Abstract Book

ISPPP - International Symposium on the Separation of Proteins, Peptides and Polynucleotides

November 6 – 9, 2016

Salzburg, Austria


novo nordisk®

octapharma

Headquartered in Lachen, Switzerland, Octapharma is one of the largest human protein products manufacturer in the world and has been committed to patient care and medical innovation since 1983. Our core business is the development and production of human proteins from human plasma and human cell-lines.

As family business till this day, Octapharma employs approximately 6,500 people worldwide to support the treatment of patients in over 100 countries with products across the following therapeutic areas: Haematology (coagulation disorders), Immunotherapy (immune disorders) and Critical Care.

Octapharma owns five state-of-the-art production facilities in Austria, France, Germany, Sweden and Mexico. Our production site in Vienna counts almost 1,000 employees and is thereby not only the largest, but also the oldest facility of our company. It houses amongst others Production, Packaging, Research & Development, International Drug Safety and International Regulatory Affaires.

For more information visit www.octapharma.com.

octapharma®

For the safe and optimal use of human proteins

We thank our sponsors

Gold Sponsor:

Boehringer Ingelheim

Silver Sponsor:

novo nordisk®

Bronze Sponsor:

octapharma®

For the safe and optimal use of human proteins

List of Exhibitors

BIO-RAD

GE imagination at work

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

POSTNOVA

WYATT
TECHNOLOGY EUROPE

ChromSword

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

TOSOH BIOSCIENCE
TOSOH

Table of contents

Conference Chair

Ales Podgornik, University of Ljubljana, Slovenia

Scientific Committee

Sonja Berensmeier, Technical University Munich, Germany
Cristina Dias-Cabral, University of Beira Interior, Portugal
Michel Eppink, University of Wageningen, Synthon, The Netherlands
Milton Hearn, Monash University, Austrialia
Sophia Hober, Royal Institute of Technology (KTH), Sweden
Jürgen Hubbuch, Karlsruhe Institute of Technology (KIT), Germany
Alois Jungbauer, Austrian Centre of Industrial Biotechnology (acib)/BOKU, Austria
Egbert Müller, Tosoh, Germany

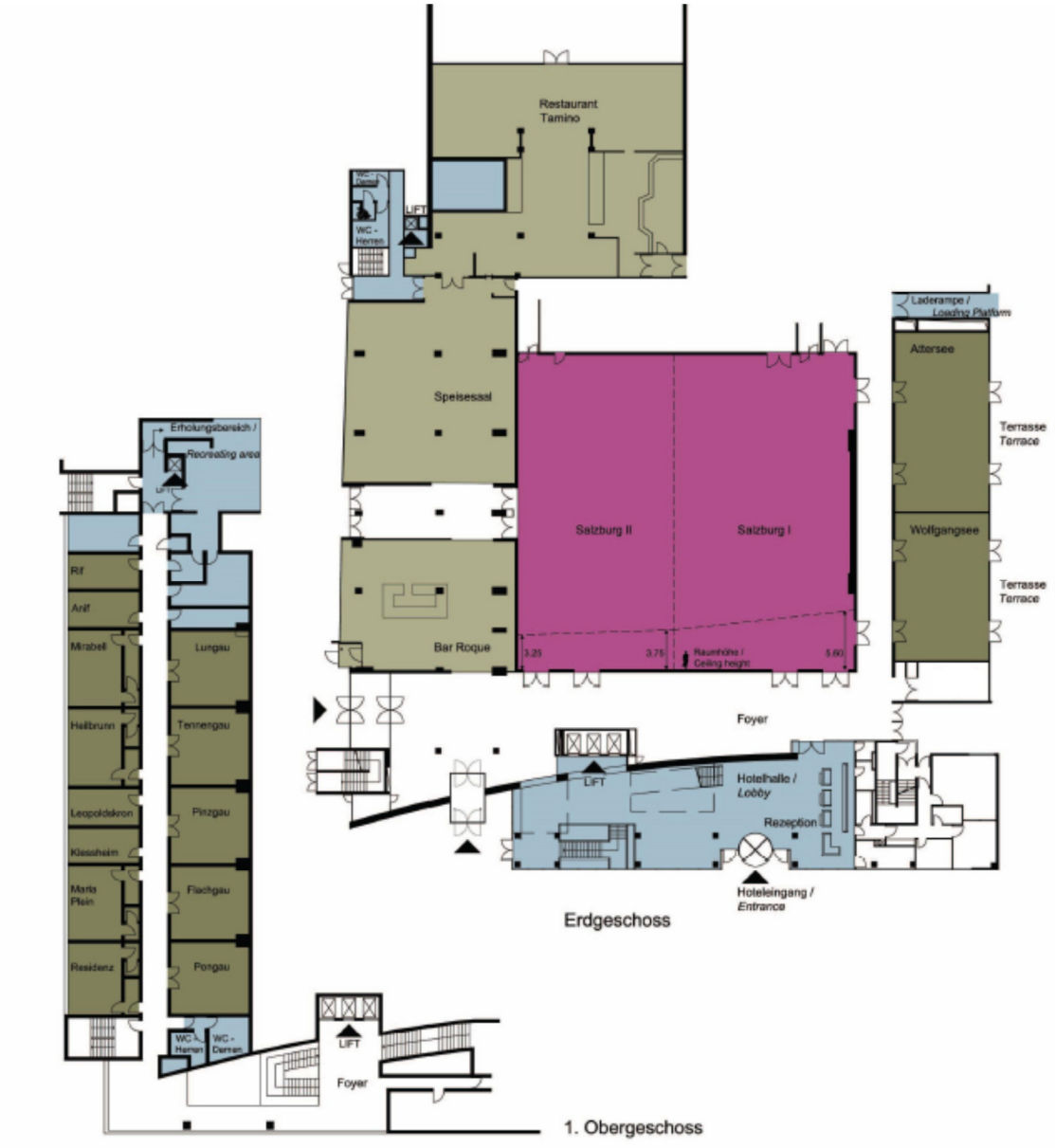
Organizational Committee

Verena Beck, Austrian Centre of Industrial Biotechnology (acib), Vienna, Austria
Aleš Podgornik, University of Ljubljana, Slovenia
Alois Jungbauer, acib/BOKU, Vienna, Austria

Venue Map

Wyndham Grand Salzburg Conference Centre Hotel
Fanny-von-Lehnert-Straße 7
5020 Salzburg, Austria

The pre-conference workshop will be held in room Flachgau (1st floor)
Lectures will be held in room Salzburg I.
Welcome Reception, refreshment breaks, poster sessions and exhibition will be held during the whole conference in room Salzburg II.
The Lunch Seminar on Monday, Nov. 7 will be held in room Wolfgangsee.



Conference Programme

Sunday, November 6, 2016

13:00 *Pre-conference Workshop for registered participants*

Start of Registration

17:00 *Welcoming Remarks*

Key Note Lecture - Chair: Alois Jungbauer

17:20	Gert Desmet	**T1:** Current & Future Improved Particle Design for HPLC

18:00 *Welcome Reception*

Monday, November 7, 2016

Key Note Lecture - Chair: Jürgen Hubbuch

08:40	Kazuki Nakanishi	**T2**: Latest applications of silica monolith to HPLC separation and purification/pre-treatments of biomolecules

Session 1: Support & Interaction I – Chair: Jürgen Hubbuch

09:20	Fabrice Gritti	**T3**: New Particle Design for High-Resolution Gradient Liquid Chromatography of Double-Stranded Deoxyribonucleic Acids
09:40	Egbert Müller	**T4:** Characterization of Resins for Biochromatography by Non-Chromatographic Methods
10:00	Christian Frech	**T5:** Modeling of Anion-Exchange Chromatography: Influence of Ligand Density on Protein Separation

10:20 *Refreshment Break*

Session 2: Support & Interaction II – Chair: Egbert Müller

10:40	Cristina Dias-Cabral	**T6:** Polymer-functionalized Ion-exchangers – an Energetic Picture from their Interaction with Biomolecules
11:00	Milan Polakovic	**T7:** Effect of grafted polymer chain length and ligand density on the protein adsorption on a cation-exchange membrane
11:20	Eva Hackemann	**T8:** Influence of Mixed Electrolytes and pH on HIC-Adsorption and Partial Molar Volume of Proteins
11:40	Goncalo Silva	**T9:** Thermodynamic overview of mAb adsorption on Protein A
12:00	Romas Skudas	**T10:** What is the most efficient Protein A resin and why?

12:20 *Break / Lunch Seminar for registered participants*

13:40 **Poster Session**

Session 3: Convective Media – Chair: Cristina Dias Cabral

14:40	Rosalinda Mazzei	**T11**: Highly selective charged UF membranes for the separation of proteins with similar molecular weight
15:00	Aleš Podgornik	**T12**: Disposable monolithic columns for PAT
15:20	Alois Jungbauer	**T13:** Structure and Flow Properties of Monoliths
15:40	Simone Dimartino	**T14:** Experimental and computational evaluation of new designs for 3D printed chromatography media
16:00	*Refreshment Break*	

Session 4: Ligands – Chair: Milton Hearn

16:20	Sophia Hober	**T15:** Optimization of an IgG-binding, Protein A-based purification matrix
16:40	Silvia Blank-Shim	**T16**: Peptide design for selective binding on biotechnology-relevant surfaces
17:00	Carsten Voss	**T17**: Platform Technologies for Non-Affinity Based Purification Schemes
17:20	Ana Azevedo	**T18**: LYTAG-driven polishing strategies for monoclonal antibodies extracts using quaternary amine as affinity matrices
17:40	Johan Nilvebrant	**T19**: Antibody Libraries based on an Autonomous Human Variable Domain
18:00	*End of Day 2*	

Tuesday, November 7, 2016

Key Note Lecture - Chair: Michel Eppink

08:40	Muriel Bardor	**T20**: Towards optimising glycan structures of plant and algae-made biopharmaceuticals

Session 5: Analytics I – Chair: Michel Eppink

9:20	Mookambeswaran Vijayalakshmi	**T21**: Identification of a Compound from Aloe Vera Extract Involved in Pancreatic Cell Regeneration for Alleviating Diabetes Mellitus
9:40	Shuichi Yamamoto	**T22**: High speed and high resolution separation of PEGylated proteins
10:00	Milton Hearn	**T23:** Tracking Host Cell Protein Contaminants During The Purification of Recombinant Proteins
10:20	*Refreshment Break*	

Session 6: Analytics II – Chair: Sophia Hober

10:40	Sophie Fröhlich	**T24**: HRMS based quantification of host cell protein networks during microbial expression as a tool for upstream process development
11:00	Gerard Rozing	**T25**: Next Generation Imaging Detection for CIEF Applications and Coupling with Mass Spectrometry
11:20	Gerhard Heinzmann	**T26**: Characterization of plasma proteins and lipoproteins using microchannel asymmetrical flow field-flow fractionation
11:40	Dierk Roessner	**T27**: Separation of Virus Like Particles and Protein-Drug-Particles using FFF and SEC
12:00	*Break*	

Session 7: Isolation I – Chair: Alois Jungbauer

13:20	Gorazd Hribar	**T28**: Next-generation biopharmaceutical downstream process
13:40	Ajoy Velayudhan	**T29**: Use of Empirical Model-Independent Optimisation for Preparative Polishing Chromatography of Proteins
14:00	Alexander Matlschweiger	**T30**: Purification of secretory immunoglobulins from whey
14:20	Emanuel Capela	**T31**: Novel Systems for the Extraction and Purification of Monoclonal Antibodies directly from CHO Cell Cultures Supernatants using Aqueous Biphasic Systems comprising Ionic Liquids
14:40	Raquel Aires-Barros	**T32**: Design of Aqueous Two-Phase Systems for Biopharmaceutical Purification in a Microfluidic Channel using Fluorescent Microscopy
15:00	*Refreshment Break*	
15:20	**Poster Session**	

Session 8: Isolation II – Chair: Raquel Barros

16:20	Peter Satzer	**T33**: Fast and easy way to boost your primary separation in mAb production: Continuous flocculation, new alternatives for old problems.
16:40	Dariusch Hekmat	**T34**: Continuous crystallization-based protein purification processes
17:00	Benedikt Ketterer	**T35**: Quasi-continuous temperature-controlled protein chromatography in downstream processing of antibodies
17:20	Marie-Therese Schermeyer	**T36**: Characterization of highly concentrated protein solutions - One step towards the predictability of long term stability
17:40	Mark Dürkop	**T37**: Destruction of proteins by cavitation and high shear
18:00	*End of session*	
19:30	*Conference Dinner*	

Wednesday, November 9, 2016

Session 9: Isolation III - Chair: Sonja Berensmeier

08:40	Joachim Kinkel	**T38**: Analytical and Preparative Separation of Peptides and Polynucleotides by Repulsion/Attraction mode on DRP-packings
09:00	Joana Valente	**T39:** Arginine-Superporous affinity chromatography for the supercoiled p53-encoding plasmid isolation
09:20	Cécile Brocard	**T40**: Manufacturing Gene Vaccines: Strategy to Fast Track supply of highly pure plasmid DNA
09:40	Sofia Carvalho	**T41**: Monitoring and improving Influenza Virus-like particles Downstream Processing using a click chemistry approach
10:00	Pavel Marichal-Gallardo	**T42**: Purification of cell culture-based influenza A virus particles with a cellulose membrane adsorber and polyethylene glycol

10:20 *Refreshment Break*

Session 10: Scaling - Chair: Aleš Podgornik

10:40	Michel Eppink	**T43**: High Throughput Screening as a tool for developing scalable purification processes of biotherapeutic proteins
11:00	Marcel Ottens	**T44**: Less is More – Miniaturization in Biopurification Process Development
11:20	Dean Harde	**T45**: HTPD Methods: Implementation in Downstream Process Development for Diverse non-MAb Protein Therapeutics
11:40	Julita Panek	**T46**: Design and key elements of qualified scale down models as an integrated part of product life cycle

12:00 *Presentation of Poster Awards and Concluding Remarks*

12:30 *End of conference*

Poster Session - Overview

P1 **Application of LamB receptor for fast detection and quantification of bacteriophage λ**

D. Nabergoj[1], B. Umek[2], M. Peterka[1], A. Podgornik[1,3]
[1]Center of Excellence for Biosensors, Instrumentation and Process Control - COBIK, Tovarniška cesta 26, 5270 Ajdovščina, Slovenia.
[2]University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[3]University of Ljubljana, Faculty of Chemistry and Chemical Technology, Večna pot 113, 1000 Ljubljana, Slovenia

P2 **Aggregation of H1N1 influenza virus during manufacturing and formulation**

Frank Hämmerling, Oliver Lorenz-Cristea, Jürgen Hubbuch
Karlsruhe Institute of Technology (KIT), Institute of Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Engler-Bunte-Ring 3, 76131 Karlsruhe, Germany

P3 **High resolution separation of virus-like particles and extracellular particles**

Petra Steppert[1], Daniel Burgstaller[1], Andres Tover[2], Alois Jungbauer[1]
[1]University of Natural Resources and Life Sciences, Vienna, Muthgasse 18, Vienna, Austria
[2]Icosagen, Eerika tee 1, Tartumaa, Estonia

P4 **Adsorption and elution of enveloped Virus-Like Particles in polymer grafted chromatography media**

Patrícia P. Aguilar[1], Tobias A. Schneider[2], Alois Jungbauer[1,2]
[1]Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 - Vienna, Austria
[2]Austrian Centre of Industrial Biotechnology, Muthgasse 11, 1190 - Vienna, Austria

P5 **Conformational stability of charge variants of antibody therapeutics**

Beate Hintersteiner[1], Nico Lingg[1], Alois Jungbauer
[1] Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Wien, Vienna, Austria

P6 **Perforated hydrophilic nanomembranes for bioseparations**

C. Schuster[1], A. Rodler[1], R. Tscheliessnig[1], A. Jungbauer[1,2]
[1]Austrian Centre of Industrial Biotechnology, Muthgasse 18, Vienna, Austria
[2]University of Natural Resources and Life Sciences, Vienna, Austria

P7 **Modeling of anion-exchange chromatography: Influence of ligand densities on Donnan equilibrium and protein separation**

Felix Wittkopp[1], Gabriela Sánchez Reyes[1], Lars Peeck[2], Christian Frech[1]
[1]Institute for Biochemistry, University of Applied Sciences Mannheim,
Paul-Wittsack-Str. 10, 68163 Mannheim, Germany
[2]Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt, Germany

P8 **Automated Development of Reversed-Phase HPLC Methods for separation of Proteins**

Oksana Rotzkaja, Sergey Galushko, Jelena Galushko
ChromSword, Germany

P9 Ultra-fast pH-Gradient Ion Exchange Chromatography for the Separation of Monoclonal Antibody Charge Variants

Robert van Ling[1], Alexander Schwahn[2], Shanhua Lin[3], Ken Cook[4], Mauro de Pra[5]
[1]Thermo Fisher Scientific, Breda, The Netherlands
[2]Thermo Fisher Scientific, Reinach, Switzerland
[3]Thermo Fisher Scientific, Sunnyvale CA, USA
[4]Thermo Fisher Scientific, Hemel Hempstead, UK
[5]Thermo Fisher Scientific, Germering, Germany

P10 Proteomic Profiling Of Aloe Vera Extract For Targeting Pancreatic Beta Cell Function

Spoorthy N Babu, Ayesha Noor, Krishnan Venkataraman, Mookambeswaran A Vijayalakshmi
CBST, VIT University, Vellore 632 014, India

P11 Characterization and manipulation of protein stabilities for downstream process development

J. Morgenstern[1], L. Galm[1], P. Baumann[1], C. Brunner[1], J. Hubbuch[1]
[1]Karlsruhe Institute Of Technology (KIT), Institute of Process Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Engler-Bunte-Ring 3, 76131Karlsruhe, Germany

P12 Application of Charged Surface Technology in the Separation of Peptides

B. Okandeji[1], M. Lauber[1], B. Alden[1], K. Wyndham[1]
[1]Waters Corporation, 34 Maple Street, Milford, Massachusetts, U.S.A

P13 Performance Comparison of Protein A Affinity Chromatography Sorbents at Different Temperatures

Walpurga Krepper[1], Peter Satzer[1], Alois Jungbauer[1,2]
[1]Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria
[2]Austrian Centre of Industrial Biotechnology (ACIB), Petersgasse 14, 8010 Graz, Austria

P14 Extending the number of LC techniques that can be interfaced with MS detection for the characterization of monoclonal antibodies

Suresh Babu C.V[2] and Linda Lloyd[1]
[2]Agilent Technologies, Inc. Bangalore, India
[1]Agilent Technologies, Church Stretton, UK

P15 Monoclonal antibody analysis: Reversed-phase LC/UV and LC/MS analysis

Suresh Babu C.V[2] and Linda Lloyd[1]
[2]Agilent Technologies, Inc. Bangalore, India
[1]Agilent Technologies, Church Stretton, UK
[1]Institute of Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering – Karlsruhe Institute of Technology (KIT), Engler-Bunte-Ring 3, 76131 Karlsruhe, Germany

P16 Reduced Baseline Noise Improves Light Scattering Detection Capabilities in Size Exclusion Chromatography of Protein Aggregates

Andy Coffey
Agilent Technologies, Church Streton UK

P17 Implementation of a Microfluidic Device for Absorption Measurements in High-Throughput Experiments

C.P. Radtke[1], M.-T. Schermeyer[1], J. Hubbuch[1]

P18 mAb N-glycosylation profiling with HILIC in respect of fucosylation and antibody dependent cellular cytotoxicity

L. Salim Abadi Ghaleh[2], W. Conze[1], J. Vajda[1], E. Mueller[1]
[1]Tosoh Bioscience GmbH, Im Leuschnerpark 4, 64347 Griesheim, Germany
[2]University of Applied Sciences, Schoefferstraße 3, 64295 Darmstadt, Germany

P19 A Smart Workflow for N-Glycan Analysis Combining Speed, Simplicity and Unrivaled Sensitivity

Marleen van Wingerden
Waters NV/SA, Belgium

P20 Novel Method to Discovery Glycol-peptides as Thrombin Inhibitors in Cancer Therapy

Hui-Ming Yu, Yu-Hsuan Chang, Yu-Hsuan Chu, Kuo-Ching Lin
Genomics Research Center, Academia Sinica, Taipei, Taiwan

P21 Development and validation of LC-MS/MS method for the quantification of polymyxin B1, polymyxin B2, polymyxin B3 and ile-polymyxin B1 in human plasma and its application to pharmacokinetics study

K.H.D. Hee[1], Y.K.J. Leaw[1], L.S.-U Lee,[1,2]
[1]National University of Singapore, MD1, 12 Science Drive 2, 117549 Singapore
[2]National University Health System, 1E Kent Ridge Road, 119228 Singapore

P22 Exploring ionic liquids for the development of a purification platform for therapeutic immunoglobulin Y (IgY) from egg yolk

Emanuel V. Capela[1], Mafalda R. Almeida[1], João A. P. Coutinho[1], Mara G. Freire[1]
[1]CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

P23 High-efficient approach for the selective separation of amino acids based on the use of aqueous biphasic systems composed of ionic liquids

Emanuel V. Capela[1], Maria V. Quental[1], João A. P. Coutinho[1], Mara G. Freire[1]
[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

P24 Membrane adsorbers for continuous processing

Helena Trnovec
Biologics Technical Development & Manufacturing, Lek Pharmaceuticals d.d., Kolodvorska 27, 1234 Menges, Slovenia

P25 Disclosing the thermodynamic phenomena behind mAbs interaction with multimodal chromatography supports: The Phenylboronic acid chromatography case study

S.A.S.L. Rosa[1], C.L. da Silva[1], M.R. Aires-Barros[1], A.M. Azevedo[1], A.C. Dias-Cabral[2, 3]
[1]iBB- Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1, 1049-001 Lisboa, Portugal
[2]CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Avenida Infante D. Henrique, 6201-506 Covilhã, Portugal
[3]Department of Chemistry, University of Beira Interior, R. Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal

P26 Developing a low-cost non-human-infective model system to study the causative agent of African sleeping sickness

W. Kipandula[1], F. Lampiao[1], T. K. Smith[2] and S. A. MacNeill[2]
[1]University of Malawi-College of Medicine P/bag 360, Blantyre, Malawi.
[2]Biomedical Sciences Research Complex B306, University of St Andrews, St Andrews KY16 9ST, United Kingdom.

