajch, Ying Li, Momenta Pharmaceuticals, Cambridge, MA, USA

4:25 PM L-132 **Developing an Automated Workflow for Disulfide Linkages Analysis of Biotherapeutics by High-Resolution LCMS.** Asish Chakraborty[1], Stephane Houel[1], Henry Shion[1], Scott Berger[1], Weibin Chen[1], Anurag Rathore[2], [1]Waters Corporation, Milford, MA, USA; [2]Indian Institute of Technology, New Delhi, Delhi, INDIA

4:45 PM L-133 **Analytical HIC for mAb Aggregate Analysis; How Does the Salt Ion Type Influence the Selectivity?** Hannah Brueck, Judith Vajda, Werner Conze, Egbert Mueller, Tosoh Bioscience GmbH, Stuttgart, GERMANY

5:05 - 7:00 PM **WELCOME MIXER in Exhibit and Poster Session Hall**
(Location: Harbor Ballroom II & III)

Thursday, July 18, 2013

7:45 AM **Symposium Registration Open**
Location: Harbor Ballroom

10:00 AM **Exhibition Open in Harbor Ballroom II & III**

Thursday Oral Session 4. Affinity and Ion Exchange Methods
Session Chair: Mark Schure, Kroungold Analytical
Location: Harbor Ballroom I

8:20 AM L-134 **Discovery and Development of Universal Fc Binders for Antibody Purification.** Marc Arnold, Holger Bittermann, Thomas Neumann, Graffinity Pharmaceuticals GmbH, Heidelberg, GERMANY

8:40 AM L-135 **Development of a Sialic Acid-specific Affinity Chromatography for the Purification and Separation of Glycoprotein Isoforms.** Matthias Meininger[1], Francisco Vito Santos da Silva[1], Samanta Cajic[1], René Hennig[1], Erdmann Rapp[1], Frank Zwanziger[2], Stefan Laufer[2], Karl-Heinz Wiesmueller[3], Heinz Rotering[4], Udo Reichl[1], Michael Wolff[1], [1]Max Planck Institut for Dynamics of Complex Technical Systems, Magdeburg, GERMANY; [2]Eberhard-Karls-University, Tuebingen, GERMANY; [3]EMC Microcollections GmbH, Tuebingen, GERMANY; [4]Merckle Biotec GmbH, Ulm, GERMANY

9:00 AM L-136 **Reversible Cyclic and Polycyclic Peptides for the Discovery of Affinity Ligands.** Stefano Menegatti, Robert Blackburn, Kevin Ward, Ruben Carbonell, North Carolina State University, Raleigh, NC, USA

9:20 AM L-137 **Understanding Ion Exchange Adsorption Mechanism.** Francisco Marques[1], Patricia Aguilar[1], Marvin Thrash Jr.[2], Cristina Dias-Cabral[1], [1]University of Beira Interior, Covilhã, PORTUGAL; [2]Central State University, Wilberforce, OH, USA

9:40 AM L-138 **Purification of a Potential Malaria Vaccine by Multimodal Ion Exchange Chromatography.** Sonja Jensen, Jessica Paul, Gesine Cornelissen, Hamburg University of Applied Sciences, Hamburg, GERMANY

10:00 AM **Break, Exhibits and Posters**
(Location: Harbor Ballroom II & III)

Thursday Oral Session 5. Monoclonal Antibody Separations
Session Chair: Robert Hodges, University of Colorado Denver
Location: Harbor Ballroom I

10:40 AM L-139 **New Tools to Achieve Enhanced Process Productivity in the Purification of Monoclonal Antibodies and Other Recombinant Proteins.** Milton Hearn, Monash University, Clayton, Victoria, AUSTRALIA

11:00 AM L-140 **Investigation of Monoclonal Antibody Purification by Aqueous Two-phase Extraction.** Jan Mündges, Shuai Shi, Tim Zeiner, TU Dortmund University, Dortmund, GERMANY

11:20 AM L-141 **Fujifilm Diosynth's and Chromatan's Investigation of Countercurrent Tangential Chromatography for Purification of Monoclonal Antibodies.** Oleg Shinkazh, Chromatan, State College, PA, USA

11:40 AM L-142 **2D-DIGE for Host Cell Protein Analysis and Antibody Process Development.** Alois Jungbauer, BOKU, Vienna, AUSTRIA

12:00 PM Lunch on own

12:00-1:30 PM **Free Vendor Seminar Sponsored by Shimadzu**
"Turning Mountains into Molehills: The New Landscape of Protein Analysis"
Location: Griffin Room
Must register at the Shimadzu exhibit by Wednesday @ 3:45 PM

12:00-1:30 PM **Free Vendor Seminar Sponsored by Knauer**
"Contichrom®: A Versatile Purification Platform for Batch/CaptureSMB/MCSGP Biochromatography"
Location: Carlton Room
Must register at the Knauer exhibit by Wednesday @ 3:45 PM

1:30 - 3:00 PM **Poster Session - II and Exhibits**
(Location: Harbor Ballroom II & III)

Thursday Poster Session II Presentations

P-Th-143 **An Alternative Capture Step for Monoclonal Antibodies: Phenyl Boronate as a New Multi-modal Ligand.** Ana M. Azevedo, Raquel dos Santos, Sara A.S.L. Rosa, M. Raquel Aires-Barros, Instituto Superior Tecnico, Lisbon, PORTUGAL

P-Th-144 **Phenyl Boronic Acid as Ligand for a Multimodal Chromatography: Adsorption Behavior Comparison between Control Pore Glass and Agarose Matrixes.** Rimenys Jr. Carvalho[1], James Woo[2], Karim A. Nakamura[2], Maria Raquel Aires-Barros[1], Ana M. Azevedo[1], Steven M. Cramer[2], [1]Instituto Superior Tecnico, Lisbon, PORTUGAL; [2]Rensselaer Polytechnic Institute, Troy, NY, USA

P-Th-145 **Chiral Separation of D,L-Phenylglycine using an Enantioselective Membrane Formed by Polycondensation of Bovine Serum Albumin with 1,6-Diisocyanatohexane on a Polysulfone Membrane.** Li-Ming Yuan, Guang-Yong Zeng, Department of Chemistry, Yunnan Normal University, Kunming, Yunnan, P.R. CHINA

P-Th-146 **Analysis of Associated Forms of Insulins.** Sara Fexby Garmer, Martin Mårtensson, Dorte Bjerre Steensgaard, Aage Hvass, Svend Havelund, Novo Nordisk A/S, Måløv, DENMARK

P-Th-147 **Lipidomics using Ion Mobility Mass Spectrometry with Transomics Informatics.** Giuseppe Astarita[1], Roy Martin[1], Giorgis Isaac[1], James Langridge[2], Weibin Chen[1], [1]Waters Corporation, Milford, MA, USA; [2]Waters Corporation, Manchester, UK

P-Th-148 **Characterization of Two Novel Analytical Chromatographic Columns for Orthogonal Analysis of Monoclonal Antibody and Protein Aggregates and their Isoforms.** Justin Steve, Atis Chakrabarti, Tosoh Bioscience LLC, King of Prussia, PA, USA

P-Th-149 **Purification of a Potential Malaria Vaccine by Multimodal Ion Exchange Chromatography.** Sonja Jensen, Jessica Paul, Gesine Cornelissen, Hamburg University of Applied Sciences, Hamburg, GERMANY

P-Th-150 **Characterization of Two Novel High Capacity Strong Ion Exchange Resins.** Chinlun Huang, J. Kevin O'Donnell, Tosoh Bioscience, King of Prussia, PA, USA

P-Th-151 **Aggregates and Particle Characterization to Support Biomanufacturing Process Development.** Yogesh Mudaliar, Rong-Rong Zhu, Tim Hanley, EMD Millipore, Bedford, MA, USA

P-Th-152 **Superficially Porous Particles for Peptide and Protein Analysis.** Barry Boyes, Joseph Kirkland, Stephanie Schuster, Brian Wagner, Joseph DeStefano, Advanced Materials Technology, Inc., Wilmington, DE, USA

P-Th-153 **Microcalorimetric Study of Linear Plasmid DNA Adsorption onto an Ion Exchange Support.** Patricia Aguilar[1], Filipa Pires[1], Marvin Thrash, Jr.[2], Cristina Dias-Cabral[1], [1]CICS-UBI-Health Sciences Research Centre, University of Beira Interior, Covilha, PORTUGAL; [2]Department of Water Resources and Environmental Engineering, College of Science and Engineering, Central State University, Wilberforce, OH, USA

P-Th-154 **Separation of DMB-Labeled Sialic Acids for the Comparison of Biosimilars to Reference Materials with an Improved Chromatographic Method.** Xiaoning Lu, Hillel Brandes, Dave Bell, Roy Eksteen, Sigma-Aldrich, Bellefonte, PA, USA

P-Th-155 **Twin Column CaptureSMB: A Novel Cyclic Process to Increase the Capacity Utilization in Protein A Chromatography.** Monica Angarita[1], Baur Daniel[1], Thomas Muller-Spath[2], Roel Lievrouw[3], Geert Lissens[3], Guido Strohlein[2], Massimo Morbidelli[1], [1]ETH Zurich, Zurich, SWITZERLAND; [2]ChromaCon AG, Zurich, SWITZERLAND; [3]JSR Life Sciences, Leuven, BELGIUM

P-Th-156 **Effects of Mobile Phase Optimization on Analyte Behaviour in Size Exclusion Chromatography of Biomolecules.** James Martosella[1], Andrew Coffey[2], [1]Agilent Technologies, Wilmington, DE, USA; [2]Agilent Technologies, Church Stretton, UK

P-Th-157 **Fast and Efficient Reversed-phase Liquid Chromatography/Mass Spectrometry Characterization of Glycosylation in the Fc Region of a Recombinant IgG(1) Therapeutic Monoclonal Antibody (mAb).** James Martosella, Phu Duong, Alex Zhu, Agilent Technologies, Wilmington, DE, USA

P-Th-158 **Combining Small-scale Purification and Analysis of Monoclonal Antibodies on One Instrument.** Sonja Schneider, Agilent Technologies, Waldbronn, GERMANY

P- Th-159 **Greater Loading Capacity and Resolution for Improved Process-scale Peptide Purification.** Jochen Saar[1], Reno Nguyen[2], Chitra Sundarajan[3], Scott Anderson[4], Dennis McCreary[5], Janine Sinck[6], [1]Worms, GERMANY; [2]Hesperia, CA, USA; [3]Hyderabad, Andrapradesh, INDIA; [4]Deerfield, IL, USA; [5]Columbia, MD, USA; [6]Allentown, PA, USA

P-Th-160 **New Wide Pore Media Improves Loading Capacity and Productivity of Peptide and Protein Purification by Flash Chromatography.** Bopanna NK, Chitra Sundararajan, Melissa Wilcox, Janine Sinck, Reno Nguyen, Grace Discovery Sciences, Deerfield, IL, USA

P-Th-161 **Comparing HILIC and RP for LC/MS Analysis of O-HexNAc Modified Peptides.** Barry Boyes[1], Stephanie Schuster[1], Alex Harvey[2], Ronald Orlando[3], [1]Advanced Materials Technology, Inc., Wilmington, DE, USA; [2]Glycoscientific, Inc., Athens, GA, USA; [3]University of Georgia, Athens, GA, USA

3:00 - 3:30 PM **Break/Social, Exhibits and Posters**
(Location: Harbor Ballroom II & III)

Thursday Oral Session 6. Protein Targets
Session Chair: Stephanie Schuster, Advanced Materials Technology
Location: Harbor Ballroom I

3:30 PM L-162 **Integration and Intensification of Downstream Bioprocessing based in Aqueous Two-phase Systems.** Ana Azevedo, Raquel Aires-Barros, Instituto Superior Tecnico, Lisbon, PORTUGAL

3:50 PM L-163 **Multi-stage Enzyme Extraction using Aqueous Two-phase Systems – Experiment and Modeling.** Axel Prinz, Katharina Koch, Tim Zeiner, TU Dortmund University, Dortmund, GERMANY

4:10 PM L-164 **Purifying and Concentrating Recovery Process Samples for Recombinant Protein Quantification.** Tanja Buch, Ian Marison, DCU, Dublin, IRELAND

4:30 PM L-165 **Predicting Protein Solubility and Crystallization Behavior based on the Second Osmotic Virial Coefficient.** Marcel Herhut, Christoph Brandenbusch, Gabriele Sadowski, Department of Biochemical and Chemical Engineering, Dortmund, GERMANY

4:50 PM **Historical Retrospective of ISPPP Conferences.** Milton Hearn, Monash University, Clayton, Victoria, AUSTRALIA

5:10 PM Pause

6:30 - 8:30 PM **Symposium Banquet (ticket required)**
Location: Burroughs room

Friday, July 19, 2013

8:15 AM **Symposium Registration Open**
Location: Harbor Ballroom

Friday Oral Session 7. Bio-Therapeutics
Session Chair: Alois Jungbauer, BOKU, Vienna
Location: Harbor Ballroom I

8:45 AM L-166 **Improved Identification and Quantitation of Host Cell Proteins in Protein Therapeutics using 2D-LC and Ion Mobility.** Weibin Chen, Catalin Doneanu, Keith Fadgen, Martha Stapels, Waters, Milford, MA, USA

9:05 AM L-167 **Additional Structural Insights on Therapeutic Glucocerebroside Variants and Impurities by Size Exclusion Chromatography.** John Thomas, Shire, Lexington, MA, USA

9:25 AM L-168 **Impact of Plasmid Size on the Purification of Model pDNA Vaccines by HIC on Phenyl Membrane Adsorbers.** Luis Raiado-Pereira, Jonathan de la Vega, D. Miguel F. Prazeres, Marilia Mateus, Instituto Superior Tecnico, Lisbon, PORTUGAL

9:45 AM L-169 **Protein Biomarker Assays for Drug Safety Assessments.** Jennifer Colangelo, Pfizer, Groton, CT, USA

10:05 AM **Break**

Friday Oral Session 8. Glycans and Complex Carbohydrates
Session Chair: Jennifer Colangelo, Pfizer
Location: Harbor Ballroom I

10:30 AM L-170 **High Temperature LC-MS of Permethylated N-Glycans Derived from Breast Cancer Cells and Human Blood Serum.** Shiyue Zhou[1], Yunli Hu[1], Tarek Shihab[1], Ahmed Hussein[2], Yehia Mechref[1], [1]Texas Tech University, Lubbock, TX, USA; [2]University of Alexandria, Alexandria, EGYPT

10:50 AM L-171 **Separations of Intact Glycoproteins by HILIC.** Barry Boyes[1], Ron Orlando[2], Joseph DeStefano[1], [1]Advanced Materials Technology, Inc., Wilmington, DE, USA; [2]University of Georgia, Athens, GA, USA

11:10 AM L-172 **Progress Towards Automated Sequencing of Heparin/Heparan Sulfate.** Joshua S. Sharp, Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA

11:30 AM L-173 **HILIC-MS of Glycans and Glycopeptides.** Joseph Zaia, Center for Biomedical Mass Spectrometry, Boston University, Boston, MA, USA

11:50 PM L-174 **Hypothesis Driven Glycomics.** Shujuan Tao[1], Yining Huang[1], Barry Boyes[2], Ron Orlando[1], [1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA; [2]Advanced Materials Technology, Inc., Wilmington, DE, USA

12:10 PM **Closing Remarks**

12:20 PM **Adjourn**

FREE VENDOR SEMINARS

Free Vendor Seminar sponsored by WATERS - Wednesday, July 17 @ 12:00-1:30PM
Must register at the Waters exhibit by Wednesday @10:30 AM

Looking at Innovator and Biosimilar Biotherapeutics through a Kaleidoscope
Characterization of biotherapeutic proteins requires the overlapping information derived from a large number of physicochemical analyses. Comprehensive physicochemical and structural characterization of these candidate biopharmaceuticals includes assessment of sequence integrity, charge variation, aggregation, glycan heterogeneity and post-translation modifications. The additional challenge of establishing comparability between an innovator and biosimilar product first requires an understanding of the variation inherent in the innovator product, and subsequent demonstration of analytical similarity with the candidate biosimilar. This seminar will illustrate how the Waters Biopharmaceutical Platform Solution with UNIFI can be applied to assess and monitor typical quality attributes of innovator and biosimilar biopharmaceuticals.

Free Vendor Seminar sponsored by SHIMADZU - Thursday, July 18 @ 12:00-1:30PM
Must register at the Shimadzu exhibit by Wednesday @ 3:45 PM

Turning Mountains into Molehills: The New Landscape of Protein Analysis
Turn your 24 hour tryptic digests into 1 minute digests. Turn your web browser into a world class bioinformatics platform. Turn your MegaDalton sized protein complexes into measurable ions. This workshop will provide your organization with the facts and critical lessons you need to know for the successful adoption and integration of ultra-fast protein digestions, ultra-fast mass spectrometry, powerful new cloud-based informatics in your laboratory and ultra-high mass detection. Please join us for this interactive workshop where you will discover new Perfinity integrated Digestion Platform (iDP) technologies that enable automated, reproducible protein digests on a sub-minute timescale, greatly improving the applicability of peptide based SRM assays. Cloud-based informatics solutions that provide a unique opportunity to improve not only laboratory data storage, but also enable on-cloud workflow execution and project sharing will also be presented. Finally, the additional challenge of analyzing high molecular weight protein complexes and aggregates with the AXIMA MegaTOF will be discussed. These tools will save your laboratory time, money and resources and will greatly improve data sharing with collaborators.

Free Vendor Seminar sponsored by KNAUER - Thursday, July 18 @ 12:00-1:30PM
Must register at the KNAUER exhibit by Wednesday @ 3:45 PM

Contichrom®: A Versatile Purification Platform for Batch/CaptureSMB/MCSGP Biochromatography
A liquid chromatographic process platform consisting of novel capture and polish processes will be presented. The novel process principles (CaptureSMB, MCSGP) offer significant benefits in discovery, process development and production and will be discussed in conjunction with the Contichrom® equipment platform being capable of performing all LC processes. Contichrom® lab is a preparative twin-column liquid chromatography platform, offering great flexibility for all process choices (batch, SMB, CaptureSMB, MCSGP, sequential flow-through) confined in a single equipment and control software. Contichrom® lab allows fast development of superior process resulting in substantial increase in processivity, yield and product purity whilst omitting large screening and process optimization efforts. Starting from a simple non-optimized batch process, the user-friendly Contichrom® lab equipment and control software allows to transform a batch process into an optimized MCSGP in a simple three step procedure.

