17th International Conference on

Biopartitioning & Purification

BPP 2013 ~ October 6 - 10, 2013

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Organization & Committees

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Frank Riske
James Van Alstine

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Welcome to BPP 2013

We are delighted to welcome you to Newport and the 17th International Conference on Biopartitioning and Purification (BPP2013). It has been two years since Marco Rito-Palomares, Juan Asenjo and Todd Przybycien organized BPP 2011 in Puerto Vallarta, Mexico. The success of that conference added to the challenge posed by the thirty-year series of BPP conferences to match the quality of the program and the venue for the next conference. We thank Marco, Juan, and Todd for their encouragement and advice, as well as several other former BPP Conference Chairs.

The first "BPP" meeting was held in Duarte, California, in 1979 and was organized by Harry Walter, Steven Flanagan and Göte Johansson. In 1981 Tim Treffrey organized the second meeting in Sheffield, England. Subsequent BPP conferences have followed the structure and flavor of those first two events. Following on its successful predecessors BPP 2013 covers a very broad range of bioseparation phenomena and downstream processing issues. We hope this diversity of topics and interests, bridged by our common scientific curiosity, will stimulate extensive discussion in the meeting sessions and beyond.

We are grateful to many people for support, in cash and in kind, that has made the conference not only possible, but also affordable - especially for students. Seed funding was provided by BPP 2011 and further funding from numerous industrial, government and academic sponsors who are recognized on the inside front cover of this program; please thank their representatives who are present at the conference. The members of the Organizing and Scientific Committees, also listed in the program, provided critical input in the form of reviews of the submitted abstracts, which ensured a high-quality scientific program. Last but not least, extensive administrative support in taking care of innumerable organizational matters was provided by University of Delaware staff, especially Cinda Younce, who will be at the registration desk during the conference.

Again, welcome to Newport, and thanks for joining us. We look forward to an exciting conference and to handing the BPP reins over to Raquel Aires-Barros, who will chair BPP 2015 in Portugal.

Jim Van Alstine
Frank Riske
Bramie Lenhoff

General Information

Venue
A floor plan of the Hyatt Regency Newport Hotel and Spa is on the next page. The oral sessions will be held in Grand Ballroom A-B and most meals in the Rose Island Room. The conference registration fee includes all meals and refreshment breaks.

Oral Presentations
Presenters should be available 30 minutes before the start of the morning or afternoon session to load their PowerPoint files onto the conference laptop and to confirm compatibility of the files.

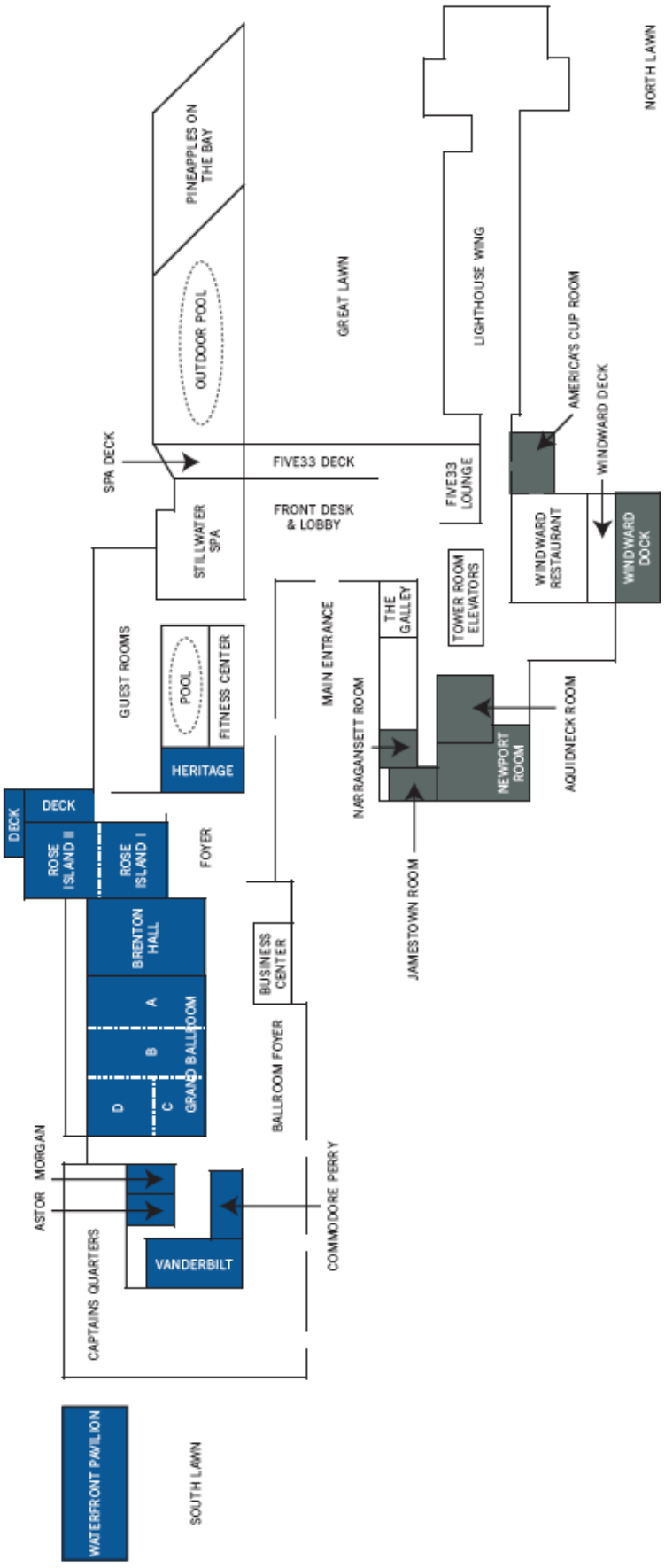
Poster Sessions
Posters should be mounted for display no later than mid-morning on Monday and should be displayed until at least 5 p.m. on Wednesday; all posters should be removed before Thursday morning. Posters should be mounted in the locations indicated by the numbers on the boards, which correspond to those in the program. Poster sessions will be held on Monday and Tuesday evenings and presenters are encouraged to be at their posters for at least a substantial portion of each session. Refreshments (including dinner on Monday and dessert on Tuesday) will be available at both sessions. In addition, each poster presenter will make a brief oral presentation summarizing the main points of the poster in the oral sessions immediately before and after lunch on Monday.

Registration Desk
The registration desk is located in the foyer in front of the Grand Ballroom. The desk will be open during the following hours:

Sunday, October 6	3:00 p.m. – 8:00 p.m.
Monday, October 7	8:15 a.m. – 6:00 p.m.
Tuesday, October 8	8:15 a.m. – 1:00 p.m.
Wednesday, October 9	8:15 a.m. – 4:00 p.m.
Thursday, October 10	8:15 a.m. – 11:00 a.m.

Notice Board
The notice board near the registration desk may be used for messages and postings of positions available or sought.

Recreational Activities
No conference activities are scheduled for Tuesday afternoon. Sign-up sheets will be available at the registration desk for possible group outings during this time. Please sign up no later than Monday lunchtime to facilitate arrangements.



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Sunday, October 6

15:00 – 20:00	Registration
15:00 – 20:00	Poster Set Up
18:00 – 20:00	Welcome Reception and Orientation
20:00 – 20:45	Introduction to Newport Caitlin Emery, Research and Interpretation Coordinator The Preservation Society of Newport County
21:00 – 22:00	Informal Poster Viewing (author attendance optional)

Monday, October 7

7:30 – 8:30	Breakfast
8:30 – 8:45	Welcome from Chairs and Outline of Program
8:45 – 11:10	***Oral Presentation Session 1: Cell Separation and Primary Recovery***
8:45 – 8:50	Introduction to Session from Session Chair, Raquel Aires-Barros
8:50 – 9:30	O-101 **Investigation of alternative separation technologies for the harvest of CHO cell culture.** Robert Piper[1], Ganesh Vedantham[1], António Grilo[2], Karol Lacki[3], and James Van Alstine[3]; Purification Process Development, Amgen Inc.[1], Instituto Superior Técnico (IST) Lisbon[2], GE Healthcare Bio-Sciences[3]
9:30 – 9:55	O-102 **Bioengineering strategies for the establishment of a novel process for the primary recovery of stem cells exploiting aqueous two-phase systems.** Mirna González-González and Marco Rito-Palomares, Centro de Biotecnología-FEMSA, Tecnológico de Monterrey. Campus Monterrey, Monterrey, MEXICO
9:55 – 10:20	Coffee Break

10:20 – 10:45 O-103 **Processing of high-density mammalian cell culture with Expanded Bed Adsorption Technology.** E.J.A.X. van de Sandt[1], P. den Boer[2]; DSM Biotechnology Center, The Netherlands[1], DSM Biologics, The Netherlands[2]

10:45 – 11:10 O-104 **Second-generation expanded bed adsorption.** Marcelo Fernández-Lahore[1], Roy N. D'Souza[1], Jürgen Fritz[1], and Rami Reddy Vennapusa[2]; Jacobs University Bremen[1], Shanta Biotechnics (Sanofi)[2]

11:15 – 12:15 ***Poster Oral Summary Session A (3 minutes each x 18 posters)***
Session Chair, Frank Riske

12:15 – 13:45 Lunch

13:45 – 15:00 ***Poster Oral Summary Session B (3 minutes each x 19 posters)***
Session Chair, Frank Riske

15:00 – 17:35 ***Oral Presentation Session 2: Molecular Design of Bioseparations***
15:00 – 15:05 Introduction to Session from Session Chair, Leif Bülow

15:05 – 15:30 O-201 **Unified super-resolution experiments and chromatographic theory reveal charge-clustering dominates protein ion exchange adsorption.** Lydia Kisley[1], Jixin Chen[1], Andrea P. Mansur[1], Bo Shuang[1], Katerina Kourentzi[2], Mohan-Vivekanandan Poongavanam[3], Wen-Hsiang Chen[2], Sagar Dhamane[3], Christy F. Landes[1] and Richard C. Willson[2, 3, 4]; Department of Chemistry, Rice University, Houston, TX[1]; Department of Chemical & Biomolecular Engineering, University of Houston, Houston, TX[2]; Department of Biology & Biochemistry, University of Houston, Houston, TX[3]; The Methodist Hospital Research Institute, Houston, TX[4]

15:30 – 15:55 O-202 **Structure-based design of purification process for a new rFVIII product.** Haleh Ahmadian[1] and Camilla Kornbeck[2]; Dept. of Protein Purification Technology, Novo Nordisk[1], Dept. of Protein Separation and Virology, Novo Nordisk[2]

15:55 – 16:20 Coffee Break

16:20 – 16:45 O-203 **Novel affinity pairs "tag-receptor" for the purification of fusion proteins.** Ana Sofia Pina[1], Christopher R. Lowe[2] and A. Cecília A. Roque[1]; REQUIMTE, FCT-UNL[1], University of Cambridge[2]

16:45 – 17:10 O-204 **RAPID selection of aptamers via multi-target microcolumn chromatography.** David Latulippe[1], Kylan Szeto[2], Abdullah Ozer[2], John Lis[2] and Harold Craighead[2]; McMaster University[1], Cornell University[2]

17:10 – 17:35 O-205 **Mutagenesis Objective Search and Selection Tool (MOSST) applied to engineer protein mutants with enhanced purification properties.** A. Olivera-Nappa, J.A. Asenjo and B.A. Andrews; Centre for Biochemical Engineering and Biotechnology, Department of Chemical Engineering and Biotechnology, University of Chile, Santiago, CHILE

18:30 – 21:00 Dinner and Poster Viewing
(Poster authors should be present for as much of the session as possible.)

Tuesday, October 8

7:30 – 8:30 Breakfast

8:30 – 11:20 ***Oral Presentation Session 3: Non-Chromatographic Separations***
8:30 – 8:35 Introduction to Session from Session Chair, Todd Przybycien

8:35 – 9:15 O-301 **Smart biopolymer affinity precipitation for mAb purification: methods development and process considerations.** Steven M. Cramer[1], Rahul Sheth[1], Mi Jin[2] and Bharat Bhut[2]; Rensselaer Polytechnic Institute[1], Bristol-Myers Squibb[2]

9:15 – 9:40 O-302 **Aqueous two-phase systems for protein separation: review and state of the art.** J.A. Asenjo and B.A. Andrews; Centre for Biochemical Engineering and Biotechnology, Department of Chemical Engineering and Biotechnology, Institute for Cell Dynamics and Biotechnology: a Centre for Systems Biology, University of Chile, Santiago, CHILE

9:40 – 10:05 O-303 **Aqueous two phase system based on ionic liquids for extracting biomolecules.** Rupali Desai[1], Michel Eppink[1, 2], Mathieu Streefland[1] and Rene Wijffels[1]; Wageningen University Netherlands[1], Synthon B.V., THE NETHERLANDS[2]

10:05 – 10:30 Coffee Break

10:30 – 10:55 O-304 **A novel approach for mAbs bioprocessing: clarification and capture by aqueous two-phase extraction.** Ana M. Azevedo[1], Marta F.F. da Silva[1] and M. Raquel Aires-Barros[1]; IBB, Instituto Superior Técnico, Lisbon, PORTUGAL[1]

10:55 – 11:20	O-305 **Scalable protein separation by selective coacervation.** Paul Dubin; Chemistry, University of Massachusetts - Amherst, Amherst, MA, USA
11:20 – 11:25	Pause
11:25 – 12:45	***Oral Presentation Session 4: Analytics***
11:25 – 11:30	Introduction to Session from Session Chair, Karol Lacki
11:30 – 11:55	O-401 **A novel microfluidic platform for high-speed process analytical technology for biotherapeutics characterization.** Charles Haynes; University of British Columbia, CANADA
11:55 – 12:20	O-402 **Cholesterol level and particle distribution as predictors of cell culture flocculation and filterability performance.** Anna Senczuk, Anne Thomas, Rob Piper, Tom McNerney, Krista Petty, John Moscariello and Yinges Yigzaw; Amgen
12:20 – 12:45	O-403 **High throughput characterization of proteins 3D structures in solution.** Larissa Mikheeva[1], Luisa Ferreira[1], Pedro Madeira[3], Leonid Breydo[2], Vladimir N. Uversky[2], Arnon Chait[1], Boris Zaslavsky[1]; Analiza, Inc., USA[1], University of South Florida, USA[2], University of Porto, PORTUGAL[3]
12:45	Box Lunch
12:45 – 18:00	Afternoon Free
18:00 – 20:00	Clambake in Waterfront Pavilion (weather permitting)
20:00 – 22:00	Free Time and Poster Viewing (Poster authors should be present for as much of the session as possible.)

