Gefördert durch
DFG Deutsche Forschungsgemeinschaft

acib austrian centre of industrial biotechnology

ISPPP 2018 BERLIN

International Symposium on the Separation of Proteins, Peptides and Polynucleotides

NOVEMBER 4 - 7, 2018 / BERLIN, GERMANY

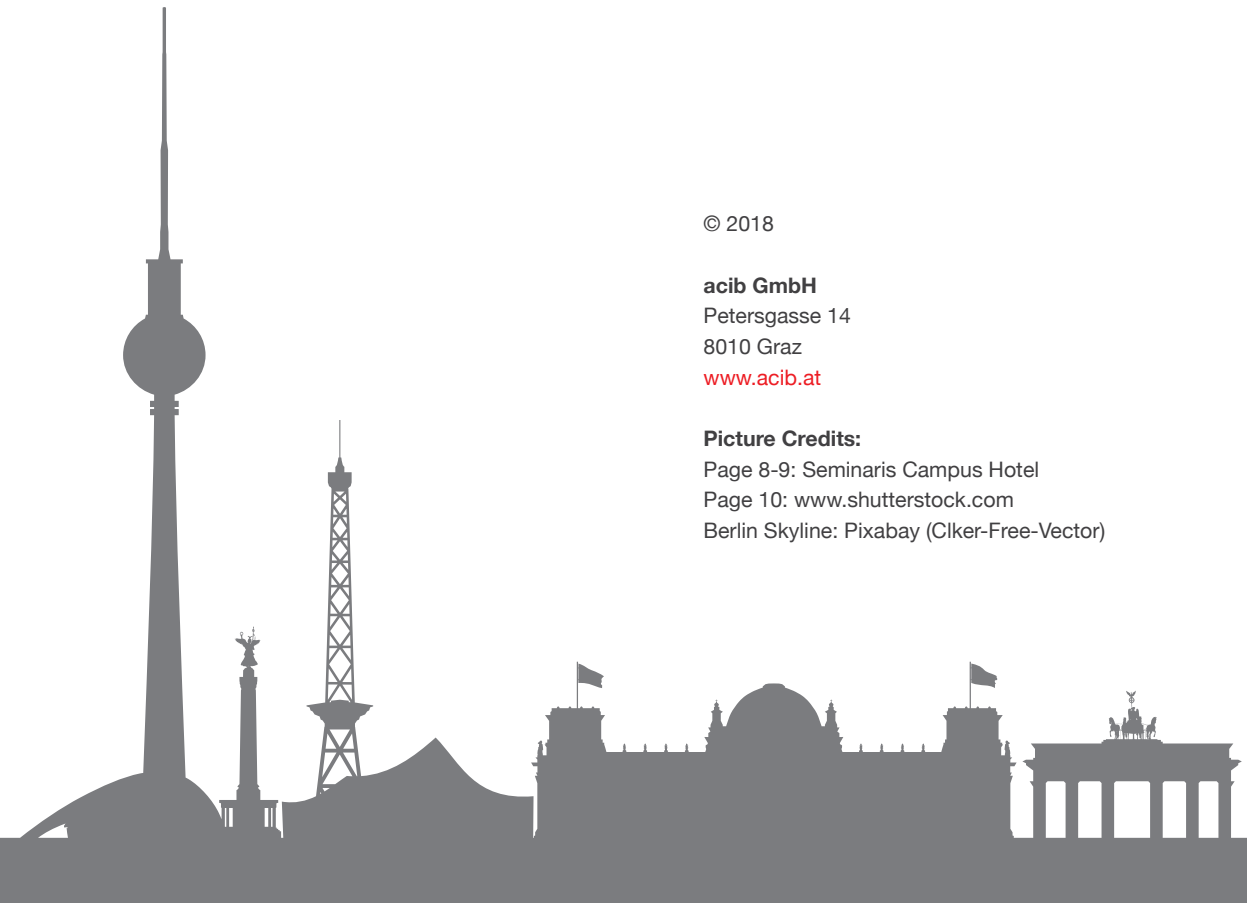
We thank our sponsors

SILVER SPONSOR

BRONZE SPONSOR

List of exhibitors

ISPPP 2018 BERLIN



acib GmbH
Petersgasse 14
8010 Graz
www.acib.at

Picture Credits:
Page 8-9: Seminaris Campus Hotel
Page 10: www.shutterstock.com
Berlin Skyline: Pixabay (Clker-Free-Vector)