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Lecture and Poster Abstracts

L = lecture P = poster

L-101 **Orthogonal Bioseparations.** Mark Schure, Kroungold Analytical, Blue Bell, PA, USA
As the sophistication and popularity of multidimensional liquid chromatography increases, it is desirable to quantify how good or bad the separation is performing with respect to the spatial distribution of peaks in the separation. Metrics for characterizing the spatial (and temporal) distribution of peaks have been advocated and studied. It is generally thought that the best separations "i.e. separations with the highest orthogonality" occupy the separation space with the minimum amount of peak overlap (i.e. to minimize peak clumping and incomplete peak resolution, a short range effect) and completely occupy the space around the separation hull (the "corners" of the separation). Uniform spacing of peaks suggests that the probability of overlap is minimized and the complete coverage of the separation space implies that there are no regions that are underutilized or chemically inaccessible. These concepts are applicable to 1D, 2D and n number of dimensions of chromatographic separation. This talk will focus on a more rigorous definition of orthogonality where both local effects of peak spacing and spatially-averaged properties are considered. It appears that separation scientists understand the concept of separation orthogonality but have not universally accepted any particular separation metric or definition. Current metrics and definitions neglect the importance of the peak distribution within the separation hull, relative to the hull size. In fact, both are important. Some rigor to a definition will be attempted by connecting metric properties to certain optimization criteria that consider both attributes. Some differences between bioseparations and "non-bio" separations will be discussed.

L-102 **New Advances in Hydrophilic Interaction Chromatography of Peptides: Comparison of HILIC/SALT to HILIC and RPC.** Colin Mant, Robert Hodges, Department of Biochemistry and Molecular Genetics, University of Colorado Denver, Aurora, CO, USA
Hydrophilic interaction chromatography (HILIC) represents a complementary, orthogonal method to reversed-phase chromatography (RPC), albeit with the latter technique still being the most widely used for peptide separations. Although HILIC offers a niche approach for the separation of very polar peptides unable to be retained and resolved by RPC packings, its potential would be better realized for peptide applications if it could be utilized for mixtures containing peptides with a wide range of varying properties (charge, structure, length, etc.), while exhibiting markedly different selectivities compared to RPC. With the relative maturity of HILIC as a separative method, coupled with the improving selections of HILIC packing materials, we believed revisiting the potential of HILIC as a generally useful peptide separation tool was overdue. Traditional HILIC of peptides involves a starting high acetonitrile concentration in the mobile phase (generally 80-90% acetonitrile), followed by a linear decreasing acetonitrile gradient to elute the peptides. An alternative approach to disrupting interactions with the hydrophilic stationary phase induced by a high background concentration of organic modifier is to effect peptide elution by a linear increasing salt gradient in the presence of a constant (isocratic) acetonitrile concentration (above 70%). We believed that such an approach would result in peptide elution and would be achieved in an analogous manner to that of traditional decreasing acetonitrile concentration (i.e., disruption of any hydrogen bonding between solute and enriched water layer; increased polarity of mobile phase relative to stationary phase), albeit with the potential of enhanced flexibility and effectiveness for resolution of peptide mixtures. Thus, this presentation describes a radical departure from the traditional HILIC elution approach, where separations are achieved via increasing salt (sodium perchlorate) gradients in the presence of high isocratic concentrations (> 80%) of acetonitrile, denoted HILIC/SALT. This initial study compares RPC, HILIC, mixed mode HILIC/CEX and HILIC/SALT for the separation of mixtures of synthetic peptide standards varying in structure (amphipathic α-helix, random coil), length (10-26 residues), number of positively charged residues (+1 to +11), hydrophilicity/hydrophobicity and peptides with the same composition and minimal sequence variation (SCMSV). Results generally showed a marked superiority of the HILIC/SALT approach compared to traditional HILIC and excellent complementarity to RPC for peptide separations. We believe these initial results offer a new dimension to HILIC, enabling it to transform from an occasional HPLC approach for peptide separations to a more generally applicable method.

L-103 **Highly Efficient LC-MS of Intact Proteins using Sub-0.5μm Particles with Slip Flow.** Zhen Wu[1], Bingchuan Wei[2], Ximo Zhang[1], Mary Wirth[1], [1]Purdue University, West Lafayette, IN, USA; [2]University of Washington, Seattle, WA, USA
Top-down proteomics has attracted increasing attention, due to its potential of characterizing the full protein sequence and post-translational modifications. Despite the rapid development in the mass spectrometry, top-down proteomics is hindered by chromatography---the separation of intact proteins. Here we report a mass spectrometry (MS) compatible nano reversed phase column with pulled tip, which is packed with 470nm silica nonporous particles. Model proteins: ribonuclease A, lysozyme, trypsin inhibitor and carbonic anhydrase were well separated by using nano-C4 reversed phase capillary in a 10 min gradient at room temperature, with a typical peak width (4σ) of 3 seconds. In addition, the soluble fraction of E. coli cell lysate was also analyzed by nano-reversed phase liquid chromatography (RPLC)-MS. The peak width (4σ) for a single protein is as small as 3s. Many factors contribute to the high efficiency of this column: i) the extremely uniform packing of colloidal

silica in capillaries reduces eddy diffusion; ii) nonporous small particles (470nm) reduce mass transfer resistance; iii) slip flow gives enhanced volume flow rates and a narrower distribution of fluid velocities. These results indicate that the capillary packed with sub-µm silica colloidal crystals with pulled tip is a good candidate for top-down proteomics.

L-104 **Capillary-channeled Polymer (C-CP) Fibers for High Throughput Analytical Separations and Desalting of Proteins Prior to MALDI and ESI-MS.** R. Kenneth Marcus[1], Abby Schadock-Hewitt[1], Benjamin T. Manard[1], Carolyn Q. Burdette[2], Marissa Pierson[1], [1]Clemson University, Clemson, SC, USA; [2]National Institute of Standards and Technology, Gaithersburg, MD, USA

Capillary-channeled polymer (C-CP) fiber stationary phases have demonstrated a number of very positive characteristics relevant to chromatographic separations of proteins as well as in solid phase extraction (SPE) applications. Fibers packed into microbore column structures provide very high permeability, while also promoting very efficient mass transfer to/from the fiber surface. The extruded polymer fibers have very low porosities (rp = ~2 nm) as determined by inverse size exclusion chromatography (iSEC) measurements. In practical terms, proteins/polypeptides having molecular weights of >10,000 Da experience virtually no van Deemter C-term broadening. As such, analytical-scale separations can be performed at linear velocities of up to 100 mm sec-1 without sacrifice of chromatographic efficiency. Melt-extrusion of C-CP fibers from simple thermopolymers (polypropylene, polyester, and nylon) present very low primary materials costs. Importantly, these base polymers present very different surface chemistries, both in terms of their native states as well as the acceptance of surface modifications. For example, nylon 6 is an excellent surface for weak anion/cation exchange separations. The surface is easily modified using simple triazine chemistries under ambient conditions. Polyester provides for a more hydrophobic surface having aromatic character, which can also be used for weak cation exchange. Finally, polypropylene provides for solely hydrophobic interactions between solutes and the fiber surface. This interaction allows for very robust affixing of capture ligands through adsorption. As such, chemistries can be affected by simply passing the ligands through the assembled column. We present here the application of C-CP fiber phases for high velocity, analytical scale protein separations and SPE isolation of proteins from complex media prior to MALDI and ESI-MS analysis. Fibers packed into 0.8 mm i.d. FEP or PEEK tubing can be implemented for discrete sample processing in a micropipette tip format (<10 µL bed volumes) or as a low-pressure, in-line microcolumn component of an HPLC injector valve. Solution processing of micropipette tips is affected by use of a benchtop centrifuge. In the simplest case, polypropylene fibers present a hydrophobic surface for protein capture, with elution in MS-friendly solvents. Protein SPE recoveries will be presented for matrices ranging from 150 mM PBS to 1 M NaCl, and mock saliva and urine, with detection based on UV-VIS absorbance, MALDI, and ESI-MS. Demonstrations of straightforward surface modifications will include a protein A phase for IgG capture, and use of a simple dye (cibacron blue) to affect an albumin depletion microcolumn.

L-105 **Increasing the Peak Capacity of Peptide Separations using Long Microcapillary Columns and Sub 2 µm Particles at 30,000+ psi.** Kaitlin Fague, Justin Godhino, Edward Franklin, Jordan Stobaugh, University of North Carolina, Chapel Hill, NC, USA

Peak capacity of one-dimensional separations for shotgun proteomics is routinely inadequate for the number of peptides in a sample. Advances in mass spectrometry (MS) and chromatography have improved the limits of detection and sensitivity problems associated with co-elution. Various multidimensional separation methodologies have been employed to improve peak capacity. Commonly, a separation of peptides through a microcapillary column is the final step before the sample is introduced to the MS. The pressure capabilities of the pump on the liquid chromatography (LC) instrument effect peak capacity by limiting the length of the column and dimensions of the sorbent. We propose a new system with a constant pressure, high temperature approach for peptide separations capable of 30,000+ psi. The system is comprised of a commercially available nanoAcquity LC instrument modified with a pneumatic pump through a configuration of tubing and valves. The ultrahigh pressure LC was coupled to a qTOF Premier for peptide analysis. To test the efficacy of the system, a complex peptide mixture was separated on a variety of microcapillary columns with sub 2 micron C18 BEH particles serving as the stationary phase. Preliminary results show while operating under the conventional UPLC conditions of 8,000 psi and 45ºC, the commercial system produced a peak capacity of 200 in two hours for a 25 cm x 75 µm ID column packed with 1.7 µm particles. On our modified system at 30,000 psi and 65ºC, the same peak capacity was achieved in only 60 minutes with a 21 cm x 75 µm ID column packed with 1 µm particles. With the same column, the observed peak capacity was nearly 300 in 90 minutes. By using a 100 cm x 75 µm ID column packed with 1.5 µm particles, peak capacity increased to 350 in 60 minutes and plateaued at 700 in 4 hours. For an E. coli proteome digest run under the same condition, the improved peak capacity resulted in a greater number of protein identifications and improved coverage.

L-106 **Application of a Robust and Modular Cartridge based NanoLC System for Proteomics.** Michael Bereman[1], Tom Corso[2], Colleen Van Pelt[2], Michael MacCoss[1], [1]University of Washington, Seattle, WA, USA; [2]CorSolutions, Ithaca, NY, USA

The characterization of a novel, vendor neutral, ultra-high pressure compatible (~10,000 psi) LC-MS source will be presented. This device makes automated connections with user-packed capillary traps, columns, and capillary emitters. The source uses plastic rectangular inserts where individual components (i.e., trap, column, or emitter) can be exchanged independent of one another in a plug and play manner. Automated robotic connections are made between the three cartridges using linear translation powered by stepper motors to axially compress each cartridge applying a well controlled constant compression force to each commercial LC fitting. The user has the versatility to tailor the separation (e.g., the length of the column, type of stationary phase, mode of separation) to the experimental design of interest in a cost-effective manner. The performance of the system will be presented using standard analytical figures of merit.

L-107 **Protein Markers for Cancer Stem Cells in Pancreatic Cancer.** David Lubman, Jianhui Zhu, University of Michigan, Ann Arbor, MI, USA

Sample heterogeneity in solid tumors represents a common problem in mass spectrometry (MS)-based analysis of tissue specimens. Combining immuno-laser capture microdissection (iLCM) and mass spectrometry (MS) provides a means to study proteins that are specific for pure cell subpopulations in complex tissues. CD24, as a cell surface marker for detecting pancreatic cancer stem cells (CSCs), is directly correlated with the development and metastasis of pancreatic cancer. Herein, we describe an in-depth proteomic profiling of frozen pancreatic CD24+ adenocarcinoma cells using iLCM and LC-MS/MS, compared with CD24- cells dissected from patient-matched normal adjacent tissues. About 40 nL tissue procured from each specimen, which corresponds to ~1.3 µg of protein material, was subjected to tandam MS analysis in triplicates. A total of 2665 proteins were identified, with 379 proteins in common that were significantly deregulated in CD24+ versus CD24- cells, with at least 2-fold change in expression. Ingenuity Pathway analysis (IPA) of these differentially expressed proteins further suggests significant involvement of signaling pathways related to cell proliferation, apoptosis resistance, and cell migration and invasion. This proteomic approach identified 27 known potential markers, including ANXA1, 14-3-3 Sigma, TGFBI, and POSTN. Five selected candidates were further evaluated in PDAC by immunohistochemistry analysis of tissue microarrays. Five interesting proteins, CD59, CD70, CD74, TGFBI, and TGM2, were further evaluated on tissue microarrays. CD74 was identified, for the first time, as highly upregulated in PDAC by using the proteomic approach, and immunohistochemical study with accumulating evidence indicates that it plays important roles in tumorigenesis and angiogenesis. These results not only demonstrate that this proteomic approach is a useful tool for marker discovery, but contribute to further understanding of pancreatic tumorigenesis and development of novel therapeutic targets to improve its treatment.

L-108 **Target-based Multiplex MRM Assays.** Christopher M. Colangelo, Lisa Chung, Shifman Mark, Abbott Thomas, Fumika Sakaue, Angus Nairn, Williams Kenneth, Yale University, New Haven, CT, USA

Targeted Proteomics was just recently selected as 2012 Nature Method of the Year and is recognized as the most sensitive and specific way to detect pre-selected components in a complex matrix such as a proteolytic digest of a plasma or tissue extract. In addition data-independent strategies have become more popular due to their potential to bridge the gap between discovery and targeted based experiments. Thus as more research laboratories implement these analysis on complex sample sets the need for accurate and reproducible HPLC/UPLC retention times is paramount. We have designed a comprehensive workflow for the development of large scale (>1500transtions/run) LC-MRM proteome assays that can be run in 90 minutes. These include improvements in assay development, data processing, and data analysis tools which greatly increase the speed and throughput of developing large scale targeted proteomic assays.

L-109 **Pretreatment of Human Fluid Samples for Trace Protein Analysis.** Frank Jahnke, Sonata Biosciences, Inc., Auburn, CA, USA

Human fluids – including plasma, serum, and cerebrospinal and pleural effusion fluids -- potentially are rich sources of protein biomarkers that can be used to indicate the presence or progression of disease or the response of disease to treatment regimens. Human fluids are enormously complex, with hundreds of thousands of different proteins, protein fragments and post-translational modifications that are present with concentrations that span ten orders of magnitude. Potential biomarkers are expected to be present at very low concentrations when compared with the abundant proteins, and the wide protein dynamic range far exceeds that of the analytical instrumentation used for the analysis, whether gel electrophoresis (such as DIGE) or bottom-up proteomic methods are used. Nine protein biomarker panels have been introduced to date, but discovery of new ones has slowed considerably. We believe that one reason is that pretreatment methods applied to human fluid samples are inadequate. We review the extensive work performed in the literature, and introduce new ones that

we are developing at Sonata Biosciences. A particular emphasis is placed on treatment of the large volumes that are required to delve deeply into the proteome to uncover dilute protein biomarkers.

P-W-110 **Epitope Mapping and the Selection of MAbs used in the Diagnostic Immunoassays.** Cheng Zhao, Bryan Tieman, Bailin Tu, Robert Ziemann, Jeffrey Fishpaugh, Carol Ramsay, Abbott Laboratories, Abbott Park, IL, USA

Epitope mapping involves the identification of the binding site of an antibody to its target protein. It is well known that identification of the epitope is a key step in the characterization of monoclonal antibody drugs required for novel antibody drugs regulatory approval by FDA and European regulatory agencies, and epitope mapping is also essential in the design of vaccines against toxins or enzymes and in the mapping of ligand binding sites in receptors using monoclonal antibodies. Then what is the role of epitope mapping in the diagnostic immunoassays? It is an important tool in the selection and characterization of antibodies used in capturing and detecting antigen biomarkers in the immunoassay, particularly where epitope similarity or dissimilarity issues are involved. Also, it is very important for intellectual property considerations of patentability, with freedom to operate consequences. X-ray co-crystallography is the golden standard method for epitope mapping. However, not every protein is easily crystallized and this technique requires large amounts of purified protein which is time-consuming and costly. More and more techniques have been developed for epitope mapping such as peptides scanning, mutagenesis, epitope mapping followed by LC/MS/MS, H/D exchange followed by high resolution MS, NMR, etc. Considering advantages and disadvantages of each method, epitope excision followed by LC/MS/MS is the epitope mapping method fitting our need and current instrumentation. The epitopes of nine MAbs with their target antigen were mapped using this method. The MAb was immobilized on the beads and the antigen was added to bind onto the MAb. After antigen binding, the enzyme was used for limited digestion of the antigen. The epitope peptide was still bound onto the MAb beads and the other peptides were washed off from the MAb beads by buffers. Finally, the epitope peptide was eluted under acidic condition and characterized by LC/MS/MS. The results demonstrated these nine MAbs belongs to different epitope groups and indicated these MAbs have different affinity to the antigen. These results help us to understand the MAbs structure and help the selection of MAbs used in the immunoassay.

P-W-111 **Aggregate Removal with Cation Exchange Chromatography (NuviaTM HR-S).** Paul K. Ng, Mark A. Snyder, Bio-Rad Laboratories, Hercules, CA, USA

Researchers in the field of chromatography have required ever more sophisticated and selective methods for removing aggregates from the monomer. Often the aggregates are at levels (1 to 10 %) that present challenges with regard to purity and clinical safety. One approach to solving such separation problem is the use of conventional chromatography resins that could be optimized for protein purification. High-capacity cation exchangers with open pore structure and high polymer strength have been available from Bio-Rad Laboratories for several years. They are most useful for direct capture or polishing of monoclonal antibodies. A recent approach we have observed with a newly improved cation exchanger (NuviaTM HR-S) allows the separation of aggregates from the monomer. From a composition of 91.1% monomer and 8.9% aggregates, we demonstrate that NuviaTM HR-S chromatography could deliver a final aggregate content of <0.3% and a monomer recovery of >80%.

P-W-112 **Optimized One-day Assay for Quantitation of Monosaccharide Content in Proteins by 2-Picoline-Borane Reductive Amination.** Nicholas Woon, Genentech, S San Francisco, CA, USA

Analysis of protein-bound sugars is typically performed by first liberating the sugar moieties through acid hydrolysis, then followed by a labeling reaction. The monosaccharides are labeled through reductive amination with a fluorophore, and thus detectable by a HPLC with a fluorescence detector. Typical monosaccharide analysis requires two days due to a dry-down step that follows acid hydrolysis, and uses toxic sodium cyanoborohydride as a reducing agent for the labeling reaction. Here, we propose to use a one-day wet assay, which incorporates 2-picoline-borane as the reducing agent as a non-toxic alternative to sodium cyanoborohydride. This alternative procedure employs green chemistry, reduces preparation time, and assures lowered toxicity. This poster will address wet assay development using Design of Experiment methodology. Development work will focus on the labeling reaction using monosaccharide standards, since hydrolysis conditions are unchanged. New UHPLC-MS separation technology will be utilized to characterize the resulting profile.