Wednesday, October 9

7:30 – 8:30	Breakfast
8:30 – 9:30	***Oral Presentation Session 5: Modeling***
8:30 – 8:35	Introduction to Session from Session Chair, Abraham Lenhoff
8:35 – 9:00	O-501 **Mass transfer and kinetics of protein renaturation applied in μ- and lab scale.** Cornelia Walther[1], Gerhard Sekot[1], Rainer Hahn[1,2], Alois Jungbauer[1,2], and Astrid Dürauer[1,2]; ACIB Austrian Centre of Industrial Biotechnology[1], University of Natural Resources and Life Sciences Vienna, Muthgasse VIENNA [2]
9:00 – 9:25	O-502 **Modeling of gradient chromatofocusing in ion exchange chromatography.** Michael Schmidt and Christian Frech; University of Applied Sciences Mannheim, GERMANY
9:25 – 9:30	Pause
9:30 – 11:45	***Oral Presentation Session 6: Novel Approaches, Targets and Materials***
9:30 – 9:35	Introduction to Session from Session Chair, Herb Lutz
9:35 – 10:00	O-601 **Microparticles for continuous capture of proteins.** Rainer Hahn[1,2] Alexander Trefilov[2], Moritz Imendoerffer[2] and Alois Jungbauer[1,2]; Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Muthgasse VIENNA[1], Austrian Centre of Industrial Biotechnology, Muthgasse VIENNA[2]
10:00 – 10:25	Coffee Break
10:25 – 10:50	O-602 **Biomagnetic nanomaterials for high gradient magnetic affinity separation.** Gil Lee[1], Conor Fields[1], James O'Mahony[1], Victoria Matias[1], Peng Li[1], Loraine M. Smith[1], and Julien Muzard[2]; School of Chemistry and Chemical Biology, University College Dublin, IRELAND[1], and Conservatoire National des Arts et Métiers, Paris, FRANCE[2]
10:50 – 11:15	O-603 **Investigation of protein partitioning in tunable aqueous polymer phase impregnated resins**. Fatma van Winssen, Juliane Merz and Gerhard Schembecker; Laboratory of plant and process design, TU Dortmund, Dortmund, GERMANY

11:15 – 11:40	O-604 Mu**ltimodal chromatography for DNA separation and purification.** Tiago Matos[1,2], João A. Queiroz[2] and Leif Bülow[1]; Pure and Applied Biochemistry, Lund University, SWEDEN[1], University of Beira Interior, Covilhã, PORTUGAL[2]
11:40 – 11:45	Announcements
11:45 – 13:30	Lunch
13:30 – 16:00	***Oral Presentation Session 7: Process Development Including HTPD***
13:30 – 13:35	Introduction to Session from Session Chair, Arne Staby
13:35 – 14:15	O-701 **High throughput process development – a door opener?** Jürgen Hubbuch; Institute of Process Engineering in Life Sciences, Karlsruhe Institute of Technology, Karlsruhe, GERMANY
14:15 – 14:40	O-702 **High throughput bio purification process development – a hybrid approach.** M. Ottens; Department of Biotechnology, Delft University of Technology, THE NETHERLANDS
14:40 – 15:05	Coffee Break
15:05 – 15:30	O-703 **High throughput optimization approach for single step polishing of monoclonal antibodies following Protein A capture.** Aleksandar Cvetkovic[1], Amit Kundu[2] and Rene Gantier[1]; Pall Life Science[1], Genmab MN, Inc.[2]
15:30 – 15:55	O-704 **Overload and elute chromatography for enhanced MAb purification.** Deepa Nadarajah, Steve Hohwald, Debola Banerjee, Kevin Shomglin, Rob van Reis, Rick St. John, Amit Mehta; Genentech Inc., San Francisco, CA, USA
15:55 – 16:00	Announcements
16:00 – 17:00	Break and Poster Viewing
17:00 – 18:00	Open Business Meeting
18:00 – 19:00	Free Time and Poster Take Down
19:00 – 21:00	Dinner

Thursday, October 10

7:30 – 8:30	Breakfast
8:30 – 10:45	***Oral Presentation Session 8: Continuous and Integrated Processing***
8:30 – 8:35	Introduction to Session from Session Chair, Phil Lester
8:35 – 9:15	O-801 **New technologies enabling an integrated mAb manufacturing process with cost and operational advantages.** Michael W. Phillips, EMD Millipore
9:15 – 9:40	O-802 **Continuous Countercurrent Tangential Chromatography using Protein A for Initial Capture of a Monoclonal Antibody.** Andrew Zydney[1], Oleg Shinkazh[2], Boris Napadensky[2], Travis Tran[2], and Achyuta Teella[2]; Penn State University[1], Chromatan, Inc.[2]
9:40 – 10:05	O-803 **Continuous chromatographic technology aimed at vaccine applications using core bead chromatography for reduction of ovalbumin impurities.** Hans Blom, Günter Jagschies and Karol Łącki; GE Healthcare Life Sciences, Uppsala, SWEDEN
10:05 – 10:30	O-804 **Purification of adenovirus by 2-column periodic countercurrent chromatography.** Piergiuseppe Nestola[1,3], Ricardo Silva[2], Cristina Peixoto1, Paula M. Alves[1, 3], Manuel Carrondo[1,2,3] and José Paulo Mota[2]; IBET - Instituto de Biologia Experimental e Tecnológica[1], Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa[2], Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa[3]
10:30 – 10:45	Conference Closing

Posters

P-101 **Continuous biological products extraction in aqueous two phase systems.** Edith Espitia-Saloma, Olga Patricia Vázquez-Villegas, Marco Antonio Rito-Palomares and Oscar Alejandro Aguilar-Jiménez, Centro de Biotecnología-FEMSA, Tec de Monterrey, Campus Monterrey, MEXICO

P-102 **Quantitative structure-property relationship for odorants by partitioning in aqueous two-phase systems.** Pedro P. Madeira [1], Ana Bessa[1], Miguel A. Teixeira[1], Luís Álvares-Ribeiro[1], Alírio E. Rodrigues[1], and Boris Zaslavsky[2], University of Porto, PORTUGAL[1], Analiza, Inc., USA[2]

P-103 **Development of a method for purifying trypsin that combines precipitation of protein-polyelectrolyte complexes with hydrophobic interaction chromatography.** Mauricio Braia[1], Gisela Tubio[1], Barbara Andrews[2], Oriana Salazar[2], María Elena Lienqueo [2] and Diana Romanini[1], Universidad Nacional de Rosario, ARGENTINA[1], Universidad de Chile[2]

P-104 **Mechanisms of protein sorption and transport in cellulosic Ion exchangers of differing salt tolerances.** James M. Angelo and Abraham M. Lenhoff, University of Delaware, Newark, DE, USA

P-105 **Diafiltration optimization for high concentration UFDF processes impacted by the Donnan effect.** Joshua Arias, Alexandra Steele, EMD Millipore, Billerica, MA, USA

P-106 **Purification strategy of AAV and scAAV vectors for gene therapy against alcoholism.** Alicia T. Lucero, Anamaría C. Sánchez, Barbara A. Andrews and Juan A. Asenjo, Centre for Biochemical Engineering and Biotechnology, Institute for Cell Dynamics and Biotechnology (ICDB): A Centre for Systems Biology, Universidad de Chile

P-107 **Biomimetic ligands for the enrichment of phosphoproteins.** Íris Luz Batalha[1], Ricardo J. F. Branco[1], Olga Iranzo[2], Christopher R. Lowe[3] and Ana C. A. Roque[1], FCT-UNL[1], ITQB-UNL[2], University of Cambridge[3]

P-108 **Effects of chemical modifications in the partition behavior of RNase A in aqueous two-phase systems.** José González-Valdez, Marco Rito-Palomares and Jorge Benavides Centro de Biotecnología FEMSA, Tecnológico de Monterrey, Campus Monterrey, MEXICO

P-109 **Primary recovery of superoxide dismutase from *Kluyveromyces marxianus* in aqueous two-phase systems.** Jesús Simental-Martínez, Marco Rito-Palomares, Jorge Benavides, Centro de Biotecnología FEMSA, Tecnológico de Monterrey, Campus

Monterrey, MEXICO

P-110 **Influence of slight variations of ion exchange media on the separation of proteins.** Simon Kluters[1], Lothar Jacob[2]; Heiner Graalfs[2] and Christian Frech[1], University of Applied Sciences Mannheim[1], Merck KGaA, Darmstadt, GERMANY[2]

P-111 **Phase system selection with fractional factorial design for purification of a recombinant protein from a hydroponic culture medium using centrifugal partition chromatography.** Łukasz Grudzien[1], Luisa Madeira[2], Derek Fisher[1], Julian Ma[2], Ian Garrard[1], Brunel Institute for Bioengineering, Brunel University, London, UK[1], St. George's Hospital Medical School, University of London, UK[2]

P-112 **Ultrafiltration behavior of low fouling zwitterionic membranes.** Mahsa Hadidi and Andrew Zydney, Pennsylvania State University, USA

P-113 **Evaluation of chaotic advection micromixers embedded in microfluidic devices for high throughput characterization of aqueous two-phase systems.** Patricia Vázquez-Villegas[1], Eric Ouellet[2], Oscar Aguilar[1], Marco Rito-Palomares[1] and Charles Haynes[2], CB - FEMSA, ITESM, Monterrey, NL, MEXICO[1], MSL, UBC, Vancouver, BC, CANADA[2]

P-114 **Applications for a pH responsive, multimodal chromatography resin.** Kristian Kallberg[1, 2], Leif Bülow[1], Kristian Becker[3] and James Van Alstine[4] Pure and Applied Biochemistry, Dept. of Chemistry, Lund University, Lund, SWEDEN[1], Takeda Pharma A/S, Copenhagen, DENMARK[2], BioInvent International AB Lund, SWEDEN[3], jim.vanalstine@telia.com[4]

P-115 **Monoclonal antibody Fc and Fab fragment self- and cross- interaction strengths.** Nicholas E. Levy, Rachael A. Lewus, and Abraham M. Lenhoff, Department of Chemical and Biomolecular Engineering, University of Delaware

P-116 **Direct recovery of lipase derived from microbial feedstock using sustainable aqueous two-phase flotation.** Pau Loke Show[1], Mohd Shamsul Anuar[2]and Tau Chuan Ling[3], Nottingham University Malaysia Campus[1] [2] Universiti Putra Malaysia[2], University of Malaya[3]

P-117 **On-column aggregation of a recombinant immunotoxin during anion exchange chromatography.** Thomas Linke, Andrew Fulton, Yang Wang and Alan K. Hunter MedImmune, Gaithersburg, MD, USA

P-118 **A new amino acid scale based on analysis of different types of amino acid-water interactions.** Pedro P. Madeira[1], Ana Bessa[1], Luís Álvares-Ribeiro[1], M. Raquel Aires-Barros[2], Alírio E. Rodrigues[1], Vladimir N. Uversky[3], and Boris Zaslavsky[4] University of Porto, PORTUGAL[1], University of Lisbon, PORTUGAL[2], University of South Florida, USA[3], Analiza, Inc., USA[4]

Poster Oral Summary Session B

P-201 **Aldehyde PEGylation of laccase from *Trametes versicolor*: Effect of reaction time on the enzyme activity.** Karla Mayolo-Deloisa , Mirna González-González, Jesús Simental-Martínez and Marco Rito-Palomares, Centro de Biotecnología-FEMSA, Instituto Tecnológico y de Estudios Superiores de Monterrey, MEXICO

P-202 **Molecular modeling study of PEG-proteins interactions using AutoDock.** Karla Mayolo-Deloisa[1], Cristian Salgado[2], Diana Ostos-Rangel[1], Gerald Zapata[3], Ma. Elena Lienqueo[2], Marco Rito-Palomares[1] and Juan A. Asenjo[2], Centro de Biotecnología-FEMSA, Tecnológico de Monterrey, MEXICO[1], Department of Chemical Engineering and Biotechnology, University of Chile,[2], Faculty of Chemical and Pharmaceutical Sciences, University of Chile[3]

P-203 **Aqueous two phase system applied for norbixin purification.** Guilherme P. Almeida, Lizzy P. Alcântara, Valéria P. R. Minim and Luis A. Minim, Department of Food Technology, Federal University of Viçosa

P-204 **Direct purification of lysozyme from low diluted egg white using a supermacroporous monolithic cryogel column.** Rafael C. I. Fontan[1], Renata C. F. Bonomo[1], Valéria P. R. Minim[2] and Luis A. Minim[2], Department of Food Engineering, Southwestern of Bahia State University[1], Department of Food Technology, Federal University of Viçosa[2]

P-205 **Extraction of cheese whey proteins using PEG/Citrate based environmentally benign aqueous two-phase system.** Rajendran Govindarajan and Muthiah Perumalsamy, National Institute of Technology, Tiruchirappalli, INDIA

P-206 **Propagation of variance in ion exchange chromatography for protein separations: Impact of mobile phase variance.** Qiyang Duan[1], Todd Przybycien[1,2] , Departments of Chemical Engineering[1] and Biomedical Engineering[2], Carnegie Mellon University, Pittsburgh, PA, USA

P-207 **Hybrid disposable cartridges for integration of downstream bioprocessing.** Poondi Rajesh Gavara[1], Marcelo Fernandez-Lahore[2], and Mariano Grasselli[3], ChiPro GmbH, GERMANY[1], Jacobs University Bremen gGmbH, GERMANY[2], Universidad Nacional de Quilmes, ARGENTINA[3]

P-208 **Partitioning of CD133 antibody in aqueous two-phase systems: In route to stem cell separation.** Mirna González-González and Marco Rito-Palomares, Centro de Biotecnología-FEMSA, Tecnológico de Monterrey. Campus Monterrey, Monterrey, MEXICO

P-209 **Scaling-up of a B-phycoerythrin production and purification bioprocess involving aqueous two-phase systems: Practical experiences.** Federico Ruiz-Ruiz[1], Jorge Benavides[1] and Marco Rito-Palomares[1], Centro de Biotecnología FEMSA, Departamento de Biotecnología e Ingeniería de Alimentos, Tecnológico de Monterrey, Campus Monterrey. Monterrey, MEXICO[1]

P-210 **Optimization of hydrodinamical and adsorptive properties of Chromatographic Gigaporose Columns.** Mirna L. Sanchez[1,2], Leandro J. Martínez1, Estefania Achilli[1], Marcelo Fernandez-Lahore[2] and Mariano Grasselli[1], Universidad Nacional de Quilmes - IMBICE (CONICET)[1], Jacobs University Bremen[2]

P-211 **Countercurrent centrifugal extraction: A new and high efficient extraction apparatus based on centrifugal partition chromatography.** Christoph Schwienheer, Juliane Merz and Gerhard Schembecker, TU-Dortmund, GERMANY

P-212 **Affinity precipitation of mAbs using stimuli responsive smart biopolymers: Methods development and process considerations.** Rahul D. Sheth [1], Bharat Bhut [2], Mi Jin[2], Jongchan Lee[2], Wilfred Chen[3], Steven M. Cramer[1], Rensselaer Polytechnic Institute[1], Bristol-Myers Squibb[2], University of Delaware[3]

P-213 **Bioanalytical method validation of a colorimetric protocol to determinate proteolytic activity of bromelain.** Diego Coelho[1], Thais Saturnino[1], Fernanda Fernandes[1], Bianca Martins[2], Beatriz Zanchetta[1], Priscila Mazzola[2], Elias Tambougi[1] and Edgar Silveira[2], FEQ - Campinas State University[1], Uberlândia Federal University[2]

P-214 **PEG/$(NH_4)_2SO_4$ aqueous two-phase systems for bromelain purification.** Diego Coelho[1], Birgit Pitzschel[2], Enoch Aguillón[3], Bianca Martins[4], Beatriz Zanchetta[1], Priscila Mazzola[1], Elias Tambougi[1] and Edgar Silveira[4], Campinas State University[1], Technischen Univeristät Clausthal[2], Inst. Tecnol. de Ciudad Madero[3], Uberlândia Federal University[4]

P-215 **Charge clustering dominates protein ion-exchange separations.** Mohan-Vivekanandan Poongavanam[1], Lydia Kisley [3], Jixin Chen[3], Andrea P. Mansur[3], Bo Shuang[3], Katerina Kourentzi[2], Sagar Dhamane[1], Wen-Hsiang Chen[2], Christy F. Landes[3,4], Richard C. Willson[1,2,5], Department of Biology & Biochemistry, University of Houston[1]; Department of Chemical & Biomolecular Engineering, University of Houston, Houston, TX[2]; Department of Chemistry, Rice University[3]; Department of Electrical and Computer Engineering, Rice University, Houston, TX[4]; The Methodist Hospital Research Institute, Houston, TX[5]

P-216 **Spermine Sepharose as a clustered-charge anion exchange adsorbent.** Sagar Dhamane[1], Federico Ruiz-Ruiz[1,2], Wen-hsiang Chen, Mohan-Vivekanandan Poongavanam[1], Katerina Kourentzi[3], Jorge Benavides[2], Marco Rito-Palomares[2], Richard C. Willson[1,3], Department of Biology & Biochemistry, University of Houston, Houston, TX, USA[1]; Centro de Biotecnología FEMSA, Departamento de Biotecnología e Ingeniería de Alimentos, Tecnológico de Monterrey, Campus Monterrey, Monterrey, Mexico[2]; Department of Chemical & Biomolecular Engineering, University of Houston, Houston, TX, USA[3]

P-217 **Polyelectrolyte precipitation: A new technique for antibody purification.** Julia Sieberz, Kerstin Wohlgemuth and Gerhard Schembecker, TU Dortmund, Dortmund, GERMANY

P-218 **A F(ab')$_2$ulous Project – Achieving cleavage of a full-length monoclonal antibody using pepsin chromatography.** Marc Wong, Anjali Srivastava, and Michelle Butler, Genentech, Inc.