Conference Chair

Sonja Berensmeier
Technical University Munich, Germany

Scientific Committee

Cristina Dias-Cabral
University of Beira Interior, Portugal

Michel Eppink
University of Wageningen, Synthon, The Netherlands

Milton Hearn
Monash University, Australia

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

Aleš Podgornik
University of Ljubljana, Slovenia

Organizational Committee

Verena Beck
Austrian Centre of Industrial Biotechnology (acib), Vienna, Austria

Sonja Berensmeier
Technical University Munich, Germany

Alois Jungbauer
acib / BOKU, Vienna, Austria


SEINÄJOKI CAMPUS HOTEL
HOTEL

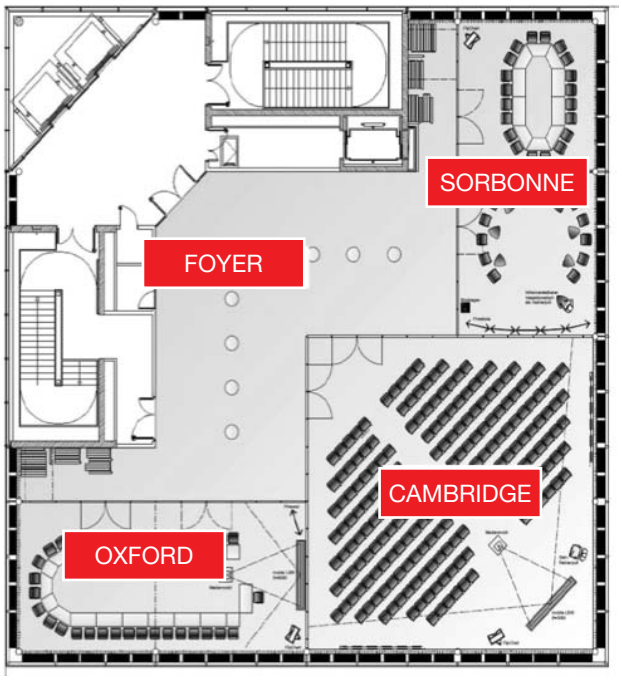
Venue Map

Seminaris Campus Hotel
Takustraße 39
14195 Berlin
GERMANY

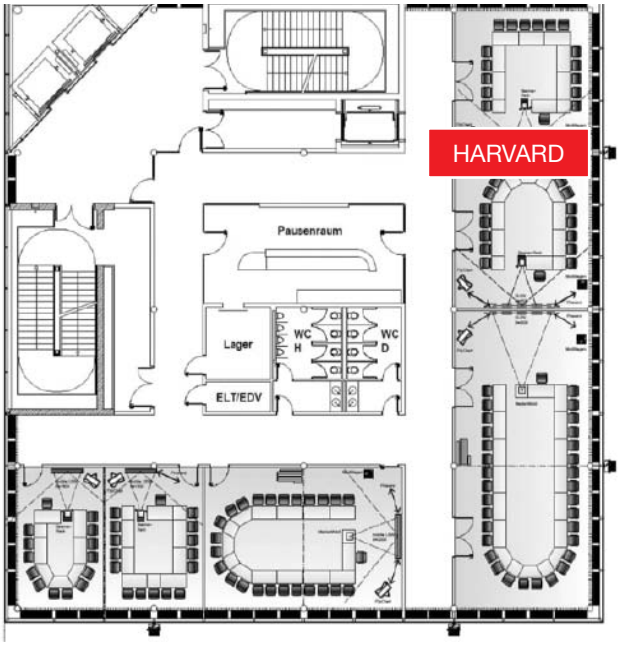
The conference will be held in Dahlem cube, 2nd floor. Lectures will be in room Cambridge.

Welcome Reception, refreshment breaks, poster sessions and exhibition will be in the Foyer including rooms Oxford and Sorbonne.

2ND FLOOR



1ST FLOOR



The pre-conference workshops will take place in Dahlem Cube, 1st floor, room Harvard.

Sunday, November 4

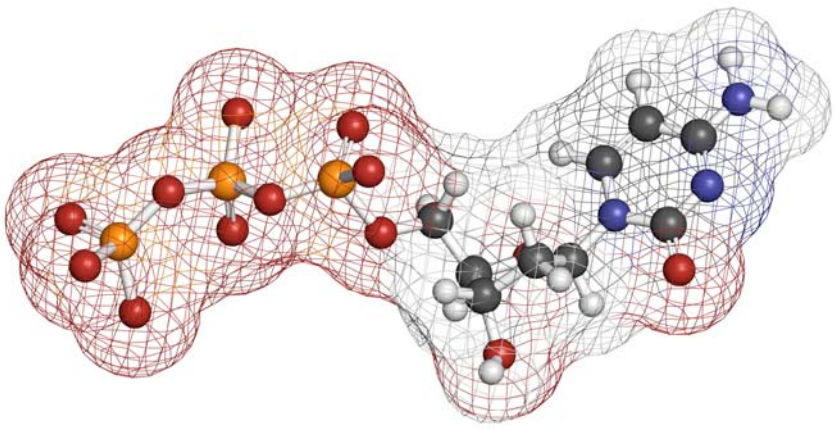
11:00	START OF REGISTRATION	

PRE-CONFERENCE WORKSHOPS FOR REGISTERED PARTICIPANTS		
11:45	Alois Jungbauer, Cristina Dias-Cabral	Mechanistic understanding of biomolecules adsorption: theory and applications
13:30	Michel Eppink, Sophia Hober	Surface plasmon resonance in biotechnical development
15:15	Sonja Berensmeier, Matthias Franzreb	Magnetic separation of proteins in the technical scale

17:00	**START OF CONFERENCE · WELCOMING REMARKS BY SONJA BERENSMEIER**	

KEY NOTE LECTURE · CHAIR: SONJA BERENSMEIER		
17:30	Rainer Bischoff	**T1:** Biomarker discovery and validation – from shotgun proteomics to targeted methods

18:30	WELCOME RECEPTION	



Monday, November 5

KEY NOTE LECTURE · CHAIR: ALOIS JUNGBAUER		
08:30	My Hedhammar	**T2:** BioSilk - A bioactive material made of recombinant silk fusion proteins

SESSION 1: BIOPHARMACEUTICALS I · CHAIR: ALOIS JUNGBAUER		
09:30	Sophia Hober	**T3:** The development of small protein domains for biotechnological and medical use
10:00	Sara Rosa	**T4:** Biothermodynamics of the adsorption of monoclonal antibodies in phenylboronic acid agarose-based supports: Affinity vs multimodal chromatography
10:20	Eva Udovic	**T5:** An optimization of Raman spectroscopy parameters and implementation of in-line Raman spectroscopy to the key steps of mAB production downstream process
10:40	Carsten Voss	**T6:** Purification of Large Biomolecules with Tailored Anion Exchangers

SESSION 2: BIOPHARMACEUTICALS II · CHAIR: SOPHIA HOBER		
11:30	Alois Jungbauer	**T7:** Virus, virus-like particles, extra cellular vesicles and gene therapy vesicles purification
12:00	Pavel Marichal-Gallardo	**T8**: A Single-Use Purification Platform For Viral Vaccines & Gene Therapy Vectors
12:20	Cristina Peixoto	**T9:** Improving oncolytic adenovirus manufacturing: designing GMP compatible bioprocesses
12:40	Leif Bülow	**T10:** One-Step Chromatographic Separations of Wild-type and Mutant Proteins from Crude Biological Fluids Using Molecularly Imprinted Polymers

Monday, November 5

SESSION 3: BIOPHARMACEUTICALS III · CHAIR: MILTON HEARN		
14:30	Egbert Müller	**T11:** Preparation and purification of protein-mimetic drug conjugate with tuneable hydrophobicity
15:00	Svenja Nicolin Bolten	**T12:** Substitution of heparin for purification of recombinant proteins
15:20	Shuichi Yamamoto	**T13:** Tuning pore size of monolithic chromatography for large biomolecule separations
15:40	Günter Allmaier	**T14:** Nano Bioparticle (Liposomes, Vlps And Viruses) Separation By Means Of Differential Mobility Analysis In Combination With Spectroscopy And Mass Spectrometry
16:00	REFRESHMENT BREAK	

SESSION 4: SHORT PRESENTATIONS · CHAIR: SONJA BERENSMEIER		
16:30	Irsyad Khairil Anuar	**SP1:** Bacterial superglue engineered for protein separation and plug-and-display nanoassembly
16:38	Lennart Kleinfeldt	**SP2:** Structuring and functionalization of iron oxide particles for continuous automated in situ protein purification
16:46	Luis Passarinha	**SP3:** Soluble catechol-O-methyltransferase capture from a Pichia pastoris X33 lysate through a new batch method with gellan microspheres
17:54	Igor Tadeu, Lazzarotto Bresolin	**SP4:** Purification of murine, rabbit and human IgG onto iminodiacetic acid immobilized onto monolithic disks
17:02	Yasmin Kaveh Baghbaderani	**SP5:** Magnetic Nanoparticles as New Stationary Phases for Antibody Affinity Purification
17:10	Jure Sencar	**SP6:** A narrow residence time incubation reactor for continuous virus inactivation based on packed beds
17:18	WELCOME RECEPTION	

Tuesday, November 6

KEY NOTE LECTURE · CHAIR: ALEŠ PODGORNIK		
08:30	Herbert Lindner	**T15:** How does CE-MS compare with LC-MS in peptide and PTM analysis?