P-W-113 **Methodology of Monoclonal Antibody Charge-variant Analysis by Ion-exchange Chromatography.** Hillel Brandes, Roy Eksteen, Sigma Aldrich / Supelco, Bellefonte, PA, USA

In recent years monoclonal antibodies (mAbs) have become a dominant class of biochemical entities in mankind's quest to develop effective and better targeted biotherapeutics to halt the spread of cancer from its place of origin to neighboring organs. While the effectiveness of approved mAbs is generally not in question,

there is a need for better characterization of these high molecular weight entities as it is known that the active ingredient in individual production batches may vary slightly in composition due to truncation, oxidation, deamidation, phosphorylation, glycosylation or other post-translational modifications. The distribution of compositional variety of a mAb is referred to as charge variants or charge heterogeneity. Thus, bioanalytical chemists are challenged to improve existing or develop new techniques to better characterize mAbs. Such improvements ultimately will lead to changes in process design that will result in higher drug purity as it is traditionally understood by chemists. Resolution of charge variants by ion-exchange, is controlled by a variety of factors: the buffer system, pH, the mode of elution, the choice of the exchangeable ion in the case of salt elution, and of course the particle design and chemistry employed in the chromatographic separation. We report on the impact of these various parameters in achieving the best results in a reasonable time frame. The study includes columns from a variety of leading vendors.

P-W-114 **2D Separation and Immunoreactive Coverage of CHO and E.coli Host Cell Proteins (HCP) by Polyclonal Antibodies – Use of Fluorescent Labeling (Cyanine Dyes) in 2D Western Blotting.** Harbhajan Dhillon, Mark Abbott, Jonathan Basch, Mark Panek, Bristol-Myers Squibb, East Syracuse, NY, USA
Cyanine fluorescent dyes (CyDyes – GE Healthcare) are widely utilized in Differential in Gel electrophoresis (DiGE) methodology to quantitatively assess relative protein levels in two samples after two dimensional (2D) electrophoretic gel separation. This study details the utilization of CyDye minimal labeling technique to visualize total host cell proteins (HCPs), obtained from Chinese Hamster Ovary (CHO) cell cultures (Mock transfected cell line) and Escherichia coli (E.coli) cultures (Mock transfected cell line as well as from biologics downstream production process) on a blot membrane and subsequent evaluation of immunodetective coverage of these proteins by CyDye labeled antibodies on the same blot. The proteins in HCP sample (CHO or E. coli HCPs) are labeled with a fluorescent dye (e.g., Cy5), separated by 2D gel electrophoresis and then transferred to a low fluorescence PVDF membrane. The anti-HCP antibodies, labeled with a second dye (e.g., Cy2) are then used to determine the proteins that are recognized by the antibodies. To evaluate the immunoreactive coverage, the images are analyzed by DeCyder software to match the spots of the HCP proteins that have been detected by the antibody. This technique harnesses the superior sensitivity of the fluorescent dyes (over the conventional protein stains) and offers the advantage of comparing images that have been generated simultaneously from a single western blot membrane to maximize spot to spot alignment, thereby providing a more accurate assessment of antibody coverage. It also eliminates the steps of secondary antibody (e.g. HRP conjugated) incubation and substrate development.

P-W-115 **N-linked Glycan Profile Comparison Between the Innovator and a Biosimilar Etanercept.** Ying Qing Yu, Weibin Chen, Waters Corporation, Milford, MA, USA
Etanercept is a biotherapeutic protein used for the treatment of autoimmune diseases. It is a fusion protein comprising of tumour necrosis factor α (TNF-α) and the FC of immunoglobulin G1. Etanercept is heavily glycosylated, with 3-N-glycosylation and 13 O-glycosylation sites. The N-glycans are highly heterogeneous. Currently, many biotechnology companies are actively developing biosimilar Etanercept. The goal of these companies is to produce highly similar Etanercept to the innovator's protein, including the N-glycan profile. In this study, we compared the N-glycan profile between the reference protein and a biosimlar protein using HILIC-LC/FLR/QTOF MS analytical instrumentation operated using the UNIFI informatics platform. Each glycan identification was done using a combination of LC retention time database along with accurate mass measurement. The reference and the biosimilar Etanercept were reduced and alkylated followed by enzymatic digestion using PNGase F to release N-linked glycans. The free glycans were labeled with 2AB Fluorescent tag. A HILIC-LC/QTof Ms system was used to acquire both the FLR and MS chromatogram in. A retention time calibration standard, 2AB-labeled dextran ladder, was used to generate a retention time calibration curve; such curve was automatically applied to the N-glycans released from Etanercept; each glycan chromatogram peak was assigned with a retention time number measured in Glucose Unit (GU). The GU value and m/z information from each glycan component form the HILIC-LC/FLR/QTOF MS chromatograms were searched inside Glycobase DB library for identification purpose. The 2AB-labeled N-glycans profile from a biosimilar Etanercept closely resembles that from the reference protein. A total of 25 glycan peaks were observed from the reference protein, relative concentration ranged from 23% to 0.1%. Same glycans were also observed from the biosimilar protein with a slight shift in relative concentration. The identification of these glycans were confirmed using a combination of peak retention time value (in GU) and m/z value that were searched against the Glycobase DB. In summary, neutral, singly sialylated and doubly sialylated glycans are observed from the reference protein, the structure of these glycans range from Mannose 5, complex type (bi- and triantanary, some of them contains the core fucose). The N-glycan profile from the biosimilar Etanercept showed a total of 14 additional peaks with very low relative intensity, < 0.2%. This could cause concerns since "undesirable" glycans could impact the drug's safety and efficacy. We interrogate these new peaks using Glycobase DB as a tool. For example, high mannose glycans, Man 6, Man 7 and Man8 were among these glycan peaks that are unique to the biosimilar protein. The

confirmation again was made by using the information from searching the GU value and accurate mass using Glycobase DB. For some biosimilar unique glycans, there are multiple possible structures returned from Glycobase DB search, because their GU and m/z values are within error tolerance. To address this issue, we applied MS/MS fragmentation and or use exoglycosidase digestion to get more confident identification.

P-W-116 **Charge Surface Modified C18 Columns for Increasing Peak Capacity in LC-MS Peptide Separations with Formic Acid Mobile Phases.** Matthew Lauber, Stephan Koza, Kenneth Fountain, Waters Corporation, Milford, MA, USA

Reversed-phase (RP) chromatography has become the separation mode of choice for peptide separations, as it offers high resolving power and can easily be paired with mass spectrometry. Peak capacity, or the maximum number of peaks that can fit into an LC-based time frame, is a measurable performance metric most relevant to gradient RP chromatography. Interestingly, chemical modification of a stationary phase can have significant impact on peak capacity. Here, we demonstrate the use of a C18 column modified with a low level, positive surface charge for peptide separations. The addition of this positive surface charge eliminates the requirement of using MS signal suppressing, ion-pairing additives, such as TFA, to achieve high peak capacity separations. A mixture of peptides, varying in both mass and polarity, was employed to benchmark the separation performance of charge surface modified C18 columns against other columns commonly used for peptide mapping. It was found that, unlike conventional C18 columns, a C18 column modified with a low level positive surface charge provides excellent peak shape with either TFA or FA-containing mobile phases. With 0.1% TFA, the charged surface modified column provided 20% greater peak capacity than either an unmodified, fully porous C18 column or a superficially porous C18 column of equal particle size. However, with 0.1% FA, the charged surface C18 column provided 90% greater peak capacity than the other columns tested. These data indicate that the performance of charge surface modified C18 columns is far less dependent on TFA, which significantly suppresses electrospray ionization. Accordingly, these columns are well-suited for LC-MS applications in which high sensitivity is desired. The charge surface modified C18 column was thus used to develop a high resolution peptide map of a Lys-C proteolytic digest of the therapeutic antibody, trastuzumab. Characterization of trastuzumab, including its disulfide bonds and deamidation at Asn55, was facilitated through the use of the charged surface column. High peak capacity separations were obtained at the mass loads needed to detect low abundance modifications, while using a mobile phase (0.02% TFA/0.08% FA) that provides high MS sensitivity. With these conditions, ESI-MS signal obtained with a QTOF mass spectrometer matched or exceeded the signal from an in-series UV detector. The advantage of applying this column technology is that the same chromatographic conditions are equally suited to both UV-based quality control analyses and MS-based characterization studies.

P-W-117 **A Quality by Design Approach: Systematic Optimization of Malaria Vaccine Purification with IMAC.** Jessica Paul, Arthur Dukart, Jasmin Zuehlke, Gesine Cornelissen, Hamburg University of Applied Sciences, Hamburg, GERMANY

In 2000 the implementation of Quality by Design was introduced by the Food and Drug Administration and described in ICH Q8 guideline. Since that time, systematic optimization strategies for purification of biopharmaceuticals gain a more important role in industrial process development. This contribution describes an optimization strategy for protein purification by immobilized metal ion affinity chromatography (IMAC). A systematic approach of the applied strategy intends to provide enhanced process understanding and thus a safe, efficient and cost-effective method development with regard to product quality. The target protein used for optimization procedure is a malaria vaccine candidate. The strategy is based on stability studies of the protein with Design of Experiments (DoE). Results were used to define an adequate range of operation for further chromatographic separation via IMAC. Batch adsorption studies in µ-scale allowed a pre-selection of column material. The DoE-based workflow for optimization started with scouting of potentially Critical Process Parameters (CPPs) and continued with a fractional factorial design screening to extract the significant impact factors. This was followed by an optimization using a central composite circumscribed design in order to determine the optimal factor settings. To evaluate appropriate models, the quality criteria maximum values of product purity and product recovery were used. An optimal design space dimensioning for a safe chromatographic separation was validated with robustness testing via DoE. Frontal analysis results potentially allow an easy up-scale for this method with optimal parameters. Stability studies facilitated the definition of an optimal workspace for optimization on the basis of the identified Critical Quality Attributes (CQAs) of the target protein. The choice of column material in µ-scale and the optimization of factor settings in milli-scale significantly minimized the costs for method development. Increased knowledge of binding kinetics allow a simplified up-scale. The applied optimization strategy enables the reduction of product losses during purification whereby product purity and product recovery will be maximized.

P-W-118 **Automating Workflows for Developing Separation and Reporting Methods for Size Exclusion Chromatography.** Thomas E. Wheat, Aparna Chavali, Patricia McConville, Waters Corporation, Milford, MA, USA

Size Exclusion Chromatography is the most common analytical tool for measuring the size of proteins in solution. This routine characterization is typically expressed in terms of molecular weights of the sample proteins by reference to a set of known and relevant proteins. It has proven particularly valuable in the development and control of biopharmaceuticals, particularly the measurement of aggregation. Many assumptions, however, are associated with the use of this technique. For example, interpretation of the results assumes that the protein has a single, consistent size in solution, and there is no interaction between the protein and the column. The distribution of packing pore size and volume must be appropriate for the Stoke's Radius of the proteins so that the elution volume is proportional to the size. And finally, both size and amount information can be extracted from the data. Each of these assumptions requires validation for a particular sample because numerous exceptions have been observed. Buffer pH and ionic strength can change both the three-dimensional shape of the protein and its interaction with the surface of the packing material. We have developed an automated set of procedures that incorporate efficient tools for developing and testing SEC analytical methods. The system combines separation chemistry, instrument operation, and data reduction software. Three different column types are available with different pore size and volumes to provide overlapping analytical ranges. The chromatographic system blends mobile phase buffers over a range of pH and salt concentrations to detect changes in protein dimensions and in surface interactions. The informatics tools can provide both the areas of the chromatographic peaks and their molecular weights in a combined report. These elements are combined in an automated protocol for screening of a sample or set of samples. The synergies among these elements of the protocol ensure a more complete analytical investigation of the properties of the sample. In this way, effects on protein structure can be better understood, and the development of biopharmaceuticals can be better controlled.

P-W-119 **Enthalpic and Entropic Contributions in Lysozyme Adsorption onto a Cation-exchange Support.** Francisco Marques[1], Goncalo Silva[1], Marvin Thrash, Jr.[2], Cristina Dias-Cabral[1], [1]CICS-UBI- Health Sciences Research Centre, University of Beira Interior, Covilha, PORTUGAL; [2]Department of Water Resources and Environmental Engineering, College of Science and Engineering, Central State University, Wilberforce, OH, USA

The greatest challenge of any chromatographic technique is predicting the adsorptive behavior of the biomolecules onto the chromatography resin. This investigation attempts to examine the complexity of lysozyme adsorption on a cation-exchange adsorbent and the role of nonspecific effects in the establishment of the adsorptive process. Flow microcalorimetry (FMC) and adsorption isotherms measurements were used to illustrate lysozyme adsorption mechanism on carboxymethyl cellulose (CMC) at both absence and presence of salt (NaCl 50mM) at a selected pH (pH 5). FMC results show that under all the studied conditions the adsorptive process is, as expected in ion exchange, enthalpically driven. FMC thermograms show three consecutive peaks, a first exothermic peak followed by other two peaks an endothermic and exothermic, evidencing nonspecific effects. Moreover, it was observed a direct correlation between microcalorimetry data and isotherm measurements. It is shown that just before the overloaded range of lysozyme surface concentration is reached ΔHads exhibits a sharp minimum. When overloaded conditions are reached, there are high repulsive forces between like charge molecules and ΔHads increases. This can also be seen on the isotherms that hit a plateau under these conditions. Also, both FMC and isotherm results show a stronger interaction between lysozyme and CMC in the absence of salt. - Work supported by FCT (Portuguese Foundation for Science and Technology), project number FCOMP-01-0124-FEDER-014750 (Ref. FCT PTDC/EBB-BIO/113576/2009) and NSF (American National Science Foundation) NSF – 1246932 (award issued by CBET division of NSF).

P-W-120 **Malaria Vaccine Purification via Expanded Bed Adsorption Chromatography Combined with an Ultrafiltration.** Sarah Schreiber, Sven Oliver Borchert, Jessica Paul, Gesine Cornelissen, University of Applied Sciences, Hamburg, GERMANY

The Research and Transfer Center for Bioprocess- Engineering and Analytical Techniques at Hamburg University of Applied Sciences provides a state of the art infrastructure for the development, investigation and optimization of biotechnological upstream and downstream processes. In this contribution an Integrated Bioprocess for production of a potential malaria vaccine has been set up. The whole process consists of the upstream in the bioreactor and subsequent protein purification steps, is fully automated. An Expanded Bed Adsorption chromatography is used as primary purification step. This method offers advantages over conventional downstream processes, such as centrifugation or filtration, because it enables cell release, concentration and protein capture by Immobilized Metal Affinity Chromatography in one step. On contrast to adsorption in fixed-bed the fluidized bed adsorption requires the application of cell containing medium, which would block a fixed bed. Afterwards an ultrafiltration step with diafiltration for buffer exchange is unavoidable to concentrate and to get the required conditions for a consecutively chromatographic step with multimodal resins.

Focus of the presented work is the optimization of the purification section via Design of Experiments to ensure a good product quality and to enhance the product recovery. Therefore the concentration of Imidazole and the pH value, which have a main influence on the target binding, were adapted. For the ultrafiltration step the process parameters transmembrane pressure and cross flow rate were optimized. Design of Experiments has emphasized itself as an applicable tool for optimization so that the optimization strategy can be easily transferred to other target proteins.

P-W-121 **Continuous-batch Protein Chromatography – Continuous Capture Coupled with Two-step Automated Batch Polish.** Peter Tiainen, Jais Rose Bjelke, Ditte Skibstrup, Haleh Ahmadian, Novo Nordisk A/S, Malov, DENMARK

This poster demonstrates a case where continuous capture in combination with an automated multi-step purification setup (two polishing steps) was applied using a conventional chromatographic system (ÄKTAexplorer). (1.) Continuous processes capturing the target protein, almost immediately after having been produced, combined with automated multi-step purification, lowers hold times and manual handling maneuvers reducing significantly the risk of protein degradation. (2.) Automation of multi-step purifications will reduce the need for operator attendance, increasing the purification throughput and the total process cost-effectiveness. It is here shown that it is possible to obtain both these features on a well-known chromatographic system, a machine found in almost every purification lab, with only limited investments. The presented setup has two alternating capture columns. While one column is being eluted, cleaned and equilibrated, the other column is loaded. The elution from the interchanging capture columns is then forwarded to the second column. Subsequently, the elution from the second column is directed to a final third column. The ability to monitor e.g. conductivity allows for adjustment of process parameters allowing for fine-tuning the protein quality produced. We have shown the utility of the method for a three step purification of a non-antibody protein of about 50 KD. Good process yields as well as consistent protein quality were obtained.

P-W-122 **Bioseparations with 3.5- and 5-Micron Wide-Pore Superficially Porous Particles.** Joseph DeStefano, Joseph Kirkland, Stephanie Schuster, Bill Johnson, Advanced Materials Technology, Inc., Wilmington, DE, USA

Columns packed with modern superficially porous (core-shell) particles (SPP) exhibit extraordinarily high performance for small-molecule separations compared to columns packed with totally porous particles of the same size. Many published reports by independent researchers verify that 2.5 – 2.7 micron SPP particles with small pores (ca. 70 – 90 Å) can produce columns with the efficiency of sub-2-micron particle columns with nearly half the back pressure of the smaller particles. The success of the SPP particles with small molecules has generated interest in the development of SPP particles with larger pore sizes for the separation of bigger molecules. The short diffusion paths provided by the thin porous shells on SPP particles are more advantageous for the separation of high molecular weight molecules that diffuse slowly compared to smaller molecules. SPP particles are now available in a variety of pore sizes from 120 Å to 400 Å for biomolecule separations. Recent introductions of nominally 3.5-micron and 5-micron larger pore SPP particles have increased the range of utility for the superficially porous particle technology. Columns of these larger-particle wide-pore SPP are demonstrated in this poster to exhibit significant performance advantages over totally porous particles of similar and smaller particle size. This report also provides examples of 3.5 and 5-micron SPP columns with different bonded phases to further demonstrate the performance and advantages for larger particle wide-pore SPP.

P-W-123 **A Novel Automated Enrichment Process for the Isolation of Product-related Impurities from Active Pharmaceutical Ingredients.** Thomas Muller-Spath, Nicole Ulmer, Lars Aumann, Guido Strohlein, Michael Bavand, ChromaCon AG, Zurich, SWITZERLAND

Isolation of product-related impurities in useful quantities for pre-clinical analysis is often tedious and requires sophisticated isolation strategies. A cyclical, continuous, twin-column chromatography process (N-richTM technology) for the isolation of product-related impurities was developed and shown to be highly efficient in isolating individual impurities in useful quantities at preparative scale. This process features simultaneous enrichment of product-related impurities and concurrent depletion of the main product, therefore allowing the isolation of highly pure and concentrated product-related impurities. The process was experimentally verified for the isolation of closely eluting strongly and weakly adsorbing product-related impurities of synthesized Fibrinopeptide A using reversed phase chromatography with a twin column Contichrom® skid. The performance of the cyclical continuous process was qualitatively compared to the performance of single column batch chromatography. This novel process principle can be operated with any kind of mode or media that is used in single column batch chromatography. Its potential lies in application fields such as lead isolation in drug discovery, product-related impurity isolations in drug development (impurity profiling), and the isolation of highly pure active pharmaceutical ingredients (API), such as synthetic peptides.