P27 Characterization of Protein and Protein Aggregates using Temperature controlled Hollow Fiber Flow Field-Flow Fractionation

Florian Meier[1], Lisa Schilder[1], Soheyl Tadjiki[2], Robert Reed[2], Thorsten Klein[1]
[1]Postnova Analytics GmbH, Landsberg am Lech, Germany
[2]Postnova Analytics Inc., Salt Lake City, UT, United States

P28 On-chip chromatography for rapid screening of multimodal ligand-target interactions

I. F. Pinto[1,2], G. Petrucci[1,4], R. R. G. Soares[1,2], V. Chu[1], M. R. Aires-Barros[2,3], J. P. Conde[1,3], A. M. Azevedo[2,3]
[1]Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologias (INESC MN) and IN – Institute of Nanoscience and Nanotechnology, Rua Alves Redol 1000-029, Lisbon, Portugal
[2]IBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1049-001, Lisbon, Portugal
[3]Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1049-001, Lisbon, Portugal
[4]Department of Information, Electronic and Telecommunication Engineering, University of Rome "La Sapienza", Via Eudossiana 18, 00184, Rome, Italy

P29 Purification of porcine gastric mucin with preservation of its native functional properties

V. Schömig[1], B. Käsdorf[2], O. Lieleg[2], S. Berensmeier[1]
[1] Bioseparation Engineering Group, Department of Mechanical Engineering, Technical University of Munich, Boltzmannstr. 15, D-85748 Garching, Germany
[2] Biological Hydrogels Group, Institute of Medical Engineering, Technical University of Munich, Boltzmannstr. 11, D-85748 Garching, Germany

P30 Host Cell Protein clearance and identification

Marius Segl[1,2], Therese Wohlschlager[1,2], Johann Holzmann[2,3], Veronika Reisinger[2,3], Christian G. Huber[1,2]

[1] University of Salzburg, Department of Molecular Biology, Division of Chemistry and Bioanalytics Salzburg, Austria

[2] Christian Doppler Laboratory for Innovative Tools for the Characterization of Biosimilars, Salzburg, Austria

[3] Analytical Characterization Biopharmaceuticals, Sandoz GmbH, Biochemiestrasse 10, 6250 Kundl, Austria

P31 High Resolution LC/MS Analysis of Therapeutic Oligonucleotides on a New Porous Polymer-based Reversed Phase Column

A. Robert van Ling[1], B. Shanhua Lin[2], Julia Baek[2], Jim Thayer[2], Hongxia Wang[2], Xiaodong Liu[2]

[1]Thermo Fisher Scientific,
Breda, The Netherlands

[2]Thermo Fisher Scientific, Sunnyvale CA, USA

P32 A quantitative FVIII assay by interferometry using camelid antibody VhH-domain

H. Engelmaier[1], N. Hammerschmidt[1], A. Jungbauer[1,2]

[1]acib - Austrian Centre of Industrial Biotechnology, Muthgasse 18, A-1190, Vienna, Austria

[2]University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology Muthgasse 19, A-1190, Vienna, Austria

P33 Selectivity behaviour of peptides with high aqueous content mobile phases using new classes of hydrophilic interaction and aqueous normal phase chromatographic materials.

Chadin Kulsing, Reinhard I. Boysen, Yuanzhong Yang and Milton T. W. Hearn
Victorian Centre for Sustainable Chemical Manufacture, School of Chemistry,
Monash University, Melbourne, Victoria 3800, Australia

P34 Characterization and quantification of monoclonal antibody oxidation variants utilizing ion-pair reversed-phase HPLC-MS/MS

Christof Regl[1,2], Therese Wohlschlager[1,2], Ines C. Forstenlehner[1,3], Silke Ruzek[1,3], Johann Holzmann[1,3], Christian G. Huber[1,2]

[1] Christian Doppler Laboratory for Innovative Tools for Biosimilar Characterization, University of Salzburg, Hellbrunnerstrasse 34, 5020 Salzburg, Austria

[2] Department of Molecular Biology, Division of Chemistry and Bioanalytics, University of Salzburg, Hellbrunnerstrasse 34, 5020 Salzburg, Austria

[3] Analytical Characterization Biopharmaceuticals, Sandoz GmbH, Biochemiestrasse 10, 6250 Kundl, Austria

P35 Novel recombinant protein purification strategy for unstable protein expressed in Yeast

M. Khatami[1,2], S. Hasannia[1], S. N. Hosseini[2]

[1] Faculty of Biological sciences, Tarbiat Modares University

[2] Production and Research complex, Pasteur Institute of Iran, Tehran, Iran

Oral Presentation Abstracts

Current & Future Improved Particle Designs for HPLC

G. Desmet[1]

[1]*Vrije Universiteit Brussel, Department of Chemical Engineering, Pleinlaan 2, 1050 Brussels, Belgium*

The recent introduction of a new generation of so-called core-shell particles (consisting of a solid core surrounded by a shell of meso-porous material) has revolutionized the speed of liquid chromatographic separations. Considering a fixed analysis time, and comparing a fully porous and a core-shell particle column, each with a properly optimized length, the latter will generally produce a significantly higher peak capacity or plate number than the former. Vice versa, the core-shell particles will need a significantly shorter time to produce a given peak capacity or theoretical plate number than their fully porous counterparts.

The reasons for this improved performance will be explained and the lessons learned will be used to discuss the possibility to even further improve the speed of chromatography. The results will be corroborated using computational fluid dynamics simulations of the band broadening and the pressure drop in 2-D and 3-D cylinder and sphere packings, as well as via experimental results obtained on micro-machined chromatography columns produced using state-of-the-art photolithographic etching

Latest applications of silica monolith to HPLC separation and purification/pre-treatments of biomolecules

K. Nakanishi[1]

[1]*Department of Chemistry, Graduate School of Science, Kyoto University*
Kitashirakawa, Sakyo-ku, Kyoto 606-8502, JAPAN

Since the invention of monolithic silica column 20 years ago [1], continuous efforts have been made to improve the performance of monolithic columns as well as to extend the chemical compositions of hierarchically porous monoliths [2,3]. Refinement of the macropore structure in terms of size and distribution resulted in the increase of plate number exceeding 200 000 plates/m [4]. Column volume has been extended to nearly 1 L (2.5 inches in diameter and 10 inches long) applicable to preparative scale applications [5]. Newly developed surface modification method using hydrosilanes [6] as well as a glass-clad formation process [4] will further contribute to fabricate high-performance, pressure-resistant monolithic columns. Highly permeable monoliths in silica and titania compositions have been commercialized as high-speed pre-treatment tools suitable for DNA-purification, concentration of phosphorylated compounds, respectively [7]. Various types of organo-siloxane macroporous monoliths have been synthesized and evaluated as solid phase extraction (SPE) devices and alkaline-resistant columns.

[1] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, *Anal. Chem.* **1996,** *68* (19), 3498-3501.
[2] K. Nakanishi and N. Tanaka, *Acc. Chem. Res.* **2007,** *40* (9), 863-873.
[3] T. Ikegami and N. Tanaka, *Ann. Rev. Anal. Chem.*, **2016**, 9, in press.
[4] K. Morisato, S. Miyazaki, M. Ohira, M. Furuno, M. Nyudo, H. Terashima, K. Nakanishi, *J. Chrom. A*, **2009**, 1216 (44), 7384-7387.
[5] R. Miyamoto, Y. Ando, C. Kurusu, H.-Z. Bai, K. Nakanishi, M. Ippommatsu, *J. Sep. Sci.*, **2013**, 36, 1890-1896.
[6] Moitra, N.; Ichii, S.; Kamei, T.; Kanamori, K.; Zhu, Y.; Takeda, K.; Nakanishi, K.; Shimada, T., *J. Am. Chem. Soc.* **2014**, *136*, 11570-11573.
[7] http://www.glsciences.com/product/sample/solid/

New Particle Design for High-Resolution Gradient Liquid Chromatography of Double-Stranded Deoxyribonucleic Acids

F. Gritti, M. Gilar, D. Walsh, K. Wyndham

Waters Corporation, 34 Maple Street, 01757 Milford, MA, USA

Plasmids are fragments of double-stranded deoxyribonucleic acid (dsDNA) that can replicate independently of chromosomal DNA and carry genes. They have become an essential tool in molecular biology and genomics because they can drive gene expression in a wide variety of living organisms. dsDNA molecules can be extremely large and slowly diffusive molecules, so, they require adequate structural designs for the packed particles used in liquid chromatography. Our objective is to propose, develop, and test new particles for high-resolution of dsDNAs.

In this presentation, we first performed the separation of 18 dsDNAs (the base pair number varies from 51 to 587) by optimizing core-shell particle technology: the impact of 1) the particle size (from 1.9 µm to 4.0 µm particles) at constant core-to-particle diameter ratio and 2) the core-to-particle diameter ratio (from 0 for standard fully porous particles to 0.85) at constant particle size on the gradient resolution was investigated. The mesopore size was fixed at 300 Å. The experimental results reveal both the advantages of operating with the smallest particle diameter and the thinnest shell thickness. In the second part of this presentation, we demonstrate theoretically the potential advantage of new particle structures including the presence of straight and radially oriented mesopores as well as the fabrication of new porous structures allowing eluent convection through the particle (perfusion chromatography). The theoretical results show that the internal mass transfer of very large analytes such as dsDNAs can be sped up by a factor 2 and beyond.

Characterization of Resins for Biochromatography by Non-Chromatographic Methods

E. Mueller

Tosoh Bioscience GmbH

Resins for protein separation are usually meso- or macroporous with particle sizes ranging from 20 to 200 µm. According to their dedicated chromatography mode, they carry different ligand types. The modes most commonly used for preparative protein separation are ion exchange, hydrophobic interaction or special affinity chromatography. Characterization of the pore structure, ligand densities and chemical structure are essential for successful application of the different chromatography resins. There are several analytical methods for resin characterization. These include:

1. Acid-base resin titration
2. Particle size measurement
3. Inverse size exclusion chromatography
4. Nitrogen adsorption, mercury intrusion

This paper will show the opportunities of the above methods and limitations for viscoelastic resin particles. In addition to that, new methods like IR and NIR,UV-VIS spectroscopy with diffusive reflection and single particle stress-strain measurements are introduced.

Modeling of anion-exchange chromatography: Influence of ligand densities on Donnan equilibrium and protein separation

Felix Wittkopp[1], Gabriela Sánchez Reyes[1], Lars Peeck[2], Christian Frech[1]

[1]Institute for Biochemistry, University of Applied Sciences Mannheim, Paul-Wittsack-Str. 10, 68163 Mannheim, Germany
[2]Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt, Germany

Anion-exchange chromatography is an important tool in downstream processing of biopharmaceuticals. It is commonly used for the removal of fragments and/or aggregates of the target protein as well as for the depletion of nucleic acids, host cell proteins and viruses. Ligand density variations are known to influence the separation and affect process robustness. Mechanistic modeling can be an effective tool to understand these effects.

Here we present the application of a Donnan ion exchange (DIX) model to linear gradient elution (LGE) modelling [1]. This model considers differences in the concentrations of charged species between the resin and buffer phase due to the Gibbs–Donnan equilibrium. The distribution of charged species between the two phases is influenced by the ligand density of the stationary phase. Our results show that by considering the Donnan effect, a global modeling of five Fractogel® EMD TMAE resins with different anion exchange ligand densities is possible. Furthermore, the results demonstrate consistent accuracy of the model for three different proteins. Finally, predictions of chromatographic separations were performed using computer simulation and compared with experimental results.

[1] M.L. Jansen, A.J.J. Straathof, L.A.M. van der Wielen, K.C.A.M. Luyben, W.J.J. van den Tweel, AIChE Journal 42 (1996) 1911.

Polymer-functionalized Ion-exchangers – an Energetic Picture from their Interaction with Biomolecules

A.C. Dias-Cabral [1,2]

[1]*CICS-UBI – Health Sciences Research Centre, University of Beira Interior, 6200-506 Covilhã, Portugal;* [2]*Department of Chemistry, University of Beira Interior, 6200-001 Covilhã, Portugal.*

It has been a long standing goal in the bioseparation field, the prediction of biomolecule adsorptive behaviour onto the chromatography resin. Needless to say, that for this a fundamental mechanistic understanding of the process is required. Thermodynamic analyses of biomolecule adsorption have helped to elucidate complex adsorption mechanisms in liquid chromatography. Particularly, calorimetric methods have proven to be a reliable methodology to elucidate these mechanisms in preparative chromatography, as they measure the heat flows caused by interactions during the adsorption process. Furthermore, flow microcalorimetry (FMC), as a dynamic means of heat signal measurement, provides extra insight about the driving forces and events occurring as the biomolecule flows through the support. A flow microcalorimeter is operated in a manner that is analogous to the operation mode of a chromatographic system and hence the results are expected to be representative of what happens in a chromatography column. Through the knowledge of the magnitude and chronology of thermal events during and after the biomolecule-adsorbent interaction, the adsorption mechanism can be elucidated. This technique has been used, with good results, to study the adsorption mechanism of different biomolecule – support systems [1, 2].

The presentation will give a short overview of the FMC technique and examples of its application in the study of protein and plasmid DNA adsorption mechanism onto different polymer-functionalized ion-exchangers. Biomolecule interaction is shown to be a complex process, controlled by a number of subprocesses with synergistic and antagonistic effects of different types of forces, dependent on the type of support studied.

[1] G.L. Silva, F.S. Marques, M.E. Thrash Jr., A.C. Dias-Cabral, J. Chromatogr. A 1352 (2014) 46-54;

[2] P. Aguilar, A. Twarda, F. Sousa, A.C. Dias-Cabral, J. Chromatogr. A 1372 (2014) 166-173.

Acknowledgements

This work is supported by FEDER funds through the POCI - COMPETE 2020 - Operational Program Competitiveness and Internationalization in Axis I - Strengthening research, technological development and innovation (Project No. 007491) and National Funds by FCT - Foundation for Science and Technology (Project UID/Multi /00709)

Effect of grafted polymer chain length and ligand density on the protein adsorption on a cation-exchange membrane

Peter Dreveňák[1], Louis Villain[2], Milan Polakovič[1]

[1]Slovak University of Technology, Bratislava, Slovakia
[2]Sartorius Stedim Biotech, Göttingen, Germany;

The dynamics of adsorption of strong cation-exchange membranes based on a cellulose support was investigated. Several membrane types were prepared using the same ligand type and grafting chemistry as the reference commercial material – Sartobind S. They differed by the length of grafted chains and ligand density per chain length. The pore structure of the grafted layer was analyzed using the inverse size exclusion chromatography method. The accessibility of dextran probes of different molecular weight was sligghtly dependent on the chaing length. The mean pore size was found to be around 10 nm. The tested protein, white egg lyzozyme was partially excluded from the grafted gel layer. This phenomenon inevitably affected also the mass transfer rate of this protein to the ligand sites in the active layer of these adsorbents. Breakthrough experiments were carried out at different inlet protein concentrations, ionic strength and flow rate. Due to a very high affinity of this protein to the ligand, minimal effect of the inlet concentration on the binding capacity was observed in the concentration range used. The binding capacity was however different for the individual membrane types. The binding capacity decreased with the ionic strength as expected. For each membrane type, no significant effect of the flow rate on the dynamic binding capacity was observed, which is typical for membrane chromatography. On the other hand, breakthrough curves were quite wide which indicated the effect of adsorption-zone broadening phenomena. Mathematical modelling showed that the shape of breakthrough curves could be described very well only when solid-phase mass transfer resistance was included into a membrane bed model. The evaluated mass transfer coefficient was dependent on the chaing length and density and those process conditions that affected the binding capacity.

Acknowledgment

This work was supported by the Slovak Research and Development Agency, grant No. APVV-14-0474.

Influence of Mixed Electrolytes and pH on HIC-Adsorption and Partial Molar Volume of Proteins

E. Hackemann, E.J. García, H. Hasse

Laboratory of Engineering Thermodynamics, University of Kaiserslautern, Erwin-Schrödinger-Str. 44, Kaiserslautern, Germany

In the present work, a systematic study on the influence of single and mixed salts on protein adsorption was carried out using different model systems. The studied solutes are lysozyme and bovine serum albumin. The salts are sodium chloride, ammonium sulfate, sodium sulfate, ammonium chloride and their mixtures. Sodium phosphate and citrate buffers were used to adjust the pH value between pH 4 and 7. The adsorbent is Toyopearl PPG-600M, a mildly hydrophobic resin. Adsorption equilibrium isotherms were measured at 298 K with a fully automated liquid handling station. Unexpected synergetic behaviour was observed, i.e. effects that cannot be described by interpolating between results for single salts. Furthermore the influence of pH was studied. Depending on the pH the influence of electrolytes on adsorption of proteins changes completely.

A mathematical model is developed to describe the influence of the different single ions and their cross-interactions on adsorption. It is shown that the specific interactions of the individual ions with particular regions of the proteins are responsible for the varying adsorption behaviour. Adsorption isotherms for aqueous solvents with mixed electrolytes at different ionic strength can be predicted.

To get a better understanding of solute-solvent interactions, and the solute structure in protein solutions the partial molar volume of the proteins in the different solutions is measured using a vibrating tube densimeter. The partial molar volume of the proteins is found to depend on the composition of the aqueous electrolyte solution. First results from a comparison of that experimental data to predictions from molecular dynamic simulation, carried out with force fields from the literature, are shown.

Thermodynamic overview of mAb adsorption on Protein A

G. L. Silva[1,2,3,4], A. Rodler[3,4], R. Tscheliessnig[3,4], A. Jungbauer[3,4], A. C. Dias-Cabral[1,2]

[1]CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique 6200-506, Covilhã, Portugal
[2]Department of Chemistry, University of Beira Interior, R. Marquês d'Ávila e Bolama 6201-001, Covilhã, Portugal
[3]Department of Biotechnology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria
[4]Austrian Centre of Industrial Biotechnology, Muthgasse 18, 1190 Vienna, Austria

Protein A affinity chromatography is the most used method for the capture of monoclonal antibodies (mAbs) in downstream processing. However, mostly due to the raise in mAbs titers, there is still a bottleneck in the purification step despite the recent increases in the dynamic binding capacities of new Protein A resins [1]. This *status quo* strongly requires for an understanding of chromatography underlying interaction mechanisms. Calorimetry techniques are excellent in the assessment of molecular interactions. More specifically, flow microcalorimetry (FMC) has been proven very useful in simulating laboratory scale chromatography operations, providing information on the thermal events associated to biomolecule adsorption on solid phase resins [2,3]. This work aimed to help elucidating the adsorption mechanism of an in-house produced mAb to different commercial Protein A resins, taking into consideration the associated energy exchange. The results indicate that antibody adsorption to Protein A is purely an exothermic event, while the desorption process has both exo- and endothermic contributions. These data suggest mAb adsorption onto Protein A as an enthalpy driven process. FMC is then useful as an *in situ* and direct technique to better understand the driving forces and mechanism involved in mAbs adsorption.

[1] E. Müller, J. Vajda, J. Chromatogr. B. 1021 (2016) 159–168.
[2] P.A. Aguilar, A. Twarda, F. Sousa, A.C. Dias-Cabral, J. Chromatogr. A. 1372 (2014) 166–173.
[3] G.L. Silva, F.S. Marques, M.E. Thrash, A.C. Dias-Cabral, J. Chromatogr. A. 1352 (2014) 46–54.

Acknowledgements

This work is supported by FEDER funds through the POCI - COMPETE 2020 - Operational Program Competitiveness and Internationalization in Axis I - Strengthening research, technological development and innovation (Project No. 007491) and National Funds by FCT - Foundation for Science and Technology (Project UID/Multi /00709).
G. L. Silva also acknowledges FCT for the PhD fellowship SFRH/BD/104498/2014. This work is also supported by the Austrian Centre of Industrial Biotechnology.