P-219 **Effect of ligand chemistry and protein surface properties on selective adsorption in multimodal chromatography systems.** James Woo[1], Siddharth Parimal[1], Mark Snyder[2] and Steven Cramer[1], Rensselaer Polytechnic Institute[1], Bio-Rad Laboratories[2]

Oral and Poster Abstracts

O = oral P= poster

O-101 **Investigation of alternative separation technologies for the harvest of CHO cell culture.**
Robert Piper[1], Ganesh Vedantham[1], António Grilo[2], Karol Lacki[3], and James Van Alstine[3]; Purification Process Development, Amgen Inc.[1], Instituto Superior Técnico (IST) Lisbon[2], GE Healthcare Bio-Sciences[3]
Continued improvements in process yields will require higher cell densities and cell mass The cellular mass generated throughout the course of production can be in excess of 20% based on packed cell volume (PCV); beyond the capabilities of a typical disk stack centrifuge to harvest.
The increased solids coupled with the desire for disposable processing have necessitated the investigation of alternative methods of CHO culture harvest. Two methods in particular have shown promise: Flocculation followed by cell settling and the use of Aqueous Two Phase Systems (ATPS). This presentation will discuss the performance of these technologies with a focus on yield, robustness, and their impact on product quality.

O-102 **Bioengineering strategies for the establishment of a novel process for the primary recovery of stem cells exploiting aqueous two-phase systems.** Mirna González-González and Marco Rito-Palomares, Centro de Biotecnología-FEMSA, Tecnológico de Monterrey. Campus Monterrey, Monterrey, MEXICO
During the past few years, stem cells have gained the attention of researchers and scientists due to the amazing potential they have shown in treating various chronic and degenerative diseases. Nevertheless, stem cell isolation and purification strategies have been set aside and conditioned to the expensive and limited existing technologies. The objective of this research is to establish the bases for the development of a novel, fast, scalable and cost-effective purification bioprocess for the selective recovery of $CD133^+$ stem cells exploiting ATPS. The proposed bioengineering strategies include the implementation of traditional polymer-polymer ATPS composed of polyethylene-glycol, dextran and/or ficoll, as well as novel systems with Ucon in order to prove the viability of $CD133^+$ stem cells and to study the partition behavior of $CD133^+$ stem cells and contaminants. Human umbilical cord blood (HUCB) was selected as the experimental matrix based upon abundance and simplicity of collection as it is a non-invasive and painless procedure to obtain suitable samples. Three independent samples were tested: (1) enriched $CD133^+$ sample, (2) whole HUCB (contaminants) and (3) complex sample ($CD133^+$ stem cells and contaminants). The results suggest that CD133+ stem cells can be recovered in the top phase when ficoll 400,000-DEX 70,000 ATPS are used. Alternatively, the systems composed of Ucon-DEX 75,000 and PEG 8,000-DEX 500,000 aided through affinity could be further exploited to concentrate the $CD133^+$ stem cells in the upper phase.

O-103 **Processing of high-density mammalian cell culture with Expanded Bed Adsorption Technology.** E.J.A.X. van de Sandt[1], P. den Boer[2]; DSM Biotechnology Center, The Netherlands[1], DSM Biologics, The Netherlands[2]
Improved Expanded Bed Adsorption Technology, RHOBUST®, has been developed to improve the recovery process. With EBA technology biomass removal and product capture is combined in one process step leading to less processing time, reduction of process steps, and lower cost of operation. A special designed rotating fluid device is able to handle high density cell cultures. Furthermore, increased density –with tungsten carbide- adsorbent media are developed with protein-A, ion-exchange or mixed mode ligands.
In this study, the following aspects were investigated:
1) scalability of EBA columns (10cm, 30, and 45 cm diameter) in terms of theoretical plate numbers (N/m), 2) effect of column flow distribution (low and high plate numbers) on chromatographic performance, 3) effect of pH and temperature of the feed stream on IgG capture, and
4) product capture and impurity removal (HCP and DNA) using high-cell density harvest (>100 million cells/ml)
It is concluded that Expanded Bed Adsorption Technology is a robust technology that may be applied to a variety of feed streams.

O-104 **Second-generation expanded bed adsorption.** Marcelo Fernández-Lahore[1], Roy N. D'Souza[1], Jürgen Fritz[1], and Rami Reddy Vennapusa[2]; Jacobs University Bremen[1], Shanta Biotechnics (Sanofi)[2]
EBA chromatography offers substantial advantages in the primary recovery of bio-pharmaceuticals from crude feedstocks by integrating clarification, concentration, and initial purification steps into one unit operation. A major challenge in this area is to minimize cell-adsorbent interactions, which not only depend on solution composition, but also upon hydrodynamic shear forces incurred at higher flow rates. The process of cell adhesion and its associated dependence on solution chemistry have been simultaneously investigated using extended DLVO colloid theory and experimentally corroborated with direct force measurements using atomic force microscopy (AFM). Efforts to minimize cell adhesion by spontaneously coating the adsorbent matrix with polymers such as

polyvinylpyrrolidone have been shown to be quite effective with bacterial feedstock. More importantly, dynamic binding capacities for model proteins remained unaffected. Combined with studies on second-generation EBA adsorbents (DSM Rhobust®) in various conditions, a streamlined approach designed to meet challenges facing EBA processes has been developed.

O-201 **Unified super-resolution experiments and chromatographic theory reveal charge-clustering dominates protein ion exchange adsorption.** Lydia Kisley[1], Jixin Chen[1], Andrea P. Mansur[1], Bo Shuang[1], Katerina Kourentzi[2], Mohan-Vivekanandan Poongavanam[3], Wen-Hsiang Chen[2], Sagar Dhamane[3], Christy F. Landes[1] and Richard C. Willson[2, 3, 4]; Department of Chemistry, Rice University, Houston, TX[1]; Department of Chemical & Biomolecular Engineering, University of Houston, Houston, TX[2]; Department of Biology & Biochemistry, University of Houston, Houston, TX[3]; The Methodist Hospital Research Institute, Houston, TX[4]
We report the use of super-resolution single molecule spectroscopy to directly study adsorption of single protein molecules on single ion exchange ligands. Additionally, we relate experimental results to the molecular-scale stochastic theory of chromatography. The combination of super-resolution spectroscopy, single adsorption site kinetic analysis, and statistical treatment allows us to establish the importance of charge clustering for stationary phase ligands. An extension of the stochastic theory to single molecule kinetics and macroscale chromatographic elution profiles shows that if it were possible to engineer clustered-charge interactions into ion exchange systems, separation plate numbers could be improved by as much as five-fold by deliberately exploiting clustered interactions that currently dominate the separation process, albeit only accidentally.

O-202 **Structure-based design of purification process for a new rFVIII product.** Haleh Ahmadian[1] and Camilla Kornbeck[2]; Dept. of Protein Purification Technology, Novo Nordisk[1], Dept. of Protein Separation and Virology, Novo Nordisk[2]
A new rFVIII, turoctocog alfa and its longer acting glycoPEGylated version are currently in the late phases of clinical development for treatment of haemophilia A. In this study, we describe the molecular design of turoctocog alfa and a structure-based approach for development of downstream processing. Full length FVIII with 2332 amino acids consists of a heavy and a light chain, which are held together by non-covalent binding. In turoctocog alfa, the 908 amino acid residue B-domain of full-length FVIII has been truncated to 21 amino acid residues, keeping the region from aa 720 to 740 required for optimal interaction with thrombin. In the immunoaffinity chromatography step, a monoclonal antibody directed towards residues aa 720-740 was chosen to ensure removal of impurities such as HC720, HC729 and other heterogeneities due to C-terminal HC truncation. The importance of this interaction site for purity will be discussed. A four step purification process was designed including use of multimodal systems for capture and immunoaffinity chromatography as the second step to obtain a highly pure product. During capture on the multimodal column, the stability of turoctocog alfa was ensured by proper combination of buffer components. The choice of keeping 21 aa in the truncated B-domain also served the design of the next generation glycoPEGylated FVIII molecule. This region contains an O-glycan that is used as the site for PEG attachment in a highly selective glycoPEGylation reaction. The process for glycoPEGylation will also be presented.

O-203 **Novel affinity pairs "tag-receptor" for the purification of fusion proteins.** Ana Sofia Pina[1], Christopher R. Lowe[2] and A. Cecília A. Roque[1]; REQUIMTE, FCT-UNL[1], University of Cambridge[2]
A popular alternative to reduce the number of purification steps of recombinant proteins employs genetically fused affinity tags that along with the respective binding partners facilitate protein purification by affinity chromatography (1). This work describes the development of novel affinity pairs "tag-receptor" for protein purification, where the tag corresponded to a peptide or small protein, and the receptor to a tailor-made synthetic affinity ligand. The affinity pairs "tag-receptor" were designed *in silico* for improved complementarity. For the production of the fusion proteins, the DNA fragments encoding for selected affinity tags fused to Green Fluorescent Protein (GFP) were designed, subcloned on a pET21c expression vector and produced in *Escherichia coli.* Simultaneously, a solid-phase combinatorial library of 64 affinity ligands was synthesized and screened towards the fusion proteins and non-tagged GFP (2). The screening strategy was carried out by micro-scale affinity chromatography on 96-well format and lead to the selection of novel putative affinity pairs. The selected ligands presented remarkable properties of selectivity towards their target tags, and robustness in a wide range of

conditions. A lead pair "tag-receptor" displaying a Ka of 2.3x105 M-1 resulted in a selective recovery of the fusion protein with 92% yield and 94% purity.
(1) Pina *et al* (2012) Methods in Molecular Biology xxx USA: Humana Press Inc (*in press*).
(2) Pina et al (2010) Separation Science and Technology, 45:15, 2187-2193

O-204 **RAPID selection of aptamers via multi-target microcolumn chromatography.** David Latulippe[1], Kylan Szeto[2], Abdullah Ozer[2], John Lis[2] and Harold Craighead[2]; McMaster University[1], Cornell University[2]
Aptamers are single-stranded oligonucleotides that fold into stable structures which bind with high affinity and specificity to a target. For affinity-based purifications, they offer a number of advantages over antibodies including increased stability to harsh elution conditions. Aptamers are identified from large libraries of random DNA/RNA sequences via an iterative, in vitro process that typically involves many selection and amplification steps; the overall process is labor intensive and time consuming. Recently, two advances have been developed that simplify the entire selection process and thus are particularly useful for the development of new aptamer-based purification strategies. The first is a microcolumn device that uses only microliter amounts of affinity chromatography resin – a condition that maximizes the enrichment of target-binding sequences. The modular device allows for multi-target selections to be done simultaneously with negative selections. The optimum selection conditions were determined using a design-of-experiments methodology and high-throughput sequencing was used to identify aptamers that were preferentially enriched after just three selection rounds. RAPID (RNA Aptamer Isolation via Dual-cycles) is a new strategy that systematically skips any unnecessary amplification steps between selection cycles. We completed a full selection for two protein targets in less than half the time required for the conventional selection method. Incredibly, high-throughput sequencing of the enriched pools revealed that the exact same sequences were identified in both selections. Additionally, a significant cost savings was achieved by eliminating the need to interconvert RNA and amplifiable DNA during each amplification cycle.

O-205 **Mutagenesis Objective Search and Selection Tool (MOSST) applied to engineer protein mutants with enhanced purification properties.** A. Olivera-Nappa, J.A. Asenjo and B.A. Andrews; Centre for Biochemical Engineering and Biotechnology, Department of Chemical Engineering and Biotechnology, Universidad de Chile
It is well known that both natural and engineered mutations in a given protein can have a tremendous impact on its affinity and binding behaviour and that these properties can in principle be modified by rationally mutating selected amino acids. Accordingly, new affinity and purification properties can be engineered into existing proteins. However, a major concern of this approach is that the rational design of mutations should alter the behaviour of target proteins in purification processes without affecting their activity. Although this strategy has been previously used, the modifications introduced in the target protein have not been exempt from deleterious effects on protein function, binding or physicochemical properties. Therefore, much finer tuned modifications should be designed in order to alter the desired purification properties of a protein and simultaneously not affect other protein properties or functions. A drawback of this is that these finer mutations usually require a thorough knowledge of the relevant structure-function relationships in the protein molecule. If no precise structure-function information is available for a protein, the number of possible amino acid mutations to be tested precludes a direct search and in many cases a directed evolution strategy cannot be successfully used to achieve the desired results applied to purification processes.
A new and powerful methodology has been developed to design mutagenesis strategies for a target protein, based only on conservation rules of physicochemical properties of amino acids extracted from a multiple alignment of a protein family to which the target protein belongs, with no need of explicit structure-function relationships. This methodology identifies amino acid positions that are putatively responsible for function, specificity, stability or molecular interactions in a family of proteins and calculates amino acid propensity distributions at each position. Not only conserved amino acid positions in a protein family can be labelled as functionally relevant, but also non-conserved amino acid positions can be identified to have a physicochemically meaningful functional effect, or even amino acid substitutions unobserved in nature. These results can be used to predict if a given mutation can have a functional implication and which is most likely to be functionally silent for a protein.
This methodology can be applied in a high-throughput approach to an entire protein and it can be combined with protein design and structural insights in the frame of an integrated AI method, to automatically suggest mutations in a recombinant protein that could enhance their large-scale purification properties and to make them amenable

to be separated by alternative purification techniques with improved recovery or purity. The method has recently been successfully used to guide the mutant construction process in enzyme engineering strategies and to protein function and affinity interaction *in vivo*. The use of this valuable and powerful technique to generate engineered protein variants where the purification properties have been tailored for specific conditions and processes will be shown and discussed in this paper.

0-301 **Smart biopolymer affinity precipitation for mAb purification: methods development and process considerations.** Steven M. Cramer[1], Rahul Sheth[1], Mi Jin[2] and Bharat Bhut[2]; Rensselaer Polytechnic Institute[1], Bristol-Myers Squibb[2]

This presentation examines methods development and process considerations for a stimuli responsive ELP-Z based affinity precipitation process for mAb purification. A multidimensional high-throughput screening (HTS) protocol is employed to determine appropriate conditions for the initial capture and co-precipitation of mAbs at high yields using ELP-Z. mAb elution from ELP-Z is then examined using HTS and the mAb yields and aggregate content of the overall process are determined. The results indicate that mAb aggregation was primarily sensitive to the elution conditions and was antibody specific and a strong function of operating temperature and elution pH. Importantly, the results indicate that room temperature operation and appropriate elution pH can be readily employed to produce both high mAb yields and low aggregate content for multiple mAbs using this approach. Results are then presented for mAb purification from an industrial harvest feed. High levels of mAb recoveries are obtained during both the initial binding step of the process as well as the subsequent elution step, with no observable mAb aggregation. The process is shown to result in more than 2 logs of HCP and more than 4 logs of DNA clearance from the harvest feed with clearance and yield values comparable or superior to Protein A chromatography. Process performance is maintained for mAb final elution concentrations up to 20 g/l, demonstrating the ability to both concentrate and purify the mAb. Effective ELP-Z cleaning is also demonstrated using 0.1 M NaOH with no adverse effect on subsequent capture efficiency. The reusability of the ELP-Z construct and robustness of the process is then demonstrated over multiple purification-cleaning cycles with minimal product and impurity carryover and high yields and purity. Finally, scale-up of the ELP-Z based mAb affinity precipitation process is successfully carried out using a combination of tangential flow microfiltration and dead end filtration for the recovery of the precipitates. This study clearly demonstrates the potential of this ELP-Z based affinity precipitation process for industrial mAb purifications.

O-302 **Aqueous two-phase systems for protein separation: review and state of the art.** J.A. Asenjo and B.A. Andrews; Centre for Biochemical Engineering and Biotechnology, Department of Chemical Engineering and Biotechnology, Institute for Cell Dynamics and Biotechnology: a Centre for Systems Biology, University of Chile, Santiago, CHILE

Aqueous two-phase systems (ATPSs) that are formed by mixing a polymer (usually polyethylene glycol, PEG) and a salt (e.g. phosphate, sulphate or citrate) or two polymers and water can be effectively used for the separation and purification of proteins. The partitioning between both phases is dependent on the surface properties of the proteins and on the composition of the two phase system. The mechanism of partitioning is complex and not easy to predict but, as this review will show, some very clear trends can be established. Hydrophobicity is the main determinant in the partitioning of proteins and can be measured in several different ways. The two methods that are more attractive, depending on the ATPS used (PEG/salt, PEG/polymer), are those that consider the 3-D structure and the hydrophobicity of aminoacids on the surface and the one based on precipitation with ammonium sulphate (parameter 1/m*). Charge has a relatively small effect on the partitioning of proteins in PEG/salt systems but is more important in PEG/dextran systems. Protein concentration can also have an important effect on the partitioning of proteins in ATPSs .