P-W-124 **Peptide Mapping of a Therapeutic Monoclonal Antibody (mAb): Optimizations for Increasing Speed and Peptide Identifications.** James Martosella[1], Ning Tang[2], Alex Zhu[1], [1]Agilent Technologies, Wilmington, DE, USA; [2]Agilent Technologies, Santa Clara, CA, USA
Peptide mapping is the common analytical technique for characterizing biologic therapeutic proteins. The protein is first digested into smaller peptides enzymatically using one or two proteases and the peptides separated by reversed-phase chromatography with UV or mass spectrometry detection. Mass spectrometry provides the masses of the peptides, which greatly enhances the information content for peptide mapping. With the additional dimension of separation by mass, co-eluting peptides can be easily identified and thus the gradient length greatly reduced to allow quicker analysis times. In addition, tandem mass spectrometry can fragment the peptides into smaller pieces and provide evidence for the amino acid sequence of the peptide and the post-translational modifications (PTM) such as glycosylation, phosphorylation, oxidation and deamidation. In this work, peptide mapping of an IgG(1) therapeutic monoclonal antibody (mAb) was performed using a novel superficially porous peptide mapping column coupled to an Agilent 6550 iFunnel Q-TOF. The unique resolving power of the peptide column to maximize resolution and efficiency in combination with the MS/MS analysis ensured confident and sensitive identification of peptides and resulted in 99% sequence coverage during a rapid runtime of 15 minutes. The iFunnel Q-TOF delivered excellent mass accuracy and sensitivity for peptide identification and the MS/MS capability helped to confirm modified peptide ID's with high confidence. Additionally, optimization of the IgG(1) peptide gradients and LC/MS/MS parameters will be explored for achieving robust, rapid and reliable mAb peptide maps.

P-W-125 **Countercurrent Tangential Chromatography for Purification of Monoclonal Antibodies.** Oleg Shinkazh, Chromatan, State College, PA, USA
Countercurrent Tangential Chromatography for Purification of Monoclonal Antibodies Chromatan's CTC is a new column-free purification technology for MABS, providing a scalable, single-use, and continuous alternative to column capture chromatography. Experimental studies were performed purifying monoclonal IgGs. We showed high-resolution antibody purification at < 20 psi operating pressure, with recovery >94%, showing great promise for this new technology.

P-W-126 **Amphoteric Ion-Exchange Separation of Biomolecules with Porous or Non-Porous Polymer-Based Resins.** Ken Tseng[1], Toshi Ono[1], Tsunehisa Hirose[2], Kazuhiro Kimata[2], [1]Nacalai USA Inc., San Diego, CA, USA; [2]Nacalai Tesque, Kyoto, JAPAN
An amphoteric ion-exchange column is applied for the biomolecule separation such as proteins and nucleotides. This hydrophilic polymer-based column has a mixed-mode anion-cation-exchange capability for the separation of both acidic and basic proteins in the same run. Any typical anion- or cation-exchange salt gradient can be used on this amphoteric ion-exchange column. The retention time shifts slightly earlier due to the electrostatic repulsion of the same charge on the stationary phase to the sample. For example, bovine serum albumin (BSA) an acidic protein was analyzed by the anion-exchange and amphoteric ion-exchange columns of the 4.6x50mm dimension, 5um particle size, and 1,000A pore size. 20 mmol/L Tris buffer at pH 8.3 with a 30-minute linear gradient to 0.5mol/L NaCl are used as the mobile phase. In the anion-exchange separation, BSA eluted at 22.5 minute. In the amphoteric ion-exchange separation, the BSA eluted at 20.5 minute. In addition to the earlier elution due to the electrostatic repulsion, the peak resolution also improved from the benefit of the electrostatic focusing. Another example of amphoteric ion-exchange separation was demonstrated with ribonuclease A (basic), cytochrome c (basic), trypsin inhibitor (acidic) and beta-galactosidase (acidic) in one chromatogram. The same chromatographic improvement in less retention time and higher resolution were observed. Further, the non-porous resins were compared with the porous resins of 1,000A pore size. As expected, the non-porous particles shorten the analysis time considerably with the added benefits of better resolution and more sensitivity. An example is shown with ribonuclease A, cytochrome c, and lysozyme in the cation exchange mode. Instead of a 30-minute gradient with a porous particle column, a better separation was achieved in a 10-minute gradient with the non-porous particle column. The sample loading capability of the amphoteric ion-exchange column was determined to be 55-75 mg/mL-resin BSA and 35-50 mg/mL-resin IgG for the porous particles. For the non-porous, it was 6-8 mg/mL-resin BSA and 5-9 mg/mL-resin IgG. Other than proteins, the amphoteric ion-exchange column was successfully applied for nucleotide separations.

P-W-127 **Isomeric Separation of Procainamide Labeled N-glycans by Using Novel Superficially Porous Particle HILIC Column.** Shujuan Tao[1], Yining Huang[1], Barry Boyes[2], Ron Orlando[1], [1]CCRC, University of Georgia, Athens, GA, USA; [2]AMT, Wilmington, DE, USA
The study of N-linked glycans is among the most challenging analytical tasks due to their complexity and variety. Hydrophilic-interaction chromatography (HILIC) is a common chromatography method for fluorescently labeled glycans. Moreover, HILIC has good compatibility with MS due to the high organic content of the mobile phase.

We have evaluated the ability of a novel superficially porous particle (Fused-core) Penta-HILIC column to chromatographically resolve N-linked glycans. Procainamide labeled dextran ladder and N-glycans from well-characterized glycoproteins (Fetuin, Asialofetuin, Ribonuclease B, Immunoglobulin G, Bovine α1-acid glycoprotein, etc.) were used to prove the separation ability of the Penta-HILIC columns. With optimized gradient and column temperature, we have been able to obtain baseline separation of structural isomers (such as the "Man7" from Ribonuclease B, linkage isomers (Galβ 1-3/1-4 of triantennary N-glycans from Asialofetuin; NeuAcα 2-3/2-6 of bi/triantennary N-glycans from Fetuin), as well as other structures with minor difference (NeuAc/NeuGc of N-glycans from Bovine α1-acid glycoprotein). The exoglycosidase studies with LC fractions of Asialofetuin triantennary N-glycans confirmed that the structures were Galβ 1-3 and Galβ 1-4 isomers as predicted. Future studies will be carried out on the separation of complex glycomic samples by using this new Fused-core HILIC column, to demonstrate its utility in the analysis of various glycan mixtures. Sequential exoglycosidase digestions will be applied to facilitate the characterization of unknown glycans.

P-W-128 **GeLC Separations: Which Dimension Plays the Most Important Role in Protein Identification Efficiency?** Darryl Johnson[1], Barry Boyes[2], Ron Orlando[1], [1]CCRC, University of Georgia, Athens, GA, USA; [2]AMT, Wilmington, DE, USA

The high degree of complexity displayed in biological systems presents a challenge for mass spectrometry based proteomic analysis, leading to the use of 2-dimensional separation techniques such as gel electrophoresis and reversed phase LC (GeLC). As separation efficiency in either dimension increases, the amount of time required for mass spectrometry analysis also increases. The aim of this work is to evaluate GeLC separations, to determine how to maximize protein identifications when mass spectrometer instrument time is kept constant. Does the highest protein identification efficiency occur with high numbers of first dimension fractions and fast LC-MS/MS analysis, few first dimension fractions and long LC-MS/MS analysis or with moderate separation efficiency in both dimensions? Soluble proteins from canine prostate tissue were subjected to SDS gel electrophoresis, and gels were cut into 3, 5, 9 or 18 fractions. In-gel digestion was preformed, with each GeLC condition analyzed using 43 hours of mass spectrometer instrument time. Data was acquired using an Agilent 1100 Capillary LC system online via a Michrom captive spray interfaced with a Thermo-Fisher LTQ mass spectrometer. Halo columns of various lengths packed with 2.7 µm diameter superficially porous particles were employed in the peptide analysis. Resulting data files were searched via Mascot, with results analyzed in ProteoIQ, where a 5% protein false discovery rate and a 0.9 peptide probability were applied to confirm proteomic identifications. We have analyzed 4 conditions; 18 gel fractions with a 21 minute LC-MS/MS analysis for each fraction, 3 gel fractions with a 140 minute LC-MS/MS analysis for each fraction, 5 gel fractions with an 83 minute LC-MS/MS analysis for each fraction and 9 gel fractions with a 45 minute LC-MS/MS analysis for each fraction. We initially thought that the highest number of protein identifications would correlate with the GeLC condition displaying the highest peak capacity, however this was not the case. Our results show the percentage of instrument dedicated to gradient elution conditions has the most significant impact on proteomic identifications. The highest numbers of protein identifications were produced with the 5 gel fraction/83 minute LC-MS/MS conditions, when the chromatographic reequilibration times constituted the lowest percentage of total experiment time. These results also show that more efficient use of instrument time can help offset the reduction in peak capacity when LC gradient time was reduced.

P-W-129 **Microfluidic CE-MS Applied to Protein, Peptide, and Small Molecule Characterization.** Gregory Roman[1], Scott Mellors[2], Martin Gilar[1], James Murphy[1], J. Michael Ramsey[2], [1]Waters Corporation, Milford, MA, USA; [2]University of North Carolina, Chapel Hill, NC, USA

We demonstrate the successful separation and identification of several intact glycoproteins, peptide degestions, small molecule basic metabolites, basic drugs, and the kinetic analysis of dynamic peptide and protein reactions using a CE-ESI microfluidic device coupled to a mass spectrometer. The CE-ESI microfluidic device offers a rapid method of injecting nL volume samples into a CE separation channel. Following separation the intact proteins are sprayed and ionized into the mass spectrometer. Since all of the necessary microfluidic components are micromachined on a planar substrate, limited dead volume enables high efficiency separations in under 2 min on a 23 cm length separation channel. In addition, integrated spray tips provide a seamless method of transporting sample from the CE separation to electrospray with limited dispersion and low dilution. Glycoprotein alpha acid, and ribonuclease A and B, have been analyzed using the microfluidic device. Mannose glycoforms have been identified by their accurate mass. We have also begun to investigate application of such devices to reaction monitoring for kinetic analysis of protein and peptide reactions.

L-130 **IgG Asparagine-linked Oligosaccharide Profiling by High-performance Anion-exchange Chromatography with Pulsed Amperometric Detection.** Jeffrey Rohrer[1], Deanna Hurum[2], Lipika Basumallick[1], [1]Thermo Fisher Scientific, Sunnyvale, CA, USA; [2]Palo Alto, CA, USA
Antibodies are among the largest class of glycoproteins studied as potential therapeutic proteins with over 30 monoclonal antibodies (mAbs) approved by the U.S. FDA. Development of mAbs, antibody-drug conjugates, and multi-functional antibodies is an active area of research. Understanding and characterizing the glycosylation of these products as a potential critical quality attribute is of importance with evidence that glycosylation may impact biological efficacy, pharmacokinetics, and cellular toxicity. Protein expression systems and reactor conditions can impact glycosylation, leading to potentially undesirable glycosylation. For example, high-mannose species may be present, which are not typical of human antibody glycosylation. The presence of these species in the Fc domain has been linked to increased serum clearance of IgG antibodies. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is an effective tool for analyzing glycans present in glycoprotein therapeutics. In this work, a method for analyzing the asparagine-linked -glycans commonly found on antibodies is described. Initial identification and mapping is performed by comparison to known glycan standards in conjunction with selective exoglycosidase digestion of both standards and released glycans to confirm identity. Both neutral and charged glycans are identified in a single injection. The neutral glycans are well resolved, including separation of high-mannose species from typical human IgG-type glycans, including the resolution of the high-mannose oligosaccharide containing five mannose residues (Man5) from the complex biantennary oligosaccharide containing no galactose residues (G0). The HPAE-PAD retentions of known glycans are indexed to the retention of maltose and a series of maltodextrin standards to define an index value characteristic of the glycan. Method ruggedness was evaluated across systems, analysts, and column lots with retention time precision RSDs between 0.63 and 4.0% and retention indices precision RSDs between 0.27 and 1.1%. These retention indices were used to aid in identification of glycans released from example monoclonal antibody samples of unknown glycosylation.

L-131 **Size-exclusion Chromatography using Multi-angle Light Scattering (SEC-MALS) for the Characterization of Polypeptide Mixtures.** Joseph Glajch, Ying Li, Momenta Pharmaceuticals, Cambridge, MA, USA
Size exclusion chromatography (SEC) is commonly used as a technique to determine the molecular weight of peptides and proteins and related materials such as aggregates and adducts. Typically the determination of molecular weight is performed using a mixture of proteins and/or peptides standards, to create a calibration curve against which the molecular weight of the material to be determined can be assessed. However, this assumes that the standards are similar in size and structure to the peptide or protein being measured, and is also typically performed on relatively monodispersed materials with a narrow distribution of molecular weight and size. Glatiramer Acetate is a polymeric, polypeptide mixture of four amino acids which is chemically synthesized using a combination of polymerization and depolymerization reactions. This results in a complex mixture with numerous individual polypeptide chains of different sizes, lengths, and compositions in a typical reaction. While SEC can be used to characterize the distribution, an accurate description of various molecular weight parameters, such as Mw and Mn, is not possible using normal standard calibration techniques and can lead to inaccurate descriptions of the molecular weight distribution. This work describes the use of multi-angle light scattering detection (MALS) in combination with an optimized SEC separation to more adequately characterize this complex mixture. A comparison to more standard techniques will be provided to demonstrate the advantage of SEC-MALS for these measurements.

L-132 **Developing an Automated Workflow for Disulfide Linkages Analysis of Biotherapeutics by High-Resolution LCMS.** Asish Chakraborty[1], Stephane Houel[1], Henry Shion[1], Scott Berger[1], Weibin Chen[1], Anurag Rathore[2], [1]Waters Corporation, Milford, MA, USA; [2]Indian Institute of Technology, New Delhi, Delhi, INDIA
Disulfide bond formation plays a critical role in stabilizing the three-dimensional folded structure and maintaining the proper biological activity of therapeutic proteins. Disulfide scrambling can occur either during the production of therapeutic proteins, or during downstream processing steps, or within the purified biotherapeutic samples exposed to environmental stress and can contribute to protein misfolding and aggregation. Comprehensive characterization is required to understand the effects of disulfide bond related structural variants on the stability, structure and biological functions of biotherapeutics. Thus, it is crucial to confirm the presence of expected disulfide linkages and to prove the absence of unexpected/scrambled disulfides in therapeutic proteins to ensure drug quality and to satisfy regulatory authorities. Here, we present an automated workflow for qualitative and quantitative analysis of expected and scrambled disulfide linkages in therapeutic proteins using high-resolution LCMS. The method used in this study successfully identified and quantified the expected and scrambled disulfides with linkage sites in recombinant human granulocyte-colony stimulating factor (rhGCSF). The presence of expected and scrambled disulfide linkages were initially detected by comparing the reduced and non-reduced peptide maps using a targeted informatics tool. Three new peaks were detected in the non-reduced

GCSF map (TIC). The first two peaks eluted between the retention time of 30 and 32 minutes contain intra-disulfide linked peptides related to position C37-C43. The peaks between retention times 68-69 minutes correspond to a homodimer related to C18 peptide formed by intermolecular disulfide bond and retention time between 98-99 minutes contains another intra-disulfide linked peptide related to position C65-C75. The identification of disulfide linked and free Sulfhydryl-containing peptides were achieved by accurate precursor ion masses and confirmed by the presence of peptide fragment ions. The relative quantity/percent of scrambled disulfides compared with their correct linkage or free forms was obtained from the deconvoluted/deisotoped mass area value from the processed maps. The (integrated) LC-TOF-MS workflow along with informatics tool presented here facilitates comprehensive disulfide bond characterization of biotherapeutics and can be used/deployed at different drug development stages or biosimilar comparison.

L-133 **Analytical HIC for mAb Aggregate Analysis; How Does the Salt Ion Type Influence the Selectivity?** Hannah Brueck, Judith Vajda, Werner Conze, Egbert Mueller, Tosoh Bioscience GmbH, Stuttgart, GERMANY

The growing demand for therapeutic and diagnostic monoclonal antibodies (mAb) has consequently led to an increased interest in analytical methods for the characterization of these molecules, especially with regards to the formation of aggregates. Evaluating the stability of a potential drug candidate during early stages of its development has gained importance, although only small amounts of the protein are available at that time. Using analytical hydrophobic interaction chromatography (HIC) is one possible approach. In our study we take advantage of 2.5 µm nonporous particles and the excellent sensitivity of fluorescence detection, providing an outstanding signal to noise ratio. This allows us to investigate the impact of different salts on the selectivity and resolution of different mAbs and mAb aggregates, while only very little sample amounts are required. In an earlier work, we have already reported improved retention behavior for sodium chloride and a mAb. Following up to this, the presented results focus on the influence of the single salt cations and anions on the selectivity of the stationary phase for various different mAbs. In order to verify the obtained results, several standard proteins have been employed.

L-134 **Discovery and Development of Universal Fc Binders for Antibody Purification.** Marc Arnold, Holger Bittermann, Thomas Neumann, Graffinity Pharmaceuticals GmbH, Heidelberg, GERMANY

Therapeutic antibodies are mainly purified by established platform processes including a Protein A affinity capture step. Although this protein ligand is accepted as the current gold standard for antibody purification it has some severe limitations like high costs, low chemical stability and leaching. Consequently, there is a demand for alternative chromatography material for the purification of mAbs. Through a unique SPR screening platform we have identified and developed small molecule affinity ligands for cost efficient affinity purification of antibodies. For the identification of the binders, several humanized therapeutic antibodies were screened against a unique and diverse library of > 116k compounds immobilized on gold chips via linkers. A tissue culture supernatant from CHO cells served as a control to identify unspecific binders. Observed antibody specific hits were then coupled to NHS-Sepharose 4 FF for chromatographic evaluation. Generic binding to the Fc region of IgG1, IgG2 and IgG4 antibodies could be demonstrated. Best ligands were further characterized by high selectivity, high capacity and fast binding kinetics comparable to Protein A. Due to the extraordinary high chemical stability and low cost of the developed ligands they might represent an attractive Protein A replacement.