What is the most efficient Protein A resin and why?

Oliver Rammo[1], Dr. Romas Skudas[1]

[1]Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany

Protein-protein based interaction, regardless its complexity, gained the biggest recognition in biopharmaceutical industry. Especially the discovery of Protein A and antibody interaction enabled to reduce the complexity of this pharmaceutical molecule group purification resulting in 5 blockbuster drugs within the top 10. And there are more to come taking into account more than 2500 clinical studies ongoing.

Regardless the robustness of this method, where remains an opened question how is this protein-protein interaction taking place and what governs the efficiency of the process if the Protein A molecule is bound to the surface of the support having limited accessibility.

We approached this challenge from user perspective, using chromatographic performance as a main criteria. Within our research we used the combined power of mass spectroscopy, quantitative amino acid analysis and thermogravimetric and standard protein analytics such as BCA/oPA and SDS-Page to distinguish the immobilized Protein A sequence, the number and origin of domains and the linkers in between them. This was followed by detecting the way of immobilization (e.g. local of multiple attachment zones) with the trace analysis of immobilized amino acid or traces thereof. Finally we added up some support physical characteristics followed by the chromatographic performance for different test molecules and operation modes.

In the presentation we will discuss the outcome of our study, concentrating on the efficiency of the chromatographic separation governed by the enhanced diffusion (e.g. outcome of physical properties from base support) and diversity of possible protein-protein interaction, originating from different Protein A domains. And finally we will come back to our question: “What is the most efficient Protein A immobilized resin available on the market and why?”

Highly selective charged UF membranes for the separation of proteins with similar molecular weight

Rosalinda Mazzei[a], Anna Maria Szymczak[a],Enrico Drioli[a], Mohamed Al-Fageeh[b], Mohammed Aljohi[b], Lidietta Giorno[a]

[a]Institute on Membrane Technology, CNR-ITM C/o University of Calabria, Via P. Bucci 17C, 87036 Rende (CS), Italy
[b]National Centre for Biotechnology, King Abdulaziz City of Science and Technology (KACST), P.O. Box 6086, Riyadh 11442, Saudi Arabia

The fractionation of proteins with similar molecular mass is an high challenge to be reached. By ultrafiltration (UF) process the complete separation is only possible for proteins that differ in molecular mass by at least a factor of 10. In the recent literature more attention was given on careful adjustment of physicochemical parameters of protein solutions, like pH or ionic strength, [1][2][3]. Important results were also achieved by charging membrane surface and promoting a transport given by electrostatic repulsion/attraction between proteins and charged membrane.

In this work a complete separation of α-lactalbumin (ALA, 14.4 kDa) from β-lactoglobulin (BLG, 18.4 kDa) with an high recovery factor was achieved starting from binary protein mixture. By properly tuning membrane surface, protein charge, ionic strength as well as optimizing fluid dynamic conditions, it was possible to obtain high selectivity and high recovery factor.

A positively charged regenerated cellulose membrane of 30 kDa was used and functionalized in order to create positively charged UF membrane. Operative conditions were firstly optimized by using single protein solutions, charged and uncharged membrane. Best conditions from the single-protein experiments (charged membranes both pH 3.4 and 7, ionic strength: 0.1M, conductivity 1.5 mS/cm) were then used to carry out binary mixture UF.

Higher selectivity of the membrane can be reached at pH 3.4 where both proteins and membrane bear same protein charge. The process permitted a complete fractionation of ALA respect to BLG with a recovery factor of about 77% after 5 hours.

[1] D.B. Burns, A.L. Zydney. Biotech Bioengineer 64 (1999) 27–37.
[2] N.S. Pujar, A.L. Zydney. Ind Eng Chem Res 33 (1994) 2473–82.
[3] A. Arunkumar, M.R. Etzel, Purification and Separation Technology, 105 (2013)121-128.

Acknowledgements

This work has been financially supported by the project “Membrane systems in regenerative medicine, tissue engineering, and biotechnology” AGREEMENT No. KACST- ITM-CNR/03.

Disposable monolithic columns for PAT

Aleš Podgornik[1,2], Tanja Mivšek[1], Shuichi Yamamoto[3]

[1] Faculty of Chemistry and Chemical technology, University of Ljubljana, Ve□na pot 113, 1000 Ljubljana, Slovenia

[2] The Centre of Excellence for Biosensors, Instrumentation and Process Control - COBIK, Tovarniška 26, 5270 Ajdovš□ina, Slovenia

[3] Bioprocess Engineering Laboratory, School of Engineering and Graduate School of Medicine, Yamaguchi University, Ube 755-8611, Japan

Monoliths are attractive stationary phases due to their convective based transport, resulting in flow unaffected separation efficiency and dynamic binding capacity combined with high porosity providing low pressure drop. Furthermore, since they can be prepared inside a column housing, their production cost can be reduced significantly. This opens possibility for preparation of inexpensive disposable monolithic columns (DMC), which can be used for process monitoring and control. To achieve high sensitivity and minimize necessary sample volume, volume of such columns should be small.

In this work we present methodology for preparation of DMC in plastic housing consisting of a single step procedure. Monoliths can be prepared via thermal or photopolymerization, affecting in this way structure of formed monolith. Volume of such columns can vary, but it is typical between 10 – 100 microliters. Monolith encased in DMC can be further modified to provide required functionality. Characterization of DMC in terms of structure and ligand density via non-invasive methods will be discussed. Finally, several applications of DMC for protein and pDNA separation will be demonstrated. As DMC can operate at flow rates up to 5 ml/min analysis time is extremely short and obtained information can be used for in-process control during USP or DSP.

Structure and flow properties of monoliths

Alois Jungbauer[1,2], Christian Jungreuthmayer[1], Petra Steppert[2], Gerhard Sekot[1], Armin Zankel[3] Herbert Reingruber[3], Juergen Zanghellini[1]

[1]*Austrian Centre of Industrial Biotechnology, Muthgasse 11, 1190 Vienna, Austria*
[2] *Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Muthgasse 18, 1190 Vienna, Austria*
[3] *Institute for Electron Microscopy and Nanoanalysis, Graz University of Technology, NAWI Graz, and Graz Centre for Electron Microscopy, Steyrergasse 17, 8010 Graz, Austria*

Organic polymer-based monoliths have excellent flow properties and exert convective mass transport. The flow in the wide channel interconnected with narrow channels is theoretically assumed to account for favorable permeability [1]. For studying pore geometry monoliths were cut into 898 slices in 50 nm distances and visualized by serial block face scanning electron microscopy (SBEM). A 3D structure was reconstructed and this was the basis for the calculation of flow profiles within the channels of the monolith, pressure drop and permeability by computational fluid dynamics (CFD). The calculated and measured permeabilities were in the same range. Small channels clearly run into wide and wide into small channels in a recurring manner which supported the hypothesis describing the favorable flow properties of polymer based monoliths. This periodic behavior was also observed in the streamline velocity profile which fluctuated. The experimental findings were corroborated by an artificial monolith which was composed of regular cells where narrow cells followed wide cells. In this model monolith, similar fluctuations were observed. Our study showed that the interconnection of small and wide pores are responsible for the excellent pressure flow properties. This study is also a guide for further design of continuous porous materials to achieve good flow properties.

[1] I Mihelič, D. Nemec, A. Podgornik, T. Koloini. Journal of Chromatography A 1065 (1), pp. 59-67

Experimental and computational evaluation of new designs for 3D printed chromatography media

Simone Dimartino[1,], Suhas Nawada[2], Fabian Dolamore[2], Anne Gordon[2], Roya Rezanavaz[2], Tim Huber[2], Don Clucas[3], Conan Fee[2]*

[1]*Institute for Bioengineering, School of Engineering, The University of Edinburgh, Mayfield Road, Edinburgh EH9 3FB, Unite Kingdom*
[2]*Biomolecular Interaction Centre and Department of Chemical & Process Engineering, University of Canterbury, Private Bag 4800, Christchurch 8040, New Zealand*
[3]*Department of Mechanical Engineering, University of Canterbury, Private Bag 4800, Christchurch 8040, New Zealand*

The use of stationary phases with ordered morphology is one of the most effective strategies to improve chromatographic performance. However, till now, the inherent packing process has made it impossible to achieve a perfectly ordered chromatographic media. However, with the advent of 3D printing, perfectly ordered structures can now be generated and further tested.

In this contribution, recent advances on 3D printed chromatographic media are presented.

First, the concept of spherical beads as the “ideal” shape for chromatographic separations is questioned. Regular packings of different geometrical particles are experimentally and computationally compared in terms of plate height and pressure drops. Interestingly, these results highlight that ordered arrangements of particle shapes such as tetrahedra and octahedra often outperform the HETP and permeability characteristics of spheres.

Second, a move from the traditional design of particles to the design of the channel network is proposed. Triply periodic minimal surfaces (TPMS) are specifically considered in this work, selected because of their optimal characteristics in terms of low permeability and optimized structural properties. These geometric entities identify two interconnecting labyrinths that are uniform, continuous and non-intersecting. Here, one network identifies the solid phase while the other the channels for the flow of the mobile phase. Residence time distribution experiments and computational fluid dynamics results will be presented on these structures. Van-Deemter plots show that several TPMS structures are endowed with lower plate height than packed spheres, whether the latter are in homogenous or random arrangement.

Finally, TPMS media printed with agarose with fully functional ion exchange moieties are tested for the separation of proteins. In particular, the design of these structures is rationally engineered to allow the direct processing of a solids-laden feed without the need of prior filtration or centrifugation steps.
The use of intricate geometries such as TPMS underlines the potential enabled by the use of 3D printing for the design of smart chromatography media, and opens a new paradigm in the operation of chromatographic bioseparations.

Optimization of an IgG-binding, Protein A-based purification matrix

Sophia Hober

School of Biotechnology, AlbaNova University Center, KTH - Royal Institute of Technology, Stockholm, Sweden

Antibodies are widely used affinity molecules in many fields of biological science. The therapeutic field for monoclonal antibodies is constantly growing and novel antibody based drugs are frequently approved. Antibodies are normally produced in mammalian cell cultures and thereafter purified by regular protein purification methods. The most common method used for purification of antibodies is Protein A affinity chromatography. This method offers high selectivity and yields pure and concentrated antibodies. However, two major issues with Protein A purification comes with the fact that the ligand is protein based. In order to be able to reuse the affinity matrix after a round of purification, a sanitization step is needed. This is normally done by a washing step using alkaline solution with high salt concentration. Moreover, when eluting the bound antibodies from the column low pH is needed. Since proteins normally are sensitive to extreme conditions, both high and low pH, these two steps cause damage to the column (alkaline pH) and the antibodies (acidic pH). Here, we have addressed these issues by protein engineering. A combination of selection and directed mutagenesis has been used to improve the behavior of an IgG-binding domain from Protein A.

Peptide design for selective binding on biotechnology-relevant surfaces

S. Blank[(1)], S.P. Schwaminger[(1)], M. Borkowska-Panek[(2)], P. Anand[(2)], P. Fraga García[(1)], Karin Fink[(2)], W. Wenzel[(2)], S. Berensmeier[(1)]

[1] *Bioseparation Engineering Group, Technical University of Munich, Germany*
[2] *Institute for Nanotechnology, Karlsruhe Institute for Technology, Germany*

The purification of therapeutic and other recombinant proteins accounts for more than 60% of their total production costs. Conventionally, purification is accomplished with the utilization of 'affinity tags´. These are peptide sequences that selectively bind to ligands immobilized on the surface of a downstream material.
The goal of the project is to develop computer-aided procedures for the rational design of surface-binding peptide tags in order to evade the cost-intensive immobilization of partially unstable ligands. These tags potentially have the ability to bind selectively to cost-effective downstream materials such as polymers and metal oxides without a further ligand modification. Consequently, synthesis costs and contamination by disassociating ligands can be reduced significantly.
Initially, technical surfaces are characterized based on their reactivity (binding sites) and electrostatic properties (surface potential, zeta potential). Maghemite and magnetite are then synthesized and characterized as adsorber materials. By optimizing the synthesis, particles with a high specific surface (>100 m^2g^{-1}) and a high saturation magnetization (>80 A m^2 kg^{-1}) can be produced. The amphiphilic surfaces of the particles can be adjusted to different peptide systems by changing the pH and the buffer.
Based on the characterization of the surfaces, biophysical models are being developed in a two-step procedure in order to model the binding of amino acids and peptides to the surface. In the first step, the simulation is performed for amino acids and small peptides by explicitly considering the solvent. Thereby, the free energy of binding of these molecules to the surface is determined as a function of the distance. In the second step, this data is used to parameterize simplified, effective models that permit the simulation of the binding behavior of larger peptides in high-throughput. In this manner, optimal sequences for the binding of the surfaces can be determined.
With respect to the technical application, an expression system in *E. coli* is being established which allow the peptides to be expressed as a fusion protein with GFP that can be easily detected. The fusion protein can be used to validate the simulation results. With the addition of selective cutting sites, it is possible to systematically characterize the peptides and their influence on the binding behavior of the fusion protein.

Acknowledgements

We especially thank the Federal Ministry of Education and Research for the financing of this project (Project number 031A173A).

Platform Technologies for Non-Affinity Based Purification Schemes

Carsten Voss

Bio-Rad Laboratories GmbH, Munich, Germany

Novell antibody formats like fragments or non-IgG molecules are gaining increased interest in biopharmaceutical development. These new targets are lacking a strong protein A binding site facilitating efficient capture and purification in a protein A affinity based platform.

New developments in ligand technology during the recent years lead to chromatographic materials with increased selectivity (mixed mode chromatography) as well as high capacities (ligand grafting).

In this presentation we demonstrate the development of a purification platform for IgM class molecules using state of the art chromatographic materials and outline the advantages in comparison to traditional approaches.

We also show the application of the purification principle to various IgM molecules differing in pI and antigen binding to outline its use as a platform for this class of proteins.

The described principles are also applicable for other classes of molecules having low or no affinity binding, e.g. scFv fragments, diabodies, nanobodies, IgE, etc.

LYTAG-driven polishing strategies for monoclonal antibodies extracts using quaternary amine as affinity matrices

I. Campos-Pinto[1], Miguel Arévalo-Rodriguez[2], Rajesh Gavara[3], Marcelo Fernández-Lahore[4], M. R. Aires-Barros[1], A. M. Azevedo[1]

[1] IBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1049-001, Lisbon, Portugal
[2] Biomedal S.L., Avda. Américo Vespucio 5E, 1º M12, 41092 Seville, Spain
[3] ChiPro GmbH, Anne-Conway-Straße, D-28359 Bremen, Germany
[4] School of Engineering & Science, Jacobs University Bremen, Campus Ring 1, D-28759 Bremen, Germany

Aqueous two-phase systems (ATPS) have been applied for decades for separation and purification of biomolecules, however, the poor predictability of a protein partition coefficient has been limiting the use of this tecnique. Nevertheless, the selectivity of systems for specific proteins can be enhanced with affinity ligands and for purification of monoclonal antibodies (mAb), in particular, several ligands have been explored[1]. In this work an affinity dual tag ligand based on a choline binding polypeptide tag (C-LytA) fused with a Z domain of protein A, LYTAG-Z, was used within a PEG/Dextran system for mAbs selective extration. C-LytA is the C-terminal region of the *Streptococcus pneumoniae* LytA amidase and enables the attachment of this enzyme to the cell wall surface. PEG is also able to bind to the choline binding site of C-LytA and this affinity has been explored for the extraction of different recombinant proteins fused to C-LytA, such as GFP and β-galactosidase, where a preferential partition of the fused protein to the PEG-rich phase allows a selective extraction of the target protein[2]. Using this dual ligand, mAbs from a CHO cell supernatant were recoverd in the top phase with an extraction yield of 89% using a system composed of 6% PEG 3350 and 7% dextran 500,000.

Moreover, since the choline binding domain of LYTAG-Z also exhibits a strong affinity for matrices containing choline analogues, such as quaternary amines, the PEG-rich phases were further processed by chromatography. Three different strong anion exchange matrices, charged with quaternary amines – CIMmultus™ QA, Hitrap Q Sepharose FF and Fibers R-SAX-F– were compared for a polishing step, as affinity matrices. A two-elution method was developed to bind all the LYTAG-Z-mAb complexes to the charged quaternary amine groups, while the remaining impurities were washed-out during column loading. By decreasing the pH to 3.5, the antibody is eluted from the Z-domain and by adding choline it is possible to recover the ligand LYTAG-Z, and recycle it for further purification runs.

[1] Azevedo, A. M. *et al.* Affinity-enhanced purification of human antibodies by aqueous two phase extraction. *Sep. Purif. Technol.* **65,** 31–39 (2009).
[2] Maestro, B. *et al.* Affinity partitioning of proteins tagged with choline-binding modules in aqueous two-phase systems. *J. Chromatogr. A* **1208,** 189–96 (2008).

Antibody libraries based on an autonomous human variable domain

A. Johan Nilvebrant[1,2], B. Sachdev Sidhu[2]

[1]Department of Protein Technology, KTH - Royal Institute of Technology, Stockholm, Sweden
[2]The Donnelly Centre, University of Toronto, 160 College Street, Room 816, Toronto, M5S 3E1, Ontario, Canada

Antibodies are tremendously useful for biotechnological applications, diagnostics and therapy. However, their complex architecture has spurred interest in smaller derivatives such as Fab and scFv that can retain the targeting specificity and be more easily produced. We have constructed two highly diverse (>10^{10}) libraries based on an autonomous human variable heavy (V_H) domain. This scaffold was generated by comprehensive mutational analysis of residues in the former light chain interface to identify structurally compatible hydrophilic substitutions that promote autonomous behavior. We integrated a CDRH1 design biased towards Asp aimed to alleviate aggregation problems that are commonly associated with human domain antibodies.

The libraries have been used to select binders to all human Eph receptors, many of which play roles in cancer. Our aim is to use these binders to investigate blocking or activation of specific Eph receptor homo- or heterodimers. In contrast to Fab fragments raised against the same antigens, the domain antibodies typically bind the ligand-binding domain and compete with ligand for binding. We have solved the structure of one EphA1-binder and propose a model for ligand blocking. Furthermore, we have analysed the influence of CDRH1 charge in a panel of EphA1 binders and also expanded this strategy to CDRH3 to enable selection of heat tolerant clones by phage display.
Moreover, binders to an intracellular GTPase implicated in Ras-dependent pancreatic cancer have been isolated and screened for potential inhibition of assembly of a signaling complex that activates Ras/MAPK. The V_H format may enable intracellular delivery to inhibit Ras-driven tumorigenic signaling.

Towards optimising glycan structures of plant and algae-made biopharmaceuticals

Muriel Bardor[1, 2]

[1]*Laboratoire Glycobiologie et Matrice Extracellulaire végétale (EA4358), Normandie Univ, UNIROUEN, 76000 Rouen, France*
[2]*Institut Universitaire de France (I.U.F.), 1, rue Descartes, 75231 Paris Cedex 05, France*

The biopharmaceuticals market includes more than 200 product types [1]. Among the various categories, monoclonal antibodies (mAbs) and their derivatives form the largest group (1). Today, Chinese Hamster Ovary (CHO) cell lines are considered as the gold-standard for the biopharmaceutical industry, due to their ability to carry out complex post-translational modifications, including human-like glycosylation [2]. However, the constantly increasing need for large quantities of biopharmaceuticals, their high production cost in CHO cells, and factors related to virus contamination have encouraged the development of new alternative production systems. Among those, there is an increasing interest for the use of plants and microalgae as alternative production systems for large-scale production of biopharmaceuticals [3, 4]. These aspects, the glycoengineering and analytical strategies developed to optimize biopharmaceuticals production and characterization in plants and microalgae will be summarized in this presentation.

[1] G. Walsh, Nat. Biotechnol. 32 (2014) 992–1000
[2] P. Hossler, S.F. Khattak, Z.J. Li, Glycobiology 19 (2009) 936–949
[3] M. Bardor, C. Burel, A. Villarejo, J.-P. Cadoret, A. Carlier, P. Lerouge Essential Reviews in Experimental Biology, volume 4, "Glycosylation in diverse cell systems – challenges and new frontiers in experimental glycobiology" Society for Experimental Biology. Ed: S. Brooks, P. Rudd and B. Apelmelk., Chapter 5 (2011) 93-118
[4] E. Mathieu-Rivet, M.-C. Kiefer-Meyer, G. Vanier, C. Ovide, C. Burel, P. Lerouge, M. Bardor Frontiers in Plant Science, section Plant physiology, Jul 28; (2014) 5:359. doi: 10.3389/fpls.2014.00359.

Acknowledgements

The authors are indebted to all coworkers and students at the Glyco-MEV laboratory who are currently contributing to the microalgae research project or did so in the past. They are also thankful to the University of Rouen, the region Haute-Normandie and the I.U.F. for their financial support.