The practical applications of ATPS have been demonstrated in many cases including a number of industrial applications with excellent levels of purity and yield. These include the purification of the enzymes chymosin and α-amylase, the sweetener thaumatin and human therapeutics tPA, IgF-1, monoclonal antibodies, interferon and α-antitrypsin. This method of separation and purification has also been successfully used for the separation of virus and virus-like particles. This presentation will review the main physico-chemical factors that affect partitioning of proteins in ATPSs as well as the main applications and important factors affecting phase separation which are critical for scale-up of this technique.

O-303 **Aqueous two phase system based on ionic liquids for extracting biomolecules.** Rupali Desai[1], Michel Eppink[1, 2], Mathieu Streefland[1] and Rene Wijffels[1]; Wageningen University Netherlands[1], Synthon B.V., THE NETHERLANDS[2]
Ionic liquids (IL) have gained considerable attention as novel tunable solvents due to their unique physical and chemical properties. They are widely used as reaction media in biocatalysis and extractions of metals. IL is capable of forming aqueous two phase systems (ATPS) when mixed with salt and / or polymer [1,2]. The main advantage of using IL based ATPS over conventional aqueous two phase system is their ability to tune the polarity by appropriate selection of anions and cations. The primary objective is to study feasibility of using ionic liquids for extraction of biomolecules and value added components from biomass. IL based ATPS was used to study the extraction of biomolecules and the effect of various parameters such as concentration of IL and salt, pH and temperature was studied. Studies were also done to investigate the stability of the biomolecules in aqueous IL solutions.
References:
1. Gutowski, K.E., et al., *Controlling the aqueous miscibility of ionic liquids: aqueous biphasic systems of water-miscible ionic liquids and water-structuring salts for recycle, metathesis, and separations.* J Am Chem Soc, 2003. **125**(22): p. 6632-3.
2. Freire, M.G., et al., *Insight into the Interactions That Control the Phase Behaviour of New Aqueous Biphasic Systems Composed of Polyethylene Glycol Polymers and Ionic Liquids.* Chemistry – A European Journal, 2012. **18**(6): p. 1831-1839.

O-304 **A novel approach for mAbs bioprocessing: clarification and capture by aqueous two-phase extraction.** Ana M. Azevedo[1], Marta F.F. da Silva[1] and M. Raquel Aires-Barros[1]; IBB, Instituto Superior Técnico, Lisbon, PORTUGAL[1]
The importance of monoclonal antibodies (mAbs) as new and highly promising biopharmaceuticals for treatment of several diseases has been increasingly recognized. MAbs are, however, amongst the most costly therapeutic options available in the market. A critical assessment of current manufacturing platforms has shown that the greatest capacity constraints are no longer found in the upstream production processes, where cell culture productivity has dramatically increased over the past decade, but in the downstream purification area. Challenges in the purification of mAbs include reducing production costs, developing robust purification processes and integrating upstream and downstream processes. The goal of this work was the intensification of mAbs downstream processing by integrating clarification and initial recovery in one step. To achieve this different polyethylene-glycol (PEG)/dextran systems were evaluated for the clarification and capture of mAbs directly from cell culture media. In this work, CD-34 mAbs have been produced using an AC133.1 hybridoma cell line. The feasibility of using PEG/dextran systems was tested at different ionic strengths (0-300 mM NaCl) and at different pH values (pH 3, pH4 and pH 7) for two distinct PEG molecular weights (3350 and 6000 Da). The highest yield was obtained with an ATPS composed by 7% PEG 6000Da, 5% dextran 500'000 Da, 150mM NaCl at pH 3. In this system, it was possible to recover 84±6.5% IgG with 0.1±0.2 % of cells in the top phase. The highest purity achieved was 86±6.8% in top phase of an ATPS composed by PEG 6000 Da/dextran, 300 mM NaCl, pH 3.

O-305 **Scalable protein separation by selective coacervation.** Paul Dubin; Chemistry, UMass Amherst, Amherst, MA, USA
Complex coacervation, involving natural or synthetic charge polymers, is an effective way to achieve separations that are non-perturbing, concentrating, scalable and inexpensive. The resultant coacervate fluids, containing 200-250 g/L of the target protein, are fully reversible by pH or ionic strength, appear to be indefinitely stable with respect to aggregation, and in the case of enzymes show no loss of activity. After redissolution by pH adjustment, the polyelectrolyte is readily removed by ultrafiltration.
The basis of selectivity is preferential formation by the target protein to give an intra-polymer complex. This can happen on both sides of the pI, but when the charges of the two macroions are opposite, charge neutralization can lead to formation of a coacervate suspension containing the target protein, forming a separate phase upon settling or centrifugation.
Global protein charge *per se* does not control protein-polyelectrolyte affinity which depends on a protein domain of opposite sign to the polyelectrolyte. Thus, BLG is selectively coacervated by a polycation from its mixture with BSA with 90% purity, even though the pI's are nearly the same (5.2 and 4.9, respectively). Selective coacervation

with the polyanion hyaluronic acid (HA) preferentially removes *BSA* with the same selectivity. The most remarkable demonstration of the effect of protein charge anisotropy is the preferential coacervation of BLGA from BLGA/BLGB isoform mixture, the two differing by only two amino acids that happen to reside in the negative "charge patch". While these results conflict with assumptions about the "non-specificity" of charge interaction, simple modifications of the polymer could enhance selectivity.

O-401 **A novel microfluidic platform for high-speed process analytical technology for biotherapeutics characterization.** Charles Haynes; University of British Columbia, CANADA
Process Analytical Technology (PAT) is increasingly applied in the pharmaceutical and chemical manufacturing industries as a means to better characterize and control a complex manufacturing process. PAT does this by linking and exploiting rapid analyte separation and analysis methods with multivariate data analysis software, process control tools, and a suitable structured queried language database to monitor in near real-time physical and chemical properties used to define a set of critical quality attributes (CQAs) at key stages of the process. These CQAs are used in conjunction with a PAT platform to create both unit-level and process-level models capable of predicting the quality of the end product. Those predictions may then be used to fine-tune the manufacturing process so as to ensure acceptable product quality and consistency. In this presentation, I will describe our recent efforts to contribute useful instrumentation for PAT through the development of a low-pressure bead packing technique for the robust integration of high-performance chromatography columns in microfluidic devices made by multilayer soft lithography. A novel column geometry is used to achieve rapid packing of multiple high-quality columns with measured plate counts of 1,650,000/m ± 7%, corresponding to a reduced plate height of h = 0.12 ± 7%. Application of the technology to PAT-based monitoring of a mAb during processing is described with emphasis placed on showing an ability to purify and separate isoforms and glycoforms of interest in less than a minute, so as to achieve very near real-time monitoring.

O-402 **Cholesterol level and particle distribution as predictors of cell culture flocculation and filterability performance.** Anna Senczuk, Anne Thomas, Rob Piper, Tom McNerney, Krista Petty, John Moscariello and Yinges Yigzaw; Amgen
Recent advances in increasing monoclonal antibody titer using high density mammalian cell culture process have led to challenges in harvest operations (centrifugation and depth/sterile filtration). Cell culture flocculation and acid precipitation have improved the performance of the centrifugation and depth filtration steps. Understanding how these flocculating agents improve clarification efficiency is important to define a robust process. Thus factors that may be contributing to filter plugging such as particle size distribution and lipids were investigated. In general, settled or centrifuged supernatants of flocculated cell culture have higher mean particle size compared to untreated cell culture fluid. Increased mean particle size consistently correlated with increased filterability. The increase in mean particle size was attributed to decreased counts in the lower particle size ranges indicating that these sizes are primarily responsible for filter fouling. Interestingly, increased filtration capacity was correlated to lower cholesterol level in the filter feed material regardless of flocculation method used. We also found that cholesterol level in cell culture correlates very well with filterability. Therefore, cholesterol is a promising filterability predictor and PAT tool.

O-403 **High throughput characterization of proteins 3D structures in solution.** Larissa Mikheeva[1], Luisa Ferreira[1], Pedro Madeira[3], Leonid Breydo[2], Vladimir N. Uversky[2], Arnon Chait[1], Boris Zaslavsky[1]; Analiza, Inc., USA[1], University of South Florida, USA[2], University of Porto, PORTUGAL[3]
Solvent Interaction Analysis method for comparison of 3D structures of proteins employs partitioning of proteins in multiple aqueous two-phase systems. The partition coefficient (K) for a protein reflects the interactions between the solvent-exposed groups of the protein with the two aqueous environments in ATPS. Hence it can be used as a numerical index to characterize the protein 3D structure. The potential lack of specificity is addressed by combining K-values for the same protein using multiple ATPS. The vector formed by a set of K-values is used as a numerical signature of the protein 3D structure.
Structural signatures for human □-synuclein, several single-point and triple-points mutants of □-synuclein are characterized and compared to those obtained for chicken egg lysozyme, ribonuclease A, □-lactoglobulin A, and cytochrome c. The structural signatures for all proteins were obtained using aqueous Dextran-PEG two-phase

systems of different ionic compositions (0.01M sodium phosphate buffer (Na-PB); 0.15 M NaCl; 0.15M $NaClO_4$, and 0.15M Na_2SO_4 – all in 0.01M Na-PB, pH 7.4).
Circular dichroism (CD) spectra in the far UV region (180-240 nm) and in the near UV region 260-320 nm) were analyzed for the proteins in the solutions of the above ionic compositions in order to explore salt effects on the protein structures, and the data obtained are discussed in terms of the crowding effects in the phases of ATPS and in regard to the relative sensitivity of the two approaches to the protein 3D characterization.

O-501 **Mass transfer and kinetics of protein renaturation applied in μ- and lab scale.** Cornelia Walther[1], Gerhard Sekot[1], Rainer Hahn[1,2], Alois Jungbauer[1,2], and Astrid Dürauer[1,2]; ACIB Austrian Centre of Industrial Biotechnology[1], University of Natural Resources and Life Sciences Vienna[2]
Inclusion bodies are a source of large quantities of protein with high purity over-expressed in *Escherichia coli*. We visualized inclusion body solubilization using high-resolution light and transmission electron microscopy. Upon solubilization, the inclusion bodies were penetrated by the solubilization agent, shrinking the densely packed cores as the protein diffused out. The solubilization could be described by a homogeneous layer model with a high order of reaction. The established model enables evaluation of the inclusion body solubilization process on a numerical level. Hence, solubilization conditions and process parameters can be related to the rate and yield of the process. Solubilization kinetics observed in shaken HTS was compared to kinetics determined in stirred laboratory scale reactor and good agreement was found between the the scales. Approximation of the two resistances potentially affecting the solubilization process, intraparticle and external mass transfer, indicated that IB solubilization is dominated mostly by the diffusion kinetics of the protein within the particles. Therefore, the solubilization process is controlled predominantly by pore diffusion. Maintaining the homogeneity of the IB suspension is sufficient for efficient solubilization, and further power input will not improve the process. The HTS on μ-scale can predict solubilization in STR over a range of 500 and can be used to determine optimal solubilization conditions for laboratory and industrial scale.

O-502 **Modeling of gradient chromatofocusing in ion exchange chromatography.** Michael Schmidt and Christian Frech; University of Applied Sciences Mannheim, GERMANY
Ion exchange chromatography (IEC) is a widely used method for the removal of contaminants like host cell proteins, aggregates or charged variants from therapeutic proteins.
Classical elution modes in ion exchange chromatography include linear salt gradients or step elutions at a fixed pH value. Use of pH as a mobile phase modifier (pH gradients) in IEC can be an effective alternative to using salt gradients. Advantages of pH gradients include the possibility of greater resolving power and elution at low ionic strengths.
In this work we present data for the separation of monoclonal antibodies, rec. proteins and peptides by externally generated linear pH gradients (gradient chromatofocusing), which is used for modeling and prediction of the pH gradient elution behavior.
The analysis is based on the linear gradient elution model of Yamamoto and allows the calculation and prediction of the elution behavior of proteins for linear salt gradients, for linear pH gradients as well as for dual linear salt and pH gradient.
The model also incorporates the pH and salt dependency of the peak compression factor in linear gradient elution chromatography which allows the prediction of the full elution profile.
An example for the adaption of the model to mixed mode chromatography will additionally be discussed.

O-601 **Microparticles for continuous capture of proteins.** Rainer Hahn[1,2] Alexander Trefilov[2], Moritz Imendoerffer[2] and Alois Jungbauer[1,2]; Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Muthgasse VIENNA[1], Austrian Centre of Industrial Biotechnology, Muthgasse VIENNA[2]
Microparticles were prepared by grinding of conventional ion exchange resins which are commonly used for desalination or waste water treatment. These microparticles with a size of 1-2µm exhibited excellent properties for protein capture, including high capacity of up to 150 mg per g particle and extremely fast binding kinetics in the range of < 30 seconds for completion of adsorption. Desorption, recovery and selectivity was comparable to conventional ion exchange media designed for protein capture. Upon protein adsorption, flocs were formed facilitating easy separation by sedimentation or centrifugation. Floc formation could be triggered by addition of small fractions of oppositely charged microparticles. Several examples for capture of proteins from crude

homogenates or cell culture supernatants will be presented along with analysis of protein recovery and impurity removal. Furthermore, the separation of flocs will be discussed and scenarios will be presented of how this system can be implemented in a continuous purification process. In advance, anion exchange microparticles could be used for disintegration of bacterial cells allowing direct extraction and capture of intracellular proteins from the cell supernatant. In this case, purity of extracted proteins was even further enhanced and endotoxin and host cell protein levels were 1-2 orders of magnitude lower compared to capture from crude homogenate. Examples for two recombinant proteins will be shown and possible mechanism of cell disintegration will be discussed.

O-602 **Biomagnetic nanomaterials for high gradient magnetic affinity separation.** Gil Lee[1], Conor Fields[1], James O'Mahony[1], Victoria Matias[1], Peng Li[1], Loraine M. Smith[1], and Julien Muzard[2]; School of Chemistry and Chemical Biology, University College Dublin, IRELAND[1], and Conservatoire National des Arts et Métiers, Paris, FRANCE[2]

The growth of biopharmaceutical and biomedical industries has created a demand for new technologies for purification of genetically engineered proteins. High gradient magnetic separation (HGMS) is a technology that has the potential to increase the speed of traditional affinity chromatography due to its use of an expanded bed of superparamagnetic microparticles (SPM). The efficiency of large scale HGMS is greatly improved if SPMs offering high binding capacity, high magnetic susceptibility, and high magnetization are employed. In this communication, we will describe strategies for synthesizing SPMs that are composed of biomolecules and viruses assembled on a superparamagnetic core. Separation of specific proteins and antibodies from high protein concentration solutions was performed in a rapid and single step. The dense SPM core of these particles makes them highly responsive to magnetic fields and the biomolecular coating produces a high binding capacity. These new biomaterials and the described technology appear to be well suited to large scale HGMS separation and promise to be cost effective.

O-603 **Investigation of protein partitioning in tunable aqueous polymer phase impregnated resins**.
Fatma van Winssen, Juliane Merz and Gerhard Schembecker; Laboratory of plant and process design, TU Dortmund, Dortmund, GERMANY

The Tunable Aqueous Polymer Phase Impregnated Resins (TAPPIR®)-Technology represents a novel alternative unit operation for Downstream Processing (DSP) of proteins. In order to face the complex and diverse DSP challenges it is motivated by overcoming Aqueous Two-Phase Extraction (ATPE) drawbacks while profiting from its advantages such as good biocompatibility. The most important drawback of ATPE is the long phase separation time due to the formation of stable emulsions. Thus, the industrial application of classical ATPE remains limited. To cope with this limitation one aqueous polymer phase is immobilized in porous particles which are suspended in the second aqueous phase. Thus, phase contact is achieved via the impregnated particles and phase emulsification and separation are superfluous.

In this work, the ability of the TAPPIR®-Technology for protein partitioning in an aqueous polyethylene glycol-citrate system is investigated. Different particle materials and sizes and the addition of sodium chloride are investigated. To evaluate the performance of the TAPPIR®-Technology, the results are compared to classical ATPE and adsorption experiments. It could be demonstrated, that the target protein could be efficiently extracted and back-extracted using the TAPPIR®-Technology. Comparing the overall recovery of target protein the TAPPIR®-Technology can absolutely compete with classical ATPE.