SESSION 5: BIOANALYTICS I · CHAIR: ALEŠ PODGORNIK		
09:30	Michel Eppink	**T16**: Microfluidics as a tool in DSP for biopharmaceutical proteins
10:00	Cédric Delvaux	**T17:** Combination of Capillary Electrophoresis and Ion mobility coupled to Mass Spectrometry and Theoretical Calculations for cysteine connectivity identification in peptides bearing two intra-molecular disulfide bonds
10:20	Anja Kristl	**T18:** Influence of pressure on gradient separations of biomolecules by ion exchange chromatography
10:40	Fátima Milhano Santos	**T19:** Development of an artificial neural network for vitreous protein profiling by bidimensional electrophoresis
11:00	REFRESHMENT BREAK	

SESSION 6: BIOANALYTICS II · CHAIR: MICHEL EPPINK		
11:30	Barry Boyes	**T20:** Protein Variant Separation Improvements using Superficially Porous Particles and Operating Condition Manipulations
12:00	Kiyohito Shimura	**T21:** Immunoaffinity Capillary Isoelectric Focusing: Two facile micro analytical platforms for charge variants of protein pharmaceuticals
12:20	Gerhard Heinzmann	**T22:** Electrical Asymmetrical Flow Field-Flow Fractionation coupled with Online Multi-Angle Light Scattering: Advanced Separation and Characterization of Charged and Polarizable Nanoparticles, Liposomes, Proteins, Antibodies and Viruses
12:40	Dierk Rössner	**T23:** Sample injection with reduced pressure-drop for size exclusion chromatography for improved protein aggregate detection
13:00	BREAK	

Tuesday, November 6

SESSION 7: SIMULATION, AUTOMATION AND MODELLING CHAIR: MATTHIAS FRANZREB		
14:15	Aleš Podgornik	**T24:** Analysis of adsorption phenomena based on pressure drop data
14:45	Nadia Galeotti	**T25:** Influence of Mixed Electrolytes on HIC-Adsorption and Prediction of Chromatographic Elution Profiles
15:05	Ajoy Velayudhan	**T26:** Analysis of modulator effects in the polishing SMB of proteins
15:25	Tatjana Turrina	**T27:** Potential-Controlled Chromatography – a Simple and Innovative Separation Technique for Charged Biomolecules
15:45	REFRESHMENT BREAK	
15:30	Poster Session	

SESSION 8: ADVANCES IN STATIONARY PHASES · CHAIR: EGBERT MÜLLER		
16:30	Sonja Berensmeier	**T28:** New stationary phases as alternatives to conventional chromatography
17:00	Milton Hearn	**T29:** Selectivity Modulation of Synthetic Peptides with Dynamic Hydrophobic Silica-based Adsorbents
17:20	Cristina Dias-Cabral	**T30:** The adsorption mechanism behind biomolecule adsorption onto a salt-tolerant anion exchanger: a calorimetric perspective.
17:40	Ursula Simon	**T31:** 3D-printed anion exchange monoliths for protein separations
18:00	End of session	
19:30	CONFERENCE DINNER	

Wednesday, November 7

SESSION 9: CONTINUOUS PROCESSING · CHAIR: CRISTINA DIAS-CABRAL		
09:00	Matthias Franzreb	**T32:** Temperature-controlled purification of monoclonal antibodies applying travelling heating zone chromatography
09:30	Chantal Brämer	**T33:** Continuous purification of industrial relevant proteins with a self-established periodic counter-current chromatography device
09:50	Hans Blom	**T34:** Productivity considerations for batch and continuous chromatography
10:10	Guido Ströhlein	**T35:** A stackable, single-use chromatography casset-te enhancing continuous antibody capture
10:30	REFRESHMENT BREAK	

SESSION 10: SCALE-UP AND SCALE-DOWN OF CHROMATOGRAPHY CHAIR: SONJA BERENSMEIER		
11:00	Giorgio Carta	**T36:** Gradient Elution Behavior of Proteins in Hydrophobic Interaction Chromatography with U-Shaped Retention Factor Curves
11:30	Dmytro Iurashev	**T37:** Computational fluid dynamics successfully predicts the scalability of chromatography columns
11:50	Yannick Krauke	**T38:** Continuous purification of xylitol by simulated moving bed (SMB) process
12:10	Presentation of Poster Awards and Concluding Remarks	
12:30	END OF CONFERENCE	

Poster Session - Overview

SP1 **Bacterial superglue engineered for protein separation and plug-and-display nanoassembly**
Khairil Anuar I[1], Howarth M[1]
[1]University of Oxford, Oxford, United Kingdom

SP2 **Structuring and functionalization of iron oxide particles for continuous automated *in situ* protein purification**
Kleinfeldt L[1,2], Gädke J[2,3,4], Biedendieck R[2,4,5], Krull R[2,3,4], Garnweitner G[1,2]
[1]Institute for Particle Technology, TU Braunschweig, Braunschweig, Germany,
[2]Center of Pharmaceutical Engineering (PVZ), Braunschweig, Germany,
[3]Institute of Biochemical Engineering, TU Braunschweig, Braunschweig, Germany,
[4]Braunschweig Integrated Centre of Systems Biology, Braunschweig, Germany,
[5]Institute of Microbiology, TU Braunschweig, Braunschweig, Germany

SP3 **Soluble catechol-O-methyltransferase capture from a Pichia pastoris X33 lysate through a new batch method with gellan microspheres.**
Passarinha L[1,2,3], Gonçalves C[2], Sousa A[2]
[1]UCIBIO@requimte, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal,
[2]CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal,
[3]Laboratório de Fármaco-Toxicologia-UBIMedical, Universidade da Beira Interior, Covilhã, Portugal

SP4 **Purification of murine, rabbit and human IgG onto iminodiacetic acid immobilized onto monolithic disks**
de Mello P[1], Lazzarotto Bresolin I[1], Rocha Antunes Pereira Bresolin I[1], Alves Bueno S[2]
[1]Federal University of São Paulo (UNIFESP), Chemical Engineering Department, Diadema, Brazil, 2University of Campinas (UNICAMP), Chemical Engineering School, Campinas, Brazil