L-135 **Development of a Sialic Acid-specific Affinity Chromatography for the Purification and Separation of Glycoprotein Isoforms.** Matthias Meininger[1], Francisco Vito Santos da Silva[1], Samanta Cajic[1], René Hennig[1], Erdmann Rapp[1], Frank Zwanziger[2], Stefan Laufer[2], Karl-Heinz Wiesmueller[3], Heinz Rotering[4], Udo Reichl[1], Michael Wolff[1], [1]Max Planck Institut for Dynamics of Complex Technical Systems, Magdeburg, GERMANY; [2]Eberhard-Karls-University, Tuebingen, GERMANY; [3]EMC Microcollections GmbH, Tuebingen, GERMANY; [4]Merckle Biotec GmbH, Ulm, GERMANY

Recombinant human (rh) glycoproteins such as erythropoietin (EPO) or follicle stimulating hormone (FSH) are of considerable pharmaceutical relevance, which goes along with a steadily growing demand. Production processes have been mainly optimized with respect to the upstream process, while several downstream processes still require attention. For the latter, not only capacity and throughput could be optimized but also the yield and specificity for glycoprotein isoforms (glycoforms). For many glycoproteins, the degree of sialylation is important for in-vivo activity. Regarding efficacy low sialylated glycoforms have to be separated from the pharmaceutically relevant high sialylated. The aim of this study was to develop an affinity chromatography method for the separation of different glycoforms depending on the degree of sialylation. Therefore, sialic acid-specific ligands, which comply with the safety regulations of human pharmaceutical production processes, had to be identified. Synthetic peptides seemed to be one attractive option with certain advantages over carbohydrate binding lectins, which are frequently toxic and economically unattractive. Potential candidates were identified via LC/MS, ELISA and literature search. Subsequent evaluation of several promising candidates

involved their immobilization onto chromatography matrices to study their separation performance in small scale affinity chromatography. In order to compare all modified matrices three parameters were investigated; dynamic binding capacity (DBC), purity of rhEPO, and separation of rhEPO glycoforms. Method optimization focused on the following process conditions: buffer composition, pH, ionic strength and step gradient period. The purity of rhEPO was determined by an EPO-specific ELISA in comparison to the total protein amount (Bradford assay). Glycoform separation performance was evaluated by a novel glycan analysis method based on multiplexed capillary gel electrophoresis with laser induced fluorescence detection (xCGE-LIF) which enables determination of differences in the EPO glycosylation pattern between the eluted fractions. Several of the tested materials showed good binding characteristics. DBCs of up to 30 mg/mlmatrix were achieved as well as a significant increase in EPO purity with recoveries up to nearly 100 %. Glycan analysis clearly illustrated differences in glycosylation patterns of EPO between the elution fractions (step gradient elution). Overall these results suggest that the established chromatography method has the potential to improve the quality of sialylated therapeutic glycoproteins if applied as a polishing step in the purification train.

L-136 **Reversible Cyclic and Polycyclic Peptides for the Discovery of Affinity Ligands.** Stefano Menegatti, Robert Blackburn, Kevin Ward, Ruben Carbonell, North Carolina State University, Raleigh, NC, USA
Cyclic peptides are a prominent class of compounds in drug research and discovery of ligands for biotechnology applications. Recent investigations point at polycyclic peptides as the next generation of peptide-based species meant for applications that require extremely high affinity and potency. Combinatorial libraries of cyclic and polycyclic peptides are very powerful tools for selecting peptide ligands against biological targets. Post-screening sequence identification, however, is usually a critical step that hinders the whole process of ligand and drug discovery. While the determination of linear sequences is straightforward, inherently more challenging is the sequencing of cyclic molecules. To overcome this issue, we propose a strategy that employs combinatorial libraries of reversible cyclic and polycyclic peptides for the discovery of highly selective ligands. Each cyclic molecule comprises a binding peptide sequence framed between two cleavable linkers. This allows extracting the peptide sequences from the selected beads by treating with an appropriate cleaving agent. The linearized peptides released from the support to the liquid phase are rapidly sequenced by single stage MS/MS. A case study is presented to illustrate the process of library screening and sequence identification. Owing to the breadth of chemical and structural diversity that these libraries can explore, the strategy herein presented show a great deal of promise for the high-throughput discovery of affinity ligands and drugs.

L-137 **Understanding Ion Exchange Adsorption Mechanism.** Francisco Marques[1], Patricia Aguilar[1], Marvin Thrash Jr.[2], Cristina Dias-Cabral[1], [1]University of Beira Interior, Covilhã, PORTUGAL; [2]Central State University, Wilberforce, OH, USA
Ion exchange chromatography (IEC) is well known for its high resolving power, speed, high capacity, and biocompatibility. It is routinely used in the biochemical field and due to its versatility, relatively low cost, and its acceptance by the regulatory authorities, has become an industrial process in the production of pharmaceutical biomolecules. As other industrial processes it needs to be optimized based on a thorough understanding of the process variables. It is also well known that the broad features of chromatographic behaviour are highly influenced by adsorption equilibrium. So, a useful description of the chromatographic process has to consider the equilibrium behaviour. The main challenge will be the adsorptive behavior prediction for any range of concentrations. The primary reason for this is that more interactions can occur than the primary interaction expected between the biomolecule and the adsorbent. It has been demonstrated that thermal events accompanying biomolecule adsorption can shed some light to the underlying mechanism. We have confirmed that flow microcalorimetry (FMC) can be used to dynamically measure the heat of biomolecule adsorption, providing invaluable insights to the biomolecule adsorption process. In this study, the ion exchange adsorption behavior accessed by FMC has been compared under different conditions. The effects of pH, ionic strength, and properties of both the biomolecule and the support have been analyzed. - Work supported by FCT (Portuguese Foundation for Science and Technology), project number FCOMP-01-0124-FEDER-014750 (Ref. FCT PTDC/EBB-BIO/113576/2009) and NSF (American National Science Foundation) NSF – 1246932 (award issued by CBET division of NSF).

L-138 **Purification of a Potential Malaria Vaccine by Multimodal Ion Exchange Chromatography.** Sonja Jensen, Jessica Paul, Gesine Cornelissen, Hamburg University of Applied Sciences, Hamburg, GERMANY
During the last several years new media for preparative chromatography have been developed. One group of these new media are the multimodal chromatography media. The ligands, used in multimodal chromatography, interact with the target molecule through multiple types of interactions. The multimodal functionality includes binding of proteins regardless of ionic strength of the loading material. This means that the medium can be used for direct loading of clarified material, without prior dilution or desalting to reduce the conductivity. In this contribution, experiments were done using Capto adhere (GE Healthcare), a weak anion exchanger, for

purification of a potential malaria vaccine. Besides the ionic interactions in conventional anion exchangers, hydrogen bonding and hydrophobic interactions are used for binding. Thus, multimodal chromatography can be utilized to solve purification problems and opens new opportunities in protein purification. After identifying the stability range of the malaria vaccine candidate, batch adsorption studies were carried out in order to define the binding capacity of the used Capto adhere. In comparison to conventional anion exchangers, the measured <120 mg ml-1 binding capacity of the multimodal resin is major for the applied conditions. The optimization strategy was carried out adopting Design of Experiments (DoE). The chromatographic system ÄKTA avant (GE Healthcare) was used for experimental runs. The protein binds at high salt conditions, and can be eluted by a step gradient shifting the pH value. It is possible to achieve a maximized purity of the product because degradation products and other contaminants do not bind at specific salt concentrations at which the product still binds to the ligands. After optimization and up-scaling of the chromatographic method, it is possible to implement this purification as a polishing step into an Integrated Bioprocess, consisting of fermentation of Pichia pastoris, protein capture via Expanded Bed Adsorption Chromatography, and an Ultrafiltraton/Diafiltration for concentration and buffer change, getting maximized product purity and recovery of the potential malaria vaccine.

L-139 **New Tools to Achieve Enhanced Process Productivity in the Purification of Monoclonal Antibodies and Other Recombinant Proteins.** Milton Hearn, Monash University, Clayton, Victoria, AUSTRALIA

A current objective in the production of proteins for use in pharmaceutical or other industrial applications is to lower the cost of manufacturing. This challenge can be addressed by reducing the number of unit operations at the upstream and downstream stages of production, provided that product yield, purity and efficacy are not sacrificed. Over the past decade, several platform technologies have emerged as a powerful framework to achieve this goal. New approaches have thus been documented for the design and implementation of novel process technologies that i) require less energy consumption; ii) reduce waste generation; iii) avoid the use of hazardous substances and conditions that cause product loss; iv) permit single use of lower cost consumables but allow some other high cost process materials to be recycled; and v) enable unique molecular attributes of each specific unit operation to be optimised, facilitating enhancement of production, purity and recovery of products. Besides the opportunities for innovation that this framework offers, the beneficial attributes of platform technologies result in the deployment of resources, infrastructure and plant/equipment in a more tangible, economic and sustainable way. In this presentation, two downstream case studies related to projects carried out within this Centre will be discussed where these principles have been captured – (i) the design, synthesis and application of new mixed mode resins based on low molecular weight heterocyclic ligands for use at the capture and purification stages with genetically engineered monoclonal antibodies (mAbs); and (ii) a new approach to achieve improved host cell protein clearance in the purification of 'tagged' recombinant proteins with a new class of tag specific affinity resins. In each case, the advantages of the use of these platform system approaches have been documented for a range of protein targets of different structures. Importantly, these technologies provide a new avenue to enhanced productivity in the purification of genetically engineered proteins.

L-140 **Investigation of Monoclonal Antibody Purification by Aqueous Two-phase Extraction.** Jan Mündges, Shuai Shi, Tim Zeiner, TU Dortmund University, Dortmund, GERMANY

Monoclonal Antibodies (mAbs) are biologically active proteins produced in response to the presence of foreign substances. During the past years, the application of mAbs for therapeutic treatment has been increasing. Several diseases like cancer, asthma or autoimmune diseases are already treated by mAbs whereas nearly all marketed are produced by mammalian cell cultures. Through an intensified research on the field of upstream-processing, the mAb concentration in the fermentation broth has significantly been increased. Hereby purification of mAbs became the most challenging step in the manufacturing process accounting for a major part of the total production costs. Since conventional unit operations like preparative chromatography are often limited by their capacity, buffer consumption and ligand cost, growing interest exists in finding alternative downstream-processing methods. Aqueous two-phase systems (ATPS) are a promising option for the improvement of mAb purification. By mixing two different phase forming components (PFCs), e.g. two hydrophilic polymers or a polymer and a salt in water at high concentration, two liquid phases can form. One of the phases is rich in PFC 1, the other is rich in PFC 2. Both phases consist of 70-80 % of water and thus have an aqueous character. Purification of the target product is achieved because of a different partitioning of target component and impurities between both phases. Especially for sensitive products ATPS are a promising alternative as they offer mild separation conditions at high, continuous capacity. In this work, an ATPS consisting of polyethylene glycol, phosphate salt and water is chosen for the purification of mAbs from Chinese Hamster Ovary cell supernatant (CHO-CS). Essential for the design of an aqueous two-phase extraction (ATPE) is a fundamental knowledge of substance properties, liquid-liquid equilibrium data as well as partitioning of target component and impurities. Several influences on the purification of mAbs like polyethylene glycol molecular weight, pH, initial concentration of CHO-CS and concentration of additional sodium chloride are analyzed in

single-stage extraction experiments. To further enhance mAb purification, ATPE is performed in multi-stage experiments in a miniplant mixer-settler battery. The feasibility of multistage ATPE at miniplant scale will be discussed with regard to different operation modes. Furthermore the purification of mAb from CHO-CS will be evaluated, paying special attention to mAb purity as well as mAb yield.

L-141 **Fujifilm Diosynth's and Chromatan's Investigation of Countercurrent Tangential Chromatography for Purification of Monoclonal Antibodies.** Oleg Shinkazh, Chromatan, State College, PA, USA
Chromatan's CTC is a new column-free purification technology for MABS and vaccines, providing a scalable, disposable, and continuous alternative to column chromatography. Experimental studies were performed purifying monoclonal IgGs. We showed high-resolution antibody purification at < 10 psi operating pressure, with recovery >94%, showing great promise for this new technology.

L-142 **2D-DIGE for Host Cell Protein Analysis and Antibody Process Development.** Alois Jungbauer, BOKU, Vienna, AUSTRIA
Host cell protein analysis is the most important analytical methods during bioprocess development. Current regulations require a reduction of HCP below 1-100 ppm depending on the intended use, route of administration of the product, and production systems. A range of immunospecific and non-specific methods are in use and have been accepted by regulatory bodies. Immunospecific methods such as ELISA are rather simple when specific antibodies are available. Non-specific methods are more complex but provide a holistic view on the HCP profile. Two Dimensional Differential in Gel Electrophoresis (2D-DIGE) has been used to characterize the feed stock (culture supernatant) of varies mammalian expression clones as well as the different flow schemes in downstream processing. In 2D-DIGE samples can be labeled with different fluorescent dyes and after combining they are separated by a single 2D-electrophresis. Hereby the gel to gel variation can be excluded. After scanning the gel at different wavelength and digitization it is possible to superimposed the separation results of the individual samples and therefore identify subtle difference. In addition a holistic view can be obtained. For antibodies and a cytokine Fc fusion the influence of cell viability and/or clone selection on the composition of the culture supernatant will be shown. Then the effect of different flow schemes on the purity and composition of the intermediate and final product will be compared and discussed. The results will be also discussed in context of stability and robustness of different purification schemes. The methods will be compared to other analytical tools for process characterization.

P-Th-143 **An Alternative Capture Step for Monoclonal Antibodies: Phenyl Boronate as a New Multi-modal Ligand.** Ana M. Azevedo, Raquel dos Santos, Sara A.S.L. Rosa, M. Raquel Aires-Barros, Instituto Superior Tecnico, Lisbon, PORTUGAL
Monoclonal antibodies (mAbs) are currently described as the shining stars of the pharmaceutical industry, with total sales expected to reach $58 billion by 2014 [1]. Over the last years, more than 30 mAbs have received regulatory approval for therapeutic and diagnostic purposes with the majority targeting diseases such as cancer and autoimmune disorders. A critical evaluation of the manufacturing bottlenecks clearly indicates that the current downstream processing technology is responsible for many capacity constrains, including inability to handle high production titers, difficulties in integrating cell culture with primary recovery steps and especially excessive cost of goods and consumables. [2]. Within this work, the feasibility of using phenyl boronate (PB) as an affinity ligand for the purification of mAbs has been investigated. The PB ligand is a useful tool for the specific capture and isolation of cis-diol-containing molecules, such as glycoproteins. Preliminary studies, have shown that PB media can bind to human antibodies, not only at strong alkaline conditions but also at acidic pH values, while typical impurities found in cell culture media (insulin and albumin), do not bind at alkaline pH. Different binding and eluting conditions were evaluated for the capture of IgG from a CHO cell supernatant and the most promising results were obtained using 20 mM HEPES at pH 8.5 as binding buffer and 1.5 M Tris-HCl as eluting buffer. Higher recovery yields could be obtained at pH 7.5 but at the expense of a lower purity [1]. In order to optimize both yield and purity, a washing step using competitive diols has been developed. References: [1] Morrow Jr., K.J. "The New Generation of Antibody Therapeutics: Current Status and Future Prospects - Overview" Insight Pharma Reports, Cambridge Healthtech Institute, Needham, MA, 2012 [2] Langer, E., "Improved Downstream Technologies Are Needed to Boost Single-Use Adoption", BioProcess International, Vol. 9, No. S2, May 2011, pp. 6–12 [3] Azevedo, A.M., Gomes, A.G., Borlido, L., Santos, I.F.S., Prazeres, D.M.F., Aires-Barros, M.R. "Capture of human monoclonal antibodies from cell culture supernatant by phenyl boronate chromatography", J. Mol. Recognit. 23 (2010) 569-576.

P-Th-144 **Phenyl Boronic Acid as Ligand for a Multimodal Chromatography: Adsorption Behavior Comparison between Control Pore Glass and Agarose Matrixes.** Rimenys Jr. Carvalho[1], James Woo[2], Karim A. Nakamura[2], Maria Raquel Aires-Barros[1], Ana M. Azevedo[1], Steven M. Cramer[2], [1]Instituto Superior Tecnico, Lisbon, PORTUGAL; [2]Rensselaer Polytechnic Institute, Troy, NY, USA

Phenylboronate (PB) chromatography has been reported as an affinity chromatography technique for the separation/identification of cis-diol containing molecules such as glycoproteins. In this work, a protein library was employed in order to investigate the various chemical interactions in this system. Adsorption experiments were carried out over a pH range from 4 to 9 and differences in retention were generally related to two protein properties, its pI value and the presence of a glycan structure. Different classes of proteins provided different trends on adsorption behavior, neutral proteins provided high interaction aroud their pI and in the acidic pH range, on the other hand, acidic proteins higher adsorption in acidic pH range (below pH 7) and basic proteins on basic pH (above pH 6) range however, glycosylated and non-glycosylated provided similar adsorption behaviors. In general, ProSep PB provided adsorption at higher pH range than P6XL in all conditions studied. Factors as steric conditions and better charge stabilization could be responsible for higher pH range of protein adsorption on ProSep PB of neutral and acidic proteins. This matrix provides bigger particles and more controlled pore size (1000 Å) than P6XL. Although the ligand density could play a role, studies using ARS dye, has showed that Agarose P6XL has higher ligand density (14.26 ± 0.47 µmol/mL) than ProSep PB (8.76 ± 1.83 µmol/mL). For basic proteins, the adsorption increases along to pH, above pH 8 for P6XL and 6 for ProSep PB due tetrahedral conformation of PBA but also from hydroxyls from silica of ProSep PB (CPG® matrix) which become negatively charged above pH 6. This study has showed that PBA interacts with proteins via several modes of interaction (electrostatics, charge transfer, hydrophobic and cis-diol interactions). Although Agarose P6XL adsorbs in lowers pH range, this could increase the selectivity of this chromatography concerning separations of different classes of proteins.

P-Th-145 **Chiral Separation of D,L-Phenylglycine using an Enantioselective Membrane Formed by Polycondensation of Bovine Serum Albumin with 1,6-Diisocyanatohexane on a Polysulfone Membrane.** Li-Ming Yuan, Guang-Yong Zeng, Department of Chemistry, Yunnan Normal University, Kunming, Yunnan, P.R. CHINA

An enantioselective composite membrane was prepared by polycondensation between bovine serum albumin (BSA) on a polysulfone support (PS) and a heptane solution of 1,6-diisocyanatohexane (1,6-DCH). The flux and permselective properties of the composite membrane were studied using D,L-phenylglycine as the feed solution. The influences of a number of parameters, such as the air-drying time of the BAS solution on PS, the time of polymerization, the operating pressure and the feed concentration of the racemate, were studied. Chemical characterization was carried out using Fourier transform infrared spectroscopy and the top surface/cross-section was analyzed by scanning electron microscopy. The results showed that when using the enantioselective composite membrane for the optical resolution of the phenylglycine racemic mixture, a separation factor of 1.2 could be obtained. The paper thus details, for the first time, how a poly(BSA crosslinked with 1,6-DCH)/PS composite membrane can be used as an optical resolution membrane material to isolate the optical isomers of D,L- phenylglycine.

P-Th-146 **Analysis of Associated Forms of Insulins.** Sara Fexby Garmer, Martin Mårtensson, Dorte Bjerre Steensgaard, Aage Hvass, Svend Havelund, Novo Nordisk A/S, Måløv, DENMARK

An analytical method based on size exclusion chromatography for determination of associated forms of insulin in drug products is presented. The association forms of insulin may provide information regarding prediction of the pharmaceutical effect and chemical stability. The content of excipients in the formulation, i.e. zinc, salt and preservatives may affect the formation of the associated forms.