O-604 Mu**ltimodal chromatography for DNA separation and purification.** Tiago Matos[1,2], João A. Queiroz[2] and Leif Bülow[1]; Pure and Applied Biochemistry, Lund University, SWEDEN[1], University of Beira Interior, Covilhã, PORTUGAL[2]

DNA molecules harbor some intrinsic chemical properties that render them suitable for chromatographic separations. These include a negatively charged phosphate backbone as well as a hydrophobic character originating mainly from the major groove of DNA which exposes the base pairs on the surface of the molecule. In addition, single stranded DNA often allows for a free exposure of the hydrophobic aromatic bases. Over the years, several different chromatographic methods have been explored for DNA purification. Particularly anion-exchange chromatography (AEX) and hydrophobic interaction chromatography (HIC) have proved most versatile. In this study, we have examined multimodal chromatography (MMC) as an alternative tool for complex separations of nucleic acids. MMC embraces more than one kind of interaction between the chromatographic ligand and the target molecules, and it has previously been successfully applied for protein and antibody purifications. The *Capto*

Adhere medium (GE Healthcare Biosciences) is a recent multimodal chromatographic material, originally developed for removal of contaminants including nucleic acids, in downstream processing of monoclonal antibodies. This ligand features strong AEX properties, which together with a hydrophobic moiety, allows the resin to combine hydrogen bonding, ionic and hydrophobic interactions. This opens up for possibilities to achieve a unique selectivity for nucleic acids compared to traditional anion exchangers.
The chemical properties of nucleic acids depend on their size and base composition. In addition, single-stranded and double-stranded DNA molecules show differences in ionic and hydrophobic characteristics. Here we have examined particularly the differences in chromatographic behavior between nucleotides and small single- and double-stranded DNAs. All DNA molecules tested bound strongly to the *Capto Adhere* resin and they could be eluted with increasing NaCl concentrations. Homopolymeric single-stranded DNA molecules resulted in a base-specific elution pattern when using a linear NaCl gradient. The elution order was poly(dA)<poly(dC)<poly(dG)<poly(dT) and this order was dependent on the secondary structure of the molecule. Such differences were not observed for small double-stranded DNAs. Due to the more hydrophobic nature of single-stranded DNA molecules they could be separated from double-stranded DNAs. Such differences of intereaction between the ligand and different nucleic acid molecules, can be explored for develping alternative and rapid purification strategies for many methods used in molecular biology, e.g. the purification of PCR products. Isolation of particulalrly PCR products are hence cumbersome often requiring isolation of the obtained DNA fragments from an agarose gel after electrophoresis. The ability of *Capto Adhere* to separate different nucleic acids has thus allowed us to isolate amplified PCR products in a single chromatographic step.

O-701 **High throughput process development – a door opener?** Jürgen Hubbuch; Institute of Process Engineering in Life Sciences, Karlsruhe Institute of Technology, Karlsruhe, GERMANY
Over the last decade, the rise of methodologies for a more rapid and concerted way to develop modern purification processes has established a new field of activity in industry and academia named high throughput process development. Starting with simple procedures of pipeting and mixing schemes, the level of sophistication has risen and converted – next to the core idea of process development – this field into an enabling technology for aspects such as molecular and mechanistic modelling, statistical experimental design, lab-on-a-chip approaches and modern analytical technologies. The paper discusses the present state of the art in the field of HTPD and highlights developments arising from this methodology focusing on modeling and analytical aspects of multi component systems.

O-702 **High throughput bio purification process development – a hybrid approach.** M. Ottens; Department of Biotechnology, Delft University of Technology, THE NETHERLANDS
In the last decade High Throughput (HT) Screening for process development (PD) saw implementation in the biopharmaceutical industry. This HT approach is an experimental “black box” method to find an optimal resin or optimal operational conditions for a chromatographic separation, capture or purification step via e.g. design of experiments (DoE). The potential of this HT approach for PD can be made more comprehensive and efficient by including mechanistic, mathematical modeling to arrive at the so-called *in silico* PD, e.g. by reducing the screening space and providing a better mechanistic understanding of processes. This presentation will show the current status and recent progress towards a High Throughput Process Development (HTPD) approach as developed in our lab in Delft, containing a hybrid miniaturized experimental and mechanistic modeling approach for fast protein purification process development [Nfor *et al*, *Chem Eng Science*, 89, 185, **2013**, *Biotech & Bioeng*, 109(12), **2012**, *Biotechnol. J*., 7, 1221, **2012**]. The focus of the presentation will lie on the development of a novel automated crude feedstock profiling to be used in HTPD. Additional examples include miniaturized HT resin screening, model based resin selection, isotherm parameter regressing using minute crude protein feed stock mixtures and subsequent process synthesis for some industrial cases. Finally, an outlook on future developments will be given.

O-703 **High throughput optimization approach for single step polishing of monoclonal antibodies following Protein A capture.** Aleksandar Cvetkovic[1], Amit Kundu[2] and Rene Gantier[1]; Pall Life Science[1], Genmab MN, Inc.[2]
Contaminants (aggregates, host cell proteins-HCP, DNA) in biotherapeutics preparation, including monoclonal antibodies (mAb), are undesirable but commonly encountered and some of them even induced under conditions used in production, purification and formulation of product. Their levels could be controlled, but not eliminated

during production. Hence, their clearance is required but can be complicated by the variety of the mechanisms involved in their interactions.
The versatility of mixed-mode chromatography makes this technique very useful for broad types of applications including intermediate and polishing steps in monoclonal antibody (mAb) purification processes.
We used various mixed-mode sorbents for single step polishing of mAbs following Protein A capture. High-throughput screening tools were employed to identify optimum conditions in both Bind/Elute and Flow through modes, and to better understand interaction mechanisms of mAb with each sorbent. The optimal conditions were transferred to column chromatography for results verification. While bind/elute mode of operations was not proven very efficient, some mixed mode sorbents used in Flow-though mode provided excellent contaminant (aggregates and HCPs) clearance with mAb loads up to 150 mg/mL of sorbent. This study confirms the applicability of mixed mode chromatography steps for post-Protein A purification of MAbs.

O-704 **Overload and elute chromatography for enhanced MAb purification.** Deepa Nadarajah, Steve Hohwald, Debola Banerjee, Kevin Shomglin, Rob van Reis, Rick St. John, Amit Mehta; Genentech Inc., San Francisco, CA, USA
Bio-pharmaceutical process development has increasingly been relying on platform processes that reduce development timelines and time to clinic. However, certain MAbs may not fit into the platform process thus requiring further development. This talk will focus on a set of MAbs that bind to the anion exchange (AEX) resin under flow-through conditions. To enable plant fit, improve yield and achieve adequate impurity clearance, a novel mode of chromatography was developed with a multimodal resin. The resin was operated in an overload mode with a step elution enabling significantly higher load density while obtaining the desired impurity clearance. Data on clearance of model viruses, host cell proteins and aggregates will be presented. High throughput screening was employed to rapidly identify process parameters for overload and elute chromatography. Data generated to develop a mechanistic understanding of product and impurity binding to the resin under the overload and elute mode of chromatography will also be presented.

O-801 **New technologies enabling an integrated mAb manufacturing process with cost and operational advantages.** Michael W. Phillips, EMD Millipore
In an evolving biopharmaceutical industry, the need for advancements in biomanufacturing becomes critical. A trend toward the use of multi-product facilities requiring flexible manufacturing solutions, increasing competition from biosimilars, and increases in upstream expression levels have focused the need for manufacturing solutions that offer increased productivity and improved economics without sacrificing process robustness. Several solutions have been proposed to address these downstream bottlenecks, including the evaluation and development of non-chromatographic purification processes, the introduction of higher capacity and more selective chromatographic media, and the investigation of highly-connected and continuous processes. In this presentation, we address each of these approaches through the development of a new downstream processing (DSP) template for mAb purification comprising polymer precipitation for clarification, continuous multi-column Protein A chromatography for product capture, and a fully-connected flow through polishing train specifically developed for removing product- and process-related impurities. Experimental data highlighting the robust performance of these individual technologies will be presented along with the rationale for selecting these solutions versus alternative options. Ultimately, holistic process considerations (ease of process integration, process economics, process robustness, facility fit) rather than unit operation considerations were the dominant drivers for technology selection.

O-802 **Continuous countercurrent tangential chromatography using Protein A for initial capture of a monoclonal antibody.** Andrew Zydney[1], Oleg Shinkazh[2], Boris Napadensky[2], Travis Tran[2], and Achyuta Teella[2]; Penn State University[1], Chromatan, Inc.[2]
There has been considerable interest in the development of continuous as well as single-use chromatographic processes for the purification of high value biological products, but the packed columns used in most of these approaches still operate primarily as “batch” operations. In contrast, Countercurrent Tangential Chromatography (CTC) can provide truly continuous product capture and purification using a column-free system that overcomes many limitations of traditional column chromatography. All operations in CTC are conducted on a moving slurry that is continuously pumped through a cascade of static mixers (to achieve binding equilibration) and hollow fiber membrane modules (to separate the fluid phase from the resin particles). Contacting in the individual steps is performed in a countercurrent fashion using multiple stages to increase throughput, reduce buffer costs, and

enhance product yield and purification. Experimental studies were performed with a harvested cell culture fluid containing a monoclonal antibody using Poros MabCapture A resin. The CTC system provided continuous and stable operation for extended periods of time by maintaining the permeate flux in each module below the critical flux. The process gave good antibody yield with better host cell protein removal than a traditional Protein A packed column. In addition, the overall productivity of the CTC system was approximately 8-fold greater than that provided by column chromatography, leading to significant cost-savings relative to conventional packed columns.

O-803 **Continuous chromatographic technology aimed at vaccine applications using core bead chromatography for reduction of ovalbumin impurities.** Hans Blom, Günter Jagschies and Karol Łącki; GE Healthcare Life Sciences, Uppsala, SWEDEN

Vaccination is the most effective prevention strategy to avoid and protect large populations against diseases like influenza infection. In order to facilitate production of the large amounts of doses required, reliable and economic vaccine production methods are key. The vast majority of influenza vaccines are still produced from allantoic fluid obtained from fertilized chicken eggs. This requires efficient removal of the main and critical impurity contaminant ovalbumin, which contributes to over 60% of the total protein content in allantoic fluid, while insufficient removal of ovalbumin can cause severe allergic reactions. Therefore, efficient and scalable ovalbumin reduction methods during egg---based vaccine manufacturing are critical. In the present work a continuous chromatography technology, using a multimodal core bead resin run in scavenger mode, has been evaluated for the reduction of ovalbumin and compared to a traditional chromatographic set up. The results demonstrate that this approach can improve the productivity in a scalable and cost effective manner.

O-804 **Purification of adenovirus by 2-column periodic countercurrent chromatography.** Piergiuseppe Nestola[1,3], Ricardo Silva[2], Cristina Peixoto1, Paula M. Alves[1, 3], Manuel Carrondo[1,2,3] and José Paulo Mota[2]; IBET - Instituto de Biologia Experimental e Tecnológica[1], Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa[2], Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa[3]

The purification of complex biopharmaceuticals, such as recombinant proteins, antibodies or vaccines, is gaining increasing importance to fulfill the requirements for a reliable downstream purification process with high purity and yield, and, most importantly, cost efficiency. Viruses, in particular, play an important role in the field of vaccines and gene therapy. The current approaches in most downstream processes are to operate the chromatographic steps using classical batch chromatography. Unlike other industries, biotechnology is late to embrace continuous processing or multicolumn processes. A possible reduction of the cost of goods (CoG) can be obtained by using multicolumn, (semi-)continuous chromatography, which gives higher throughput, lower buffer consumption, higher capacity of utilization of the stationary phase or smaller column volume, and hence increased productivity.

In the present work the use of a simple two-column, semi-continuous, open-loop simulated moving-bed chromatographic process was studied. The separation of adenovirus serotype 5 (Ad5) as model virus was successfully accomplished using a size exclusion resin under isocratic elution conditions. Clearance of impurities, namely DNA and host cell protein (HCP), were assessed. Our results showed a virus recovery yield of 86%, and a clearance of 90% and 89% for DNA and HCP, respectively. These figures compare very favorably against single-column batch chromatography. In conclusion, the main drawbacks of size-exclusion chromatography, namely lower productivity and scale up difficulties, were easily overcome by an innovative two-column configuration that keeps the mixed fractions inside the system at all times.

P-101 **Continuous biological products extraction in aqueous two phase systems.** Edith Espitia-Saloma, Olga Patricia Vázquez-Villegas, Marco Antonio Rito-Palomares and Oscar Alejandro Aguilar-Jiménez; Centro de Biotecnología-FEMSA, Tec de Monterrey, Campus Monterrey, MEXICO

Continuous operation advantages are more than acknowledged within industrial processes: process time and cost decreases and yield increases. Notable research has been performed concerning batch aqueous two-phase systems (ATPS), nevertheless continuous operation showing industrial potential, have been traditionally left behind. In the present work, a continuous device capable to process ATPS at pilot scale was implemented and characterized (mass balances, mixing evaluation, coalescence and separation profiles). Such novel patented prototype was scaled-up from a capacity of 50 mL/min to 1 L/min and semi automatized. The scaled-up system

performance was tested for crystal violet and whey protein isolate (WPI). Spent brewer's yeast suspension was chosen as a complex cell matrix model for the recovery of three industrially relevant enzymes: α-amylase, protease, and invertase. A central composite design was performed in order to model and optimize the operation process parameters. Critical parameters were Top and Bottom phase feeding flow, number of static mixers, and sample load. Mass transfer was improved in more than 150% in Gentian violet and WPI in the top phase when compared to batch experiments. Differential partitioning was evidenced by recovery of α-amylase in the top phase, and invertase at the interphase. Continuous operation showed noteworthy advantages over batch operation regarding partitioning. Separation time was reduced in approx. 10 min when compared with batch processes. Results show that a continuous large-scale ATPS represent a viable and reproducible extraction/purification system at industrial scale.

P-102 **Quantitative structure-property relationship for odorants by partitioning in aqueous two-phase systems.** Pedro P. Madeira [1], Ana Bessa[1], Miguel A. Teixeira[1], Luís Álvares-Ribeiro[1], Alírio E. Rodrigues[1], and Boris Zaslavsky[2] ; University of Porto, PORTUGAL[1], Analiza, Inc., USA[2]
It has been suggested some years ago that the distribution of a compound (e.g. drug) in vivo, in the absence of active transport mechanisms, may be approximated by equilibrium partitioning between different aqueous environments. Aqueous two phase systems (ATPS) being formed by two or more distinct aqueous phases with different solvent properties can be adequately used as a model to simulate processes occurring in vivo.
In the present work partition ratios of 9 odorants (p-anisaldehyde; methyl anthranilate; coumarin; trans-cinnamyl alcohol; vanillin; aniline; indole; acetophenone; and 4-nitrophenol) were measured in ten different polymer/polymer ATPS, all at physiological pH. The data was well described by the modified solvatochromic equation using only three descriptors (solute dipole-dipole, hydrogen bonding and electrostatic interactions with aqueous environment) and was used to obtain their corresponding solute-specific coefficients .
The results obtained in the present work show that linear combinations of these aroma compounds-aqueous environment interactions can be used in a quantitative-structure relationship to describe their odor detection threshold. They also suggest that *(i)* the receptor (or receptors) involved in the mechanism of perception of these compounds is strongly charged or has a strong dipole; *(ii)* the receptor-solute dipole-dipole interactions have opposite effects to the receptor-solute charge redistribution interactions in the odor perception, and *(iii)* the compounds in the present study interact similarly with odorant receptors.