SP5 **Magnetic Nanoparticles as New Stationary Phases for Antibody Affinity Purification**
Kaveh Baghbaderani Y[1], Schwaminger S[1], Fraga-García P[1], Berensmeier S[1]
[1]Bioseparation Engineering Group, Technical University of Munich, Garching, Germany

SP6 **A narrow residence time incubation reactor for continuous virus inactivation based on packed beds**
Senčar J[1], Hammerschmidt N[1], Lima Mantins D[1], Jungbauer A[1,2]
[1]Austrian Centre for Industrial Biotechnology, Vienna, Austria,
[2]Department of Biotechnology, University of Natural Resources and Life Sciences, Austria

P1 **Protein adsorption behavior in multimodal cation exchange resin**
Adamíková J[1], Očková M[1], Antošová M[1], Polakovič M[1]
[1]Faculty of Chemical and Food Technology STU in Bratislava, Bratislava, Slovakia

P2 **Effect of process conditions on recombinant human erythropoietin separation by multimodal chromatography**
Molnar T[1], Antosova M[1], Adamíková J[1], Polakovic M
[1]Faculty of Chemical And Food Technology Stu In Bratislava, Bratislava, Slovakia

P3 **Making Hydrophobic Interaction Chromatography compatible with MS by 2D-LC for the analysis of mAbs and ADCs**
Arendt M[1]
[1]Agilent Technologies, Waldbronn, Germany

P4 **Opportunities and challenges of nanoparticle based magnetic separation for biomolecule recovery**
Fraga-García P[1], Schwaminger S[1], Blank-Shim S[1], Brammen M[1], Berensmeier S[1]
[1]Bioseparation Engineering Group, Technical University of Munich, Garching, Germany

P5 Internal structure of chromatographic membranes analyzed by microscopic techniques
Gramblicka M[1], Kurak T[1], Polakovic M[1]
[1]Slovak University of Technology, Bratislava, Slovakia

P6 FFF-MALS: A Powerful Technology for Separation and Quantification of Proteins, Antibodies, Viruses and High Molar Mass Aggregates
Heinzmann G[1], Meier F[1], Klein T[1]
[1]Postnova Analytics GmbH, Landsberg, Germany

P7 A novel range of monodisperse agarose beads designed for large-scale manufacturing
Johansson H[1], Gilbert P[1]
[1]Purolite, Llantrissant, UK

P8 Development of monolithic silica capillary columns for high resolution separation of intact proteins
Kobayashi H[1], Wada H[1]
[1]Shinwa Chemical Industries, Ltd., Kyoto, Japan

P9 Development of laser-induced fluorescence detector for analysis of prostate specific antigen by capillary electrophoresis-based immunofluorescence assay
Krenkova J[1], Prikryl J[1], Vaclavek T[1], Foret F[1]
[1]Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic

P10 Simplify your purification with AZURA FPLC. Examples for automated two step purification.
Krop U[1], Krauke Y[1], Monks K
[1]Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany

P11 Unconventional purification of His-tagged proteins using TiO_2 Nanotubes Decorated by Fe_3O_4 Nanoparticles
Kupcik R[1], Macak J[2], Rehulka P[3], Sopha H[2], Bilkova Z[1]
[1]Department of Biological and Biochemical Sciences, University of Pardubice, Pardubice, Czech Republic,
[2]Center of Materials and Nanotechnologies, University of Pardubice, Pardubice, Czech Republic,
[3]Department of Molecular Pathology and Biology, University of Defence, Hradec Kralove, Czech Republic

P12 Continuous virus inactivation process using a novel packed-bed reactor
Martins D[1,2], Sencar J[1,2], Hammerschmidt N[1,2], Tille B[3], Kindermann J[3], Kreil T[3], Jungbauer A[1,2]
[1]Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Vienna, Austria,
[2]Austria Center for Industrial Biotechnology, Vienna, Austria,
[3]Global Pathogen Safety, Shire, Vienna, Austria

P13 A fast and scalable purification technique for vaccines and viral vectors: Purification of parapoxvirus particles using steric exclusion chromatography
Lothert K[1], Pagallies F[2], Feger T[2], Amann R[2], Wolff M[1,3]
[1]Institute of Bioprocess Engineering and Pharmaceutical Technology -Technische Hochschule Mittelhessen, Gießen, Germany,
[2]Department of Imnunology - University of Tuebingen, Tuebingen, Germany,
[3]Bioprocess Engineering - Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany

P14 Formulation development of Biosimilar drugs: A multi-technique analytical approach using DLS, DSC and SEC for in-depth stability profiling
Makwana H[1], Stenson J[1], Ross A1, Pothecary M[2] and Markova N[1]
[1]Malvern Panalytical Ltd, Malvern, UK
[2]Malvern Panalytical Ltd. Westborough, USA

P15 Investigation of biosimilars and innovator monoclonal antibodies using multidetection SEC
Marenchino M[1], Stenson J[2], Ross A[2], Pothecary M[3]
[1]Malvern Panalytical GmbH, Kassel, Germany,
[2]Malvern Panalytical Limited, Malvern, United Kingdom,
[3]Malvern Panalytical Inc., Westborough, USA

P16 HILIC separation of positively charged compounds
Moravcová D[1], Křenková J[1], Foret F[1]
[1]Institute of Analytical Chemistry of the CAS, Brno, Czech Republic

P17 Process integration and optimization for food enzyme production including large scale purification
den Hollander J[1], van Beckhoven R[1], van de Sandt E[1], Neves C[1]
[1]DSM Biotechnology Center, PO Box 1, 2600 MA Delft, The Netherlands

P18 Enhancing Subunit-Level Profiling of mAbs and ADCs with MS-Quality Difluoroacetic Acid
Nguyen J[1], Smith J[3], Friese O[3], Rouse J[4], Walsh D[2], Lauber M[2]
[1]University Of Copenhagen, Frederiksberg, Denmark,
[2]Waters Corporation, Milford, USA,
[3]Biotherapeutics Pharm. Sci., Pfizer WRD, St. Louis, USA,
[4]Biotherapeutics Pharm. Sci., Pfizer WRD, Andover, USA

P19 µSEC-MALS adds Multi-Angle Light Scattering to UHP-SEC for Measurement of Absolute Molecular Weight and Size
Roessner D[1]
[1]Wyatt Technology, Dernbach, Germany