Identification of a Compound from Aloe Vera Extract Involved in Pancreatic Cell Regeneration for Alleviating Diabetes Mellitus

Neha Deora, Prasanna Raja, Agamudi S. Kamalanathan, Krishnan Venkataraman, Mookambeswaran Vijayalakshmi

CBST, VIT University, Vellore, 632014, INDIA

Diabetes is the most prevalent endocrine disorder and has become huge social and economic problem. Defect in Beta cell mass or its function results in diminished or inappropriate secretion of insulin eventually leading to long term complications. Previous work in our laboratory has shown that there has been a quantitative and qualitative regeneration of 85% of pancreatic beta cells. Hence, it was important to elicit the pathway by which pancreatic cell mass and its function was restored. One of the major pathways of islet cell regeneration is through increased half-life of GLP-1 by inhibition of DPP4. Dipetidyl peptidase-4 (DPP-4),a serine proteases that normally inactivates GLP-1. As a result half- life of GLP-1 is short (~2 min) and its function is perverted. This study demonstrates that Aloe Vera extract has the ability to inhibit DPP4 enzyme in biochemical assays. Subsequently, a small molecule belonging to pyrrole family responsible for the DPP-IV inhibition was successfully isolated by activity guided fractionation through 3 chromatographic steps involvingC-18 RP-HPLC, LH-20 followed by one more step of C-18 RP-HPLC. Further, this molecule was characterized for its structure and the mechanism of inhibition. Currently, studies are underway to evaluate the effect of purified DPP4 inhibitor on pancreatic cell regeneration and the safety and efficacy of this molecule in alleviating diabetes in animal models.

Fast and high resolution separation of PEGylated proteins

Noriko Yoshimoto[1], Shuichi Yamamoto[1], Ales Podgornik[2]

[1] *Bio-Process Engineering Laboratory, Biomedical Engineering Center (YUBEC) Yamaguchi University, Tokiwadai, Ube, 755-8611, Japan*
[2] *University of Ljubljana, Ljubljana, Slovenia*

PEGylation reaction mixtures usually contain various unwanted species, such as PEGylated isoforms (positional isomers), over PEGylated proteins, the native protein, non-reacted PEGs and more. Electrostatic interaction based chromatography (ion-exchange chromatography, IEC) is known to be most efficient for PEGylated protein separations. Although PEGylated proteins were much more weakly retained in IEC, the number of binding sites for mono-PEGylated proteins was similar to the value for native proteins. Weak retention of PEGylated proteins was likely to be due to a steric hindrance between the ion-exchange ligand and the charges of PEGylated proteins. Good correlation was found between the peak shift and the calculated thickness of PEG layer. Because of large hydrodynamic size of PEGylated proteins, slow mass transfer (low pore diffusivity) lowers the performance of chromatography. By choosing a suitable mobile phase pH and gradient slope, a large number of PEGylated isoforms can be successfully separated by salt linear gradient elution on monolithic disk IEC.

[1] Yu Isakari, Ales Podgornik, Noriko Yoshimoto, Shuichi Yamamoto, Monolith disk chromatography can separates a large number of PEGylated protein positional isoforms within minutes at low pressure, Biotechnology Journal, 2016, 11, 100–106

Tracking Host Cell Protein Contaminants During The Purification of Recombinant Proteins

Reinhard I. Boysen, Yuanzhong Yang and Milton T. W. Hearn

Victorian Centre for Sustainable Chemical Manufacture, School of Chemistry, Monash University, Melbourne, Victoria 3800, Australia

Traditionally, detection of host cell protein (HCPs) contaminants in a purified recombinant protein, expressed in a prokaryotic or eukaryotic cell host, has been based on ELISA methods. Although limits of detection can be high, several fundamental questions remain with such antibody-based methods, including (i) what is the extent of molecular diversity of the HCPs that are detected; (ii) are the same HCPs always present when a platform purification technology is employed; (iii) how much variation in HCPs populations occurs with different load, wash and elution chromatographic steps; (iv) do chromatographic resins which notionally operate with similar adsorption mechanisms carry forward HCPs in a similar or different manner; (v) how much promiscuity HCP binding occurs with recombinant proteins of different molecular structure and conformation? In this investigation, a general approach has been developed based on LC-MSn procedures to address these questions and provide solutions that distinguish between these alternative types of HCP behaviours with tagged recombinant proteins, expressed in genetically engineered E. coli cells.

HRMS based quantification of host cell protein networks during microbial expression as a tool for upstream process development

S. Fröhlich[1], J. Jarmer[1,2], H. Exl[1], A. Dürauer[2], M. Allmer[1]

[1]Boehringer Ingelheim RCV GmbH & Co KG, Dr. Boehringer-Gasse 5-11, 1121 Vienna, Austria
[2]Department of Biotechnology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria

Early stage quantitative analysis of host cell protein (HCP) variations is challenging yet necessary for comprehensive bioprocess development. High resolution mass spectrometry (HRMS) provides a high-end technology for accurate identification alongside with quantitative information. Hereby we describe a flexible HRMS assay platform to quantify HCPs relevant in microbial expression systems such as *E. Coli*.

Cell pellets were lysed and proteins extracted before applying the SMART tryptic digest kit. Peptides separation was optimized using an RP-UHPLC separation platform. HRMS-MSMS analysis was conducted on an Orbitrap Velos Elite applying CID. Quantification strategies covered TMT and iTRAQ labelling compared to label-free normalization approaches for peptide level comparison. Results were analyzed using ProteomeDiscoverer 2.0, PepFinder 2.0, SIEVE 2.0 (Thermo Fisher Scientific) and SIMCA (Umetrics AG).

The developed HRMS platform was applied to an *E. Coli* expression set with varying productivity. HCPs known for relevant biological mechanisms, e.g. riboswitch technology, were successfully quantified within the fmol range. Analysing HCP networks based on pattern analysis facilitated low level quantification and enhanced validity. This approach is of high relevance for high-throughput screening experiments during upstream development, e.g. for titer determination, dynamic HCP network analysis or product characterization

Next Generation Imaging Detection for CIEF Applications and Coupling with Mass Spectrometry

Tiemin Huang[1], Gerard Rozing[2], Martin Donker[3]

[1.] *Advanced Electrophoresis Solutions Ltd., Cambridge, ON, N3H4W5, Canada*
[2.] *ROZING.COM Consulting, Karlsruhe, Germany, gerard@rozing.com*
[3.] *Isogen Life Science, De Meern, the Netherlands.*

Capillary IsoElectric Focusing (CIEF) has become an indispensable tool in life science research, in the development of new biopharmaceutical products. CIEF is the preferred method for study of post translational modifications of recombinant proteins, for protein degradation studies, study of the variability in glycosylation, quality control of mAB's and biopharmaceuticals and for instance in profiling and authentication of food products.

Despite great efforts in study of fundamental aspects of CIEF, the method has remained cumbersome in execution, difficult to optimize and time consuming. Capillary isoelectric focusing with whole column imaging detection (*i*CIEF) was introduced on the bioanalysis market some years ago. This technique resolves these issues by providing faster method development, control of practical operation, better reliability and robustness, and higher analytical throughput of CIEF.

Based on this technology, the authors have made some important breakthroughs such as high sensitivity imaging with 200 µm i.d. separation capillary, a modern optical system, and proprietary carrier ampholytes (CAs) and pI standards optimized for CIEF. The system was recently commercialized in a new CE instrument system.

High resolution, high sensitivity CIEF separations of therapeutic proteins such as antibody drug conjugates (ADC), monoclonal antibodies (mAb's), and fusion proteins with CEInfinite system have been demonstrated successfully and will be discussed.
In addition, the system allows high resolution preparative *i*CIEF. As an example, fractionation of haemoglobin control sample will be illustrated by a recorded live execution. Fractions can be collected into individual vials, and e.g. remixed with carrier ampholytes, and their purity checked with *i*CIEF. The preparative mode will also allow easy coupling with mass spectrometry through MALDI or ESI interfacing techniques.

Characterization of plasma proteins and lipoproteins using microchannel asymmetrical flow field-flow fractionation

Florian Meier[1], Lisa Schilder[1], Soheyl Tadjiki[2], Robert Reed[2], Thorsten Klein[1]

[1]Postnova Analytics GmbH, Landsberg am Lech, Germany
[2]Postnova Analytics Inc., Salt Lake City, UT, United States

Field-flow fractionation (FFF) [1] is an elution-based separation technique which is capable of the rapid and high efficient separation of macromolecules, colloids and particles. Asymmetrical flow FFF (AF4) is one of the FFF sub-techniques that has been broadly used in characterization of complex biological and pharmaceutical products [2,3]. The commercial AF4 system commonly employs flat channels with different dimensions and aspect ratios (10-28 cm long and 2-10 cm wide). Annular channels (hollow fiber) have been also used in the AF4 system as an alternative geometry. The AF4 channel could be further downscaled to reduce channel volume (less dilution), sample consumption and operating cost. A miniaturized flat AF4 channel (microchannel) with a size smaller than a credit card was constructed and examined for characterization of biological samples spanning a wide molecular weight and diameter range. The performance of the microchannel was tested using a mixture of plasma proteins. The resolution and reproducibility of the microchannel were found to be similar to those of standard channel. The microchannel was used to fractionate Human Serum Albumin (HSA) from different lipoprotein fractions. Baseline separation was also achieved between the high-density and low density lipoproteins. An AF4 microchannel is an alternative to full-size channels which allows use of smaller sample mass, and reduces the amount of dilution in the channel, allowing more efficient detection.

[1] J.C. Giddings, F.J. Yang, M. N. Myers, Anal. Biochem. 81(2) (1977) 395-407
[2] C.R.M. Bria, J. Jones, A. Charlesworth, S.K.R. Williams, J. Pharm. Sci. 1 (2016) 31-39
[3] C. John, T. Herz, J. Boos, K. Langer, G. Hemper, Talanta 146 (2016) 335-339

Separation of Virus Like Particles and Protein-Drug-Particles using FFF and SEC

Dr. Dierk Roessner, Dr. Christoph Johann

Wyatt Technology Europe GmbH, Dernbach, Germany

to characterize the 60 nm particles. Both methods dilute the sample during elution. We Size Exclusion Chromatography (SEC) is the gold standard for the size based separation of macromolecules. But for biological particles and superstructures Field Flow Fractionation (FFF) is an interesting alternative because of the absence of a stationary phase. This allows separation of biological particles and superstructures up to 1 µm or samples which interact with a stationary phase.

In this presentation we discuss the separation of Virus Like Particles (VLP) in two applications:

- Separation and characterization of VLP and aggregates
- Separation and characterization of two VLPs with 180 and 240 subunits

The separation was monitored using online Multi Angle Light Scattering (MALS) for molar mass and rms radius measurement as well as online Dynamic Light Scattering (DLS) for hydrodynamic radius measurement. The results were compared with batch (no separation) DLS and simulated using the FFF theory to determine if the separation process is ideal or if membrane interaction plays a role.

The third application is the separation of a complex formed by a protein and a hydrophobic small drug molecule, forming 60 nm particles in aqueous solution. The 60 nm particles dis-associate if the concentration drops below a critical concentration. Both, SEC and FFF separation were performed will show that it is possible to separate and characterize the particles using FFF and discuss the differences to SEC. Results were compared with batch DLS results.

Next-generation biopharmaceutical downstream process

Gorazd Hribar

Biologics Technical Development & Manufacturing, Lek Pharmaceuticals d.d., Kolodvorska 27, 1234 Menges, Slovenia

The aim of the talk is to present current status and updates within nextBioPharmDSP project - Next-generation biopharmaceutical downstream process, where the main focus is to develop fully continuous approach for purification of monoclonal antibodies. The specific topics, on which the focus will be on are different approaches for primary separation, such as flocculation or ATF/TFF in perfusion, continuous chromatography, several flow-through options for polishing steps and advanced analytical tools, which will enable in-line detection of quality.

Acknowledgements:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635557

Use of Empirical Model-Independent Optimisation for Preparative Polishing Chromatography of Proteins

Ajoy Velayudhan[1], *Spyridion Konstantinidis*[1], *Kosma Jurlewicz*[1]

[1]*University College London, Department of Biochemical Engineering, Gordon Street, London, United Kingdom WC1H 0AH*

Work in our laboratory has shown that a model-independent empirical optimisation method is effective in identifying near-optimal operating conditions for preparative polishing chromatography steps. (It is assumed that the feed mixture consists at least of three components: the desired product, a 'weak impurity' that binds less strongly than the product, and a 'strong impurity' that binds more strongly than the product.) We have refined this method, based on the popular numerical method of Nelder and Mead [1], in various ways, e.g., to allow for a discrete grid of independent variables [2]. This refined method has been demonstrated to be more efficient in finding effective operating conditions for a single polishing step than traditional design of experiment (DoE) methods. Further, it was shown that our method was extremely effective at identifying global operating conditions in complex problems, which was usually far beyond the ability of DoE methods, even with high-dimensional regressions [3]. Here, we present results on multi-objective criteria, to demonstrate that our version of the simplex method can address multiple objectives of differing weights. These results address the traditional objection that simplex methods cannot cope with multi-objective criteria. The method is applied to a variety of complex separation problems, both from industry and from our own laboratory, and is shown to be efficient and adaptive. It is suggested that this refined simplex method is a much more natural and effective optimisation method than DoE for complex polishing chromatography steps, and is ideally suited to such optimisation in early-phase development.

[1] J.A. Nelder, R. Mead, Computer J. 7 (1965) 309-313
[2] S. Konstantinidis, S. Chhatre, A. Velayudhan, E. Heldin, N.J. Titchener-Hooker, Analyt. Chim. Acta 743 (2012) 19-32
[3] S. Konstantinidis, J.P Welsh, E.F. D.J. Roush, A. Velayudhan, Biotechnol. Prog. 32(2) (2016) 404-419

Purification of secretory immunoglobulins from whey

A. Matlschweiger[1], H. Engelmaier[1], G. Himmler[2], R. Hahn[1]

[1]Department of Biotechnology, BOKU Vienna, Muthgasse 18, Vienna, Austria
[2]Angothera GesmbH, Donau-Oder-Kanal IV SW 80, Groß-Enzersdorf, Austria

Immunoglobulin A (IgA) is the most abundant antibody class in the human body. In its secretory form (SIgA) it is the main effector of the mucosal immune system. We have studied the isolation of SIg (SIgA and SIgM) from animal whey applying standard unit operations for protein purification, including membrane separations, precipitation and chromatographic methods. Due to the large molecular weight of SIg with 400 and 900 kDa, respectively, and also the low titer, ultra/diafiltration was the obvious first step in the purification sequence. Low transmembrane pressure was found to be most important operating parameter, whereas the membrane cut-off was a less dominating factor. In an optimized process, ultrafiltration was followed by fractional precipitation with 3 and 7% PEG 10000 and the dissolved precipitate was then subjected to diafiltration resulting in a purity of ~ 75%. To obtain high purity of > 95% a combination of two anion exchange steps was established. In the first step on Q-Sepharose FF albumin and IgG were captured and SIg was collected in the flow-through. This step utilized the low effective pore diffusion coefficient of SIg in the small-pore medium, an effect that was further enhanced when operated under conditions where the impurities were bound. The flow through was then supplemented with sodium chloride and directly loaded onto a POROS 50 HQ column. Under these conditions the residual IgG did not bind and SIg could be captured at capacities of 20 mg/mL. The presented study highlights many of the critical steps and bottlenecks associated with purification of such complex molecules

Novel Systems for the Extraction and Purification of Monoclonal Antibodies directly from CHO Cell Cultures Supernatants using Aqueous Biphasic Systems comprising Ionic Liquids

Emanuel V. Capela[1], Isabel Campos-Pinto[2], Sara A. S. L. Rosa[2], João A. P. Coutinho[1], M. Raquel Aires-Barros[2], Ana M. Azevedo[2], Mara G. Freire[1]

[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.
[2] Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal.

Currently, monoclonal antibodies (mAbs) are the most prevalent class of recombinant protein therapeutics for the treatment of several diseases (e.g. cancer and autoimmune disorders) [1]. The outstanding improvements achieved at upstream of their manufacturing process, shifted the bottleneck towards downstream, especially due to the high cost and low capacity of standard purification processes, mainly based on protein A chromatography capture [2]. Aqueous biphasic systems (ABS) can be foreseen as a valuable alternative to the established chromatographic purification platforms presenting promising advantages as high loading capacity, ability to operate in a continuous mode and easiness in scalability. ABS based on ionic liquids (ILs) emerged, recently, as suitable alternatives to the traditional polymer-based ABS for the extraction and purification of a wide plethora of (bio)molecules, allowing improved and selective extractions by the manipulation of phases' polarities [3]. In this work, several ILs were tested as adjuvants, at different concentrations, in conventional PEG/Dextran ABS for the capture/purification of mAbs directly from Chinese Hamster Ovary (CHO) cell cultures supernatants. According to the obtained results, imidazolium-based ILs revealed to increase extraction and selectivity potential of ABS towards mAbs. Moreover, with higher concentrations of IL, it is possible to extract 82% of mAbs to the PEG-rich phase, with a purity of 64%, only in a single-step. In this context, novel ABS composed of IL as adjuvant is proposed as an effective platform for the extraction/purification of value-added mAbs directly from CHO cell cultures supernatants, and their application at a large scale is thus envisaged.

[1] J. G. Elvin, R. G. Couston, C. F. van der Walle, International Journal of Pharmaceutics, 440 (2013) 83–98;
[2] J. M. Reichert, C. J. Rosensweig, L. B. Faden, M. C. Dewitz, Nature Biotechnology, 23 (2005) 1073;
[3] M. G. Freire, A. F. M. Cláudio, J. M. M. Araújo, J. A. P. Coutinho, I. M. Marrucho, J. N. Canongia Lopes, L. P. N. Rebelo, Chemical Society Review, 41(14) (2012) 4966-4995.

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. M. G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ERC-2013-StG-3377.

Design of Aqueous Two-Phase Systems for Biopharmaceutical Purification in a Microfluidic Channel using Fluorescent Microscopy

D.F.C. Silva[1,2], V. Chu[2],
A. M. Azevedo[1], J. P. Conde[2], M. R. Aires-Barros[1]

[1]IBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Department of Bioengineering, Universidade de Lisboa, Avenida Rovisco Pais 1049-001, Lisbon, Portugal
[2]Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologias (INESC MN) and IN – Institute of Nanoscience and Nanotechnology, Rua Alves Redol 1000-029, Lisbon, Portugal

ATPS process design and optimization is time consuming and requires large material volumes, limiting its application to the current downstream processing. In this work, a fast and novel technique based on a microfluidic platform was used for screening and optimizing mAbs extraction conditions in ATPS, combining the process efficiency of ATPS with the reduced times and volumes associated with microfluidics [1]. This method consists of using a microfluidic device with a single microchannel and three inlets. In two of the inlets solutions containing ATPS components were loaded while in the third milli-Q water containing a FITC tagged biomolecule was fed. Different phases of a PEG/phosphate buffer ATPS were introduced in parallel in a 150 µm width, 20 µm height and 16,8 cm long microchannel along with FITC marked mAbs. Using fluorescent microscopy, it was possible to follow the FITC tagged mAbs and, by simply varying the solutions pumping rates, to test a wide variety of ATPS compositions. This process allows for faster screening of extraction conditions and partition coefficients using only a few microliters of material for each ATPS composition and the possible automation of the process is another advantage. The microfluidic device also allowed to determine the binodal curves of different ATPS phase diagrams [2].

[1] D.F.C. Silva et al "Design of a microfluidic platform for monoclonal antibody extraction using an aqueous two-phase system. J. Chromat. A 1249, 1-7. (2012).

[2] D.F.C. Silva et al. "Determination of Aqueous Two Phase System Binodal Curves using a Microfluidic Device" J. Chromat A, 1370, 115–120 (2014).

Fast and easy way to boost your primary separation in mAb production: Continuous flocculation, new alternatives for old problems.