P-103 **Development of a method for purifying trypsin that combines precipitation of protein-polyelectrolyte complexes with hydrophobic interaction chromatography.** Mauricio Braia[1], Gisela Tubio[1], Barbara Andrews[2], Oriana Salazar[2], María Elena Lienqueo [2] and Diana Romanini[1]; Universidad Nacional de Rosario, ARGENTINA[1], Universidad de Chile[2]
Trypsin (TRP) is a serine protease widely used in the food and pharmaceutical industries and in molecular biology. Alginate (ALG) is an anionic polylectrolyte obtained from brown algae which has been extensively used for many biomedical applications due to its biocompatibility, low toxicity, relatively low cost and gelation capacity. Proteins and polyelectrolytes form insoluble complexes via electrostatic interactions that might be useful to isolate and immobilize enzymes. Hydrophobic interaction chromatography (HIC) is a powerful technique used for separating proteins based on their hydrophobic properties. The combination of precipitation of protein-polyelectrolyte complexes and HIC is presented as a novel strategy for purifying proteins. The aim of this work was to develop a method for purifying TRP that combines precipitation of TRP-ALG complexes and HIC purification. At pH 3.5 TRP and ALG interact to form an insoluble complex that can be precipitated, obtaining a recovery of 93 %. Then, the precipitated complex was dissolved in buffer TRIS 50 mM pH 8.00 NaCl 1 M and injected into a Phenyl-sepharose column. The recovery was 57 % and we are working to improve it. The most important feature is that it allowed separation of the TRP from the ALG.
Authors would like to thank MINCyT (Argentina) and CONICyT (Chile) for the financial support (CH/11/16).

P-104 **Mechanisms of protein sorption and transport in cellulosic ion exchangers of differing salt tolerances.** James M. Angelo and Abraham M. Lenhoff; University of Delaware, Newark, DE, USA
Cellulosic ion exchange materials have recently been developed that allow high loading capacity and rapid uptake of proteins in addition to displaying a low sensitivity to heightened total ionic strengths (TIS) of the solution, making protein retention and separation of undiluted feed streams much more feasible. The functional

characteristics of three cellulosic ion exchange resins were investigated: S HyperCel for CEX and Q HyperCel and a salt-tolerant derivative, STAR AX HyperCel, for AEX. Mechanistic insight was sought using confocal microcopy to gain a physical understanding of protein uptake profiles. Time–series confocal imaging of particle cross-sections elucidated the type of intraparticle transport in which fluorescently labeled protein traversed to the particle core. Elution profiles at varied TIS of the solution were performed to assess optimal conditions for particle unloading, as well as displacement experiments using different combinations of model proteins. Adsorption capacity, protein uptake rates and retention at differing ionic strengths were also assessed for each of the stationary phases. The salt-tolerant AEX material displayed higher adsorptive capacities than the standard strong ion exchange resins at elevated TIS and similarly presented rapid uptake of protein measured via batch kinetics. Column loading and unloading work was carried out using similar conditions that were employed during confocal microscopy to comparatively test performance across the range of ion exchangers and to corroborate that the uptake and elution behavior observed during microscopic flow experiments persisted following scale-up.

P-105 **Diafiltration optimization for high concentration UFDF processes impacted by the Donnan effect.**
Joshua Arias, Alexandra Steele; EMD Millipore, Billerica, MA, USA
The goal of a formulation TFF process is to bring the product to its final concentration and to perform a buffer exchange so as to achieve the target product formulation. Eight to ten diavolumes is a standard recommendation an acceptable buffer exchange in a typical final formulation UFDF process. This recommendation comes from the theory that during the DF process, buffer excipients will flow freely through the UF membrane, having an R (retention) value of 0. Given this assumption at 10 diavolumes, 99.995% of the original buffer would be removed from the system; this percentage is considered an acceptable exchange by industry standards. The following study details how the Donnan effect affects the R value of a system (i.e. the removal of excipients), changing the number of diavolumes required to perform a complete exchange. It was observed in this study that if the MAb and buffer excipient(s) that is being removed are oppositely charged, the R value will increase. The higher the R values the more diavolumes will be required to achieve an acceptable exchange. Conversely if the MAb and buffer (excipient) that is being removed are congruently charged, the R value will be negative and less diavolumes will be required. Furthermore, the study showed that the concentration at which the DF is performed also affects the R value since a higher concentration involves more interaction between the mAb and charged excipients. Based off of the specific data generated from this study a methodology was developed for determining the optimal concentration at which to perform the diafiltration exchange. This methodology could be used to determine optimums for individual systems. Using knowledge about the Donnan effect and how it can affect buffer removal, better recommendations can be made for developing formulation TFF processes.

P-106 **Purification strategy of AAV and scAAV vectors for gene therapy against alcoholism.** Alicia T. Lucero, Anamaría C. Sánchez, Barbara A. Andrews and Juan A. Asenjo; Centre for Biochemical Engineering and Biotechnology, Institute for Cell Dynamics and Biotechnology (ICDB): A Centre for Systems Biology, Universidad de Chile
In the context of viral vectors as therapeutic pharmaceuticals, preclinical and clinical trials require the use of good manufacturing practice in the preparation of these agents. Hence, scale up and GMP production requires a downstream purification process, such as liquid chromatography. This procedure is one of the most efficient methods in a cost-benefit aspect, which is essential for public health in the treatment of alcoholism.
The present study describes recombinant AAV and scAAV serotype 2 and 8 purification using FPLC for gene therapy in alcoholism treatment.
The vectors were produced in HEK293 cells growing in DMEM/F12 media with 5% FBS. Viral vectors were purified using a CIM QA-1 tube monolithic column, an FPLC system was used for the chromatography. The CIM QA-1 tube monolithic column supports higher pressures and can be used at higher flow rates. Comparison of the purification was made for different flow rates and different gradients of elution. The elution fractions of the purification was quantified by RT-PCR and analyzed by SDS-Page. The purified vectors were structurally intact by electron microscopy analysis. An improved separation of vectors from cell lysate contaminants was achieved using strong anion-exchange columns using a CIM QA-1 tube monolithic column by 3-step gradient chromatography for different flow rates. The purified vectors will be used for infectivity and therapeutic activity studies in cell culture.

P-107 **Biomimetic ligands for the enrichment of phosphoproteins.** Íris Luz Batalha[1], Ricardo J. F. Branco[1], Olga Iranzo[2], Christopher R. Lowe[3] and Ana C. A. Roque[1]; FCT-UNL[1], ITQB-UNL[2], University of Cambridge[3]
There is enormous interest to identify, isolate and quantify phosphoproteins (p-Pr) and related kinases, which are potential biomarkers and drug targets. However, due to their scarcity in biological samples, there is a need to enrich samples in p-Pr prior to further Mass Spectrometry analysis [1]. The key amino acid residues and three-dimensional constraints characteristic of the interaction between natural human p-Pr-binding domains and their phosphorylated targets were identified *in silico*, and used for the *de novo* design of two classes of p-Pr-binding biomimetic ligands, including fully synthetic affinity ligands and a peptide-based affinity ligand. Three different libraries of robust and low-cost synthetic ligands consisting of 232 small molecules were synthesized on solid-phase (agarose) by high-throughput combinatorial chemistry. Two lead ligands were identified, presenting selective binding towards phosphorylated species, high binding capacities and up to 70% recovery yields upon elution. A cyclic 14-mer β-hairpin scaffold mimicking the BRCT domain, which is strongly associated with breast and ovarian cancers, was also synthesized using standard Fmoc solid-phase chemistry, purified and characterized. Both classes of ligands were successfully immobilized onto polymer-coated magnetic nanoparticles, and the affinity and selectivity toward p-Pr were confirmed. [1] Batalha *et al* (2012) Trends Biotechnol 30 (2), 100-110

P-108 **Effects of chemical modifications in the partition behavior of RNase A in aqueous two-phase systems.** José González-Valdez, Marco Rito-Palomares and Jorge Benavides; Centro de Biotecnología FEMSA, Tecnológico de Monterrey, Campus Monterrey, MEXICO
Chemical modifications confer proteins improvements in properties and a broader range of applications. Once modified, several strategies need to be applied to isolate the conjugates of interest. Aqueous Two-Phase Systems (ATPS) have demonstrated to be an attractive alternative for the primary recovery of proteins and their conjugates. However, to better understand the biochemical parameters that affect partition behavior, it becomes necessary to characterize partition of different species. In this work, the fractionation of Ribonuclease A (RNase A) in its native form was compared to that of mono-PEGylated RNase A and chemically modified forms of the protein with Uniblue A, Dabsyl Chloride and Direct Red 83 in polyethylene glycol (PEG) – potassium phosphate ATPS. Results suggest that while system parameters (i.e. Tie-Line Length (TLL) and PEG molecular weight) govern the partition of native RNase A, the behavior of the modified species is more influenced by the physicochemical characteristics of the modifying molecules such as hydrophobicity and molecular weight promoting partition towards the top polymer rich phase with recovery percentages as high as 86%. In fact, it has been found that as hydrophobicity increases partition is promoted towards the PEG-rich phase counterbalancing the effect of the molecular weight of the modifying molecules that tends to shift partition towards the salt rich phase.

P-109 **Primary recovery of superoxide dismutase from *Kluyveromyces marxianus* in aqueous two-phase systems.** Jesús Simental-Martínez, Marco Rito-Palomares, Jorge Benavides; Centro de Biotecnología FEMSA, Tecnológico de Monterrey, Campus Monterrey, MEXICO
The increasing interest in the production of biomolecules and products from natural sources has triggered the need to develop and design adequate bioprocesses for their efficient purification. In this context, aqueous two-phase systems (ATPS) represent a good choice for the recovery of biomacromolecules, particularly proteins. The aim of this study was to recover a superoxide dismutase (SOD; EC 1.15.1.1) naturally expressed in *Kluyveromyces marxianus* using polymer–polymer, polymer–salt and alcohol–salt ATPS. The effect of the system parameters on the recovery of the enzyme was characterized. Polyethylene glycol (PEG)-phosphate ATPS showed potential for SOD recovery. Systems prepared with low-molecular weight PEG (400 g/mol) attained the recovery > 70% on the top phase, while high molecular weight PEG (8000 g/mol) systems allowed the recovery of SOD at the bottom phase (65%). The enzyme retained at least 90% of its initial activity. In the polymer–polymer systems the enzyme distributed more evenly between phases. Ethanol-salt systems showed good results, recovery values are over 70%; nevertheless the enzyme lost more than 35% of its initial activity. This study demonstrates the potential of aqueous two-phase systems for the primary recovery of SOD from yeast.

P-110 **Influence of slight variations of ion exchange media on the separation of proteins.** Simon Kluters[1], Lothar Jacob[2]; Heiner Graalfs[2] and Christian Frech[1]; University of Applied Sciences Mannheim[1], Merck KGaA, Darmstadt, GERMANY[2]
Ion-exchange chromatography (IEC) is a very versatile protein separation method, and widely used as production scale protein purification process. When IEC is used for high resolution separation of proteins, retention and peak broadening are not always reproducible and are very sensitive to small changes in typical operating factors such as pH, ionic strength and stationary phase ion-exchange capacity.
As lot to lot variations of ion-exchange capacity (Λ) are unavoidable the influence of the variation of Λ was investigated.
Gradient elution data of monoclonal antibodies at different pH values on two strong anion exchange materials (Fractogel TMAE, Fractogel TMAE HiCap) are used to evaluate the influence of ionic capacity on protein retention. Retention models for ion exchange chromatography based on the work of Mollerup and Yamamoto are used to calculate their model parameters and to predict the influence on critical process parameter like ionic strength of elution, elution volume and binding capacity of the final product.

P-111 **Phase system selection with fractional factorial design for purification of a recombinant protein from a hydroponic culture medium using centrifugal partition chromatography.** Łukasz Grudzien[1], Luisa Madeira[2], Derek Fisher[1], Julian Ma[2], Ian Garrard[1]; Brunel Institute for Bioengineering, Brunel University, London, UK[1], St. George's Hospital Medical School, University of London, UK[2]
Design of experiment (DoE) software was successfully used to optimise parameters of aqueous two-phase system (ATPS), which allowed purification of a recombinant protein, by centrifugal partition chromatography (CPC). The protein, cyanovirin-N (CV-N), was purified from other proteins which were co-secreted into a hydroponic plant medium in a rhizosecretion process. To achieve satisfactory protein concentration, the purification was preceded by ultrafiltration performed on a 5 kDa filter. ATPS, because of their gentle nature, were selected as the phase system for CPC. A systematic phase system selection was applied. This involved studying the effect of seven parameters of ATPS: polymer type, salt type, the polymer and salt concentration, the polymer molecular weight, pH, and presence of two additional salts; NaCl and NaClO4, which all together gave 320 combinations. DoE software allowed the reduction of this number to 46. Having tested partitioning of cyanovirin-N and impurities in 46 ATPS, the three best potential phase systems generated by the programme were then tested on the CPC. Out of these three, 13/13% PEG4000 sodium phosphate, pH 3.0, proved to be most effective phase system in the purification of cyanovirin-N, judged by ELISA and SDS-PAGE analysis, as it eliminated most of the impurities from the final cyanovirin-N preparation.

P-112 **Ultrafiltration behavior of low fouling zwitterionic membranes.** Mahsa Hadidi and Andrew Zydney; Pennsylvania State University, USA
High performance ultrafiltration membranes are of growing interest in the biotechnology industry for protein concentration and purification. Several recent studies have shown that zwitterionic membranes containing both positive and negative charge groups can provide a very low fouling surface while retaining some of the benefits of electrically-charged membranes in terms of their high permeability and selectivity. However, there have yet be any detailed comparisons of the performance of these zwitterionic membranes with both charged and neutral membranes under controlled experimental conditions. Membranes were generated by chemical modification of a cellulosic membrane using epichlorohydrin activation followed by reaction with ligands having approximately the same length but different end-group functionality. The extent of modification was evaluated by X-ray photoelectron spectroscopy, and the membrane surface characteristics were determined from contact angle and streaming potential measurements. Protein fouling studies were performed using human serum IgG. The zwitterionic membranes showed the lowest amount of protein adsorption and membrane fouling, with less than a 12% reduction in permeability over a wide range of solution pH. The greatest amount of fouling was seen with the positively- and negatively-charged membranes under conditions where the protein and membrane had opposite surface charge due to the attractive electrostatic interactions. Membrane hydrophobicity also had a significant effect on the fouling behavior. The zwitterionic membranes also showed high degrees of protein retention. These results provide important insights into the behavior of these zwitterionic membranes and their potential in high performance ultrafiltration applications.

P-113 **Evaluation of chaotic advection micromixers embedded in microfluidic devices for high throughput characterization of aqueous two-phase systems.** Patricia Vázquez-Villegas[1], Eric Ouellet[2], Oscar Aguilar[1], Marco Rito-Palomares[1] and Charles Haynes[2]; CB -FEMSA, ITESM, Monterrey, NL, MEXICO[1], MSL, UBC, Vancouver, BC, CANADA[2]

While aqueous two-phase systems (ATPS) are traditionally used as a bioseparation technique at a macroscale, microfluidic ATPS devices have recently been described for various applications. Currently, these microfluidic approaches involve flowing streams of immiscible fluids in a continuous fashion to separate biomolecules by diffusion across a microchannel. This study aims to validate a new micro-fluidic platform for the high throughput characterization of ATPS and biomolecules partitioning behavior. Chaotic advection micromixers embedded within the device allowed for the sequential and rapid preparation of two-phase systems with varying compositions by changing the volumetric flow rate for each phase component (0.001 to 10 □L/min). Combinations of poly(ethylene glycol) (PEG) and phosphate or PEG and dextran solutions were used for the proof of concept of micro-device functioning and partitioning of different experimental models (organic dyes, fluorescent proteins and microbeads). Recovered VR varied 1.53% from the correspondent batch systems of 200 □L. Both rapid mixing of phase components and fast separation were achieved via a combination of advective and diffusive forces rather than diffusion alone. Despite flow thresholds due to phase densities, causing back flow and Plateau–Rayleigh instabilities appear, the weight percent of phase components can be altered on-demand. This microfluidic platform could be useful for the rapid generation of phase diagrams for unknown samples in shorter amount of times than by traditional methods.

P-114 **Applications for a pH responsive, multimodal chromatography resin.** Kristian Kallberg[1, 2], Leif Bülow[1], Kristian Becker[3] and James Van Alstine[4]; Pure and Applied Biochemistry, Dept. of Chemistry, Lund University, Lund, SWEDEN[1], Takeda Pharma A/S, Copenhagen, DENMARK[2], BioInvent International AB Lund, SWEDEN[3], jim.vanalstine@telia.com[4]

Over the past few years an experimental pH responsive, multimodal chromatography resin (1) has been tested in regard to applications calling for a very selective, non-fouling chromatography resin. The applications included: A. Resolving of protein test mixtures, B. Resolving recombinant proteins only differing in a few neutral amino acids, C. Resolving protein PEGylation reaction mixtures, D. Resolving of protein samples differing in glycosylation, and E. Resolving monoclonal antibody fractions of differing glycan composition, formed under varied eukaryotic host cell culture conditions. Parts A and B of this work have been published previously, while D and E have recently been submitted for publication. This presentation will summarize the above work in regard to what it tells us about the potential for such resins to be used in the above and other challenging applications.