P20 Affinity-free purification process for a single-chain variable fragment developed using DoE and proteomics approach
Sakhnini L[1,2], K. Pedersen A[1], R. León I[1], J. Greisen P[1], Hansen J[1], B. Vester-Christensen M[1], Bülow L[2], Dainiak M[1]
[1]Novo Nordisk A/S, Måloev, Denmark,
[2]Lund University, Lund, Sweden

P21 Charge Variant Analysis of Therapeutic Proteins Using a Novel Weak Cation Exchange Stationary Phase
Schwahn A[1], Lin S[2], Bechler S[2], Baek J[2], Liu X[2]
[1]Thermo Fisher Scientific, Reinach, Switzerland,
[2]Thermo Fisher Scientific, Sunnyvale, USA

P22 Charge Variant Method Design for Analysis of Monoclonal Antibodies
Schwahn A[1], Lin S[2], Baek J[2], Bechler S[2]
[1]Thermo Fisher Scientific, Reinach, Switzerland,
[2]Thermo Fisher Scientific, Sunnyvale, USA

P23 New concepts for protein purification: magnetic separation with bare iron oxide nanoparticles
Schwaminger S[1], Blank-Shim S[1], Fraga-García P[1], Rauwolf S[1], Zanker A[1], Berensmeier S[1]
[1]Bioseparation Engineering Group, Garching, Germany

P24 Miniaturized and simple gradient liquid chromatography system for capillary columns
Šesták J[1], Kahle V[1]
[1]Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic

P25 Intensification of mAb downstream processes using modular innovative purification technologies
Trapp A[1], Kössler V[1], Faude A[1], Schubert S[1]
[1]Rentschler Biopharma SE, Laupheim, Germany

P26 Micro Pillar Array Columns: A novel robust chromatography platform for deep and reproducible proteome coverage
van Ling R[1], Op de Beeck J[1], Van Mol K[1], Claerebout B[1], Van Landuyt N[1], De Malsche W[2], Desmet G[2], Jacobs P[1]
[1]PharmaFluidics, Gent, Belgium, 2Free University Brussels (VUB), Brussels, Belgium

P27 Accelerated downstream process development: Mechanistic modeling of LMW, HMW and HCP separation from product on a multimodal resin
Wittkopp F[1], Kraus J[1], Isik E[1], von Hirschheydt T[1], Schaubmar A[1]
[1]Roche Diagnostics GmbH, Pharma Research and Early Development (pRED), Large Molecule Research (LMR), Roche Innovation Center Munich, Penzberg, Germany

P28 Exploring a 2um non-porous ODS column for proteins and peptides separation
Yazawa I[1]
[1]Imtakt Corp., Kyoto, Japan

P29 A proteome wide approach to understand chromatography behavior on hydrophobic adsorbents
Yelemane V[1], Ali N[1], Fernández-Lahore M[1]
[1]Jacobs University Bremen Ggmbh, Bremen, Germany

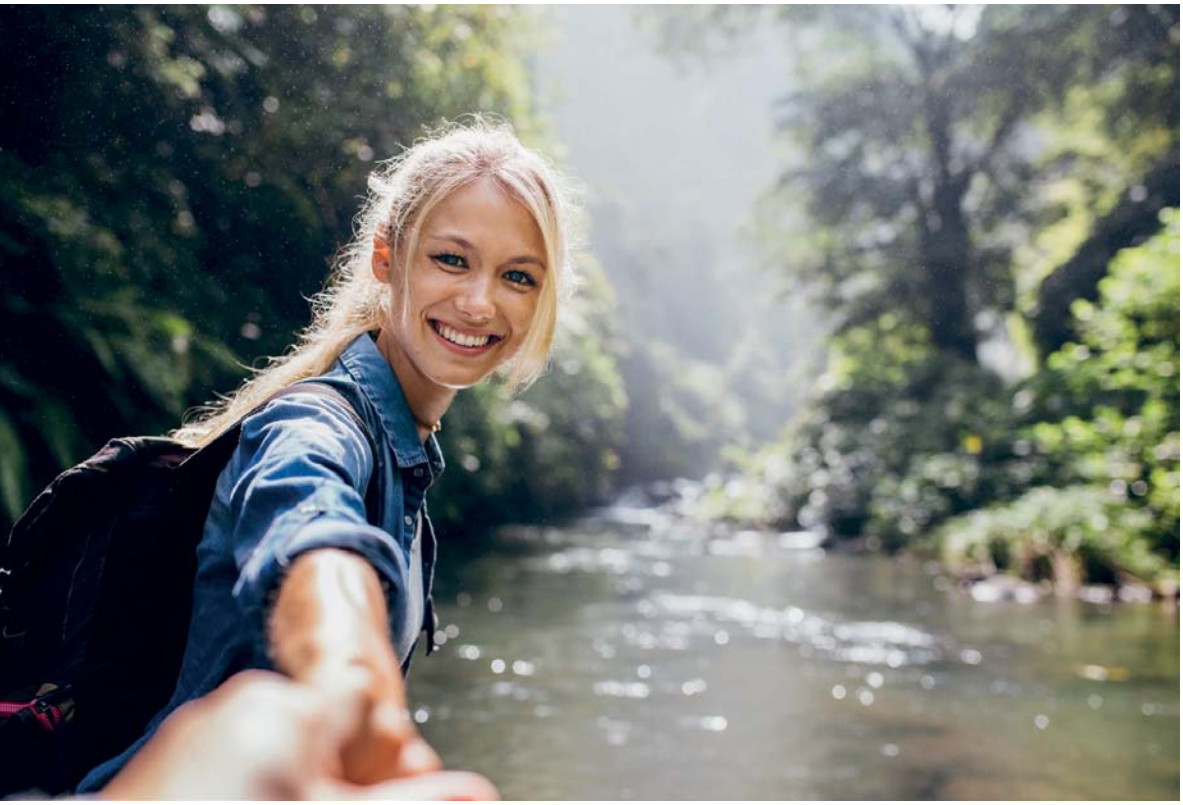
P30 Identification of High-Affinity Peptide Scaffold from *In vitro* and *In silico* Selection Process
Yun S[1], Lee S[1], Choo J[1], Lee E[1]
[1]Hanyang University, Ansan-si, South Korea

P31 Potential Application of Taiwan Actinomycetes Broth in Treatment of Alzheimer's Disease
Hui-Ming Yu[1], Yu-Hsuan Chu[1], Kuo-Ching Lin[1]
[1]Genomics Research Center, Academia Sinica, Taipei, Taiwan

NOTES

valneva

Advancing vaccines for better lives



Valneva is a fully integrated, commercial stage biotech company focused on developing innovative life-saving vaccines.
Our vision is to contribute to a world in which no one dies or suffers from a vaccine-preventable disease.