P. Satzer [1], D. Burgstaller [1], W. Krepper [1], A. Cataldo [1], J. Haas [2], M. Maszelin [2], J. Mohoric [3], K. Pajnic [3], A. Jungbauer [1,4]

[1] Department of Biotechnology, University of Natural Resources and Life Sciences, 1190 Vienna, Austria
[2] Biomanufacturing Sciences Network, Merck, 67120 Molsheim, France
[3] LEK, a Sandoz company, 1000 Ljubljana, Slovenia
[4] Austrian Centre of Industrial Biotechnology (ACIB), Muthgasse 18, 1190 Vienna, Austria

We present the development of new flocculation strategies for removal of impurities during primary separation while simultaneously improving filterability of the harvest. We tested different flocculation strategies using salt flocculation by Calcium Chloride and polymer based flocculation using Polydiallyldimethylammonium Chloride (pDADMAC) or benzyl modified Polyallylamine (mPAA). While pDADMAC showed the biggest benefit in regards to necessary filtration area for depth filtration, all methods had different additional benefits that can be utilized in regards to other shortcomings in the downstream-train. pDADMAC and Calcium Chloride were able to remove virtually all DNA during flocculation, while mPAA and to a certain extent Calcium Chloride were able to reduce aggregate content with negligible yield losses given the right conditions. Novel analytical tools such as Focus Beam Reflectance Measurements (FBRM) made it possible to observe the flocculation kinetics. This information makes the design of a continuous step, not a matter of best guess, but of informed choice. We tested the suitability for batch and continuous mode of operation for flocculation by parallelization of the depth filtration. We were able to continuously induce efficient flocculation in a feed stream of cell culture and divert the stream to one of two filters for filtration. Using three scenarios, classical batch depth filtration, depth filtration coupled with flocculation, and continuous flocculation we evaluated the economic benefits of the flocculation and the continuous operation

Continuous crystallization-based protein purification processes

D. Hekmat, C. Lohse, N. von den Eichen, D. Weuster-Botz

Institute of Biochemical Engineering, Technical University of Munich, Garching, Germany

Large-scale crystallization has been shown to represent an interesting alternative to preparative chromatography for purification of proteins [1]. This is due to the fact that crystallization processes do not represent a downstream processing bottleneck and do not require costly consumables like chromatography resins. Fast and easily scalable batch crystallization processes of macromolecular biopharmaceuticals, e.g. an antibody fragment and a whole monoclonal antibody have been developed [2, 3]. Due to undergoing efforts of the pharmaceutical industry to apply continuous downstream processing, the feasibility of continuous crystallization was investigated. A novel continuous protein crystallization process will be presented which consists of a stirred-tank crystallizer (100 mL) in series with a tubular crystallizer (30 mL) with recycle. Continuous crystallization in this novel combined system was examined using two exemplary proteins, lysozyme and a whole therapeutic monoclonal antibody. Reproducible and stable continuous crystallization with high yields (up to 94 %) and productivities (4.4 g L^{-1} h^{-1}) was possible. Adequate crystal morphologies with narrow crystal size distributions were obtained. It will be shown that the combined system enabled a classified removal of larger protein crystals in the stirred-tank crystallizer outlet. The tubular crystallizer with recycle of the suspension of smaller protein crystals facilitated an advantageous reduction of the operating temperature resulting in a higher yield of the process. The crystallization rates in the continuous crystallization system were comparable to batch crystallization rates. The prospect of a fully continuous chromatography-free biomanufacturing process by integrating upstream processing with downstream processing via crystallization will be discussed.

[1] D. Hekmat, Bioprocess Biosyst. Eng. 38 (2015) 1209-1231
[2] D. Hebel, S. Huber, B. Stanislawski, D. Hekmat, J. Biotechnol. 166 (2013) 206-211
[3] B. Smejkal, N. Agrawal, B. Helk, H. Schulz, M. Giffard, M. Mechelke, F. Ortner, P. Heckmeier, B. Trout, D. Hekmat, Biotechnol. Bioeng. 110 (2013) 2452-2461

Quasi-continuous temperature-controlled protein chromatography in downstream processing of antibodies

B. Ketterer, H. W. Ooi, M. Franzreb

Karlsruhe Institute of Technology (KIT), Institute of Functional Interfaces (IFG), Eggenstein-Leopoldshafen, Germany

Liquid chromatography nowadays plays a major role in downstream processing of biopharmaceuticals, whereby binding/elution of the product is controlled by changing the composition of the mobile phase. The large amounts of buffers needed are an important cost factor in a process. In addition, regeneration steps and idle states are generally required.

By the use of thermoresponsive resins, binding/elution is controlled by temperature changes. Therefore, no change in the composition of the liquid phase is needed, hence saving the need for an elution buffer and consecutive buffer-exchange steps. In addition, combining the temperature-controlled chromatography with the traveling cooling zone reactor (TCZR) principle enables quasi-continuous processes. With temperature-controlled chromatography, binding/elution can be decoupled from the mobile phase velocity. The column can be loaded continuously, whereas the external temperature field can lead to a peak wise elution of the target protein. A steady state can be reached, whereby regeneration steps as well as idle times can be eliminated.

Up to now, thermoresponsive ion-exchange resins have been used with the TCZR solely for the concentration of lactoferrin. A better understanding of the process as well as novel thermoresponsive mixed-mode resins open up new applications for continuous chromatography with the TCZR.

We will demonstrate the applicability of novel thermoresponsive resins for antibodies, a commercially particular important product. The applicability of the novel resins in a quasi-continuous thermoresponsive chromatography process will be assessed. Experimental data of quasi-continuous operation will reveal different applications of the TCZR.

Characterization of highly concentrated protein solutions - One step towards the predictability of protein long term stability

Marie-Therese Schermeyer[1], Katharina C. Bauer[1], Anna K. Wöll[1], Kristina Schleining[1], Jürgen Hubbuch[1]

[1] Institute of Engineering in Life Sciences, Research Unit IV: Biomolecular Separation Engineering, Karlsruhe Institute of Technology, Engler-Bunte-Ring 3, 76131 Karlsruhe, Germany

A bottleneck in the development of novel dosage forms is the long-term stability of highly concentrated protein solutions. To study formulation stability long-range as well as short range protein-protein interactions and the formation of complex network structures have to be taken into account. The interplay of described protein interactions is not well understood and therefore unwanted aggregation a serious issue.

This work deals with the suitability of conventional and novel analytical tools for the characterization of highly concentrated protein solutions aiming the prediction of protein long term stability. For this purpose model proteins as well as monoclonal antibodies in a concentration range of 60-200 mg/ml were screened under varying solution conditions. Melting and aggregation temperatures were determined, the zeta potential and the dynamic viscosity measured as well as high frequency rheometry used to determine the storage (G') and loss modulus (G'') of given samples. To correlate the determined parameters with the phase behavior, the long-term stability of identical samples was studied in an automated microbatch format. The correlation indicate that particularly the rheological determined parameters, G' and G'' provide information about the influence of varying solution conditions on highly concentrated protein solution. Determined complex rheological parameters in combination with calculated dynamic viscosities and the difference of obtained melting and aggregation temperature (T_m-T_{agg}) allowed accurate predictions of the long-term stability of studied protein solutions. This study provides a better understanding of highly concentrated protein solution characteristics and thereby a further step towards the predictability of stable and save formulations

Destruction of proteins by cavitation and high shear

[1]M. Dürkop, [1]E. Berger, [1,2]A. Dürauer, [1,2]A. Jungbauer

[1]Austrian Centre of Industrial Biotechnology, Muthgasse 18, 1190 Vienna, Austria
[2] University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria

The influence of shear stress on the destruction of proteins is a conflicting topic in literature. Some claim that cavitation might be responsible for mechanical destruction of proteins and not shear stress. At high flow rates the local static pressure can fall below the vapour pressure of the liquid and hence gas bubbles form. This effect is called cavitation and leads to several protein relevant effect. Growing gas cavities provide additional phase boundaries to the proteins while the implosion of the cavities lead to formation of hydroxyl radicals which can affect the protein structure. To address the topic of cavitation we compared the influence of simple cavitation derived from an ultra-sonication homogenizer with the combined effect of cavitation and extreme shear stress (> 107 s -1) obtained from a micro orifice (< 100 µm) integrated into an ÄKTA system to the response of different proteins. We were able to both describe the flow regime inside the micro orifice with computational fluid dynamics to calculate shear stress and on the other hand determine the amount of generated hydroxyl radicals by using a sensitive and specific Terephthalic acid dosimeter. Several different proteins, among those also highly pharmaceutically relevant proteins like IgG or FGF-2, were tested in both systems. We analyzed secondary as well as quaternary structure before and after treatment with circular dichroism and size-exclusion chromatography respectively. Although we expected the vast majority of these proteins to show relevant protein loss after the treatment we could only clearly state that 3 proteins reacted on the treatment. In a further analysis we strengthened the theory that the additional phase boundary is responsible for protein aggregation and not the occurrence of hydroxyl radicals or shear stress, which did not harm the proteins at all at the prevailing concentration.

Analytical and Preparative Separation of Peptides and Polynucleotides By Repulsion/Attraction Mode On DRP-Packings

Joachim Kinkel[1], *Juergen Machielse* [2], *Andrea Wild*[1]

1) Faculty of Applied Chemistry , Analytical Chemistry and Separation Technology Laboratory, Technical University Nuremberg, Prinzregentenufer 47, D-90489 Nuremberg, Germany
2) R&D, ZEOCHEM AG, CH-8707 Uetikon am See, Suisse

Analysis and preparative purification of API's based on synthetic peptides is one of the major areas of interest in pharmaceutical research. This task typically involves several purification steps where different chromatographic interaction modes like Ion-Exchange, Reversed Phase Chromatography and others are combined mainly by gradient elution conditions.

With the availability of Doped Reversed phase packings [1], [2], which combine electrostatic repulsion and hydrophobic attraction, new 2-dimensional selectivity patterns for charged biomolecules like peptides, polynucleotides and proteins are obtained.

For all separations of peptides under repulsion-attraction conditions on DRP packings tested so far, observed selectivities and chromatographic resolution were equal or better compared to those results obtained by RP-chromatography alone. The preparative results were superior to those obtained under optimized IEX or RP conditions alone or in combination.

The paper will describe the chromatographic separation conditions, the optimization strategy, and the application of such Doped Reversed Phase –packings in analytical chromatography and their importance for preparative separations of crude peptides under isocratic and gradient elution conditions in batch, recycle and SMB technology.

[1] PCT/CH2013/000050, Doped Materials for Reversed Phase Chromatography
[2] Khalaf R, Forrer N, Buffolino G, Gétaz D, Bernardi S, Butté A, Morbidelli M.:Doping reversed-phase media for improved peptide purification. J. Chromatogr. A , 1407, (2015) 169-175

Arginine-Superporous affinity chromatography for the supercoiled p53-encoding plasmid isolation

J.F.A. Valente[1], A. Sousa[1], C. Cruz[1], J.A. Queiroz[1] and F. Sousa [1]

[1]CICS-UBI- Health Science Research Centre, Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

P53 is a tumor suppressor gene that has been explored for gene therapy as a possible alternative to the common cancer treatments. The use of plasmid DNA (pDNA) in gene therapy is gaining interest and the maintenance of the supercoiled (sc) structure is required for eliciting an effective therapeutic gene expression and to induce the re-establishment of the protein expression [1].

To promote the biorecognition of pDNA and the sc plasmid purification, amino acids-based affinity chromatography, by using the arginine ligand immobilized in monoliths and agarose based matrices, has been successfully applied by our research group [2,3].

Concerning that, in this research work, different methods were used for L-arginine immobilization into superporous beads. The resulting supports were morphological and chemically characterized through SEM, EDX, FTIR and NMR. For the specific purification of sc pDNA, different parameters were evaluated, namely the buffer composition, salt concentration, pH and temperature, in order to achieve the best binding/elution conditions. The pDNA isoforms were separated by using an increasing stepwise gradient comprising a first step of 10 mM Tris-HCl with 10 mM EDTA, pH 8.0, a second step of 310 mM NaCl and finally a third step of 1 M NaCl in the same buffer, at 4 °C, where the sc isoform was eluted. The dynamic binding capacity (DBC) of the superporous arginine matrix was also evaluated and the results revealed an improvement in comparison with the arginine-agarose based matrix. To understand the robustness of this new matrix, other plasmids with different molecular weights and composition were also tested, being verified its applicability for the purification of pUC19 (2.7kbp) and HPV-16 E6/E7 (8.7kpb) plasmids.

[1] Valente, J., et al., Selective purification of supercoiled p53-encoding pDNA with l-methionine–agarose matrix. Analytical biochemistry, 2014. 459: p. 61-69.
[2] Almeida, A. M., et al. "Optimization of supercoiled HPV-16 E6/E7 plasmid DNA purification with arginine monolith using design of experiments." Journal of Chromatography B 978 (2015): 145-150.
[3] Sousa, F., et al., Specific recognition of supercoiled plasmid DNA in arginine affinity chromatography. Analytical biochemistry, 2008. 374(2): p. 432-434.

Acknowledgements

The authors would like to thank to Dr. Thomas Roberts and Dr. Karl Münger for providing the pcDNA3–FLAG–p53 and HPV-16 E6/E7 plasmids through Addgene, ref: 10838 and 8641, respectively, to Tosoh bioscience for kindly provide us the 650M epoxy matrix and to Eng. Ana Paula for her help in the SEM and EDX analysis.
J.F.A. Valente and A. Sousa also acknowledge Ph.D. and Postdoctoral fellowships (Ref SFRH/BD/96809/2013 and Ref SFRH/BPD/102716/2014, respectively).

Use of HTS process development chains to shorten timelines and increase process understanding

C. Walther[1,2], M. Berkemeyer[2], C. Brocard[2], A. Dürauer[1]

[1]University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria
[2]Boehringer-Ingelheim RCV, Dr. Boehringer-Gasse 5-11, 1121 Vienna, Austria

Whereas for downstream processing of mAbs derived from mammalians cell culture platform technologies are already established and commonly in use higher flexibility is required for products derived from microbials. The general demand for process development is to increase productivity and efficacy, shorten timelines and enhance flexibility as needed for highly diverse products. HTS technologies clearly address these requirements consuming less time and material. As additional benefit valuable process understanding is gained during development. Since each unit operation affects the subsequent process steps a HTS representing the entire process chain enable a more holistic approach of process development.

In accordance to product requirements we combine single operation units for harvest, recovery and purification interlinked to evaluate their interactions and impact on the overall process outcome. Due to its flexibility this set up can cover multistep processes such as product recovery from IBS as well as already existing platform processes. Applications range from early stage support for bioprocess development to post licence process evaluation. Examples will be shown for the first scenario and the scalability to bench scale.

Monitoring and improving Influenza Virus-like particles Downstream Processing using a click chemistry approach

Sofia B. Carvalho[1,2], João Freire[3], Mafalda Moleirinho[1,2], Francisca Monteiro[1,2], Diana Gaspar[3], Miguel Castanho[3], Manuel J.T. Carrondo[1,2,4], Paula M. Alves[1,2], Gonçalo Bernardes[3,5], Cristina Peixoto[1,2]

[1]ITQB – Instituto de Tecnologia Química e Biológica, Av. da República Estação Agronómica Nacional 2780-157, Oeiras, Portugal
[2]IBET – Instituto de Biologia Experimental e Tecnológica, Apartado 12 2780-901, Oeiras, Portugal
[3]IMM – Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa Av. Professor Egas Moniz 1649-028 Lisboa, Portugal
[4]FCT – Faculdade de Ciencias e Tecnologia, 2829-516 Caparica, Portugal
[5]University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom

Virus-like particles (VLPs) constitute a promising platform in vaccine development and targeted drug-delivery. However, most applications use non-enveloped VLPs that present less technical challenges, not only to produce and purify, but also in terms of characterization, compared to enveloped VLPs. Recent advances in upstream processing, new product quality requirements and other regulatory issues, as well as the search for more cost-effective processes, led to the need to develop more efficient downstream processes for biopharmaceuticals [1]. Thus, new monitoring and product characterization methods, applicable at all stages of downstream processing, are needed.

Here is reported a valuable platform used for the downstream processing and monitoring of the in vivo production of site-specifically functionalized enveloped Influenza VLPs. This strategy involves a two-step procedure that consists of residue-specific replacement of methionine by an analog azidohomolanine that enabled for post-expression functionalization with a fluorophore [2]. This platform does not impact VLP production/purification processes, and allows functionalization without deleterious effect on hemagglutinin biological function. As a proof of concept a complete downstream processing was performed, including clarification, capture and polishing steps. A flow cytometry analysis step was added to achieve a refined discrimination and separation between VLPs and baculovirus - the major impurity of the process [3]. This was further confirmed using atomic force microscopy. The developed platform allows an accurate product monitoring, enabling higher product recovery yields and higher impurity removal levels. The presented system is broadly applicable to the production of functionalized enveloped VLPs, for vaccine design, targeted drug delivery and molecular imaging.

[1] Cramer et al., Curr Opin Chem Eng, 1 (2011) 27-37
[2] Banerjee et al., J. Virol., 85 (2011) 7546-7554
[3] Van der Vlist et al., Nature Protoc, 7.7 (2012) 1311-1326

Purification of cell culture-based influenza A virus particles with a cellulose membrane adsorber and polyethylene glycol

P. Marichal-Gallardo [1], *M. M. Pieler* [1], *M. Wolff* [1,2], *U. Reichl* [1,2]

[1] *Bioprocess Engineering, Max Planck Institute for Dynamics of Complex Technical Systems, Sandtorstr. 1, 39106, Magdeburg, Germany*
[2] *Chair of Bioprocess Engineering, Otto-von-Guericke University Magdeburg, Universitaetsplatz 2, 39106, Magdeburg, Germany*

The variety of techniques for industrial-scale purification of large biomolecules such as viruses, virus-like particles, and gene vectors is often restricted to chromatography and filtration-based operations. Steric exclusion chromatography (SXC), whose selectivity is mainly based on target size, was described in the past for purifying proteins and viruses using monoliths. The target species are captured by the mutual steric exclusion of PEG between the product and the matrix. The product is desorbed by reducing the polymer concentration and can be recovered in virtually any buffer.

Here, we show that regenerated cellulose filters are a single use alternative to monoliths for SXC. The starting material containing inactivated influenza A virus particles was produced in 5 L bioreactor MDCK cell suspension cultures. Product recovery based on a hemagglutination activity assay and mass recovery of the hemagglutinin (HA) antigen estimated by single radial immunodiffusion assay were both above 95%. The size of the purified virus particles was 84 nm. Maximum DNA and total protein depletion achieved were 99.7% and 92.4%, respectively. DNA depletion exceeded 99.9% when a nuclease treatment was made before SXC. It was possible to purify 250 mL of virus broth in around 40 min. The surface productivity was 600–1000 trivalent doses·m^{-2}·h^{-1} (3×15 µg HA) depending on the feed. The dynamic binding capacity at 5% breakthrough of purified virus particles was 3.36 $\mu g_{HA} \cdot cm^{-2}$.

Overall, SXC with cellulose filters seems to be more economical than using monoliths and is a promising method for commercial production of large biologics.

High Throughput Screening as a tool for developing scalable purification processes of biotherapeutic proteins: Approach for developing purification processes from micro to column scale

M. Eppink, D. van Wijk, G. de Roo, K. Burgers, B. Kokke

Synthon Biopharmaceuticals BV, Microweg 22, Nijmegen, The Netherlands

Development of purification processes has become in recent years a more structure based approach by using high throughput screening technologies and statistical design. Different companies/universities explored a large variety of conditions at microscale format developing more robust purification processes for biologicals with low product amounts. One of the challenges in these smart developments concerns the translation of purification processes from microscale format in microtiter plates towards labscale format in columns which will be explained in this presentation.

[1] X. Li, G. de Roo, K. Burgers, M. Ottens, M. Eppink (2012) Self-packed filter plates: a good alternative for pre-packed filter plates for developing purification processes of therapeutic proteins. *Biotechnology Journal,* Oct;7(10):1269-76

Less is More – Miniaturization in Biopurification Process Development

Marcel Ottens

Department of Biotechnology, Delft University of Technology, Delft, The Netherlands

The vast experimental space that needs to be explored during the development of a biopharmaceutical purification process has led to the widespread adoption of High-Throughput (HT) technologies. These HT technologies can also be used to support knowledge-based development of chromatographic separation processes, allowing to step away from traditional platform processes. Such an approach requires efficient techniques to determine properties of the product and the impurities to be removed (i.e. charge, hydrophobicity, size, and adsorption isotherms). In order to be fast and efficient, small quantities of material should be used and this leads to miniaturization of experimental techniques and devices. This paper will present a state-of-the-art High-Throughput Process Development (HTPD) approach, emphasizing miniaturized, recently developed approaches (including chip based) for the purification process development for the production of biopharmaceuticals.

HTPD Methods: Implementation in Downstream Process Development for Diverse non-MAb Protein Therapeutics

Dean Harde[1], *Cornelia Walther*[2], *Lars Demmel*[3]

[1]*Boehringer Ingelheim RCV, Doktor-Boehringer-Gasse 5-11, 1120 Vienna, Austria*
[2]*BOKU, Gregor Mendel Strasse 33, A-1180 Vienna, Austria*
[3] *Boehringer Ingelheim RCV, Doktor-Boehringer-Gasse 5-11, 1120 Vienna, Austria*

Boehringer-Ingelheim RCV (Vienna, Austria) has developed an automated HTPD chromatography platform to accelerate the design of downstream purification processes for diverse non-MAb protein therapeutics (FAb, scaffold, Vhh domain antibodies, etc.).

HTPD chromatography workflows are used for early stage process development, for late stage process characterization, and for non-chromatographic methods such as, protein refold screening, inclusion body solubilization screening, and fermentation productivity screening. The advantage of using HTPD processes is the ability to experimentally examine a radically increased design space.

We use both, 96-well batch binding HTPD chromatography processes, and small volume (0.6 ml) mini-column chromatography using suitable liquid handling systems.

For new chromatography processes work begins with small volume HTPD screens. Resin and condition screening, especially for IEX, has proven that optimal conditions are highly non-obvious. We have therefore devised a flexible and broad screening protocol, and will present example screening data.