(1) The resin was from GE Healthcare (GEHC), 751 82 Uppsala, Sweden and is described in WO2004082801.

P-115 **Monoclonal antibody Fc and Fab fragment self- and cross- interaction strengths.** Nicholas E. Levy, Rachael A. Lewus and Abraham M. Lenhoff; Department of Chemical and Biomolecular Engineering, University of Delaware

Protein self-interactions and phase behavior are involved in numerous aspects of downstream processing, either by design as in crystallization or precipitation processes, or as an undesired effect, such as aggregation. The self-interaction strengths – expressed as second osmotic virial coefficients – and instantaneous phase behaviors of eight monoclonal antibodies (mAbs) were previously determined. It was found that the majority of mAbs have similar phase boundaries and interaction strengths, but there are a few highly attractive and non-attractive outliers. The interactions of Fc and Fab fragments for two of these mAbs were studied to better understand the different behaviors observed. The first mAb was found to have self-interaction strengths, phase boundaries and oligomerization characteristics similar to those of most mAbs studied previously. The second mAb has much more attractive self-interactions, significantly shifted phase boundaries and higher-order oligomerization states than those of any other mAb studied. Fab and Fc domain self- and cross-interaction strengths were measured using self- and cross-interaction chromatography in various solution conditions. In this work it was determined that the attractive nature and unusual oligomerization behavior of the second mAb are primarily due to very strong Fab-Fab interactions compared to the moderately attractive interactions seen for all fragment interactions of the first mAb.

P-116 **Direct recovery of lipase derived from microbial feedstock using sustainable aqueous two-phase flotation.** Pau Loke Show[1], Mohd Shamsul Anuar[2]and Tau Chuan Ling[3]; Nottingham University Malaysia Campus[1] [2] Universiti Putra Malaysia[2], University of Malaya[3]
In this study, lipase derived from *B. cenopacia* ST8 was directly recovered from unclarified microbial feedstock using sustainable aqueous two-phase flotation (ATPF) technique. ATPF is an integrated technique which combines of hydrophilic organic solvent/inorganic salt aqueous two-phase system (ATPS) and solvent sublation (SS) methods. The ATPF technique composed of 2-propanol and potassium phosphate. A purification factor of 14 and a recovery yield of 99% were obtained in the integrated operation. This study demonstrated that the average recovery of organic solvent (2-propanol) and inorganic salt (potassium phosphate) were up to 70% and 61%, respectively. The generic application of this integrated technique has been evaluated.

P-117 **On-column aggregation of a recombinant immunotoxin during anion exchange chromatography.** Thomas Linke, Andrew Fulton, Yang Wang and Alan K. Hunter; MedImmune, Gaithersburg, MD, USA
This work describes the identification and subsequent resolution of on-column aggregation of a recombinant immunotoxin produced in E coli. The crude product is isolated as inclusion bodies, refolded and purified by column chromatography. Strong anion exchange chromatography in gradient elution mode was used as a final polishing step with a focus on removing residual process-related contaminants. However, a mass balance analysis demonstrated that a significant amount of protein was lost during this step. The root cause for this loss was identified as on-column aggregation of the recombinant immunotoxin. High molecular weight aggregates were recovered from the column with a high salt wash. SDS-PAGE analysis demonstrated that the high molecular weight aggregates consisted of disulfide-linked recombinant immunotoxin. On-column aggregation was minimized by adjusting the conductivity of the column load and equilibration buffer, resulting in improved step and overall process yields while maintaining product quality. Models are proposed to explain on-column aggregation behavior at a molecular level.

P-118 **A new amino acid scale based on analysis of different types of amino acid-water interactions.** Pedro P. Madeira[1], Ana Bessa[1], Luís Álvares-Ribeiro[1], M. Raquel Aires- Barros[2], Alírio E. Rodrigues[1], Vladimir N. Uversky[3], and Boris Zaslavsky[4]; University of Porto, PORTUGAL[1], University of Lisbon, PORTUGAL[2], University of South Florida, USA[3], Analiza, Inc., USA[4]
It is well known that the aqueous environment plays an active role in protein folding, maintaining the protein structure and protein function *in vivo*. Therefore, studies of protein-water interactions are important from both theoretical and practical viewpoints.
Amino acids are the building blocks of proteins and hence analysis of their interactions with aqueous media is necessary to gain better insight into protein-water interactions.
In order to study interactions of biological molecules with aqueous environment we suggested previously to apply the solvatochromic comparison method to characterize solvent properties of the media in the coexisting phases in multiple aqueous two-phase systems (ATPS) with a set of selected solvatochromic probes.
In this study the solute-specific coefficients representing the solute dipole-dipole, hydrogen bonding and electrostatic interactions with the aqueous environment of the amino acids were determined by multiple linear regression analysis using the modified linear solvation energy relationship and were used in Quantitative Structure-Property Relationship (QSPR) analysis.
It is shown that linear combinations of these solute-specific coefficients are correlated well with various physicochemical, structural, and biological properties of amino acids.

P-201 **Aldehyde PEGylation of laccase from *Trametes versicolor*: Effect of reaction time on the enzyme activity.** Karla Mayolo-Deloisa, Mirna González-González, Jesús Simental-Martínez and Marco Rito-Palomares; Centro de Biotecnología-FEMSA, Instituto Tecnológico y de Estudios Superiores de Monterrey, MEXICO
Laccase is a multi-copper oxidase that catalyzes the oxidation of phenolic compounds. Laccase can be used in bioremediation, beverage (wine, fruit juice and beer) processing, ascorbic acid determination, sugar beet pectin gelation baking and as a biosensor. Recently, the anti-proliferative activity of laccase towards tumor cells has been reported. Due to the potential application of the enzyme, the efforts for enhancing and stabilizing the activity have been increased. Thus, the PEGylation of laccase can be an alternative. PEGylation is the covalent attachment of one or more molecules of methoxy polyethylene-glycol (mPEG) to a protein. During the PEGylation reaction the

activity is reduced but the stability increases, thus it is important to minimize the loss of activity. In this work, the influence of time of the PEGylation reaction on the total enzyme activity of laccase was analyzed after 4 and 17 hours. The activity was measured using three substrates: ABTS, syringaldazine and 2,6-dimethoxyphenol. Additionally, different molecular weights of mPEGs were used: 20, 30, 40 kDa and 40kDa-branched. The PEGylation of laccase has not been extensively explored so it is important to analyze the effects of this bioconjugation.

P-202 **Molecular modeling study of PEG-proteins interactions using AutoDock.** Karla Mayolo-Deloisa[1], Cristian Salgado[2], Diana Ostos-Rangel[1], Gerald Zapata[3], Ma. Elena Lienqueo[2], Marco Rito-Palomares[1] and Juan A. Asenjo[2]; Centro de Biotecnología-FEMSA, Tecnológico de Monterrey, MEXICO[1], Department of Chemical Engineering and Biotechnology, University of Chile,[2], Faculty of Chemical and Pharmaceutical Sciences, University of Chile[3]
The polyethylene-glycol (PEG) plays an important role in the stabilization of proteins. PEG is used as a macromolecular crowding agent, as a component of aqueous two phase systems and to produce PEGylated proteins. PEGylation is the covalent attachment of one or more molecules of PEG to a protein. Docking experiments between the proteins (RNase A, lysozyme and β-lactalbumin) and the PEG were conducted in order to contribute to understanding the potential molecular interactions between the PEG strand and the PEGylated proteins. It considered that the molecular interactions between the PEG and the protein are given by the interaction of the ethylene glycol group with the protein surface. The PEG molecule was represented by 2, 4, 6 and 8 repetition groups. Molecular docking experiments were performed using ADT and conducted using the software AutoDock v4.2. In all experiments, the PEG molecule was considered as flexible and the maximum number of torsions was taken into account in order to explore its conformational space. The proteins were considered as rigid. The results permit to identify the main aminoacids involved in the interaction with the protein.

P-203 **Aqueous two phase system applied for norbixin purification.** Guilherme P. Almeida, Lizzy P. Alcântara, Valéria P. R. Minim and Luis A. Minim; Department of Food Technology, Federal University of Viçosa
Annatto is a natural dye obtained from the seeds of Bixa orellana L and the main pigment extracted from the seeds is the bixin, a fat-soluble carotenoid. Bixin is used as coloring agent in the food, pharmaceutical and cosmetic industries and is known for its antioxidant capacity. Norbixin is the water-soluble form resultant from the saponified bixin in sodium or potassium alkali solutions. Both carotenoids are considered to promote human health, such as cell protection against free radicals and reactive oxygen species scavenging, due to their antioxidant activity. Liquid–liquid extraction using organic solvents is usually used to extract and concentrate bixin. However, this technique is disadvantageous once that organic solvents are used which are not environmentally friendly and may cause health problems due to its toxicity, flammability, and carcinogenicity. In the last decade, researchers have launched to exploit Aqueous Two-Phase Systems (ATPS) as an alternative technique for dye purification. Recent studies have been carried out to apply ATPS for the extraction of natural dyes. In this work, a different and more complete approach was conducted to purify norbixin. Initially, different conditions of different types of ATPS were studied. Based on the partition coefficient of norbixin, potassium phosphate and PEG (4000 g/mol) at pH 11.0 and 25oC was selected which presented a partition coefficient of 140. Subsequently, the liquid-liquid equilibrium (LLE) data of norbixin was determined for that system. Finally, a study based on the precipitation of norbixin and consequent separation was developed. Norbixin was successfully separated by precipitation from the PEG phase at pH 4.0.

P-204 **Direct purification of lysozyme from low diluted egg white using a supermacroporous monolithic cryogel column.** Rafael C. I. Fontan[1], Renata C. F. Bonomo[1], Valéria P. R. Minim[2] and Luis A. Minim[2]; Department of Food Engineering, Southwestern of Bahia State University[1], Department of Food Technology, Federal University of Viçosa[2]
Supermacroporous cryogels are an alternative support for proteins purification, allowing the use of more concentrated or less clarified feedstock due to its high permeability and porosity. Lysozyme is an enzyme with antimicrobial activity that is generally used in the pharmaceutical and food industries. A faster and cheaper purification process, with less environmental impact is of great interest. The potential of cryogels has not yet been fully explored for protein purification from concentrated solutions. So, the objective of this study was to produce a supermacroporous cryogel for one-step purification of lysozyme from low diluted chicken egg white. A cation-exchange cryogel column (10 mm diameter, 94 mm length) was produced by cryogenic polymerization (-12 °C) using acrylamide, bis-acrylamide and allyl glycidyl ether monomers, and grafted with sulfo functional group. The

column presented porosity of 91% and ionic capacity of 25.7 mmolNa+/Lbed. Egg white ovomucin free was diluted three fold in sodium phosphate buffer 0.02 mol L-1 pH 7.2 and was circulated through the column. The adsorbed proteins were eluted with NaCl 1.0 mol L-1 in phosphate buffer. The eluted peak was dialyzed and lyophilized, presenting 78% purity. This work demonstrated that supermacroporous cryogel could be used for lysozyme purification directly from low diluted egg white.

P-205 **Extraction of cheese whey proteins using PEG/Citrate based environmentally benign aqueous two-phase system.** Rajendran Govindarajan and Muthiah Perumalsamy; National Institute of Technology, Tiruchirappalli, INDIA

Cheese whey is a liquid or milk serum remaining after the separation of casein and fat during milk coagulation. Historically, cheese whey has been considered as a waste product and is used as an animal feed or discarded as an effluent without treatment. It is an inexpensive and abundant nutrient source, rich in proteins, vitamins, minerals and salts etc. During recent decades, interest has grown in the nutritional utility of whey proteins in infant formula and in human health foods. Hence, the separation and purification of nutritional and functional whey proteins is considered to be highly essential. Extraction of biomolecules from complex mixtures using aqueous two phase systems (ATPSs) has several advantages over conventional purification methods such as short processing time, simple scale-up, provide gentle environment for biomolecules. In the present study, the extraction of cheese whey proteins using environmentally benign aqueous two-phase system comprising polyethylene glycol (PEG) 4000/triammonium citrate was studied. The addition of cheese whey has influenced the position of the binodal curve and is displaced towards the origin. This displacement was attributed to the presence of cheese whey proteins and whey salts, contributing to the two-phase formation. Thus, the threshold concentration required for two-phase formation decreases, thereby reducing the amount of phase forming chemicals. The effects of PEG concentration, salt concentration, volume ratio and pH on the partitioning of cheese whey proteins were studied in detail.

P-206 **Propagation of variance in ion exchange chromatography for protein separations: Impact of mobile phase variance.** Qiyang Duan[1], Todd Przybycien[1,2]; Departments of Chemical Engineering[1] and Biomedical Engineering[2], Carnegie Mellon University, Pittsburgh, PA, USA

Ion exchange chromatography is widely used in the downstream processing of biologicals and the performance of this separation operation is exquisitely dependent on mobile phase characteristics. We have examined the impact of mobile phase variance – simultaneous variation in composition, gradient slope, gradient shape, and flow rate – on chromatographic performance for a model protein separation system. We have used a mass transport model of ion exchange chromatography and several common ion exchange protein isotherm models together with a Monte Carlo simulation technique to propagate mobile phase variance through to the three classic chromatographic performance metrics: target yield, target purity and throughput. Ultimately, we suggest guidelines for the selection of standard versus high performance mobile phase delivery skids.

P-207 **Hybrid disposable cartridges for integration of downstream bioprocessing.** Poondi Rajesh Gavara[1], Marcelo Fernandez-Lahore[2], and Mariano Grasselli[3]; ChiPro GmbH, GERMANY[1], Jacobs University Bremen gGmbH, GERMANY[2], Universidad Nacional de Quilmes, ARGENTINA[3]

Conventional multistep downstream processing (DSP) of bioengineered therapeutic production account for more than 80% of the manufacturing costs. Hybrid Disposable Cartridges (HDC), an approach created by merging existing textile technology with material / polymer chemistry will be used to generate a gaining productivity, cost efficiency and sustainability in the DSP of bio products by Integration and Intensification strategies. A HDC system, gPore NW materials, based on a delicate combination of functional and bi-component fibres which are integrated into a disposable element will allow customized flow distribution properties and efficiently used for primary recovery and purification of proteins and monoclonal antibodies. A model HDC system, gPore NW is presented here to support an integration effect when utilized for early recovery of intracellular recombinant protein (~50 kDa). The chromatographic results demonstrated that partial pure product was captured from a disrupted whole cell extract containing 12% (w/v) and recovery was > 90%. The system shows almost no pressure and no clumps, indicating high biomass compatibility and high binding specificity with high process flow rates (300 cm/h). This in turn makes drastic reduction of process time, buffer volume and energy expenditure. The novel adsorbent cartridges exhibited

superior performance during early protein capture so as to introduce a high degree of integration in downstream purification.
http://intensoproject.eu/project-outline/state-of-the-art/hybrid-disposable-cartridges/

P-208 **Partitioning of CD133 antibody in aqueous two-phase systems: In route to stem cell separation.** Mirna González-González and Marco Rito-Palomares; Centro de Biotecnología-FEMSA, Tecnológico de Monterrey. Campus Monterrey, Monterrey, MEXICO
Immunoaffinity aqueous two phase systems represent a promising alternative for the isolation of stem cells due to the multiple advantages they possess, including, biocompatibility, scalability, low processing times and relatively low cost. Moreover, if the antibodies employed are PEGylated, a novel strategy for the purification of $CD133^+$ stem cells could be achieved due to the valuable properties conferred through the PEGylation reaction. The aim of this study is to first determine the partition preference of the native CD133 antibody in three different polymer-polymer aqueous two phase systems: ficoll 400,000-Dextran 70,000, Ucon-Dextran 75,000 and PEG 8,000-Dextran 500,000. Afterwards, the fractionation of the PEGylated CD133 antibody in these same systems will be investigated to compare their performance with the native antibody. The CD133-biotin antibody is PEGylated exploiting the streptavidin-biotin conjugation and two different modified polyethylene glycols (PEGs) will be employed: COOH-PEG-Biotin and NH2-PEG-Biotin. The fractionation of the native and PEGylated antibody in these polymer-polymer ATPS would help to elucidate the feasibility of employing these systems for the future recovery of $CD133^+$ stem cells.