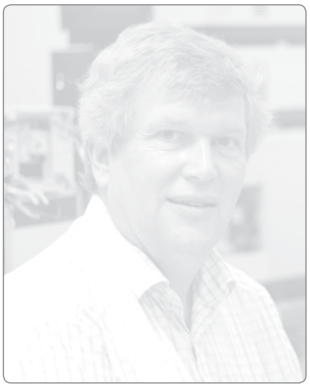
www.valneva.com

octapharma

Octapharma is the largest, privately-owned human protein products manufacturer worldwide 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.

Octapharma employs over 7,600 people worldwide to support the treatment of patients in over 100 countries with products across the following therapeutic areas: Haematology, Immunotherapy and Critical Care. Octapharma owns 6 state-of-the-art production facilities in Austria, France, Germany, Sweden and Mexico.
For more information visit www.octapharma.com.

Oral Presentation Abstracts



T1 RAINER BISCHOFF

Biomarker discovery and validation - from shotgun proteomics to targeted methods

Klont F[1], Pouwels S[2], Hadderingh M[1], Joosten M[1], Güzel C[3], Hermans J[1], Govorukhina N[1], Stingl C[3], Dekker L[3], Wisman B[4], van der Zee A[4], Hollema H[5], Guryev V[6], van de Merbel N[1,7], Wolters J[8], Horvatovich P[1], Luider T[3], ten Hacken N[2], Bischoff R[1]

[1] *University of Groningen, Department of Analytical Biochemistry, Groningen, Netherlands*
[2] *Department of Pulmonary Diseases, University Medical Center Groningen, University of Groningen, Groningen, Netherlands,*
[3] *Laboratory of Neuro-Oncology, Clinical and Cancer Proteomics, Department of Neurology, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands,*
[4] *Department of Gynecologic Oncology, Cancer Research Center Groningen, University of Groningen, University Medical Center Groningen, Groningen, Netherlands,*
[5] *Department of Pathology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands,*
[6] *European Research Institute for the Biology of Ageing, University Medical Center Groningen, University of Groningen, Groningen, Netherlands,*
[7] *Bioanalytical Laboratory, PRA Health Sciences, Early Development Services, Assen, Netherlands,*
[8] *Department of Pediatrics, Center for Liver, Digestive and Metabolic Diseases, University of Groningen, University Medical Center Groningen, Groningen, Netherlands*

Biomarkers are an indispensable part of the toolbox of modern medicine. Biomarkers may indicate the physiological state of an individual and predict pathophysiological deviations from homeostasis. While many biomarkers are currently in use, further developments are needed to address unmet clinical needs, notably in predicting response to therapy on an individualized level (personalized or precision medicine).

In this lecture, I will cover fundamental aspects of biomarker discovery by bottom-up (also referred to as “shotgun”) proteomics on the example of tissue analysis of biopsies from cervical cancer patients after laser capture microdissection (LCM). This will be followed by a discussion of biomarker prioritization for Chronic Obstructive Pulmonary Disease (COPD) from existing literature data, one of the most prevalent diseases worldwide with a high level of mortality and morbidity. Finally I will provide an overview of different targeted LC-MS/MS methods to quantify the soluble receptor of advanced glycation end products (sRAGE), a candidate biomarker for emphysema development during COPD that is currently under consideration for qualification by the US Food and Drug Administration (FDA). This methodological comparison serves to discuss differences in the obtained results and with respect to a commercial enzyme-linked immunosorbent assay (ELISA). I will conclude with future perspectives on how multiplex, targeted LC-MS/MS assays may be used to quantify a range of proteins using concatenated, stable-isotope-labelled internal standards.

T2 MY HEDHAMMAR

BioSilk - A bioactive material made of recombinant silk fusion proteins

Hedhammar M[1]

[1] *KTH, Royal Institute Of Technology, Stockholm, Sweden*

Spider silk is composed of special proteins, spidroins, arranged in intricate structures that give unique material properties of combined strength and elasticity. Characteristic features of spidroins are their highly repetitive amino acid sequences rich in glycine and alanine, flanked by conserved domains at each end. We have used recombinant DNA technology to produce partial variants of spidroins that can self-assemble into macroscopic fibrillar materials that mimic the properties of silk.
By genetic engineering we are also able to incorporate foreign elements in a site-specific manner that add desired biochemical functions to the material. For example, we have constructed silk decorated with cell adhesion sites, antimicrobial functions, enzymatic activity and selective affinity.
This novel type of hybrid bio-silk material possesses favorable properties otherwise only found in synthetic materials (stability and easy manufacturing) combined with essential features of living systems (cell compatibility and biochemically functional molecules).

I will describe the methods used for constructing bioactive silk materials, and demonstrate some examples of how this can be used for various biomedical applications.

T3 SOPHIA HOBER

The development of small protein domains for biotechnological and medical use

Hober S[1]

[1] *KTH-Royal Institute of Technology, Stockholm, Sweden*

Binding proteins are utilized in many applications, both within biotechnology and medicine. In nature, antibodies are the primary affinity proteins and their usefulness has led to a widespread use both in basic and applied research but also in therapy and diagnostics. By using combinatorial protein engineering and protein library technologies, smaller antibody fragments and alternative non-immunoglobulin protein scaffolds can be engineered for various functions based on molecular recognition. Our research is focused on the development of protein-based systems for purification and detection. We are developing small, well characterized and folded domains from bacterial surface proteins to achieve protein domains with different characteristics usable in a range of applications. Specific and effective domains have been developed to render covalent modification of antibodies possible. Protein domains with bispecific binding usable for diagnostic and therapeutic purposes has been developed. Moreover, we have optimized protein domains for stringent protein purification leading to mild purification strategies or/and stable purification matrices. Here, the development as well as the use of novel affinity domains will be discussed.

T4 SARA ROSA

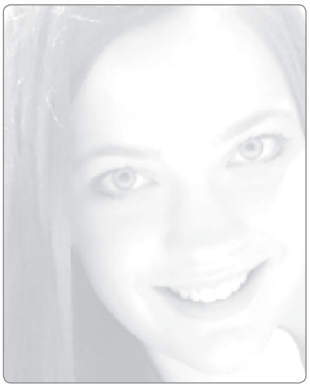
Biothermodynamics of the adsorption of monoclonal antibodies in phenylboronic acid agarose-based supports: Affinity vs multimodal chromatography

Rosa S[1], da Silva C[1,2], Aires-Barros M[1], Dias-Cabral A[3,4], Azevedo A[1]

[1] *iBB- Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal,*
[2] *The Discoveries Centre for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal,*
[3] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal,*
[4] *Department of Chemistry, University of Beira Interior, Covilhã, Portugal*

The increasing market needs involving therapeutic monoclonal (mAb) products, the developments achieved at process upstream, and the emergence of biosimilars fostered the development of new downstream technologies and/or the improvement of existing ones. In this context, novel and cost-effective synthetic ligands are evolving being important to fully characterize them as the interactions that occur with mAbs.