For the characterization of established processes, process qualities are identified which can be accurately duplicated with a small scale mini-column purification train. We show examples of process quality measures across scales.

In addition, we use HTPD workflows for non-chromatographic process steps such as protein refold screening. HTPD methods here both increase experimental through-put and the quality of the data read out. Examples of refold optimization screen data will be shown.

Design and key elements of qualified scale down models as an integrated part of product life cycle

Julita Panek, Meinhard Hasslacher

Shire, Process Development & Technical Services, Vienna, Austria

Qualified scale down models are an integrated part of product life cycle management. The key elements (input process parameters, intermediate attributes, material attributes and equipment characteristics) are compared between manufacturing scale and the scale down model. The differences between scales are evaluated and assessed by a risk assessment approach. The limitations of the scale down model are discussed with regards to the purpose of the model (e.g. column packing, tubing diameters, pumps). The output attributes are selected based on risk assessments and are related to the product quality (Critical Quality Attributes) and process performance (Process Consistency Indicators). Furthermore, for qualification of the scale down model, the experimental design including number of runs, acceptance criteria and statistical data evaluation are defined.

A case study for qualification of scale down model, meant to ensure predictability of the results and their correlation to the manufacturing scale, will be presented.

Poster Presentation Abstracts

Application of LamB receptor for fast detection and quantification of bacteriophage λ

D. Nabergoj[1], B. Umek[2], M. Peterka[1], A. Podgornik[1,3]

[1] Center of Excellence for Biosensors, Instrumentation and Process Control - COBIK, Tovarniška cesta 26, 5270 Ajdovščina, Slovenia.
[2] University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[3] University of Ljubljana, Faculty of Chemistry and Chemical Technology, Večna pot 113, 1000 Ljubljana, Slovenia

Plaque assay (PA) is traditional method for quantification of bacteriophages which is referred as »gold standard« and is nowadays still widely used regardless of time and labor consumption and potential inaccuracy due to subjective interpretation of the results [1]. In order to overcome major drawbacks of PA, there is a huge need to establish fast and reliable method for quantification of infectious bacteriophages. One such method could be based on spectrophotometric quantification of ejected bacteriophage DNA and could determine concentration of bacteriophages within minutes. The principle of the method is simple and it consists of two steps. First, bacteriophages bind to their protein receptors which are attached to a carrier. It was already demonstrated that bacteriophage DNA ejection occurs when phages bind to their receptors in aqueous solution [2, 3]. Second, fluorescent nucleic acid stain is added which enables spectrophotometric measurement of ejected DNA and by that determination of bacteriophage titer as well. We used bacteriophage λ and its host *E. coli* as model for testing the proof of concept. Bacteriophage λ specifically binds to LamB, outer membrane protein, located at host cell surface [4]. LamB was isolated from *E. coli* cells according to protocol [5], with minor changes. Cells were disrupted by sonication and ultracentrifuged in order to separate membranes from cytoplasmic proteins [6]. Membrane-rich pellets were incubated with detergent and several purification steps (ion and size exclusion chromatography) were needed to obtain purified LamB. Finally, LamB was specifically attached to carrier without losing its activity as bacteriophage receptor.

[1] A.M. Kropinski, A. Mazzocco, T.E. Waddell, E. Lingohr, R.P. Johnson, Methods Mol Biol. 501 (2009) 69-76
[2] S.L. Novick, J.D. Baldeschwieler, Biochemistry 27(20) (1988) 7919-7924
[3] A. Evilevitch, L. Lavelle, C.M. Knobler, E. Raspaud, W.M. Gelbart, PNAS 100(16) (2003) 9292-9295
[4] S. Chatterjee, E. Rothenberg, Viruses 4(11) (2012) 3162-3178
[5] H. Luckey, H. Nikaido, PNAS 77(1) (1980) 167-171
[6] K. Beis, C. Whitfield, I. Booth, J.H. Naismith, International Journal of Biological Macromolecules 39(1-3) (2006) 167-171

Aggregation of H1N1 influenza virus during manufacturing and formulation

Frank Hämmerling, Oliver Lorenz-Cristea, Jürgen Hubbuch

Karlsruhe Institute of Technology (KIT), Institute of Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Engler-Bunte-Ring 3, 76131 Karlsruhe, Germany

Influenza epidemics emerge seasonally and have an annual attack rate of 5 – 10 % in adults worldwide. There are about 3 to 5 million cases of severe illness per year with up to 500.000 deaths. Vaccination still remains the best strategy to control the spread of influenza infection [1].

Nowadays most vaccine formulations require a continuous cold chain which is not available in many developing countries [2]. Therefore on-going research focuses on an increased stability and extended shelf-life for vaccine formulations at moderate temperatures.

In this study the phase behavior and the aggregation propensity of H1N1 influenza A viruses was systematically investigated. Phase diagrams were generated in a microbatch format using an automated liquid handling station to evaluate the influence of parameters such as pH, ionic strength, type of salt, and the influence of excipients and additives on virus solubility.

The results were verified by an orthogonal analytical strategy using hydrophobicity determination in terms of surface tension measurements of viruses. As a second methodology for addressing stability, precipitation of viruses by polyethylene glycol was assessed for the potential to be used as a predictive tool for characterization of colloidal stability of influenza virus formulations. The presented work allows to gain a deepened understanding of the parameters and mechanisms leading to aggregation of influenza viruses.

[1]http://www.who.int/mediacentre/factsheets/fs211/en
[2] Amorij, J-P. et al., Pharmaceutical Research 25(6) (2008) 1256-73

High resolution separation of virus-like particles and extracellular particles

Petra Steppert[1], Daniel Burgstaller[1], Andres Tover[2], Alois Jungbauer[1]

[1]University of Natural Resources and Life Sciences, Vienna, Muthgasse 18, Vienna, Austria
[2]Icosagen, Eerika tee 1, Tartumaa, Estonia

Enveloped HIV-1 gag virus-like particles (VLPs) are macromolecular assemblies that consist of viral proteins which are surrounded by a host cell derived membrane and lack a viral genome. They are approximately 100 to 150 nm in size, mimic viral structures and are able to elicit strong immune response. Consequently they are promising vaccine candidates or can be used in immuno- or gene therapy. During the production process in a mammalian host correctly assembled VLPs bud from the cell membrane besides other similar sized extracellular particles, such as microvesicles or exosomes. Additionally, the feedstock is contaminated with dsDNA, free viral or host cell proteins and virus-like structures that could contain genetic material. We developed an efficient purification strategy based on anion-exchange chromatography using monoliths as stationary phase to separate VLPs from these main impurities. The VLPs were captured directly form to the clarified and filter cell culture supernatant and eluted into two fractions by linear or step gradient elution. One fraction was enriched with impurity particles while the second fraction contained the majority of pure VLPs. The process was optimized to be operated at a flow rate of 5 CV/min which enabled the purification of 2000 vaccination doses within 47 min using a 1 mL lab-scale monolith. This suggests that this method is useful for large-scale purification of pandemic vaccines.

Adsorption and elution of enveloped Virus-Like Particles in polymer grafted chromatography media

Patrícia P. Aguilar[1], Tobias A. Schneider[2], Alois Jungbauer[1,2]

[1]Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 - Vienna, Austria
[2]Austrian Centre of Industrial Biotechnology, Muthgasse 11, 1190 - Vienna, Austria

Virus-Like Particles (VLPs) are non-infectious self-assembled protein structures which mimic native viruses. Their ability to stimulate humoral and cellular immune responses combined with their inability to replicate and proliferate turns them into key candidates for modern vaccines and drug delivery. Especially for gene therapy applications, high purity is requested. The present VLPs purification processes use complex and time consuming unit operations, such as density gradient centrifugation and ultrafiltration. Therefore, there is a demand for new, fast, cost efficient and scalable purifications strategies. Charged hydrogels or chromatography beads in form of polymer-grafted media have a very high protein binding capacity and they also bind large biomolecules such as plasmids and viruses. Nevertheless, for large biomolecules the separation mechanisms are still not clear and this lack of knowledge hinders process development and optimization. To overcome this, our aim is to elucidate how VLPs, large proteins and protein superstructures partition into highly charged hydrogels and polymer-grafted media. The binding and elution of HIV-1 VLPs, produced in CHO cells, have been measured for different polymer grafted media to elucidate the effect of the charged polymer. Adsorption isotherms were measured in microtiter plates and the binding and elution studies were performed in batch mode and in 1 mL columns. The effect of salt during the binding was evaluated and different elution strategies were explored. The VLPs binding to the chromatography material was visualized by confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM).

Conformational stability of charge variants of antibody therapeutics

Beate Hintersteiner[1], Nico Lingg[1], Alois Jungbauer

[1] Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Wien, Vienna, Austria

IgG variants commonly arise due to post-translational modifications of amino acid side chains as well as modifications during the production process or during product storage of therapeutic antibodies. They sometimes show slightly different biophysical and biochemical characteristics, which may even result in altered biological activities. Separation of such IgG variants is possible using a high resolution cation-exchange chromatography method, employing pH gradients for elution, which was previously developed in our group. As model proteins material from several blockbuster antibody therapeutic products (Arzerra, Avastin, Erbitux, Herceptin, and Xolair) was obtained for this study. At a preparative scale, utilizing a ProPac WCX-10, 22x250 column, the separation method allowed the isolation of charge variant fractions of these antibody products in quantities sufficient for further analysis. Using Differential Scanning Calorimetry the thermal unfolding of the separated variants was measured. The thermal stability determined by this method is commonly accepted as an indicator for the overall conformational stability of proteins. The measured thermograms varied in their shapes for the different mAbs, but could all be fitted to a model consisting of three transition peaks. The obtained melting temperatures attributed to different structural domains were comparable for all mAb products with variations of no more than 3°C. Comparison of this thermal unfolding data for the first time allows for an assessment of the impact of antibody microheterogeneity on the conformational stability of antibody therapeutics.

Perforated hydrophilic nanomembranes for bioseparations

C. Schuster[1], A. Rodler[1], R. Tscheliessnig[1], A. Jungbauer[1,2]

[1]Austrian Centre of Industrial Biotechnology, Petersgasse 14, Graz, Austria
[2]University of Natural Resources and Life Sciences, Vienna, Austria

The implementation of nanomembranes for bioseparation purposes is being sought with great effort since they promise outstanding selectivity and efficiency. However, the applicability of current sufficiently stable nanomembranes is generally restricted by poor biocompatibility imposed by their hydrophobicity. We demonstrate the facile fabrication of hydrophilic and perforated nanomembranes and their use for the separation of biomolecules. A selected epoxy resin is mixed with a branched polyamine as the curing agent and a polylactide-co-glycolide as porogen. After spin coating the ultrathin epoxy film is cured to form a covalently crosslinked polymer network. Hydrophilic, perforated and self-supporting nanomembranes with a thickness less than 100 nm can be released after selective solvent etching of the porogen. Bulging tests on the cm scale reveal their outstanding mechanical properties such as biaxial moduli around 400 MPa and ultimate tensile strengths of up to 13 MPa. This translates to theoretical transmembrane pressures of more than 3 bar when free-standing over 3 µm openings of a porous support. We observe structural integrity and fast, selective separation of model proteins from TiO_2-nanoparticles based on their size evidenced in real-time with a simple spectrophotometric setup. Nanomembranes have the potential to induce a paradigm shift in bioseparations and the introduction of our hydrophilic, perforated nanomembranes paves the way for industrial applicability.

Modeling of anion-exchange chromatography: Influence of ligand densities on Donnan equilibrium and protein separation

*Felix Wittkopp[1], Gabriela Sánchez Reyes[1], Lars Peeck[2], Christian Frech[1]**

[1]Institute for Biochemistry, University of Applied Sciences Mannheim, Paul-Wittsack-Str. 10, 68163 Mannheim, Germany
[2]Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt, Germany

Anion-exchange chromatography is an important tool in downstream processing of biopharmaceuticals. It is commonly used for the removal of fragments and/or aggregates of the target protein as well as for the depletion of nucleic acids, host cell proteins and viruses. Ligand density variations are known to influence the separation and affect process robustness. Mechanistic modeling can be an effective tool to understand these effects.

Here we present the application of a Donnan ion exchange (DIX) model to linear gradient elution (LGE) modelling [1]. This model considers differences in the concentrations of charged species between the resin and buffer phase due to the Gibbs–Donnan equilibrium. The distribution of charged species between the two phases is influenced by the ligand density of the stationary phase. Our results show that by considering the Donnan effect, a global modeling of five Fractogel® EMD TMAE resins with different anion exchange ligand densities is possible. Furthermore, the results demonstrate consistent accuracy of the model for three different proteins. Finally, predictions of chromatographic separations were performed using computer simulation and compared with experimental results.

[1] M.L. Jansen, A.J.J. Straathof, L.A.M. van der Wielen, K.C.A.M. Luyben, W.J.J. van den Tweel, AIChE Journal 42 (1996) 1911.

Automated Development of Reversed-Phase HPLC Methods for separation of Proteins

Oksana Rotzkaja, Sergey Galushko, Jelena Galushko

ChromSword, Germany

Fully automated strategy for RP-HPLC method development is demonstrated for separation of protein mixtures. The strategy involves: (1) the automated screening of RP protein columns, (2) rapid gradient optimization for different combinations of solvent, temperature, flow rates, (3) detailed sample profiling and gradient optimization with the best column/solvent/temperature/flow rate combination, and (4) robustness tests of the final method. All steps of the method development including report generation are automated with method development software reducing method development time. Case studies of method development for different protein mixtures will be demonstrated.

Ultra-fast pH-Gradient Ion Exchange Chromatography for the Separation of Monoclonal Antibody Charge Variants

Robert van Ling[1], Alexander Schwahn[2], Shanhua Lin[3], Ken Cook[4], and Mauro de Pra[5]

[1]Thermo Fisher Scientific, Breda, The Netherlands
[2] Thermo Fisher Scientific, Reinach, Switzerland
[3] Thermo Fisher Scientific, Sunnyvale CA, USA
[4]Thermo Fisher Scientific, Hemel Hempstead, UK
[5]Thermo Fisher Scientific, Germering, Germany

Monoclonal antibodies (mAbs) are prone to modifications such as sialylation, deamidation or C-terminal lysine truncation. Traditionally, salt gradient cation exchange chromatography has successfully been used for the assessment of the mAb charge variant profile. However, significant efforts are often required to tailor salt gradient methods to individual mAbs and generally long run times are needed to achieve the desired resolution. In the fast-paced drug development environment, rapid and robust platform methods are desirable, accommodating the majority of mAbs analyzed.

Here, we present the charge variant profile of top-selling mAbs, analyzed by strong cation exchange with a linear pH gradient method utilizing next generation UHPLC technology. The pH gradient method serves as a platform method for the mAb charge variant analysis, covering a pH range from 5.6 – 10.2, allowing to determine the pI value of the charge variants when combined with an on-line pH monitor. Bevacizumab, Cetuximab, Infliximab, and Trastuzumab were analyzed on a small particle MAbPac SCX-10 column using a full pH gradient of 10 min. Separations of multiple charge variants was achieved for all mAbs analyzed. Additional resolution improvements and a significant shortening of the analysis time was achieved by optimizing the utilized pH range for each mAb in conjunction with the application of an elevated flow rate to further decrease the applied gradient slope. This easy and fast method optimization approach allowed the registration of the charge variant profile for each mAb within 5 min while retaining the high-resolution separation normally only associated with longer gradient runs

Proteomic Profiling Of Aloe Vera Extract For Targeting Pancreatic Beta Cell Function

*Spoorthy N Babu, Ayesha Noor, Krishnan Venkataraman, Mookambeswaran A Vijayalakshmi**

CBST, VIT University, Vellore 632 014, India

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from dysfunction of pancreatic beta cells and has become huge socio economic burden. Previous studies in our laboratory has shown that *Aloe vera* extract alleviated diabetes and there has been qualitative and quantitative rejuvenation of pancreatic beta cells. To further gain insights into the mechanism of action of rejuvenation of pancreatic islet cells, group wise fractionation of *Aloe vera* extract was carried out. One of the major pathways is through increased half-life of GLP-1 through Dipeptidyl peptidase-4 (DPP-4) inhibition. This study demonstrates that polypeptide enriched *Aloe vera* extract has the ability to decrease fasting plasma glucose levels in streptozotocin induced diabetic rats. And also it showed significant DPP-4 inhibition activity in biochemical assay. Further, the separation of polypeptides was carried out by RP-HPLC and will be subsequently analyzed by Mass spectrometry. The specific peptides/polypeptides responsible will be identified by DPP-4 inhibitory activity using human serum.

Characterization and manipulation of protein stabilities for downstream process development

J. Morgenstern[1], L. Galm[1], P.Baumann[1], C. Brunner[1], J. Hubbuch[1]

[1]Karlsruhe Institute Of Technology (KIT), Institute of Process Engineering in Life Sciences Section IV: Biomolecular Separation Engineering, Engler-Bunte-Ring 3, 76131Karlsruhe, Germany

Compared to low molecular weight pharmaceuticals, protein therapeutics have a significantly higher potential to cure human diseases because they can catalyze biochemical reactions, form receptors and channels in membranes and transport molecules within a cell or from one organ to another. The preservation of the complex three-dimensional structure is crucial for the highly selective functionality of proteins and the acceptance of the drug by the patient's immune system. However, during purification, formulation, and storage protein therapeutics face solution conditions that are unfavorable for their conformational and colloidal stability, e.g. extreme pH changes, high ionic strengths or agitation stress due to stirring, pumping, and shaking.

Therefore, the characterization of the main influencing factors promoting undesired changes of protein conformation and aggregation as well as the manipulation and selective control of protein stability are crucially important to biopharmaceutical research and process development.

In this work, high-throughput techniques are presented which allow a rapid evaluation of the influence of a large matrix of possible solution conditions on protein stabilities. Conformational changes are thereby detected by means of Fourier transform infrared spectroscopy (FTIR) and intrinsic protein fluorescence measurements. The colloidal protein stability is studied using a liquid-handling- station in combination with an automated imaging system for the generation of protein phase diagrams. Using these techniques, the suitability of both protein conjugation (covalent attachment of Polyethylene glycol) and the addition of particular additives (polymers and osmolytes) for the stabilization of pharmaceutical relevant proteins is investigated.

P12

Application of Charged Surface Technology in the Separation of Peptides

B. Okandeji[1], M. Lauber[1], B. Alden[1], K. Wyndham[1]

[1]Waters Corporation, 34 Maple Street, Milford, Massachusetts, U.S.A

The choice of chromatographic stationary phase, method and instrumentation can have profound effects on the quality of separation of peptides. For example, peptides often exhibit broad tailing peaks due to overloading when the separation is performed on columns packed with traditional SiO2 stationary phases using low ionic strength acidic mobile phases. While this situation is often mitigated with the use of high ionic strength mobile phases and/or ion pairing agents, a drawback of this approach is a significant loss of MS sensitivity.

This talk outlines the development of charged surface stationary phases that significantly reduce the need for a trade-off between peak shape of peptides and MS sensitivity. The stationary phases were developed by incorporating a very low level of an ionizable silane that confers a surface charge. By carefully controlling the surface charge on the stationary phases, we found an optimal combination of parameters that afford significantly improved peak shapes for peptides in low ionic strength acidic mobile phases that are preferred for mass spectrometric analysis.

Performance Comparison of Protein A Affinity Chromatography Sorbents at Different Temperatures

Walpurga Krepper[1], Peter Satzer[1], Alois Jungbauer[1,2]

[1]Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria
[2]Austrian Centre of Industrial Biotechnology (ACIB), Petersgasse 14, 8010 Graz, Austria

Innovative renewals in the field of downstream processing are required to keep up with the increasing demand for antibody treatments and improvements in upstream process economy. Despite high costs, protein A affinity chromatography remains by far the most employed system because of its excellent selectivity towards antibodies of the IgG class. One key limitation of conventional protein A chromatography is, however, the necessity to carry out product elution at low pH which can give rise to the formation of soluble high molecular weight aggregates and/or insoluble precipitates. Byzen Pro® is a novel chromatography medium based on a thermosensitive mutant type of protein A which enables elution at constant pH by changing the temperature from binding (5˚C) to elution conditions (40˚C).

We investigated mAb purification performances of two well-established protein A resins (*MabSelect SuRe™, GE Healthcare* and *TOYOPEARL AF-rProtein A HC-650F, Tosoh Bioscience LLC*) at different temperatures and compared them with the thermo-responsive protein A medium *Byzen Pro®* from *Nomadic Bioscience Co., Ltd.*. As the general basis of this evaluation, we present adsorption isotherms of batch experiments at different temperatures. The binding capacities under operating conditions (DBC) are currently assessed and will be presented in the near future.