P-Th-147 **Lipidomics using Ion Mobility Mass Spectrometry with Transomics Informatics.** Giuseppe Astarita[1], Roy Martin[1], Giorgis Isaac[1], James Langridge[2], Weibin Chen[1], [1]Waters Corporation, Milford, MA, USA; [2]Waters Corporation, Manchester, UK

Lipids play essential roles in health and disease. Alterations in lipid metabolites are associated with various human diseases including obesity, heart disease, and diabetes. Many lipids are currently used as biomarkers of health and diseases, including cholesterol, triglycerides, vitamin D, testosterone and free fatty acids. The discovery of novel alterations in lipid levels related to human diseases could lead to the development of novel biomarkers and future diagnostic testing. The challenge with global lipid analysis — lipidomics — is the chemical complexity and the large range of concentrations of thousands of lipid species that are present in biological samples. New liquid chromatography and mass spectrometry-based tools allow to accurately and rapidly measure hundreds of individual molecular species providing the opportunity to use more complex lipid profiles for disease diagnostics. In this study, we present a robust workflow for lipidomics, which employs UPLC/ion

mobility/time-of-flight (TOF) with the TransOmics informatics solution for biomarker discovery and automated identification of lipids in complex biological matrices.

P-Th-148 **Characterization of Two Novel Analytical Chromatographic Columns for Orthogonal Analysis of Monoclonal Antibody and Protein Aggregates and their Isoforms.** Justin Steve, Atis Chakrabarti, Tosoh Bioscience LLC, King of Prussia, PA, USA

TSKgel UltraSW Aggregate 7.8 mm ID × 30 cm analytical columns are novel columns packed with 30 nm pore size, 3 µm silica particles. Larger pore size with the estimated exclusion limit of ~4 x 106 Da provides improved separation and quantitation of protein aggregates and oligomers, particularly for the separation of the monoclonal antibody aggregates. For safe bio therapeutic application separation of the pure monomer needs to be very well resolved from its dimer and higher order aggregates. Similarly for quality control and regulatory purpose the separation of antibody fragments is also very much essential. The species other than the monomer might induce toxic side effects to the body if not removed. Orthogonal chromatographic separation of protein is necessary for evaluation of the purity of the fraction obtained by a particular chromatographic mode. A well resolved symmetric pure protein peak from size exclusion chromatography need to be checked by another mode for the presence of any isoforms. TSKgel Protein C4-300 columns, consisting 3 µm spherical silica gel with 30 nm pore size and a short polymeric butyl (C4) alkyl groups are the newest reversed phase columns designed for the optimal recovery and resolution of proteins. Furthermore, the large pore size, allowing macromolecules to enter the interior of the pore, provides higher peak capacities than reversed phase columns with 10 nm pore size. Ferritin is a 440 kDa globular protein complex consisting of 24 protein subunits and is the primary intracellular iron-storage protein in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. Ferritin that is not combined with iron is called apoferritin. Structurally, the molecule resembles a small icosahedral virus, shaped from a multimeric protein shell, apoferritin, within which variable amounts of iron may be stored. Ferritin and apoferritin are widely used for the calibration of gel filtration columns. Here we report the use of Ferritin and monoclonal antibody to demonstrate the important features of the new SEC and RPC column. This report also demonstrates the superior performance of mAb/protein separation in comparison to conventional columns. We also report the efficient separation of aggregates induced by forced denaturation using these columns. The two columns are also used for the orthogonal separation to detect protein heterogeneity.

P-Th-149 **Purification of a Potential Malaria Vaccine by Multimodal Ion Exchange Chromatography.** Sonja Jensen, Jessica Paul, Gesine Cornelissen, Hamburg University of Applied Sciences, Hamburg, GERMANY

During the last several years new media for preparative chromatography have been developed. One group of these new media are the multimodal chromatography media. The ligands, used in multimodal chromatography, interact with the target molecule through multiple types of interactions. The multimodal functionality includes binding of proteins regardless of ionic strength of the loading material. This means that the medium can be used for direct loading of clarified material, without prior dilution or desalting to reduce the conductivity. In this contribution, experiments were done using Capto adhere (GE Healthcare), a weak anion exchanger, for purification of a potential malaria vaccine. Besides the ionic interactions in conventional anion exchangers, hydrogen bonding and hydrophobic interactions are used for binding. Thus, multimodal chromatography can be utilized to solve purification problems and opens new opportunities in protein purification. After identifying the stability range of the malaria vaccine candidate, batch adsorption studies were carried out in order to define the binding capacity of the used Capto adhere. In comparison to conventional anion exchangers, the measured <120 mg ml-1 binding capacity of the multimodal resin is major for the applied conditions. The optimization strategy was carried out adopting Design of Experiments (DoE). The chromatographic system ÄKTA avant (GE Healthcare) was used for experimental runs. The protein binds at high salt conditions, and can be eluted by a step gradient shifting the pH value. It is possible to achieve a maximized purity of the product because degradation products and other contaminants do not bind at specific salt concentrations at which the product still binds to the ligands. After optimization and up-scaling of the chromatographic method, it is possible to implement this purification as a polishing step into an Integrated Bioprocess, consisting of fermentation of Pichia pastoris, protein capture via Expanded Bed Adsorption Chromatography, and an Ultrafiltraton/Diafiltration for concentration and buffer change, getting maximized product purity and recovery of the potential malaria vaccine.

P-Th-150 **Characterization of Two Novel High Capacity Strong Ion Exchange Resins.** Chinlun Huang, J. Kevin O'Donnell, Tosoh Bioscience, King of Prussia, PA, USA

Ion exchange resins with increased selectivity and capacity are in great demand. The TOYOPEARL GigaCap® resins were developed by Tosoh Corporation in response to this demand. Two new high capacity and high resolution experimental ion exchange resins were recently developed, and this poster focuses on these resins and their ability to purify proteins and other biomolecules, such as oligonucleotides. The high capacity strong ion

exchange resins utilize a well-known size exclusion chromatography resin as the base bead. The base resin is chemically modified with either quaternary amine or sulfonic groups to provide a high level of binding sites. The strong anion exchanger, TOYOPEARL GigaCap Q-650S has a dynamic binding capacity (DBC) of greater than 100 g/L for bovine serum albumin (BSA) at both 5% and 10% breakthrough. The strong cation exchanger, TOYOPEARL GigaCap S-650S has a DBC of 130 g/L for lysozyme at 5% and 10% breakthrough. These two new strong ion exchange resins can effectively separate ovalbumin and trypsin inhibitor even when loaded to 96 g/L. The new resins were also compared to other available resins and demonstrated excellent selectivity under increasing loading conditions. The strong anion exchange resin was also able to purify a crude oligonucleotide to greater than 95% in one step. The purity of the main peak indicates the resin is not yet loaded to capacity at 5 mg. In addition, these resins are stable at high linear velocities with good pressure flow characteristics. The resins have a pressure rating of 3 bar and are stable in the pH range of 3-13. In addition, due to the smaller particle size of 30 µm, increased resolution can be achieved. The higher capacity and resolution of these resins also maintain excellent binding and elution characteristics, result in narrower peak elutions and more efficient protein purifications, allowing for increased throughput in downstream purification steps.

P-Th-151 **Aggregates and Particle Characterization to Support Biomanufacturing Process Development.** Yogesh Mudaliar, Rong-Rong Zhu, Tim Hanley, EMD Millipore, Bedford, MA, USA
Aggregates and particles can form through the life cycle of biotherapeutics from upstream and downstream processes to drug storage. Aggregates and particles should be closely monitored and eliminated during manufacturing process. SEC-HPLC has been the most common method used for aggregate quantification because of its robustness and high-throughput. However, there are limitations with SEC-HPLC for aggregate detection, such as it cannot detect insoluble aggregates, sub-visible and visible particles. Orthogonal analytical methods (SEC-HPLC-UV and MALS, Dynamic Light Scattering (DLS) and Dynamic Imaging Analysis (DIA)) have been implemented in aggregates and particle characterization, through mAb and vaccine manufacturing processes. By implementing DLS and DIA as complementary methods to SEC-HPLC for aggregates and particle characterization, we are able to improve the biotherapeutic and vaccine manufacturing processes.

P-Th-152 **Superficially Porous Particles for Peptide and Protein Analysis.** Barry Boyes, Joseph Kirkland, Stephanie Schuster, Brian Wagner, Joseph DeStefano, Advanced Materials Technology, Inc., Wilmington, DE, USA
The original 2.7 µm superficially porous particles (SPP) introduced in 2006 were created with an average pore size of 90 Å, which was suitable for small molecule analytical separations. This SPP particle technology now has been expanded to include wider pore sizes and larger particle sizes that are specifically designed for larger biomolecules. Novel particle designs with specially selected bonded phases for peptide and protein separations are described. This presentation includes fast separations of peptides and intact protein mixtures, as well as examples of very high resolution separations of larger proteins and associated variants and contaminants. Columns with specially engineered bonded phases for these particles demonstrate high temperature stability, which is ideally suited for the conditions that are often used for analytical and small scale preparative biomolecular separations. Protein recovery and sample loading investigations are included. The optimized shell thickness of the new 400 Å SPP particles represents a compromise between a short diffusion path versus adequate retention and mass load tolerance. Examples of high molecular weight protein separations highlight the advantages of using columns of superficially porous particles with wider pores. Some comparisons with conventional totally porous particles are also shown.

P-Th-153 **Microcalorimetric Study of Linear Plasmid DNA Adsorption onto an Ion Exchange Support.** Patricia Aguilar[1], Filipa Pires[1], Marvin Thrash, Jr.[2], Cristina Dias-Cabral[1], [1]CICS-UBI-Health Sciences Research Centre, University of Beira Interior, Covilha, PORTUGAL; [2]Department of Water Resources and Environmental Engineering, College of Science and Engineering, Central State University, Wilberforce, OH, USA
Recent research sustains the potential of gene therapy in the cure of several diseases. This type of therapy requires a large scale production of gene vectors such as plasmid DNA (pDNA) in a high purity level. Ion exchange chromatography is a crucial technique in the purification process of pDNA, whereby is often used in its large scale production. Industrial scale needs more insight knowledge of the adsorption mechanisms, avoiding the huge time and resources consumption of the trial and error methods. Using flow microcalorimetry (FMC), the aim of this investigation was twofold. The first aim was to study the pDNA adsorption mechanism on an anion exchange support and the second was to study the effect of nonspecific interactions on the enthalpy variation. FMC results for the adsorption of linear pVAX1-lacZ plasmid onto Q-Sepharose showed that the adsorptive process is, as expected, enthalpy driven, however, the presence of nonspecific effects were evident. An examination of the FMC thermograms revealed the presence of an endothermic peak followed by a single exothermic peak that was independent of the injected pDNA mass. However, when the injection loop volume was increased to change the residence time, it was observed that the endothermic peak was followed by two

mostly separate exothermic peaks. Accordingly with previous published isotherms, FMC assays where performed under linear and overloaded conditions. Isothermal Titration Microcalorimetry (ITM) assays were also done to investigate the effect of the flow on the FMC results, the process of dilution and the interaction of buffer with the adsorbent. - Work supported by FCT (Portuguese Foundation for Science and Technology), project number FCOMP-01-0124-FEDER-014750 (Ref. FCT PTDC/EBB-BIO/113576/2009) and NSF (American National Science Foundation) NSF – 1246932 (award issued by CBET division of NSF).

P-Th-154 **Separation of DMB-Labeled Sialic Acids for the Comparison of Biosimilars to Reference Materials with an Improved Chromatographic Method.** Xiaoning Lu, Hillel Brandes, Dave Bell, Roy Eksteen, Sigma-Aldrich, Bellefonte, PA, USA

Purpose: Sialic acids affect the bioavailability, function, stability, and metabolism of glycoproteins. Two forms of sialic acid are commonly present in therapeutic glycoproteins: N-acetylneuraminic acid (NANA) and N-glycolylneuraminic acid (NGNA). One of the most common quantification methods involves releasing sialic acids from the glycoprotein, derivatizing NANA and NGNA with 1,2-diamino-4, 5-methylenedioxybenzene (DMB), and analyzing by C18-HPLC with fluorescence detection. This procedure is subject to interference from peaks originating from excess reagent and other derivatized impurities, limiting sensitivity and reproducibility. The objectives of this study were to develop a significantly improved HPLC-fluorescence method for DMB-NANA and DMB-NGNA, and to apply this method to compare two candidate biosimilar therapeutic proteins to their respective reference materials. Methods: LC optimization was performed using standard mixtures of DMB-NANA and DMB-NGNA and an HPLC with a fluorescence detector. Four columns of the same dimensions were evaluated: C18, F5, RP-Amide (reversed-phased columns), and bare silica (HILIC column). Flow rate and column temperature were constant. Mobile phase composition varied, but A and B solvents were always 0.1% formic acid in water, and 0.1% formic acid in acetonitrile, respectively. Sialic acids were released from glycoproteins, derivatized with DMB, and then analyzed with the optimum chromatographic conditions. Data were fit to external calibration curves. Results were expressed as the ratio of (mol of sialic acid) : (mol of protein). Results: Among the screened columns, the RP-Amide column provided the best selectivity. The optimized HPLC separation with the RP-Amide column resolved the DMB-NANA and DMB-NGNA as well as excess reagent and other derivatized interferences. The new method takes 12 minutes and uses a simple mobile phase and gradient (6-20% B). This is an improvement over the existing 25-minute isocratic method using a C18 column and a more complicated mobile phase composition (water/acetonitrile/methanol). DMB-NANA and DMB-NGNA are now baseline resolved, and the analytes are free from interfering peaks.Conclusion: An improved HPLC method has been established for analysis of DMB-NANA and DMB-NGNA. The method employs a RP-Amide column and offers improved selectivity, sensitivity, ruggedness, and resolution in shorter run time.

P-Th-155 **Twin Column CaptureSMB: A Novel Cyclic Process to Increase the Capacity Utilization in Protein A Chromatography.** Monica Angarita[1], Baur Daniel[1], Thomas Muller-Spath[2], Roel Lievrouw[3], Geert Lissens[3], Guido Strohlein[2], Massimo Morbidelli[1], [1]ETH Zurich, Zurich, SWITZERLAND; [2]ChromaCon AG, Zurich, SWITZERLAND; [3]JSR Life Sciences, Leuven, BELGIUM

Protein A-affinity chromatography of monoclonal antibodies (mAbs) is the state-of-the-art purification platform in industry for capture of mAbs from cell culture harvest. The use of multi-column sequential loading processes for increase of stationary phase capacity utilization and productivity has gained significant interest in the last years. However, the available process solutions require at least 3-4 columns, introducing hardware complexity and increased downtime risk. To overcome these drawbacks a novel twin-column countercurrent sequential capture process has been developed. In this work we introduce the method design as well as experimental results using AmsphereTM Protein A JWT203, showing the significant advantages over batch chromatography in terms of higher column loading, higher productivity and lower buffer consumption. In addition, simulation results are presented, showing a performance comparison to conventional 3- and 4-column capture processes.

P-Th-156 **Effects of Mobile Phase Optimization on Analyte Behaviour in Size Exclusion Chromatography of Biomolecules.** James Martosella[1], Andrew Coffey[2], [1]Agilent Technologies, Wilmington, DE, USA; [2]Agilent Technologies, Church Stretton, UK

Systematic approaches to method development are an essential part of modern chromatography. Ensuring a method is fit for purpose and not simply a result of ad-hoc changes to your experimental procedure is a time consuming but essential part of the process. Size exclusion chromatography is often considered to be the simplest of separation techniques since the analyte should not interact with the stationary phase at all. The correct choice of pore size remains the most critical aspect for separation based on molecular size, however it is important to remember that molecular weight does not necessarily reflect the size in solution. Furthermore, the process of optimizing the mobile phase to reduce any unwanted interactions between the molecules of interest and the stationary phase can result in changes to the sample itself. The molecule can potentially change shape

by adopting a different confirmation, become denatured and unfold under high salt concentrations, or perhaps begin to aggregate; all of which will influence the retention time. In this poster we investigate some of the key parameters to be considered when developing a size exclusion chromatography method. Of particular interest is the ability to use quaternary pumps to dynamically control mobile phase parameters and how the use of multiple detectors, including concentration detectors such as differential refractive index detectors, and UV or diode array detectors together with low angle laser light scattering detectors can give greater insight into the behavior of the analyte.

P-Th-157 **Fast and Efficient Reversed-phase Liquid Chromatography/Mass Spectrometry Characterization of Glycosylation in the Fc Region of a Recombinant IgG(1) Therapeutic Monoclonal Antibody (mAb).** James Martosella, Phu Duong, Alex Zhu, Agilent Technologies, Wilmington, DE, USA

Monoclonal antibodies (mAbs) are used increasingly as therapeutic agents to target various diseases, and form a large fraction of therapeutic proteins commercially approved or currently under development. With an increasing number of mAbs moving to market, the need for rapid analytical methods for their detailed characterization has dramatically increased. For example, during mAb development it is important to find the recombinant production system that offers optimal productivity with rapid turnaround times to meet process development demands. Assessing the glycosylation profiles in shortened analysis times is thus vital to mAb development and the need to have rapid and reliable LC/MS glycoprofiling methods are growing. This poster describes a rapid online reversed-phase (RP) LC/MS approach to profile glycosylation for a therapeutic mAb (IgG1). Separations of intact IgG1 and papain-cleaved IgG1, to yield Fc and Fab fragments, were optimized for rapid analysis using a 5 µm, 300A C3 superficially porous column. Specifically, intact IgG1 was characterized to obtain glycoform and accurate glycan mass information. The intact IgG1 was then subjected to papain digestion and the glycosylation containing Fc portion analyzed by time-of-flight (TOF) mass spectrometry to determine glyco-specific modifications. The methods were developed to provide essential glycoform profile information using rapid LC/MS procedures for achieving quick data turnaround times. For further validation of the Fc glycan-specific assignments from the TOF analysis, the intact mAb was then subjected to a fully automated rapid glycan analysis. This was achieved using an mAb-glyco chip kit to establish a glycan accurate-mass database. The methods described herein are useful for cell line and clone selection processes, and cell culture process optimizations where absolute quantitation is not necessary, but where rapid, reliable and efficient characterizations are desired.

P-Th-158 **Combining Small-scale Purification and Analysis of Monoclonal Antibodies on One Instrument.** Sonja Schneider, Agilent Technologies, Waldbronn, GERMANY

Preparative and analytical methods for protein purification and analysis require two different instrument setups: Protein preparation requires large injection volumes and high flow rates, whereas analytical methods require small injection volumes and lower flow rates. Traditionally, preparation and analysis are done on two instruments specifically designed for these purposes. Here, we show a two-step purification procedure on a single instrument for monoclonal antibodies (mAb) out of cell lysate. As a capturing step, Protein A affinity chromatography followed by a size exclusion polishing step was performed. In the subsequent analytical step the purified mAb fraction was identified and quantified with Protein A affinity chromatography, and the charge variants were characterized with ion exchange chromatography (IEX). The analysis of the purified mAb after the SEC polishing step is an essential part of the workflow to determine purity and quantity. Especially for therapeutic mAbs, it is extremely important to thoroughly characterize the biomolecules to ensure efficacy and safety of the drug. One part of the characterization procedure, besides peptide mapping and aggregate analysis, is the determination of charge variants of the mAb, usually carried out by ion exchange chromatography. In this case, the analysis of the purified mAb includes, besides identification using Protein A chromatography, also quantification. The characterization of the mAb in terms of charge variants was carried out using weak cation exchange chromatography in a 4-component ionic strength gradient. Here, we present an application solution for the small-scale purification of a monoclonal antibody from a cell lysate and the subsequent analysis on a single LC instrument. Purification and polishing steps using preparative columns for Protein A purification and size exclusion chromatography were combined with high-volume injection. Analysis of the purified mAb was demonstrated using a Protein A Monolith for quantification and identification. In addition, charge variant analysis was carried out by a four-component ionic strength gradient using ion exchange chromatography.