The aim of this work was to investigate the complex phenomena underneath the adsorption of an anti-human IL-8 monoclonal antibody from a clarified supernatant obtained from Chinese Hamster Ovary cell cultures, towards phenyl boronic acid (PBA) ligands by Flow Microcalorimetry (FMC), and understand the role of non-specific effects in the adsorption process. Results obtained showed that anti-human IL-8 mAb adsorption on P6XL resin is enthalpically driven (ΔHads<0), as expected for the predominant reversible esterification reaction between boronic acids or boronate ligands and cis-diols containing molecules. For all the pH conditions under study (7.5, 8.5, 9.0, 9.5 and 10.0), thermograms presented a first exothermic peak characteristic of the pair of covalent bonds formed between mAb (pI≥9.3) and m-APBA (pKa=8.8), with the exception of pH 9.0 in the presence of salt. ΔHads obtained for conditions where cis-diol interactions are predominant (or unique) was approximately -0.28 ± 0.06 kJ/mmol against -0.08 ± 0.01 kJ/mmol for the pH 9.0, 150 mM NaCl condition, at which was proved that electrostatic interactions are prevalent. Moreover, whereas the affinity-based binding of the PBA ligand to mAb has been described for decades, our study demonstrates the multimodal behavior of this interaction.



T5 EVA UDOVIC

An optimization of Raman spectroscopy parameters and implementation of in-line Raman spectroscopy to the key steps of mAB production downstream process

Udovic E[1]

[1] *Faculty Of Chemistry And Chemical Technology, Ljubljana, Slovenia*

Lately, Raman spectroscopy is becoming more and more popular in the field of biopharmaceutical industry due to its ability to support monitoring and control of upstream (USP) and downstream process (DSP) critical quality attributes (CQA). This way of providing (near) real-time process information is a guide idea of process analytical technology (PAT). So far, literature published a lot of work related to implementation of Raman spectroscopy in the USP applications where the ability to measure nutrients, cell viability and metabolites is demonstrated.
Therefore, an optimization of Raman spectroscopy parameters and a concept of implementation of in-line Raman spectroscopy to the key steps of DSP were developed in collaboration with Novartis Technical Development Biologics Menges, Lek d.d.. First, a setup with pump and flow cell was performed to test flow behaviour and find optimal Raman setup parameters under different flow rates, types of material and different constructions of flow cell. As DSP consists of several steps comprising of capture step, viral inactivation, polishing steps, viral filtration and ultrafiltration/diafiltration (UF/DF) step, methodology of implementation in-line Raman measurements was developed to monitor output of individual step representing the input to the next DSP step and regulate process when necessary. The mathematical model was built to predict particular CQAs such as aggregation, buffer components, protein concentration and HCP (Host Cell Protein) content by implementing multivariate data analysis (MVDA).

T6 CARSTEN VOSS

Purification of Large Biomolecules with Tailored Anion Exchangers

Voss C[1]

[1] *Bio-rad Laboratories Gmbh, Munich, Germany*

Large proteins (> 300 kDa) and comparable biomolecules like nucleic acids, viruses, and virus like particles pose substantial challenges to chromatographic purification. Because of their large size these molecules cannot efficiently penetrate the pores of traditional chromatographic materials. Large pore particles that could accommodate these molecules on the other hand have a reduced binding capacity.
Bio-Rad has developed an anion exchanger for the purification of large biomolecules combining medium particle size of 50 µm with large poresize of 0.5 µm to accommodate larger biomolecules, etc. The surface extender technology utilizing grafted polymer to increase DBC of this large pore size resin is also optimized for the binding of larger molecules.
In this presentation, we demonstrate the versatile application of this new anion exchaner in the purification of large biomolecules (IgM, VLP/viruses) and outline how resin characteristics like the ligand density of grafted polymer influence the dynamic binding capacity and selectivity of the resin.

T7 ALOIS JUNGBAUER

Virus, virus-like particles, extra cellular vesicles and gene therapy vesicles purification

Jungbauer A[1,2]

[1] *Boku, Wien, Austria,*
[2] *Austrian Centre of Industrial Biotechnology, Vienna, Austria*

Bionanoparticles such as recombinant viruses, virus-like particles and extra cellular particles are considered as the next generation biopharmaceutical. They have a distinct cell tropism and in addition they can be loaded with other drugs. Recombinant viruses and virus-like particles have the potential of a platform pandemic vaccine for emerging infections. Purification to high degree of purity is a challenge for this next generation of biopharmaceuticals. Purity requirements are much higher compared to existing vaccine, which often only concentrate a clarified culture supernatant. This class of bionanoparticles are contaminated with other extra cellular particles, cell debris, and organelles. They have similar size and often very similar surface properties and thus very difficult to separate from each other, which may be required in future due to the biological function; often detrimental to the product. An overview will be given for the separation of these next generation biopharmaceuticals. Design of purification trains will be demonstrated by purification of enveloped virus-like particles. Purification strategies with flow through principles, packed beds and convective media will be shown. The successful separation of other bionanoparticles from will be demonstrated and the biological impact demonstrated.

T8 PAVEL MARICHAL-GALLARDO

A single-use purification platform for viral vaccines & gene therapy vectors

Marichal-Gallardo P[1], Börner K[2,3], Grimm D[2,3], Genzel Y[1], Wolff M[1,4], Reichl U[1,5]

[1] *Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany,*
[2] *Heidelberg University, Heidelberg, Germany,*
[3] *German Center for Infection Research (DZIF), Heidelberg, Germany,*
[4] *Institute of Bioprocess Engineering and Pharmaceutical Technology, Technische Hochschule Mittelhessen, Giessen, Germany,*
[5] *Chair of Bioprocess Engineering, Otto-von-Guericke University Magdeburg, Magdeburg, Germany*

In steric exclusion chromatography (SXC), a raw sample is mixed with polyethylene glycol (PEG) and fed onto a single-use cellulose column: selectivity is strongly influenced by the target species' size, so SXC is particularly convenient for virus purification. The product is recovered at physiological pH and conductivity. We have observed recoveries ≥95% for several cell culture-based viruses: influenza, yellow fever, Modified Vaccinia Ankara (MVA), and adeno-associated virus (AAV). Host cell DNA and protein depletion are typically ≥90%.