Extending the number of LC techniques that can be interfaced with MS detection for the characterization of monoclonal antibodies

Suresh Babu C.V[2] and Linda Lloyd[1]

[2]Agilent Technologies, Inc. Bangalore, India
[1]Agilent Technologies, Church Stretton, UK

Characterization of monoclonal antibodies requires a number of bioanalytical tools to characterize heterogeneity. Liquid chromatography/mass spectrometry (LC/MS) is a routine technology that enables identification of the heterogeneity. However, for some LC techniques, such as reversed phase, it is possible to use mobile phases that are directly compatible with MS detection but others, such as ion exchange or affinity, use non-volatile salts and additives which are not compatible with MS. To use MS with these techniques it has been necessary to collect fractions, remove the salt/additives and then do the MS analysis on the purified fraction.

In this study, a new desalting cartridge is evaluated for LC/MS based protein applications which enables the on-line removal of sample additives. The reverse phase LC method for desalting was developed using a polymeric based desalting material. Various mAb preparations that contain buffer salts were tested for effective desalting. The desalting approach described in this work demonstrates that using the desalting cartridge prior to the MS detector enables more LC techniques to be interfaced with MS detection and improves the quality of data obtained.

Monoclonal antibody analysis: Reversed-phase LC/UV and LC/MS analysis

Suresh Babu C.V[2] and Linda Lloyd[1]

[2]Agilent Technologies, Inc. Bangalore, India
[1]Agilent Technologies, Church Stretton, UK

In recent years, monoclonal antibodies (mAbs') represent the largest class of biotherapeutics. The drug discovery pipeline comprises a series of meticulously controlled and evaluated steps, demanding monitoring and measuring the impact of each variable on the product critical quality attributes. Therefore, a comprehensive characterization of mAbs at every stage is required in order to develop a biologic that at commercialization is fully understood and has the required efficacy and acceptable impurity profile.
Reversed-phase chromatography with UV and mass spectrometry detection is the method of choice for the primary characterization of mAbs. The correct choice of reversed-phase column and method is critical to achieve fast analysis times and reproducible high-resolution separations. In this presentation we have evaluated the performance of **polymeric and** silica based **reversed phase chromatography columns for mAb analysis using LC/UV and LC/MS. Different sample types, including intact mAbs and mAb fragments, were used to probe column performance characteristics and the separation efficiency. In** addition, repeatability and carry-over tests were conducted between the two columns. The unique features of polymeric and silica based columns will be discussed.

For Research Use Only. Not for use in diagnostic procedures.

Reduced Baseline Noise Improves Light Scattering Detection Capabilities in Size Exclusion Chromatography of Protein Aggregates

Andy Coffey

Agilent Technologies, Church Streton UK

Combining light scattering (LS) detection with aqueous size exclusion chromatography (SEC) enables additional information such as absolute molecular weight and hydrodynamic radius to be determined. However, LS detection is particularly sensitive to particulate noise, and proteins are often considered rather small to provide an adequate signal with an LS detector.

Advancements in size exclusion column manufacturing techniques, in particular the use of hydrophilic polymer coatings to overcome non-specific interactions, leads to an improvement in the cleanliness of SEC columns. In turn this reduces background noise and significantly reduces the time typically required to flush a column before it becomes useable with an LS detector.

Furthermore, method optimisation is possible using the capabilities provided by a bio-inert LC system equipped with quaternary LC pump. By using Buffer Advisor software, a wide range of mobile phase conditions can be explored and optimized, even with LS detection.
For Research Use Only. Not for use in diagnostic procedures

Implementation of a Microfluidic Device for Absorption Measurements in High-Throughput Experiments

C.P. Radtke[1], M.-T. Schermeyer[1], J.Hubbuch[1]

[1]Institute of Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering – Karlsruhe Institute of Technology (KIT), Engler-Bunte-Ring 3, 76131 Karlsruhe, Germany

The use of microwell plates is common practice in the high-throughput process development. Standard lab equipment like automated liquid handling stations (LHS) and photometers are tailored for the dimension and structure of microwell plates. This allows the execution of an increased number of experiments in parallel with a reduced amount of sample volume. But the application of microwell plates in experiments can be limited by the absorption strength of the analyte or the influence of meniscus formation in microwells.

Here we implement a novel microfluidic device developed for accurate absorption measurements in high-throughput format. The presented device allows parallel absorption measurements of 96 samples with a volume of 36 µL. Each of the 96 measurement channels has four chambers with increasing height. This allows the determination of photometrical detectable analytes like dyes or proteins in a wide concentration range (e.g. lysozyme up to 100 mg/mL) independent of the used optical path length, respectively chamber height. Additionally, due to this structure the extinction coefficient can be determined by a direct four-point calibration with no need of a dilution series. Also the impact of a meniscus can be excluded because of the closed measurement chambers. Due to the construction of the device according to the microwell plate format the microfluidic device is fully compatible with established lab equipment like plate photometers and LHS.

The implementation of the presented microfluidic device has the potential to significantly accelerate the absorption measurements in high-throughput experiments with validated accuracy.

mAb N-glycosylation profiling with HILIC in respect of fucosylation and antibody dependent cellular cytotoxicity

L. Salim Abadi Ghaleh[2], W. Conze[1], J. Vajda[1], E. Mueller[1]

[1]Tosoh Bioscience GmbH, Im Leuschnerpark 4, 64347 Griesheim, Germany
[2]University of Applied Sciences, Schoefferstraße 3, 64295 Darmstadt, Germany

Immunoglobulin G (IgG) glycosylation in the Fc region is essential for the binding affinity of Fcγ receptor and thus for cancer cell regression. Fragment, crystallizable region receptor and glycosylation play an important role for the antibody-dependent cellular cytotoxicity (ADCC). On the other hand the absence of fucose in monoclonal antibody increases the affinity of Fcγ receptor and ADCC [1]. Therefore the glycosylation analysis and identification of fucosylated and non-fucosylated antibodies is necessary for understanding therapeutic Monoclonal Antibody (mAb) function. This Study focuses on comparison of N-glycosylation profiling of IgG antibodies from each other. Hydrophilic Interaction Liquid Chromatography (HILIC) is an analytical chromatography which was used for the separation of fluorescent-labeled N-glycan of mAbs. With the help of our results from HILIC chromatogram, it is easy to differentiate between fucosylated and non-fucosylated antibodies, due to the fact that they exhibit different N-glycanstructure of mAb to HILIC column. This information can be used for the construction of new antibodies with increased affinity of Fcγ receptor and enhanced therapeutic function.

[1] T. Mizushima et al., Genes Cells. Nov;16(11) (2011)1071-80

A Smart Workflow for N-Glycan Analysis Combining Speed, Simplicity and Unrivaled Sensitivity

Marleen van Wingerden

Waters NV/SA, Belgium
Business Development Manager, Europe Biopharmaceutical Market & Separation Technologies

The glycan profile of a biopharmaceutical is commonly defined as a critical quality attribute, since it can be a measure of efficacy and immunogenicity as well as be an indicator of manufacturing conditions. Unfortunately, conventional workflows for glycan analyses are either laborious and time-consuming or require compromises in sensitivity. This limits the throughput or detail by which the glycosylation of a biopharmaceutical can be characterized or monitored. To address these shortcomings, the new GlycoWorks *Rapi*Fluor-MS™ kit enables unprecedented sensitivity for glycan detection while also improving the throughput of N-glycan sample preparation. An analyst can complete an N-glycan sample preparation, from glycoprotein to ready-to-analyze sample, in just 30 minutes while also using the sensitivity enhancing *Rapi*Fluor-MS labeling reagent.

During this presentation we will:

1. Show a sample preparation workflow that integrates fast deglycosylation, rapid labeling and a robust sample cleanup that has been rigorously developed to provide quantitative recovery of glycans (from neutral to tetrasialylated species) and to facilitate immediate analysis of samples.
2. Introduce the chemistry behind a novel labeling reagent, *Rapi*Fluor-MS, that has been synthesized to rapidly react with glycosylamines upon their release from glycoproteins. In a 5 minute reaction, N-glycans are labeled with *Rapi*Fluor-MS™.
3. Show the impact that unrivaled sensitivity of serial fluorescence and mass spectrometric detection for characterization can have on the assignment of low abundance glycans.

Highlight the enhanced ionization characteristics of *Rapi*Fluor-MS™ that allow glycans to be detected by the ACQUITY QDa mass detector and to thereby increase confidence in routine monitoring.

Novel Method to Discovery Glycol-peptides as Thrombin Inhibitors in Cancer Therapy

Hui-Ming Yu, Yu-Hsuan Chang, Yu-Hsuan Chu, Kuo-Ching Lin*

Genomics Research Center, Academia Sinica, Taipei, Taiwan

A novel substrate for detection the activity of PAR-1 degrading enzyme, such as thrombin, associated with potential molecular target for cancer therapy, is provided. In previously studies, thrombin will recognizes the N-terminal exodomain of the G-protein-coupled thrombin receptor PAR-1. According to this concept, a peptide substrate which sequence was adopted from the N-terminal ten residues of the PAR-1 was synthesized to detect the activity of thrombin. The high performance liquid chromatography (HPLC) system was performed to detect the change of signal peak as the peptide substrate was degraded by thrombin could provide a rapid assay system to screen compounds which are able to decrease the activity of thrombin. Further optimized glycol-peptides (HS series) were been design and performed to this screening system to value the more potent leads. These leads were test by animal model with human lung cancer. In this report, we success apply the HPLC screening method to identified thrombin inhibitors. Two glycol-peptides, HS-14 and HS-16, have revealed the well potential for the cancer therapy.

Development and validation of LC-MS/MS method for the quantification of polymyxin B1, polymyxin B2, polymyxin B3 and ile-polymyxin B1 in human plasma and its application to pharmacokinetics study

K.H.D. Hee[1], Y.K.J. Leaw[1], L.S.-U Lee[1,2]

[1]National University of Singapore, MD1, 12 Science Drive 2, 117549 Singapore
[2]National University Health System, 1E Kent Ridge Road, 119228 Singapore

Polymyxin B (PB) is antibiotic consisting of a cyclic heptapeptide and a tripeptide side chain used in treatment of infections caused by Gram-negative bacteria [1]. Commercial formulations of PB contain multiple structurally related components with major constituents of PB1, PB2, PB3 and ile-PB1 [2]. To understand the pharmacokinetics of these major components, we have developed and validated a LC-MS/MS method to quantify PB1, PB2, PB3 and ile-PB1 in human plasma. PB was extracted from plasma by protein precipitation using trichloroacetic acid followed by chromatographic separation on Zorbax Bonus-RP column (100mm × 2.1mm, 1.8µm) using stepwise gradient elution of water containing 0.1% formic acid and 0.1% trichloroacetic acid (mobile phase A) and 90% acetonitrile with 0.1% formic acid (mobile phase B). Despite structural similarities, these PBs were completely resolved in the analytical run time of 6.5 min. Detection and quantification of PBs were performed by multiple reaction monitoring (MRM) under positive ionization mode in the mass spectrometer. Separation of PB1 and ile-PB1 as well as PB2 and PB3 before quantification is crucial because they are structural isomers sharing the same MRM detection. Excellent linearity was achieved (r^2 > 0.99) in the calibration curves of PBs. The developed method was accurate (95.3–111.7%) and precise (CV < 5.1%). Recovery of PBs from the extraction was between 53–76% and reproducible (CV < 4.5%). This methodology has been successfully applied to clinical study of subjects infused with PB and should enable a better understanding of the pharmacokinetics of PB1, PB2, PB3 and ile-PB1.

[1] A.P. Zavascki, L.Z. Goldani, L. Li, R.L. Nation, J Antimicrob Chemother 60(6) (2007) 1206-1215

[2] J. He, K.R. Ledesma, W.Y. Lam, D.A. Figueroa, T.P. Lim, D.S. Chow, V.H. Tam, Int J Antimicrob Agents 35(3) (2010) 308-310

Exploring ionic liquids for the development of a purification platform for therapeutic immunoglobulin Y (IgY) from egg yolk

Emanuel V. Capela[1], Mafalda R. Almeida[1], João A. P. Coutinho[1], Mara G. Freire[1]

[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Biopharmaceuticals, and in particular antibodies, have greatly improved the treatment of many diseases and sometimes are the only approved therapies available for a particular disease [1]. Although the largest part of research has been focused on mammal antibodies, immunoglobulin Y (IgY) present in egg yolk has also been reported as a viable alternative to mammal immunoglobulins, since they are produced in higher quantities (100mg/egg) [2]. However, IgY still is very expensive due to the absence of an effective purification technique able to separate IgY from other contaminant proteins present in egg yolk [3]. Therefore, in this work, three types of aqueous biphasic systems (ABS) were studied, namely constituted by a polymer and a salt, a polymer, a salt and an ionic liquid (IL) as adjuvant and by an IL and a salt, as alternative liquid-liquid systems for the selective extraction, and thus purification, of IgY from egg yolk. According to the obtained results, systems composed of imidazolium-based ILs achieved protein extraction efficiencies in the range between 88% and 98%. Moreover, systems composed of ILs and salts allow the selective extraction of β-livetin (the major contaminant protein) for one phase while retaining IgY in the opposite layer. Based on these promising results, it can be concluded that a cost-effective platform using ABS for the purification of the value-added IgY from egg yolk could be developed, fomenting the purification of this biopharmaceutical by the pharmaceutical industry in the near future.

[1] J. Kovacs-Nolan, Y. Mine, Annual Review of Food Science and Technology, 3 (2012) 163-182;
[2] M. Tini, U. R. Jewell, G. Camenisch, D. Chilov, M. Gassman, Comparative Biochemistry and Physiology -Molecular and Integrative Physiology, 131 (2002) 569-574;
[3] M. Taha, M. R. Almeida, P. Domingues, S. P. Ventura, J. A. P. Coutinho, M. G. Freire, Chemistry - A European Journal, 21 (2015) 4781-4788.

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. M. G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ERC-2013-StG-3377.

High-efficient approach for the selective separation of amino acids based on the use of aqueous biphasic systems composed of ionic liquids

Emanuel V. Capela[1], Maria V. Quental[1], João A. P. Coutinho[1], Mara G. Freire[1]

[1] CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Aromatic amino acids, such as L-tryptophan, L-phenylalanine and L-tyrosine, can be produced by bacterial fermentation [1]. In particular, L-tryptophan is difficult to obtain at a high production yield, and several approaches have been attempted in the past few years to improve the amino acids production, which includes the continuous removal of the target amino acid from the fermentative medium [2]. In this context, the application of ionic-liquid-based aqueous biphasic systems (IL-based ABS) could be seen as a valuable alternative to guarantee a continuous extraction and purification of aromatic amino acids during fermentation. Recently, it has been demonstrated that aliphatic amino acids can behave as salting-out agents when employed in IL-based ABS, allowing thus the replacement of the mostly employed non-biocompatible inorganic salts [3]. Based on this possibility, in the current work, we investigated the ability of forming ABS combining phosphonium-based ILs with aliphatic amino acids, namely L-lysine and L-proline, and we then used these systems to separate aromatic amino acids (L-phenylalanine, L-tyrosine and L-tryptophan). The liquid-liquid phase diagrams, tie-lines and tie-line lengths of the ABS were firstly determined at 25ºC, and then used for the design of aqueous two-phase extraction routes envisaging the selective separation of aromatic and aliphatic amino acids. According to the gathered results, selective extraction efficiencies up to 85 % for opposite phases were attained in a single-step. The results obtained reveal that IL-based ABS are promising approaches for the selective extraction of mixtures of amino acids, being the selectivity highly dependent on the IL employed.

[1] M. Ikeda, Applied Microbiology and Biotechnology, 69 (2005) 615–626;
[2] A. Marín-Sanguino, N. V. Torres, Biotechnology Progress, 16 (2000) 133–145;
[3] M. Domínguez-Pérez, L. I. N. Tomé, M. G. Freire, I. M. Marrucho, O. Cabeza and J. A. P. Coutinho, Separation and Purification Technology, 72 (2010) 85-91.

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. M. G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ERC-2013-StG-3377.

Membrane adsorbers for continuous processing

Helena Trnovec

Biologics Technical Development & Manufacturing, Lek Pharmaceuticals d.d., Kolodvorska 27, 1234 Menges, Slovenia

Nowadays, more and more pharmaceutical companies are focused on development of new biological drugs. Monoclonal antibodies (mAb) are biological drugs that are used for treatment of autoimmune diseases and various types of cancer and represent the biggest group of biologics. Costs of production of new mAb are very high, therefore pharmaceutical industry is searching for new solutions to decrease production costs and development of continuous processes can be one of the solutions.

For purification of mAb affinity resins with protein A as a ligand are usual choice for capture step. After protein A chromatography there is usually virus inactivation step for virus removal and other chromatographic steps (Cation exchange chromatography, Anion exchange chromatography, Mixed mode chromatography) for further removal of process and product related impurities (host cell proteins (HCP), DNA, leached protein A, aggregates, …). We focused on the use of membrane adsorbers for the polishing steps in the setup that enables continuous processing.

Membrane absorbers hold some benefits in comparison with classical chromatography resins. Due to their microporous membrane structure, ranging from 0.8 to 3 µm, diffusion related mass transfer effects are reduced. Membrane chromatography devices can efficiently remove impurities even at higher flow rates. One of the requirement for development of continuous processes is, that chromatographic steps are performed as fast as possible. With application of higher flow rates in membrane chromatography devices this requirement can be achieved. Additionally, contaminants, such as HCP, DNA, endotoxin and viruses can be efficiently removed due to their open pore structure.

In scope of nextBioPharmDSP project majority of anion exchange membrane absorbers available on market were tested. Some observations will be presented on this paper.

Acknowledgements:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635557.

Disclosing the thermodynamic phenomena behind mAbs interaction with multimodal chromatography supports: The Phenylboronic acid chromatography case study

S.A.S.L. Rosa[1], C.L. da Silva[1], M.R. Aires-Barros[1], A.M. Azevedo[1], A.C. Dias-Cabral[2, 3]

[1]iBB- Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1, 1049-001 Lisboa, Portugal
[2]CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Avenida Infante D. Henrique, 6201-506 Covilhã, Portugal
[3]Department of Chemistry, University of Beira Interior, R. Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal

Monoclonal antibodies (mAbs) are currently playing the lead role in the biopharmaceutical industry and hold great promise as new therapeutic agents against cancer and autoimmune diseases such as rheumatoid arthritis [1]. The increasing market demand, the drastic improvements achieved, at process upstream, and the emergence of biosimilars has turned mandatory the demand for new downstream technologies and/or the improvement of existing ones. Therefore, novel and cost-effective multimodal ligands that can selectively capture mAbs from complex feedstocks are emerging [2,3].

In this work, the main interest is on investigating the complex phenomena underneath the purification of an anti-human IL-8 mAb from a clarified supernatant of Chinese Hamster Ovary (CHO) cell cultures, by multimodal and affinity chromatography, as stated in [3,4], and, on showing the important role of nonspecific effects in the establishment of such processes.

For this purpose, an emergent technique - Flow Microcalorimetry (FMC) Chromatography [5] – using the synthetic ligand phenylboronic acid, under different pH conditions, is being exploited as a dynamic and on-line method considering instantaneous heat energy transfers in order to understand the thermodynamics and surface transport phenomena underneath the mAb adsorption and desorption events and the different chromatographic supports.

The application of the FMC to these studies could (i) significantly shorten process development times since models can be applied to better fit and predict the biomolecular phenomena and (ii) create an on-line monitoring of chromatographic supports conditions. In sum, the integration of this system in the existing ones could level up the comprehension beneath chromatography science.

[1] J.M. Reichert, C.J. Rosenswieg, L.B. Faden, M.C. Dewitz, *Nature Biotechnol.* 23(9) (2005), 1073-1078.
[2] K.A. Kaleas, M. Tripodi, S. Revelli, V. Sharma, S.A. Pizarro, *J. Chromatogr. B.* 969 (2014) 256-63.
[3] R. dos Santos, S.A.S.L. Rosa, M.R. Aires-Barros, Andres Tover, A.M Azevedo, *J. Chromatogr. A.* 1355 (2014), 115-124
[4] S.A.S.L. Rosa, R. dos Santos, M.R. Aires-Barros, A.M Azevedo, *Sep Purif Technol.* 160 (2016), 43-50
[5] G.L. Silva, F.S. Marques, M.E. Thrash, A.C. Dias-Cabral, *J. Chromatogr. A.* 1352 (2014), 46–54

Acknowledgements

This work was supported by National Funds by FCT – Fundação para a Ciência e Tecnologia - (Project PTDC/QEQ-PRS/0286/2014). Rosa S.A.S.L. and Azevedo A.M. acknowledge FCT for the PhD fellowship SFRH/BD/102574/2014 and research contract under the program ‘‘Investigador FCT 2014” (IF/00048/2014/CP1214/CT0010), respectively. iBB and CICS-UBI also acknowledges FCT for funding through the research contract UID/BIO/04565/2013 and Project UID/Multi /00709, respectively. CICS-UBI acknowledges FEDER funds through the POCI - COMPETE 2020 – Operational Programme for Competitiveness and Internationalization in Axis I – Strengthening research, technological development and innovation (Project No. 007491).