P- Th-159 **Greater Loading Capacity and Resolution for Improved Process-scale Peptide Purification.** Jochen Saar[1], Reno Nguyen[2], Chitra Sundarajan[3], Scott Anderson[4], Dennis McCreary[5], Janine Sinck[6], [1]Worms, GERMANY; [2]Hesperia, CA, USA; [3]Hyderabad, Andrapradesh, INDIA; [4]Deerfield, IL, USA; [5]Columbia, MD, USA; [6]Allentown, PA, USA

The demand for high purity peptides is increasing. Small synthetic peptides to large cellular produced peptides are being investigated for possible therapeutic benefits. Both can be difficult to purify to high levels, >98%, because of the very similar products, many times differing by only one amino acid. Optimized purification techniques are required to meet these high purity demands in an economical manner. Reversed-phase chromatography, because of its high resolving power, has been the technique of choice for achieving the high level of purity necessary in the pharmaceutical industry. For industrial purification, important consideration and selection of particle size, pore size, and stationary phase in relation to the peptide can optimize purification. We illustrate how a new 150A reversed-phase media is highly effective at purifying peptides with greater loading capacity and improved productivity compared to competitive media. The media has unique selectivity that can reveal peaks masked by other C18 phases and improves resolution of closely related peptides and impurities for higher purity target peptides. The bulk media incorporates bonded phase chemistries identical to those used in analytical and prep columns, thereby assuring economical method development and reliable scale-up for preparative and process purification.

P-Th-160 **New Wide Pore Media Improves Loading Capacity and Productivity of Peptide and Protein Purification by Flash Chromatography.** Bopanna NK, Chitra Sundararajan, Melissa Wilcox, Janine Sinck, Reno Nguyen, Grace Discovery Sciences, Deerfield, IL, USA

Peptides and proteins are becoming increasingly popular for their potential use as therapeutic drugs. To earn and maintain a share in the fast-growing peptide market, peptide researchers and manufacturers are constantly trying to improve and optimize the various steps in peptide synthesis. One of the main bottlenecks in peptide synthesis is the purification step. Techniques such as FPLC and preparative HPLC are limited by small loading amounts, long separation times, poor recoveries and high costs. Here, we demonstrate that flash chromatography can be a powerful tool in the fast and efficient purification of a diverse range of peptides. A new wide pore C18 phase expands flash purification capabilities to peptides and proteins approaching 70000 MW, while providing better resolution based on the smaller particle size. We present data to show the benefits of higher loading and faster purifications in peptide purification. This rapid purification technique ensures less degradation of peptides and provides better recovery, yield and purity.

P-Th-161 **Comparing HILIC and RP for LC/MS Analysis of O-HexNAc Modified Peptides.** Barry Boyes[1], Stephanie Schuster[1], Alex Harvey[2], Ronald Orlando[3], [1]Advanced Materials Technology, Inc., Wilmington, DE, USA; [2]Glycoscientific, Inc., Athens, GA, USA; [3]University of Georgia, Athens, GA, USA

Modification of serine or threonine residues of proteins by β-D-N-acetylglucosamine (GlcNAc) has emerged as a significant biological signaling mechanism. O-GlcNAcylation of relevant sites can involve meaningful cross-talk with phosphorylation targets, both nearby and at distant sequences. For a variety of purposes, LC/MS methods to qualify the purity and identities of O-GlcNAc peptides, and similarly of O-GalNAc modified peptides, (herein referred to collectively as O-HexNAc modifications) are needed to investigate the biochemistry and biology of such modifications. Hydrophilic-interaction chromatography (HILIC) and reversed-phase chromatography (RPC) separations are compared for the ability to resolve peptide/glycopeptide pairs. Under comparable conditions of separation, RP and HILIC exhibited nearly identical peak widths, but overall HILIC separations had superior selectivity differences, leading to much better resolution. In all cases examined to date, the glycopeptide preceded peptide elution in RP, and the opposite was true in HILIC. The selectivity differences between the O-HexNAc- modified/unmodified peptide pairs is sufficiently high in HILIC, relative to RP, to lead to better predictive ability for retention of the O-HexNAc peptide, once the retention of the peptide is known. Thus, we conclude that HILIC separations of complex mixtures from protein digests may be usefully interrogated by LC/MS to uncover novel sites of protein modifications.

L-162 **Integration and Intensification of Downstream Bioprocessing based in Aqueous Two-phase Systems.** Ana Azevedo, Raquel Aires-Barros, Instituto Superior Tecnico, Lisbon, PORTUGAL

The number of biotechnology-based pharmaceuticals in the late-stage pipeline has been increasing more than ever in particular monoclonal antibodies (mAbs) represent a quarter of all biopharmaceuticals in clinical trials. As a result, there is an enhanced demand for more efficient and cost-effective processes. During the last years, the upstream technology for the production of biopharmaceuticals has been considerably improved. Continuous discoveries in molecular biology and genetics, combined with new advances in media and feed development, have significantly increased the production titers. In order to keep up this gain, it is now essential to design new, as well as to improve the existing downstream processes that remain an unresolved bottleneck. Aqueous two-phase systems (ATPS) is a potential alternative to the currently used platforms for the downstream processing

of biopharmaceuticals, which can combine a high recovery, selectivity and biocompatibility with an easy scale-up and a continuous operation mode [1,2]. Furthermore ATPS enables process integration and the large-scale processing of high quantities of biological substances [3, 4]. An innovative downstream process based on ATPS, for the purification of mAbs from a complex medium, is being designed, comprising cell separation and antibody selective extraction, envisaging process integration and intensification. This extraction based technology can also be extended for the intensification and cost reduction of production processes of other high-value biomolecules, like recombinant proteins, VLPs and vaccines, p-DNA and RNA. Microscale process techniques are used as effective tools for expediting bioprocess design in a cost-effective manner [5]. Furthermore, microfluidic devices bring high-throughput, lab-scale process optimization closer to the large-scale processes. [1] P.A.J. Rosa, I.F. Ferreira, A.M. Azevedo, M.R. Aires-Barros. "Aqueous two-phase systems: A viable platform in the manufacturing of biopharmaceuticals J. Chromatogr. A 1217, 2296-2305 (2010). [2] P.A.J. Rosa, A.M. Azevedo, I.F. Ferreira, S. Sommerfeld, W. Bäcker, M.R. Aires-Barros. "Downstream processing of antibodies: Single-stage versus multi-stage aqueous two-phase extraction", J. Chromatogr. A 1216, 8741-8749 (2009). [3] A.M. Azevedo, P.A.J. Rosa, I.F. Ferreira, M.R. Aires-Barros. "Chromatography-free recovery of biopharmaceuticals through aqueous two-phase processing", Trends Biotechnol. 27, 240-247 (2009). [4] P.A.J. Rosa, A.M. Azevedo, S. Sommerfeld, W. Bäcker, M.R. Aires-Barros. "Aqueous two-phase extraction as a platform in the biomanufacturing industry: Economical and environmental sustainability", Biotechnology Advances 29, 559–567 (2011). [5] da Silva, D.F.C., Azevedo, A.M., Fernandes, P Chu V., Conde, J.P., Aires-Barros, M.R., "Design of a microfluidic platform for monoclonal antibody extraction using an aqueous two-phase system", J. Chromatogr. A, 1249, 1-7 (2012).

L-163 **Multi-stage Enzyme Extraction using Aqueous Two-phase Systems – Experiment and Modeling.**
Axel Prinz, Katharina Koch, Tim Zeiner, TU Dortmund University, Dortmund, GERMANY
Chromatography based downstream processing of fermentative produced biomolecules is limited due to capacity as well as scale-up issues. Classical extraction as known form chemical engineering could overcome this capacity problem. Besides, it is already well known that aqueous two-phase systems (ATPS) can be applied for the extraction of biomolecules. ATPS can be formed by mixing a hydrophilic polymer and a salt above a critical concentration. Often the partitioning behavior of solutes in ATPS can be influenced by an additional solute such as sodium chloride. The major advantage of ATPS compared to aqueous-organic systems lies in the aqueous character of both phases leading to an increasing biocompatibility. Until now there exist only few works [1] investigating the aqueous two phase extraction in multi stage experiments. In the work ATPS consisting of polyethylene glycol 3000 (PEG3000) and phosphate are applied to purify the enzyme laccase from a Pleurotus sapidus (P.s.) culture supernatant from contaminating proteins in a multi-stage extraction process. In addition to the purification the separation of laccase of P.s. from laccase from Trametes versicolor (T.v.) is investigated. Using multi-stage aqueous two-phase extraction both enzymes could be separated. The extraction experiments were performed in three stages of a mixer-settler-extraction-unit with a total throughput of 0.5 kg per hour. The influence of feed position (PEG phase inlet or phosphate phase inlet) and sodium chloride concentration (0% and 2.5%) have been varied and the extraction behavior of the enzymes investigated. Depending on the mode of operation either two laccase have been separated from each other or both laccases were separated from contaminating proteins. In addition the extraction process has been modeled. In single-stage experiments partitioning influencing parameters and physical data have been measured. The correlations were implemented into a multi-stage extraction model. Finally, the results of the equilibrium based extraction model for the extraction of laccase from culture supernatant was compared to the multistage results. [1] P.A.J. Rosa, A.M. Azevedo, S. Sommerfeld, M. Mutter, W. Bäcker, M.R. Aires-Barros, Continuous purification of antibodies from cell culture supernatant with aqueous two-phase systems: From concept to process; Biotechnology Journal (2013) in press

L-164 **Purifying and Concentrating Recovery Process Samples for Recombinant Protein Quantification.**
Tanja Buch, Ian Marison, DCU, Dublin, IRELAND
Purifying and concentrating recovery process samples for recombinant protein quantification Detection and quantification of specific recombinant proteins in a protein mixture can be considered as a keystone in process characterization. Recombinant proteins are widely produced in batch and fed-batch fermentations at industrial scale using microbial cells. Besides the recombinant protein also different isoforms of the protein of interest are produced during the fermentation. Protein isoforms are different forms of the same protein which have similar characteristics but different functionality. The different isoform concentrations during fermentation and the primary recovery are known to be only a small fraction of the overall total protein concentration. Therefore it is indispensable to purify and concentrate these process samples in order to detect and quantify the recombinant protein and its isoforms. Ion-exchange chromatography is known to be a method of choice for the purification and concentration of process samples prior quantification per HPLC. Unfortunately pH settings are mandatory for this method which can change the distribution of the recombinant protein and its isoforms. Therefore a

method needed to be developed to purify and concentrate the recombinant protein and its isoforms without changing the pH or other process conditions. Primary recovery process samples were purified and concentrated using ultrafiltration devices with different cut-offs. Main challenge is the specific purification and avoidance of protein – protein interactions. The advantage of detergents which are known to disrupt hydrophobic – hydrophilic interactions was used to prevent non-specific bindings between proteins. As a result, the recombinant protein and its isoforms were able to be concentrated and successfully purified in order to detect and quantify each isoform per HPLC. Keywords: preparative purification, selective separation

L-165 **Predicting Protein Solubility and Crystallization Behavior based on the Second Osmotic Virial Coefficient.** Marcel Herhut, Christoph Brandenbusch, Gabriele Sadowski, Department of Biochemical and Chemical Engineering, Dortmund, GERMANY

The biocatalytic production of proteins has gained an increased industrial interest especially in the field of red biotechnology, where complex pharmaceuticals (e.g. monoclonal antibodies) are produced. Within these processes, the costs for downstream processing can cover up to 80 % of the total production costs. In state of the art engineering, protein purification is usually achieved by a series of cost intensive chromatographic steps. Hence the demand for other cost economic alternatives is increasing. One interesting alternative to the conventional processing is protein precipitation, which is applicable as a first capture step, and crystallization for final product polishing. In order to develop a precipitation or crystallization step, the solubility of the target protein in aqueous solutions and the crystallization behavior are crucial information. In order to supply this data, within this work, an innovative and simple technique to measure and predict the protein solubility and crystallization behavior based on the second osmotic virial coefficient (B22) is developed. The value of B22 gives an indication on the crystallization or precipitation behavior of the protein under the applied conditions. B22 herein serves as an ideal measure, as it combines important influence factors such as temperature, systems pH, type of precipitant and precipitant concentration. By relating B22 to the rational activity coefficient, the solubility of a protein in solution, consisting of solvent, protein, and additive, can be calculated. Experimental data of B22 for two proteins in water at varying conditions were measured by static light scattering. The further use of a potential-of-mean-force model (DLVO) with parameters fitted to B22 data at low additives concentration, allows for the prediction of B22 at high additives concentration. In combination with the solubility model, protein solubility can be predicted over a broad concentration range. The results were compared to experimental and literature data. The results point to the possibility of qualitative and quantitative prediction of the protein solubility in aqueous solution based on two B22 and two solubility concentrations. This model supports a cost efficient development of crystallization steps.

L-166 **Improved Identification and Quantitation of Host Cell Proteins in Protein Therapeutics using 2D-LC and Ion Mobility.** Weibin Chen, Catalin Doneanu, Keith Fadgen, Martha Stapels, Waters, Milford, MA, USA

Residual host cell proteins (HCPs) are ppm level contaminants in biotherapeutics that may elicit an unpredictable immune response and need to be monitored as part of regulatory guidelines. Data-independent analysis with 2D chromatography has been used to measure HCPs over 5 orders of magnitude in concentration (1). Adding ion mobility into this analysis inserts an orthogonal separation in the gas phase, in the millisecond timescale, between chromatographic and mass spectral analyses. In this study, 2D chromatography was combined with ion mobility to identify and quantify HCPs in biotherapeutic samples with increased throughput. Purified biotherapeutic proteins (e.g. mAbs) were spiked with standard proteins (albumin, enolase, glycogen phosphorylase, and alcohol dehydrogenase) in known amounts and then reduced, alkylated, and digested in-solution with trypsin. Samples were injected in triplicate onto a microscale liquid chromatography system and analyzed with a data-independent method using alternating low and elevated collision energy on a quadrupole time of flight instrument with ion mobility. Multidimensional chromatographic methods were employed using high-low pH RP-RP (2) with discontinuous step gradients. Standard proteins were spiked into a mAb sample (trastuzumab) at levels ranging from 8 to 1000 ppm in order to assess the ability of the system to identify and quantify proteins at typical HCP levels in a protein therapeutic. Increasing the number of LC fractions in the first dimension from 5 to 10 improved the limit of detection from 80 to 8 ppm, while doubling the analysis time. The same benefit was achieved by incorporating ion mobility into the analysis, with no additional instrument time. Ion mobility also yielded more reproducible and accurate quantitation of the lowest abundance proteins. A comparison was made between a traditional 2D method and a faster technique that utilized simultaneous gradients in both dimensions and multiple trapping columns in order to increase throughput. The faster technique took 70% of the time of the traditional 5-fraction method and the percent savings in time will increase as the number of desired fractions increases. The precision and accuracy of HCP determination will be compared and contrasted with the increase in effective peak capacity, both in the liquid and gas phases. Measurement of the HCPs in a variety of protein therapeutics will also be presented. References: 1. Schenauer, M.R. et. al. Analytical Biochemistry. 2012 (428):150-157. 2. Gilar M. et. al. J. Sep. Sci. 2005 (28):1694-1703.

L-167 **Additional Structural Insights on Therapeutic Glucocerebroside Variants and Impurities by Size Exclusion Chromatography.** John Thomas, Shire, Lexington, MA, USA
Size exclusion chromatography (SEC) is a methodology for assessing relative size distribution of species in protein therapeutics. While the standard practice is to minimize analyte interactions with the stationary phase, these interactions can be modulated to separate protein variants. Here we describe the use of SEC to separate and characterize variants of recombinant Glucocerebrosidase (GCB), a monomeric enzyme responsible for the catabolism of glucocerebroside. By enhancing the interactions between GCB and the stationary phase two GCB monomeric variants were isolated. These isolated GCB variants were then characterized by orthogonal methods. SEC was performed using a silica-based Waters Protein Pak 3000 SW column and a phosphate-sulfate buffered mobile phase (pH 6.1-6.5). A column temperature of 12.5°C was used in these studies and was one of the critical parameters for separating the GCB variants. The separation was monitored by UV and Multi-Angle Light Scattering MALS. The separated species were collected, concentrated, proteolytically digested, and analyzed by Liquid Chromatography Mass Spectrometry (LC-MS). Computational molecular models of the GCB variants were assessed using Maestro software through the BioLuminate interface. SEC analysis of GCB resolved low levels of two shoulder peaks (two GCB variants), a high-molecular weight species, as well as a large peak for the expected monomeric form. Resolution of the shoulder peaks required an unusually low column temperature along with pH adjustment and/or changes to the sulfate concentration. SEC-MALS analysis confirmed that the main peak and its shoulder peaks consist of monomeric forms of GCB. In contrast, due to the retention time of the monomeric GCB the experimentally determined molecular weight, relative to a protein standard calibration curve, resulted in an apparent molecular weight significantly lower than the known molecular weight of the GCB (ca. 62.9 kDa). This later than expected retention time suggests an interaction with the column. LC-MS peptide mapping studies revealed that the main peak was composed of unmodified GCB and the shoulder peaks were composed of oxidized forms of the molecule. The chromatographic retention suggests that non-oxidized and oxidized Cys contribute to the level of column interaction. Molecular modeling illustrated differences in the hydrophobic protein surface, suggesting potential regions for protein to column interactions. The cumulative data from this study reveals an apparent relationship between protein variants and the SEC elution characteristics for GCB.