P-209 **Scaling-up of a B-phycoerythrin production and purification bioprocess involving aqueous two-phase systems: Practical experiences.** Federico Ruiz-Ruiz[1], Jorge Benavides[1] and Marco Rito-Palomares[1]; Centro de Biotecnología FEMSA, Departamento de Biotecnología e Ingeniería de Alimentos, Tecnológico de Monterrey, Campus Monterrey. Monterrey, MEXICO[1]
One of the most attractive segments in food and cosmetic industries is that of natural pigments. Since some synthetic pigments have been reported to be hazardous for humans, natural pigments obtained through biological sources represent an attractive alternative. Our research group has previously worked on the development of an aqueous two-phase system (ATPS)-based prototype process for the recovery of B-Phycoerythrin (BPE), a natural high-value pigment obtained from *Porphyridium cruentum*. Studies describing the scaling up of ATPS processes from bench to pilot plant scale are not common. In this research experiences derived from the scale-up of a previously developed process for production and recovery of highly purified (purity defined as the absorbance ratio A545/A280 > 4) BPE are described, where a scale-up factor up to 850X was considered. Characterization of cell disruption with a pilot-scale bead mill allowed efficient BPE release at 2900 rpm, 10% (w/v) sample load, 60% (v/v) bead load with 0.5 mm glass beads and 22 min of residence time with a yield of 1.35 mg BPE/g wet biomass. BPE was recovered and purified using a strategy comprising isoelectric precipitation, aqueous two-phase fractionation and ultrafiltration. A 54% global BPE recovery yield, with final purity of 4.1, was achieved under optimal process conditions. Considering total costs for raw materials and energy expenditures for one batch it was determined that the production cost of BPE was of $1.17 USD/mg, while the commercial price of a BPE standard of the same purity is >30 USD/mg.

P-210 **Optimization of hydrodinamical and adsorptive properties of Chromatographic Gigaporose Columns.** Mirna L. Sanchez[1,2], Leandro J. Martínez1, Estefania Achilli[1], Marcelo Fernandez-Lahore[2] and Mariano Grasselli[1]; Universidad Nacional de Quilmes - IMBICE (CONICET)[1], Jacobs University Bremen[2]
[1] Universidad Nacional de Quilmes - IMBICE (CONICET). [2] Jacobs University Bremen.
Modern biotechnology heavily depends on the availability of efficient processes, which have to generate competitive products in terms of quality and cost. Most commonly, the purification of bioproducts is based on chromatographic platforms. However, the low productivity imposed by diffusional limitations, the need for multiple separation steps, and the requirement of extensive solids separation, drives to a technological situation characterised by low global bioproduct yields [1].
In a previous work, we fabricated porous, opened, and reticulated polyurethane foams supporting a functional hydrogel layer. Preliminary studies showed that the adsorptive properties of these materials allow the reversibly binding of proteins by ion exchange mechanisms.

Dynamic binding capacity tests (DBC) were performed employing lysozyme (HEWL) on a cation exchanger matrix. DBC experiments were carried out as a function column length, compression degree and flow velocity. Furthermore, bed hydrodynamics was studied via residence time experiments and correlated to the observed amount of protein captured.
Experimental data indicated that optimum performance, as judged by Pe and Qdyn, was obtained when 6 column length, 3X compaction degree, and an 212 cm/h linear flow was utilized.
The novel cation exchange material shows promise for the direct capture and purification of a range of biotechnology-derived products.

P-211 **Countercurrent centrifugal extraction: A new and high efficient extraction apparatus based on centrifugal partition chromatography.** Christoph Schwienheer, Juliane Merz and Gerhard Schembecker; TU-Dortmund, GERMANY
Countercurrent centrifugal extraction (CCE) is a new developed and patented (PCT/EP2012/068999) extraction apparatus, where the phase separation and phase mixing is supported by a centrifugal field. The construction is based on Centrifugal Partition Chromatography, where one liquid phase is kept stationary in a cascade of chambers by a centrifugal field while a second liquid phase is pumped through as a mobile phase. With CCE it is possible to pump both liquid phases simultaneously through each chamber and along the whole cascade in counter flow direction, allowing a continuous separation. The flow is achieved by connecting the chambers with two channels, one for each liquid phase. Specially arranged valves at the inlet and outlet of the chamber cascade control the cross flow. A substance mixture can be feed continuously at the middle of the chamber cascade. According to the distribution coefficients between the two liquid phases the separation of the substances occurs in direction of the heavy or light phase flow. A particularly advantage of CCE compared to classical extraction processes is the design of combined “mixer-settler-units” in small scale. By use of the centrifugal field it is possible to use instable phase systems, which form more stable emulsions with long settling times. Additionally, CCE provides a high number of separation stages, resulting in a good separation selectivity. In the presentation the construction and working principle of CCE will be explained and experiments which demonstrate the separation efficiency of the newly developed CCE technique are shown.

P-212 **Affinity precipitation of mAbs using stimuli responsive smart biopolymers: Methods development and process considerations.** Rahul D. Sheth [1], Bharat Bhut [2], Mi Jin[2], Jongchan Lee[2], Wilfred Chen[3], Steven M. Cramer[1]; Rensselaer Polytechnic Institute[1], Bristol-Myers Squibb[2], University of Delaware[3]
This work provides a detailed investigation into the development of a robust and scalable mAb affinity precipitation process using Elastin like polypeptides (ELPs) fused to the mAb binding Z domain. A multidimensional high-throughput screening (HTS) protocol was initially employed to determine initial capture and co-precipitation of pure model mAbs at high yields by the ELP-Z. mAb elution from the ELP-Z-mAb complex was subsequently determined using another HTS screen and mAb yields and aggregate content for the entire process were determined. High mAb yields with low aggregate content were obtained using mild elution conditions at a room temperature operation. Findings from the HTS studies were then used to guide studies for mAb purification from a mAb harvest feed. The process resulted in more than 2 logs of HCP and more than 4 logs of DNA clearance from the harvest feed, which was comparable or superior to Protein A chromatography for that mAb. Process performance was maintained for mAb final elution concentrations up to 20 g/l. Effective ELP-Z using NaOH and reusability over multiple purification cycles was demonstrated. Finally, process scalability was evaluated using scaled-down Tangential flow microfiltration (TFF-MF) and Dead end filtration approaches and it resulted in complete precipitate recoveries, high mAb yields and quality at high volumetric throughputs. This work demonstrates the potential of this ELP-Z based affinity precipitation process for industrial mAb purification.

P-213 **Bioanalytical method validation of a colorimetric protocol to determinate proteolytic activity of bromelain.** Diego Coelho[1], Thais Saturnino[1], Fernanda Fernandes[1], Bianca Martins[2], Beatriz Zanchetta[1], Priscila Mazzola[2], Elias Tambougi[1] and Edgar Silveira[2]; FEQ - Campinas State University[1], Uberlândia Federal University[2]
The choice of selective and sensitive bioanalytical methods is critical in the development and evaluation of drugs and plays an important role on quality of its studies, once it is directly related to the quality of underlying bioanalytical data. The importance of validation, at least of routine bioanalytical methods, can therefore hardly be overestimated. This is especially true in the context of quality management and accreditation, which have become matters of increasing importance in the requirements of peer reviewed scientific journals concerning method validation. This work aimed to demonstrate, using fundamental validation parameters (such as accuracy, precision, selectivity, sensitivity, reproducibility, and stability) and using specific laboratory investigations, that colorimetric method based on azocasein digestion is suitable and reliable for the proteolytic activity determination of bromelain. The obtained results showed that substrate can be stored up to two months and may undergo up to three cycles of freezing and thawing with no influence in its performance. The best results of activity was achieved at 50°C and pH 9.

P-214 **PEG/$(NH_4)_2SO_4$ aqueous two-phase systems for bromelain purification.** Diego Coelho[1], Birgit Pitzschel[2], Enoch Aguillón[3], Bianca Martins[4], Beatriz Zanchetta[1], Priscila Mazzola[1], Elias Tambougi[1] and Edgar Silveira[4]; Campinas State University[1], Technischen Univeristät Clausthal[2], Inst. Tecnol. de Ciudad Madero[3], Uberlândia Federal University[4]
Downstream process is defined as a sequence of procedures that, when followed, results in a purified product. Fractional precipitation, membrane separation, fluidized bed sorption and aqueous two-phase systems extraction are well described downstream processes. The fractional precipitation are often used in early processual stages, and can reduce costs by concentrating and purifying the target molecule. Extraction in aqueous two-phase systems (ATPSs) is a suitable technology for the first step of a separation procedure and to partially replace chromatographic steps. This separation procedure is influenced by phase system parameters, such as the molecular weight of the polymer, pH, type and concentration of phase-forming components. However, the application of ammonium sulphate in the purification of proteins through fractional precipitation integrated to poly(ethylene glycol)/salt ATPS, results in a medium-resolution downstream technique. This work describes the binodal curves and tie-lines for PEG/$(NH_4)_2SO_4$ ATPS, and its use for bromelain purification. Results showed that bromelain was best recovered with PEG 2000/$(NH_4)_2SO_4$ ATPS with 99.63% yield and a purification factor of 5.49 fold.

P-215 **Charge clustering dominates protein ion-exchange separations.** Mohan-Vivekanandan Poongavanam[1], Lydia Kisley [3], Jixin Chen[3], Andrea P. Mansur[3], Bo Shuang[3], Katerina Kourentzi[2], Sagar Dhamane[1], Wen-Hsiang Chen[2], Christy F. Landes[3,4], Richard C. Willson[1,2,5]; Department of Biology & Biochemistry, University of Houston[1]; Department of Chemical & Biomolecular Engineering, University of Houston, Houston, TX[2]; Department of Chemistry, Rice University[3]; Department of Electrical and Computer Engineering, Rice University, Houston, TX[4]; The Methodist Hospital Research Institute, Houston, TX[5]
We are employing single-molecule methods to study the complex mechanistic details involved in protein chromatography. We have proposed that ion-exchange separations on classical ion-exchange matrices are dominated by adsorption to stochastically clustered sites with minimal contribution of the isolated charges. We applied a combination of super-resolution optical methods and stochastic theoretical models to single-protein adsorption on a realistic, agarose-supported stationary phase. Most importantly, we conclusively demonstrated that localized protein adsorption only occurs at clusters of charges. Adsorption of proteins to individual singly-charged ligands was unobservable under our experimental conditions, unless the ligands were randomly clustered at high surface loadings. By extending the stochastic theory to model macroscale observables, the observed single molecule kinetics were used to simulate ensemble chromatographic elution profiles for ensembles of individual and clustered charges, and for individual types of sites. We believe these methods can be extended to competitive adsorption, affinity interactions such as Protein A, and potentially to thin sections of commercial adsorbents.

P-216 **Spermine Sepharose as a clustered-charge anion exchange adsorbent.** Sagar Dhamane[1], Federico Ruiz-Ruiz[1,2], Wen-hsiang Chen, Mohan-Vivekanandan Poongavanam[1], Katerina Kourentzi[3], Jorge Benavides[2], Marco Rito-Palomares[2], Richard C. Willson[1,3]; Department of Biology & Biochemistry, University of Houston, Houston, TX, USA[1]; Centro de Biotecnología FEMSA, Departamento de Biotecnología e Ingeniería de Alimentos, Tecnológico de Monterrey, Campus Monterrey, Monterrey, Mexico[2]; Department of Chemical & Biomolecular Engineering, University of Houston, Houston, TX, USA[3]

Ion-exchange matrices are prepared by random chemical processes which result in a heterogeneous charge distribution. Previous work from our group has shown that penta-argininamide and penta-lysininamide clustered-charge adsorbents show superior selectivity and affinity compared to non-clustered ones, especially for biomolecules with inherent charge clusters. The work presented here establishes spermine as an economical ligand alternative to peptide based clustered-charge ligands. Calcium-depleted α-lactalbumin showed higher affinity towards spermine Sepharose than towards the commercially available anion exchange adsorbents GE DEAE and Qiagen DEAE. It was also observed that spermine Sepharose displayed stronger dependence of binding affinity on salt concentration than the commercial Qiagen DEAE adsorbent, potentially implying higher resolution in some elution chromatography applications. The apparent number of interaction sites between protein and ligand, given by the *Z* parameter, was higher for the clustered-charge spermine Sepharose (*Z*= 1.7) than for the Qiagen DEAE adsorbent (*Z* = 0.5).

P-217 **Polyelectrolyte precipitation: A new technique for antibody purification.** Julia Sieberz, Kerstin Wohlgemuth and Gerhard Schembecker; TU Dortmund, Dortmund, GERMANY

Due to the growing demand for monoclonal antibodies (mAbs) in therapeutic and diagnostic applications, the development of cost-efficient purification methods is of high interest. A new and promising method is the precipitation with polyelectrolytes. Under certain conditions, these charged polymers are able to form complexes with proteins. The complexes formed can aggregate to insoluble particles, which can be separated form impurities remaining in supernatant. The small quantity of polyelectrolytes needed for precipitation and the reversible complex formation makes it a cheap and gentle method for the purification of mAbs. In this study, the separation of a mAb by precipitation with anionic polyelectrolytes was studied and optimized. Different types and molecular weights of anionic polyelectrolytes were screened for their capability as precipitation agents. The influences of the parameters pH value as well as polyelectrolyte, salt and impurity concentration were systematically investigated by Design of experiments (DoE). Through the application of DoE the influence of parameter effects and their interactions on yield and selectivity could be determined. These results were used to perform a precipitation of a mAb from a complex fermentation broth resulting in a yield up to 92 % (62 % purity) and selectivity up to 75 % (81 % recovery). To integrate the polyelectrolyte precipitation in a state of the art mAb downstream process, the combination of precipitation and generally used purification steps, like anion and cation exchange chromatography were investigated. The additional ion exchange steps lead to an overall yield of 76 % and a purity of 99.9 %.

P-218 **A F(ab')$_2$ulous Project – Achieving cleavage of a full-length monoclonal antibody using pepsin chromatography.** Marc Wong, Anjali Srivastava, and Michelle Butler; Genentech, Inc.

Pepsin is an acidic endopeptidase that preferentially cleaves at the carboxyl side of leucine and aromatic amino acids. Pepsin is commonly used to generate F(ab')$_2$ fragments from intact IgG monoclonal antibodies. At Genentech, an immobilized pepsin column was implemented to cleave a full-length monoclonal antibody and produce F(ab')$_2$ fragments. To develop a robust and efficient pepsin cleavage step temperature, flow rate, load concentration, pH and conductivity were investigated to determine the parameter impact on IgG cleaving efficiency. Optimal cleaving conditions were found and a robust and effective pepsin chromatography method was successfully developed to generate F(ab')$_2$. This poster highlights the key experiments implemented to select process conditions and understand parameter impact on cleaving efficiency.

P-219 **Effect of ligand chemistry and protein surface properties on selective adsorption in multimodal chromatography systems.** James Woo[1], Siddharth Parimal[1], Mark Snyder[2] and Steven Cramer[1]; Rensselaer Polytechnic Institute[1], Bio-Rad Laboratories[2]

Multimodal ligands represent a unique class of chromatographic materials, with several advantages, including increased salt tolerance, general ion-exchange behavior and unique selectivities. In this study, we compare the retention of a library of common proteins on several multi-modal cation-exchange ligands, including Capto MMC and Nuvia cPrime. Despite containing similar functional groups (a phenyl ring, amide bond and carboxylic acid), the retention of many proteins proved to be sensitive to subtle changes in the ligand chemistry and geometrical presentation of the hydrophobic phenyl moiety. These differences in retention behavior were most pronounced for proteins with a strong hydrophobic character. Molecular dynamics (MD) simulations with several proteins also showed that the preferred ligand-binding regions are qualitatively different in these two systems. Stronger adsorption on Capto MMC was correlated with the presence of large aliphatic residue clusters on a protein's surface. Proteins with clusters of surface-exposed aromatic residues were similarly retained on both resins. Finally, the insights from this work were used to generate new classes of protein surface descriptors which were shown to effectively predict the retention of proteins on several multimodal cation exchange systems ($R2 > 0.9$) using Quantitative Structure-Activity Relationship models.