Developing a low-cost non-human-infective model system to study the causative agent of African sleeping sickness

W. Kipandula[1], F. Lampiao[1], T. K. Smith[2] and S. A. MacNeill[2]

[1]University of Malawi-College of Medicine P/bag 360, Blantyre, Malawi.
[2]Biomedical Sciences Research Complex B306, University of St Andrews, St Andrews KY16 9ST, United Kingdom.

Introduction

The parasitic protozoan *Trypanosoma brucei* the causative agent of Human African trypanosomiasis, also known as sleeping sickness, is a neglected tropical disease that has a significant impact on the human populations in Sub-Saharan Africa. There is an urgent need for more research in this area, to better understand the biology of this organism and identify novel drug targets as current therapeutics are largely ineffective. Studies on human pathogenic trypanosomatids, has been hampered by the need to culture these organisms safely in a laboratory, requiring dedicated containment facilities. However, these are far more expensive to build, maintain and equip, (particularly in a resource-limited African country like Malawi) as experimental apparatus cannot be moved in and out of the containment without rigorous decontamination, which consequently renders research in this field unattractive and impractical.

Objectives

The aim of this project is to develop a model system that can be used to study trypanosome/kinetoplastid biology without the need for costly facilities and the accompanying safety issues. The related non-human-infective trypanosomatid parasite *Crithidia fasciculata* is a suitable model-system. Unlike other trypanosomatids, the protozoan *C. fasciculata* can be easily and inexpensively grown in liquid culture in a standard laboratory without safety concerns and is amenable to molecular genetics and biochemical analysis. The complete genome sequence of *C. fasciculata* has been determined and is publically available to facilitate genome studies of the organism.

Methods

We have identified (by bioinformatic analysis) high value target proteins as components of key protein complexes in a range of biological functions complexes within *C .fasciculata.* Corresponding plasmid vectors have been constructed and *C. fasciculata* transfected with them, allowing us to start purifying these protein complexes by tandem affinity purification (TAP) and identify protein binding partners by mass spectrometry.

Results and significance of the work

We anticipate identifying novel and trypanosome-unique proteins as part of these complexes, which will be functionally characterised and validated as potential drug targets in *T. brucei*. We hope this project will revolutionise and develop capacity for safe, effective and low-cost trypanosomal research, not only in Malawi, but in Africa as whole.

Characterization of Protein and Protein Aggregates using Temperature controlled Hollow Fiber Flow Field-Flow Fractionation

Florian Meier[1], Lisa Schilder[1], Soheyl Tadjiki[2], Robert Reed[2], Thorsten Klein[1]

[1]Postnova Analytics GmbH, Landsberg am Lech, Germany
[2]Postnova Analytics Inc., Salt Lake City, UT, United States

Asymmetrical Flow Field-Flow Fractionation (AF4) is a powerful separation and characterization technique for biological macromolecules, lipids, viruses and cell particulates. In AF4 the separation takes place in an open channel and is based on the hydrodynamic diameter or the diffusion coefficient of the respective sample components [1]. The AF4 channel consists of two parallel walls with a thickness of 0.0190 - 0.0500 cm. Sample components are pushed toward the semi-permeable lower channel wall by the separation field (cross flow) and transported along the channel at different flow velocities via a secondary laminar flow (channel flow). The AF4 channel can also be made of a semi-permeable ultrafiltration hollow fiber, where the cross flow exits radially through the fiber wall and the channel flow moves axially along the fiber length [2]. Resolution, reproducibility and sample recovery of the hollow fiber cartridge was studied using a Bovine Serum Albumin (BSA) standard for more than 100 consecutive injections. The average retention time RSD of the monomer and dimer peaks was found to be below 1%. The hollow fiber flow FFF system was hyphenated with a dynamic light scattering (DLS) detector in order to characterize protein aggregates. Aggregation of BSA and gamma globulin standards was triggered by heat-induced stress at low pH buffer condition. The aggregated samples were subsequently analyzed by the hollow fiber flow FFF-DLS system. The results showed the separation of the large protein aggregates from a few tens to hundreds of nanometers.

[1] K.G. Wahlund, J.C. Giddings, Anal. Chem. 59(9) (1987) 1332-1339
[2] R. Zhu, W. Frankema, Y. Huo, W.T. Kok, Anal. Chem. 77(14) (2005) 4581-4586

On-chip chromatography for rapid screening of multimodal ligand-target interactions

I. F. Pinto[1,2], G. Petrucci[1,4], R. R. G. Soares[1,2], V. Chu[1], M. R. Aires-Barros[2,3], J. P. Conde[1,3], A. M. Azevedo[2,3]

[1]Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologias (INESC MN) and IN – Institute of Nanoscience and Nanotechnology, Rua Alves Redol 1000-029, Lisbon, Portugal

[2]IBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1049-001, Lisbon, Portugal

[3]Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1049-001, Lisbon, Portugal

[4]Department of Information, Electronic and Telecommunication Engineering, University of Rome "La Sapienza", Via Eudossiana 18, 00184, Rome, Italy

Chromatography has been the standard method of choice for the purification of a wide range of compounds with high commercial value. Monoclonal antibodies (mAbs) in particular are one of the most prominent molecules among biopharmaceuticals and are typically required in high doses over large periods of time, increasing the demand for highly pure molecules at affordable prices. In this context, multimodal chromatography, which combines multiple types of molecular interactions, has emerged as an alternative to the "pure" conventional modes [1]. The implementation of multimodal chromatography, however, relies on predicting the optimal operating window for a particular target, which is not straightforward and requires several optimization attempts including the development of high-throughput screening platforms.

This work presents a novel microfluidic platform for the rapid screening of chromatography operating conditions. In this platform, a chromatographic ligand (Capto MMC) was studied for the capture and elution of a fluorescent conjugate mAb-Alexa 430 under different conductivities and buffer pH conditions. Micro-columns (210 nL) were fabricated in PDMS for packing agarose chromatographic beads and the binding kinetics were measured in real time at resin level by fluorescence microscopy. The platform with integrated pneumatic valves allowed for the automated real-time monitoring of adsorption, elution, regeneration and equilibration of the microfluidic column, within 2-3 min total assay time per condition. Summarizing, we report a versatile, scalable and relatively inexpensive method to perform chromatographic screening studies, with very low reagent consumption and rapid output of results, potentially extendable to every type of chromatographic beads and target molecule.

[1] I.F. Pinto, M.R. Aires-Barros, A.M. Azevedo, Pharm Bioprocess(3) (2015) 263-279.

Purification of porcine gastric mucin with preservation of its native functional properties

V. Schömig[1], B. Käsdorf[2], O. Lieleg[2], S. Berensmeier[1]

[1] Bioseparation Engineering Group, Department of Mechanical Engineering, Technical University of Munich, Boltzmannstr. 15, D-85748 Garching, Germany
[2] Biological Hydrogels Group, Institute of Medical Engineering, Technical University of Munich, Boltzmannstr. 11, D-85748 Garching, Germany

Porcine gastric mucin shows promising properties in respect to lubrication and hydrogel formation and has gained much attention regarding the coating of biomaterials and antiviral supplements. Mucins are high molecular weight glycoproteins and are predominantly found in the gastrointestinal tract and bronchia of animals and humans and serve as a selective barrier for molecules and protection of underlying tissue from pathogens. Because the naturally formation of a protein hydrogel at acidic pH is lost for commercially available purified porcine mucins we established a purification process for mucin from pig stomach (mainly Muc5AC) that maintains the desired properties. We focused on volume reduction for increased concentration in the early purification stage, functionality of the protein in terms of gel formation and the increase of protein yield and productivity. Different cross-flow module systems for pre-concentration of the mucus from pig stomach were evaluated using mass transfer relations. The mucus was successfully concentrated with 100 kDa MWCO membranes. Subsequent size exclusion chromatography resulted in a total recovery of the gel forming Muc5AC. Further diafiltration to remove salts was essential for preserving the functionality in terms of gel formation. The scale-up by factor 10 was successfully implemented for each process unit and allowed us to purify 65 mg mucin per pig stomach with an overall productivity of 0.15 mg mucin per mL crude mucus and hour. The desired key properties of mucin were confirmed by rheological measurements, lubrication studies and colocalization experiments. The purification method is applicable to other high molecular weight glycoproteins such as human salivary mucins.

Host Cell Protein clearance and identification

Marius Segl[*1,2], *Therese Wohlschlager*[1,2], *Johann Holzmann*[2,3], *Veronika Reisinger*[2,3], *Christian G. Huber*[1,2]

[1] *University of Salzburg, Department of Molecular Biology, Division of Chemistry and Bioanalytics Salzburg, Austria*
[2] *Christian Doppler Laboratory for Innovative Tools for the Characterization of Biosimilars, Salzburg, Austria*
[3] *Analytical Characterization Biopharmaceuticals, Sandoz GmbH, Biochemiestrasse 10, 6250 Kundl, Austria*

Biotherapeutics, especially monoclonal antibodies (mAb) and biotherapeutic fusion proteins, represent a class of drugs, which are on the rise for treating human diseases like cancer, allergies and autoimmune diseases [1], [2]. The development and production of those complex molecules is very cost and labour intensive and besides in-depth characterization of the drug itself, its purity plays an important role in order to fulfill the requirements of regulatory authorities. This is also true for Biosimilars, which are follow-on products of already accredited biotherapeutics. A very common problem in this context is the presence of so called host cell proteins (HCPs). HCPs are expressed alongside with the active pharmaceutical ingredient (API) and are a common source for impurities. Although there is a rigid product clean-up from upstream to downstream processing, some of those HCPs are still present in the final product in low ppm amounts. They have to be quantified as they could elicit unwanted side effects such as immune responses. Certain HCP species are capable of binding to the product itself via hydrophobic and electrostatic interactions [3] and hence can bypass product clean-up.

High performance liquid chromatography (HPLC) hyphenated to mass spectrometry (MS) offers a versatile platform for analyses complementary to the enzyme-linked immunosorbent assay (ELISA) which is still the gold standard for HCP quantification [4]. Although ELISA is a very sensitive method it is discriminatory when it comes to detection of HCPs. In the work presented we pursue a discovery-based approach for HCP identification using HPLC-MS/MS. Due to the high dynamic range (4-6 orders of magnitude) between HCP and API content the analysis is very challenging. Thus, the API - which contains an Fc part of an IgG_1 class antibody - is depleted before the analysis using protein A affinity-chromatography. To address unspecific binding of certain HCPs to the API itself, different washing solutions are tested to determine their efficacy to disrupt those interactions. To gain sensitivity, analysis is conducted via a “bottom-up” approach, where HCPs are enzymatically digested into peptides and are then identified through fragmentation in the gas phase. Tandem mass spectra and possible identifications are then evaluated using suitable database searching routines.

[1] J. M. Reichert, “Marketed therapeutic antibodies compendium,” *MAbs*, vol. 4, no. 3, pp. 413–415, May 2012.
[2] G. WALSH, “Biopharmaceutical benchmarks 2010,” *Nat. Biotechnol.*, vol. 28, no. 9, pp. 917–924.
[3] A. A. Shukla and P. Hinckley, “Host cell protein clearance during protein a chromatography: Development of an improved column wash Step,” *Biotechnol. Prog.*, vol. 24, no. 5, pp. 1115–1121, 2008.
[4] J. H. Thompson, W. K. Chung, M. Zhu, L. Tie, Y. Lu, N. Aboulaich, R. Strouse, and W. D. Mo, “Improved detection of host cell proteins (HCPs) in a mammalian cell-derived antibody drug using liquid chromatography/mass spectrometry in conjunction with an HCP-enrichment strategy,” *Rapid Commun. Mass Spectrom.*, vol. 28, no. 8, pp. 855–860, 2014.

High Resolution LC/MS Analysis of Therapeutic Oligonucleotides on a New Porous Polymer-based Reversed Phase Column

A. Robert van Ling[1], B. Shanhua Lin[2], Julia Baek[2], Jim Thayer[2], Hongxia Wang[2], Xiaodong Liu[2]

[1]*Thermo Fisher Scientific, Breda, The Netherlands*
[2]*Thermo Fisher Scientific, Sunnyvale CA, USA*

Synthetic oligonucleotides (ONs) with different functionalities including antisense ONs, small interfering RNAs (siRNAs), aptamers and immunostimulatory RNAs (isRNAs) are promising therapeutic agents due to their specificity, and well-established synthesis and modification technologies. Thorough characterization is required to demonstrate that efficacy and safety of these therapeutic ONs are ensured. This includes characterization of modifications to the base, sugar and backbone linkages, as these are commonly employed to decrease in vivo degradation and increase therapeutic efficacy. High performance LC/MS and LC/MS/MS are the preferred tools for these analyses, and are often used for more common ON purity assessments. Ion-pair reversed phase LC, with volatile mobile phase components, can be directly coupled to MS.

Here we introduce a new wide-pore polymeric reversed phase column and ionpair methods for LC and LC/MS ON analysis. The new resin is stable to 110 °C and up to pH 14, and coupling the column to HRAM mass spectrometry successfully identified overlapping failure sequences, ONs with characteristic methylation, phosphorothioate diastereoisomers and 5'-phosphorylated DNAs. Short gradients allowed for the separation of mixed-base 21-mer and its n-1 failure sequence, and MS identified n-1 sequences where guanine, cytosine and thymine failed to couple during synthesis.Separation of diasteroisomers of several siRNA molecules with one or two phosphorothioate linkages was achieved, and MS analysis revealed identical mass values to within 9 ppm. These results demonstrate the use of DNAPac RP and HRAM mass spectrometry for critical analyses of modified oligonucleotides.

A quantitative FVIII assay by interferometry using camelid antibody VhH-domain

H. Engelmaier[1], N. Hammerschmidt[1], A. Jungbauer[1,2]

[1]acib - Austrian Centre of Industrial Biotechnology, Muthgasse 18, A-1190, Vienna, Austria
[2]University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology Muthgasse 19, A-1190, Vienna, Austria

FVIII is required for the treatment of Haemophilia A the most common inherited blood coagulation disorder. Quantification during the production process is usually performed by antigen ELISA and chromogenic activity assays. These methods are labour-intensive, time-consuming and suffer from high variability. We have developed a bio-layer interferometry assay on the Octet® that is based on a camelid antibody VhH-domain specific for FVIII. Recombinant FVIII can be detected from cell-culture supernatant as well as other process intermediates. This method allows for convenient and robust determination of FVIII without the need for tedious sample preparation. By optimizing regeneration conditions, sensor tips can be re-used. The Octet's 96-well based format allows for high throughput and is ideally suited for screening large numbers of purification conditions. This assay can be used as a valuable tool during process development as well as for in process control, providing a fast and straightforward method for FVIII quantification.

Selectivity behaviour of peptides with high aqueous content mobile phases using new classes of hydrophilic interaction and aqueous normal phase chromatographic materials.

Chadin Kulsing, Reinhard I. Boysen, Yuanzhong Yang and Milton T. W. Hearn

Victorian Centre for Sustainable Chemical Manufacture, School of Chemistry, Monash University, Melbourne, Victoria 3800, Australia

A general experimental approach has been developed to differentiate the individual free energy contributions to peptide retention with silica-based chromatographic stationary phases capable of manifesting hydrophilic interaction and aqueous normal phase behaviour. To this end, the contributions from electrostatic, dipolar and hydrogen bond interactions between different sets of peptides and these stationary phase in the presence of water-organic mobile phases of varying (low) organic modifier content, and containing different percentages of formic or acetic acid, were assessed in terms of linear solvation energy relationships and dipole-moment fluctuation suppression theory. The magnitude of the electrostatic contributions was calculated from the peptide charges and the measured zeta potential values of the stationary phases as a function of mobile phase compositions. With acidic mobile phases, and depending on the peptide structure, a significant proportion of the change in free energy associated with peptide retention with these new stationary phases can be attributed to electrostatic attractive interactions, but that this is not associated with the effect of residual silanol groups. The remainder of the free energy change associated with retention was dominated by the effects of hydrogen bond basicity of the basic functionalities, as well as the amide backbone N-H bonds, of the peptides in their interaction with these stationary phases. The general applicability of these methods is expected to significantly broaden the application scope, stationary phase design potential and the interpretation of the retention behaviour of peptides and other compound classes separated with silica-based hydrophilic interaction and aqueous normal phase stationary phases.

Characterization and quantification of monoclonal antibody oxidation variants utilizing ion-pair reversed-phase HPLC-MS/MS

Christof Regl[1,2], Therese Wohlschlager[1,2], Ines C. Forstenlehner[1,3], Silke Ruzek[1,3], Johann Holzmann[1,3], Christian G. Huber[1,2]

[1]1Christian Doppler Laboratory for Innovative Tools for Biosimilar Characterization, University of Salzburg, Hellbrunnerstrasse 34, 5020 Salzburg, Austria
[2] Department of Molecular Biology, Division of Chemistry and Bioanalytics, University of Salzburg, Hellbrunnerstrasse 34, 5020 Salzburg, Austria
[3] Analytical Characterization Biopharmaceuticals, Sandoz GmbH, Biochemiestrasse 10, 6250 Kundl, Austria

Oxidation of methionine residues induced by reactive oxygen due to inappropriate storage conditions is a major shelf-life-limiting factor for biopharmaceuticals [1]. It has been shown for therapeutic IgG monoclonal antibodies that methionine oxidation within the Fc region may not only lead to decreased bioactivity, but also cause faster plasma clearance [2]. Peptide mapping is the state-of-the-art method for analysis of monoclonal antibody (mAb) oxidation variants. However, top-down or middle-down analysis would not only enable faster analysis and provide information on individual proteoforms, but also avoid artifacts occurring during the extensive sample preparation of a peptide map [3].

In order to develop an analytical workflow for the characterization of oxidized therapeutic antibodies, IgG1 antibody Rituximab was stressed by exposure to hydrogen peroxide. Limited proteolysis utilizing the IdeS enzyme was applied in a middle-down approach to generate mAb Fc/2 fragments. We achieved full chromatographic separation of single, double and non-oxidized Fc/2 species by ion-pair reversed-phase HPLC on a diphenyl column. Furthermore, methionine oxidation site assignment was accomplished upon higher-energy collisional dissociation fragmentation in a quadrupole-Orbitrap mass spectrometer. Based on these results, absolute quantification of oxidation by means of UV-spectroscopy as well as full scan MS, was performed following a previously described protocol [4].

[1] Houde, D., et al., Determination of protein oxidation by mass spectrometry and method transfer to quality control. J Chromatogr A, 2006. 1123(2): p. 189-98.
[2] Stracke, J., et al., A novel approach to investigate the effect of methionine oxidation on pharmacokinetic properties of therapeutic antibodies. MAbs, 2014. 6(5): p. 1229-42.
[3] An, Y., et al., A new tool for monoclonal antibody analysis: application of IdeS proteolysis in IgG domain-specific characterization. MAbs, 2014. 6(4): p. 879-93.
[4] Forstenlehner, I.C., et al., Site-specific characterization and absolute quantification of pegfilgrastim oxidation by top-down high-performance liquid chromatography-mass spectrometry. Anal Chem, 2015. 87(18): p. 9336-43.

Novel recombinant protein purification strategy for unstable protein expressed in Yeast

M. Khatami[1,2], S. Hasannia[1], S. N. Hosseini[2]

[1] Faculty of Biological sciences, Tarbiat Modares University
[2] Production and Research complex, Pasteur Institute of Iran, Tehran, Iran

Growth speed, easy genetic manipulation, low cost media, high cell density make yeast useful for production of recombinant proteins.

Recombinant proteins in yeast can be over-expressed so the product is secreted from the cell and available for recovery in the fermentation solution. Secretory protein passes through the ER and Golgi to obtain complete glycosylation pattern.

Glycosylation is essential for biological activity of some pharmaceutical protein like alpha 1 antitrypsin (AAT) which has 55KD molecular weight.

By considering that Yeast as the eukaryotic host is not able to secrete protein larger than 20KD for obtaining completely glycosylated pharmaceutical recombinant protein that has molecular weight more than 20KD a new strategy must be stablished.

By respect to molecular weight of AAT and its correct pattern of glycosylation to guarantee its functionality and also decrease downstream processes to prevent it from oligomerization a new approach must be considered.

As vesicular fusion with a target membrane distribute to SNARE protein we decided to prevent protein secretion by focusing on SNARE proteins.

Although Secretion of pharmaceutical protein to the medium ease the downstream processes by decreasing purification steps to keep AAT inside cell to prevent it's oligomerization we tried to prevent our desired protein from secretion by studying SNARE complex formation.

When the desired secretory protein passes the secretory pathway and reaches to membrane by inhibiting SNARE complex formation that play important role in fusion and secretion , protein endosome trapped inside yeast.

Co-expression of our desired protein and cytoplasmic domain of V-SNARE lead to accumulation of alpha-1 antitrypsin (AAT) endosomes inside yeast.

Ultracentrifugation provides us one step purification to collect AAT endosomes.

There are many advantages for this method,

1. Completely glycosylated proteins achieved as secretory pathway leading to correct protein processing and post-translational modifications.

2. Less complicated purification step was needed by processing endosomes inside yeast.

3.Oligomerization of Alpha-1 antitrypsin was prevented as protein trapped inside yeast cell and be purified quickly.

Index of Authors