L-168 **Impact of Plasmid Size on the Purification of Model pDNA Vaccines by HIC on Phenyl Membrane Adsorbers.** Luis Raiado-Pereira, Jonathan de la Vega, D. Miguel F. Prazeres, Marilia Mateus, Instituto Superior Tecnico, Lisbon, PORTUGAL
Plasmid DNA offers a versatile platform for development of new pharmaceuticals able to target a myriad of diseases. This versatility also adds in variability among plasmid products most of the times sharing only the same basic molecular structure. This work highlights the existence of different interactions strengths among differently sized plasmids and their respective isoforms with HIC membrane adsorbers. These were exploited to increase the purity of supercoiled pDNA isoform by optimization of the purification strategies on a membrane chromatography performed with Sartorius® Phenyl 3 mL spiral cartridge. The relative strengths of binding of the different isoforms were studied using stepwise elution strategy of decreasing buffer conductivities. Open circular (OC) isoforms of both 3.70 kbp and 6.05 kbp plasmids are eluted earlier with a conductivity difference of 16.0 mS/cm and 22.5 mS/cm, respectively, than their supercoiled (SC) counterparts. Furthermore, the relative binding strengths of the OC over SC isoforms were 1.09 and 1.14 for respective plasmids. The isoforms of the bigger pCEP4 plasmid (10.4 kbp) did not display this characteristic binding difference, hindering the reduction of OC pDNA in the final plasmid formulation. Nonetheless, the purified and pooled plasmid solutions were assayed and demonstrated high degree of purity, compliant with regulatory agencies criteria: over 99% RNA removal, endotoxin levels below 20 EU/mg pDNA and undetectable protein content by BCA assay. The overall step yield was above 85% for all plasmids. The pooled fractions of the 3.70 kbp pVax1/GFP were further concentrated and diafiltrated through a 50 kDa centrifugal membrane device and used in a successful CHO cell transfection (88% ± 8% efficiency), which consolidates the membrane HIC polishing methodology.

L-169 **Protein Biomarker Assays for Drug Safety Assessments.** Jennifer Colangelo, Pfizer, Groton, CT, USA
Safety biomarkers are an integral part of the decision-making process for drug development at all stages, aiding in compound selection for early pre-clinical studies and ensuring patient safety in clinical trials. Recently, many novel proteins have been proposed as potential markers of disease states, so the demand for robust, quantitative assays has increased as more and more proteins are being investigated and deployed as biomarkers. Biomarker assays for proteins are often built off of antibody technologies, which have benefits and disadvantages. The challenge comes in finding reagents for novel biomarkers or for multiple preclinical species. Sometimes reagents may not be robust, may demonstrate lot to lot differences, or may not be available on a consistent basis. For these reasons, other technologies, such as chromatography and mass spectrometry, have been employed to fill this gap. For LC-MS assays, specific antibodies are not necessary to develop a

quantitative assay; sensitivity is on par with antibody based platforms; and these platforms are now found in clinical hospital laboratories and other similar settings. These technologies provide another advantage in that they are often used for proteomic experiments, which then provide starting conditions for assay development, streamlining the process. This presentation will focus on analytical methodologies in mass spectrometry for protein analysis that do not require highly specific antibodies and that are amenable to translation between pre-clinical and clinical models. The advantages and disadvantages of applying these techniques to biomarker development, qualification, and deployment will be discussed. The examples provided will focus on toxicity biomarker applications in the drug development process.

L-170 **High Temperature LC-MS of Permethylated N-Glycans Derived from Breast Cancer Cells and Human Blood Serum**. Shiyue Zhou[1], Yunli Hu[1], Tarek Shihab[1], Ahmed Hussein[2], Yehia Mechref[1], [1]Texas Tech University, Lubbock, TX, USA; [2]University of Alexandria, Alexandria, EGYPT
Permethylation is currently considered the most routine derivatization methods in MS-based glycomics. This derivatization improves the ionization efficiency of glycans and allows separation on reversed-phase liquid chromatography (RPLC). However, RPLC separation of permethylated glycans at ambient temperatures is not efficient, partially because of the low mass transfer between stationary phase and mobile phase. This is limited by the intermolecular interactions among the methyl groups present on permethylated glycan branches. Performing the RPLC separation at high temperatures (45-75oC) improved separation efficiency and selectivity. Better peak symmetry was observed at the elevated temperatures. For example, the peak asymmetry factor of Man9 glycan improved from 3.63 to 1.19 as the column temperature increased from ambient to 55oC. The chromatographic resolving power was also improved at high temperatures, especially in the case of biological samples. This was especially true for high molecular-weight branched glycans derived from human blood serum samples. The peak width decreased significantly at higher temperatures with no loss in peak area. For example, the triantennary monosialylated glycan has isomeric peak overlapping at ambient temperature under optimized gradient elution conditions. However, all isomers were resolved at temperatures higher than 45oC. The resolution of isomeric peaks at ambient temperature was only 0.58, which was much lower than the 1.81 achieved at 55 oC. Improved resolution reduces peak overlapping, which diminishes competitive ionization in an ESI source and generates more reliable quantitative data. This high temperature LC-MS of permethylated glycans permitted the isomeric separation of glycans derived from different breast cancer cells, thus helping in better understanding breast cancer brain metastasis.

L-171 **Separations of Intact Glycoproteins by HILIC.** Barry Boyes[1], Ron Orlando[2], Joseph DeStefano[1], [1]Advanced Materials Technology, Inc., Wilmington, DE, USA; [2]University of Georgia, Athens, GA, USA
HILIC separations of intact proteins and protein mixtures has had surprisingly limited application. The examples of HILIC protein separations reported to date use combinations of stationary and mobile phases that do not demonstrate general utility for separating a broad variety of proteins. In part this may be due to solubility problems with the high concentrations of organic modifiers typical for HILIC separations, and may also result from the application of consensus conditions favored for reversed-phase separations, such as the use of dilute trifluoroacetic acid or formic acid mobile phases with water/acetonitrile mixtures. Improved separations of intact proteins are obtained using acidic buffered gradient elution conditions with a HILIC column packing material prepared using a highly hydroxylated bonded-phase on wider pore superficially porous Fused-Core silica particles. With appropriate conditions for gradient elution, high resolution separations of proteins and glycoproteins are achieved. The selected conditions permit on-line mass spectral (MS) analysis of intact proteins. Consistent with recent observations in our studies of glycosylated peptides, protein retention in HILIC is strongly affected by glycosylation. In some instances, separations of intact protein glycoforms can be obtained, with selectivity driven by the extent and nature of protein-linked glycan structure(s). The ability to recover specific protein glycoforms may permit insights on the structure-function relationships of the glycan portion of glycoproteins.

L-172 **Progress Towards Automated Sequencing of Heparin/Heparan Sulfate.** Joshua S. Sharp, Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA
Heparin/heparan sulfate (HS) is a linear polysaccharide that is heterogeneously modified by O- and N-sulfation, as well as by N-acetylation. Changes in the patterns of sulfation has been implicated in a wide variety of developmental and disease processes. To fully understand the regulation of HS modification and the biological function of HS through its interactions with protein ligands, it is essential to know the specific HS sequences present. However, the sequencing of mixtures of HS oligosaccharides presents major challenges due to the lability of the sulfate modifications, as well as difficulties in separating isomeric HS chains. Here, we apply a sequential chemical derivatization strategy involving permethylation, desulfation and trideuteroperacetylation to label original sulfation sites with stable and hydrophobic trideuteroacetyl groups. The derivatization chemistry differentiates between all possible heparin/HS sequences solely by glycosidic bond cleavages, without the need

to generate cross-ring cleavages. A method for sequencing complex heparin/HS oligosaccharide mixtures from affinity purification strategies using a derivatization strategy coupled with data-dependent LC-MS/MS was developed and applied to Robo1, a heparin/HS binding protein involved in neuronal development. We also report the ongoing development of an automated data analysis package for interpretation of LC-MS/MS data from our derivatization scheme.

L-173 **HILIC-MS of Glycans and Glycopeptides.** Joseph Zaia, Center for Biomedical Mass Spectrometry, Boston University, Boston, MA, USA

Heparin, a member of the glycosaminoglycan (GAG) family of polysaccharides, is a $ multibillion dollar anticoagulant drug. Heparan sulfate, a closely related GAG, is expressed on every animal cell surface as well as in extracellular matrices. Low molecular weight heparins are pharmaceutical products that have been engineered for use to treat specific clinical indications including deep vein thrombosis. Because several of these products are now off patent, there has been an effort to develop generic low molecular weight heparin products. Two such products have been approved by the U.S. Food and Drug Administration, and others are under development. This activity demonstrates a strong need for effective means of analyzing heparin and other GAGs for pharmaceutical products. Effective analytics enable reverse engineering of complex biologic drugs pursuant to the development of generics. Such generics are expected to reduce costs to the public. One of the key steps in heparin analysis is the determination of the composition with respect to disaccharide repeating units. This task is made complicated by the fact that there are several isomeric heparin disaccharides. The analytical challenges are such that neither liquid chromatography alone or mass spectrometry alone is capable of fully characterizing GAG disaccharides. This is because some disaccharides are not easily resolved chromatographically and others cannot be differentiated directly using MS or tandem MS. As a result, there is clear need for LC-MS methods for disaccharide analysis. This presentation will summarize the state-of-the-art in GAG disaccharide analysis, with emphasis given to emerging LC-MS methods. Results acquired using graphitized carbon chromatography and hydrophilic interaction chromatography in LC-MS methods will be compared and contrasted.

L-174 **Hypothesis Driven Glycomics.** Shujuan Tao[1], Yining Huang[1], Barry Boyes[2], Ron Orlando[1], [1]Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA; [2]Advanced Materials Technology, Inc., Wilmington, DE, USA

Changes in glycan structures have been shown to occur with the state/condition of the cell in which the proteins are produced. For example, it has been known for over fifty years that tumor cells attach different N-linked glycans than the corresponding “normal” cells from the same tissue/organ.

A particularly challenging aspect is the identification and quantitation of low abundance glycans that are present as minor components in complex isomeric mixtures. We have developed an approach, which we call Hypothesis Driven Glycomics, to overcome these limitations. Basically, this approach is the reverse of the typical glycomics workflow, which focuses on the identification of all glycans present. In Hypothesis Driven Glycomics, one asks if glycans with selected masses are present in the sample being analyzed. This is possible because there appears to be a finite number of glycan masses, particularly true for N-linked glycans which are predominately composed from five monoscaharides if one excludes stereochemistry, i.e., hexoses, de-oxy hexoses, N-acetylated hexoses, N-Acetylneuraminic acid, and N-Glycolylneuraminic acid. By combining these five different molecular weights with the biosynthetic pathways by which these glycans are created, one finds that only certain glycan masses are possible. A second key to Hypothesis Driven Glycomics was the development of a model that allows glycan retention times to be accurately predicted from their monosaccharide composition. The final step was the development of a method to predict precursor and fragment ion transitions. Combined these allowed us to develop a selected reaction monitoring (SRM) LC-MS/MS experiment for every currently known N-linked glycan composition. An SRM approach was selected because it has a lower limit of detection, larger dynamic range that other MS approaches, and is more amenable to quantitation. The focus of this presentation will be to highlight this approach, to demonstrate its utility for the detection and quantitation of individual glycans in complex mixtures.

Author Index

L = lecture P = poster W = Wednesday Th = Thursday

A

Abbott, M	P-W-114
Abbott, T	L-108
Aguilar, P	L-137
Aguilar, P	P-Th-153
Ahmadian, H	P-W-121
Aires-Barros, M R	P-Th-143
Aires-Barros, M R	P-Th-144
Aires-Barros, M R	L-162
Amari, J V	L-167
Amster, J	L-172
Anderson, S	P-Th-159
Angarita, M	P-Th-155
Arnold, M	L-134
Astarita, G	P-Th-147
Aumann, L	P-W-123
Azadi, P	L-172
Azevedo, A	L-162
Azevedo, A M	P-Th-143
Azevedo, A M	P-Th-144

B

Basch, J	P-W-114
Basumallick, L	L-130
Baur, D	P-Th-155
Bavand, M	P-W-123
Bell, D	P-Th-154
Bereman, M	L-106
Berger, S	L-132
Bittermann, H	L-134
Blackburn, R	L-136
Borchert, S O	P-W-120
Boyes, B	L-171
Boyes, B	L-174
Boyes, B	P-Th-152
Boyes, B	P-Th-161
Boyes, B	P-W-127
Boyes, B	P-W-128
Brandenbusch, C	L-165
Brandes, H	P-Th-154
Brandes, H	P-W-113
Brueck, H	L-133
Buch, T	L-164
Burdette, C Q	L-104

C

Cajic, S	L-135
Carbonell, R	L-136
Carvalho Jr, R	P-Th-144
Chakrabarti, A	P-Th-148
Chakraborty, A	L-132
Chavali, A	P-W-118
Chen, W	L-132
Chen, W	L-166
Chen, W	P-Th-147
Chen, W	P-W-115
Chiu, Y	L-172
Chung, L	L-108
Coffey, A	P-Th-156
Colangelo, C	L-108
Colangelo, J	L-169
Condac, E	L-172
Conze, W	L-133
Cornelissen, G	L-138
Cornelissen, G	P-Th-149
Cornelissen, G	P-W-117
Cornelissen, G	P-W-120
Corso, T	L-106
Cramer, S M	P-Th-144

D

DeStefano, J	L-171
Destefano, J	P-Th-152
Destefano, J	P-W-122
Dhillon, H	P-W-114
Dias-Cabral, C	L-137
Dias-Cabral, C	P-Th-153
Dias-Cabral, C	P-W-119
Doneanu, C	L-166
dos Santos, R	P-Th-143
Dukart, A	P-W-117
Duong, P	P-Th-157
Eksteen, R	P-Th-154
Eksteen, R	P-W-113

F

Fadgen, K	L-166
Fague, K	L-105
Fishpaugh, J	P-W-110
Fountain, K	P-W-116
Franklin, E	L-105

G

Garmer, S F	P-Th-146
Gilar, M	P-W-129
Glajch, J	L-131
Godhino, J	L-105

H

Hanley, T	P-Th-151
Hardy, M	L-167
Harvey, A	P-Th-161
Havelund, S	P-Th-146
Hearn, M	L-139
Heiss, C	L-172

Hennig, R L-135
Herhut, M L-165
Hirose, T P-W-126
Hodges, R L-102
Houel, S L-132
Hu, Y L-170
Huang, C P-Th-150
Huang, R L-172
Huang, Y L-174
Huang, Y P-W-127
Hurum, D L-130
Hussein, A L-170
Hvass, A P-Th-146

I

Isaac, G P-Th-147
Ishihara, M L-172
Jahnke, F L-109
Jensen, S L-138
Jensen, S P-Th-149
Johnson, B P-W-122
Johnson, D P-W-128
Jorgenson, J L-105
Jungbauer, A L-142

K

Kailemia, M J L-172
Khatri, K L-173
Kimata, K P-W-126
Kirkland, J P-Th-152
Kirkland, J P-W-122
Koch, K L-163
Koza, S P-W-116

L

Langridge, J P-Th-147
Lauber, M P-W-116
Laufer, S L-135
Leymarie, N L-173
Li, Y L-131
Lievrouw, R P-Th-155
Lissens, G P-Th-155
Liu, J L-172
Lu, X P-Th-154
Lubman, D L-107

M

MacCoss, M L-106
Manard, B T L-104
Mant, C L-102
Marcus, R K L-104
Marison, I L-164
Marques, F L-137
Marques, F P-W-119
Mårtensson, M P-Th-146
Martin, R P-Th-147
Martosella, J P-Th-156
Martosella, J P-Th-157
Martosella, J P-W-124
Mateus, M L-168
McConville, P P-W-118
McCreary, D P-Th-159
Mechref, Y L-170
Meininger, M L-135
Mellors, S P-W-129
Menegatti, S L-136
Morbidelli, M P-Th-155
Mudaliar, Y P-Th-151
Mueller, E L-133
Muller-Spath, T P-Th-155
Muller-Spath, T P-W-123
Mündges, J L-140
Murphy, J P-W-129

N

Nairn, A L-108
Nakamura, K A P-Th-144
Neumann, T L-134
Ng, P K P-W-111
Nguyen, R P-Th-159
Nguyen, R P-Th-160
NK, B P-Th-160

O

O'Donnell, J K P-Th-150
Ono, T P-W-126
Orlando, R L-171
Orlando, R L-174
Orlando, R P-Th-161
Orlando, R P-W-127
Orlando, R P-W-128

P

Panek, M P-W-114
Paul, J L-138
Paul, J P-Th-149
Paul, J P-W-117
Paul, J P-W-120
Pierson, M L-104
Pires, F P-Th-153
Prazeres, D M F L-168
Prinz, A L-163

R

Raiado-Pereira, L L-168
Ramsay, C P-W-110
Ramsey, J M P-W-129
Rapp, E L-135
Rathore, A L-132
Reichl, U L-135
Rohrer, J L-130
Roman, G P-W-129
Rosa, S A S L P-Th-143
Rose Bjelke, J P-W-121
Ross, P L-109
Rotering, H L-135

S

Saar, J	P-Th-159
Sadowski, G	L-165
Sakaue, F	L-108
Salinas, P	L-167
Santos da Silva, F V	L-135
Savickas, P	L-167
Schadock-Hewitt, A	L-104
Schneider, S	P-Th-158
Schreiber, S	P-W-120
Schure, M	L-101
Schuster, S	P-Th-152
Schuster, S	P-Th-161
Schuster, S	P-W-122
Shao, C	L-173
Sharp, J S	L-172
Shi, S	L-140
Shifman, M	L-108
Shihab, T	L-170
Shinkazh, O	L-141
Shinkazh, O	P-W-125
Shion, H	L-132
Silva, G	P-W-119
Sinck, J	P-Th-159
Sinck, J	P-Th-160
Skibstrup, D	P-W-121
Snyder, M A	P-W-111
Stapels, M	L-166
Steensgaard, D B	P-Th-146
Steve, J	P-Th-148
Stobaugh, J	L-105
Strohlein, G	P-Th-155
Strohlein, G	P-W-123
Sundarajan, C	P-Th-159
Sundararajan, C	P-Th-160

T

Tang, N	P-W-124
Tao, S	L-174
Tao, S	P-W-127
Thomas, J J	L-167
Thrash Jr., M	L-137
Thrash Jr., M	P-Th-153
Thrash Jr., M	P-W-119
Tiainen, P	P-W-121
Tieman, B	P-W-110
Tseng, K	P-W-126
Tu, B	P-W-110

U

Ulmer, N	P-W-123

V

Vajda, J	L-133
Van Pelt, C	L-106
Varghese, J	L-167
Vega, J	L-168

W

Wagner, B	P-Th-152
Wang, L	L-172
Wang, Q	L-173
Ward, K	L-136
Wei, B T	L-103
Wheat, T E	P-W-118
Wiesmüller, K-H	L-135
Wilcox, M	P-Th-160
Williams, K	L-108
Wirth, M	L-103
Wolff, M	L-135
Woo, J	P-Th-144
Woon, N	P-W-112
Wu, Z	L-103

Y

Yan, G	L-167
Yu, Y Q	P-W-115
Yuan, L-M	P-Th-145

Z

Zaia, J	L-173
Zeiner, T	L-140
Zeiner, T	L-163
Zeng, G-Y	P-Th-145
Zhang, X	L-103
Zhao, C	P-W-110
Zhou, S	L-170
Zhu, A	P-Th-157
Zhu, A	P-W-124
Zhu, J	L-107
Zhu, R-R	P-Th-151
Ziemann, R	P-W-110
Zuehlke, J	P-W-117
Zwanziger, F	L-135