For influenza virus, four strains were produced in MDCK cells. Full recovery of all strains was observed using identical SXC conditions for both infectious and chemically inactivated viruses. The column capacity in terms of the viral hemagglutinin antigen was ≥50 $mg \cdot m^{-2}$. In the case of yellow fever virus, two attenuated strains were produced in Vero cells. Here, full recovery of infective titers was also achieved: the elution fraction was concentrated >100-fold to a titer of $>6\times10^{9}$ plaque forming units (≈100 000 doses). Elution pools from MVA virus produced in continuous bioreactors with an avian cell line contained $\approx3.7\times10^{9}$ $TCID_{50}$. Lastly, several AAV serotypes and display mutants were produced using HEK cells and purified with ≤95% recovery. Elution fractions had $\leq2\times10^{14}$ viral genomes$\cdot L^{-1}$.

It seems that capture with PEG and unmodified cellulose membranes performs very well for a broad range of viruses from different processes. SXC can drastically reduce process development and the high recoveries obtained so far allow adding polishing operations without risking overall low process yields.

T9 CRISTINA PEIXOTO

Improving oncolytic adenovirus manufacturing: designing GMP compatible bioprocesses

Peixoto C[1], Moleirinho M[1,2], Silva R[1], Hagner-McWhirter Å[3], Ahlén G[3], Lundgren M[3], M. Alves P[1,2], J.T. Carrondo M[1,4]

[1] *iBET, Oeiras, Portugal,*
[2] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157, Oeiras, Portugal,*
[3] *GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 , Uppsala, Sweden,*
[4] *Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 , Monte da Caparica, Portugal*

Oncolytic virotherapy represents a new class of cancer drugs that can be used to improve the traditional treatments. Adenovirus is a well-known and extensively characterised oncolytic agent. The rise of the number of clinical trials using this virus generates the demands to develop an improved purification platform to lower costs of treatments.
In phase I and II clinical trials, six unit operations are often utilized for adenovirus purification at 10-50L scale: cell lysis and nuclease treatment; clarification; ultrafiltration; intermediate purification and polishing. The use of Triton X-100 detergent in the lysis step is a popular approach, since it is easily scalable and well described. However, Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation prohibits its use, without authorisation, from 4 January 2021.
In this work, we report an improved oncolytic adenovirus purification process for phase I and II clinical trials. We proposed a 6-steps process using state-of-the-art materials, such as high-throughput Capto Q ImpRes media for intermediate chromatography purification. We also investigated the use of Polysorbate 20, for cell lysis and its effect in product recovery and impurities removal throughout all the steps of the purification process. Overall, over 40% of total and infectious particles was recovered and 99% of the impurities (host cell protein and dsDNA) were removed. Additionally, we will present a case on the use of Polysorb 20 as a replacement for Triton X-100 during cell lysis. Product recovery, potency, purity and the effect of manufacturing holding points will be discussed.

T10 LEIF BÜLOW

One-Step Chromatographic Separations of Wildtype and Mutant Proteins from Crude Biological Fluids Using Molecularly Imprinted Polymers

Bülow L[1], Zhang K[1], Ye L[1]

[1] *Lund University, Lund, Sweden*

Due to the high stability and cost effectiveness of Molecularly Imprinted Polymers (MIPs), they have found applications in a range of fields. However to date, molecular imprinting has been successful mainly for low molecular weight compounds, e.g. drugs, metal ions, sugars, steroids and amino acid derivatives. In the case of biological macromolecules such as proteins, the use of molecular imprinting is still a challenging task. The challenge is largely due to the low and heterogeneous binding capacity as well as slow mass transfer due to the large size of many proteins. Furthermore, proteins are often vulnerable to harsh conditions, such as high/low pH, high temperatures and salt concentrations, which are often used to synthesize MIPs. However, because of the high demands for protein-selective materials in the areas of biology and life science, the development of protein imprinting has shown very rapid progress recently.
The protein-MIP resins can be prepared by several alternative polymerisation methods but particularly Pickering emulsion techniques have resulted in excellent protein binding capacities, often higher than the corresponding immunosorbents. Several different monomers have been examined to enhance the useful pH range of the material. The obtained MIP particles are heavily crosslinked and very robust. This allows for sterilisation by autoclavation without any detrimental effects on column capacity.

The aim of this presentation is to describe the potentials of MIPs as a chromatographic resin to enable a specific isolation of proteins directly from crude and complex biological solutions.

Preparation and Purification of Protein-Mimetic Drug Conjugate with tunable Hydrophobicity

Müller E[1], Endres P[1], Sevilla M[2], Huck T[2]

[1] *Tosoh Bioscience Gmbh, Griesheim, Germany,*
[2] *Karlsruher Institut of Technology, Karlsruhe, Germany*

Antibody Drug Conjugates (ADC) are highly potent biopharmaceuticals with immense potentials as targeting therapy for the treatment of cancer. To date only four ADC's have been approved by FDA, but more than 70 are currently in clinical pipeline.
ADC's are antibodies carrying different toxic drugs (payload) with low molecular weight connected by a cleavable or non- cleavable spacer. The immobilization of the payloads can be performed site-directed or randomized. Despite the chemical structure, payloads greatly vary in their main property of very low aqueous solubility. This influences aggregate formation and overall hydrophobicity of the ADC. The chromatographic purification of ADC's is consequently dependent on the hydrophobicity ratio of payload to antibody.
To investigate this attributes model system was developed based on fluorescein isothiocyanate (FITC) as mimetic payload immobilized onto proteins. The solubility of fluorescein in aqueous solution is strongly dependent on pH; therefore, with FITC immobilized onto a protein the overall hydrophobicity can be changed by the pH. This effect influences binding capacities and recoveries in hydrophobic interaction chromatography. Separation of mimetic ADC's with high resolution non-porous resins in analytical HPLC – as well as with preparative Toyopearl HIC resins – will be discussed.

T12 SVENJA NICOLIN BOLTEN

Substitution of heparin for purification of recombinant proteins

Bolten S[1], Rinas U[1], Scheper T[1]

[1] *Institute For Technical Chemistry, Leibniz University Hannover, Hannover, Germany*

The highly sulfated polysaccharide heparin belongs to the family of glycosaminoglycans. Its major biological purpose is the inhibition of the coagulation cascade to maintain the blood flow in the vasculature. Therefore, heparin is used in therapeutic applications as anticoagulant especially to cure and prevent thrombosis. Heparin is also involved in further biological processes. These activities are associated with heparin's interaction to diverse proteins.

These heparin-protein interactions take not only an advantage in therapeutic applications but also in biotechnological applications such as in the purification process of recombinant proteins. The application of heparin affinity chromatography is successfully used for protein purification. It is a simple method to purify different types of proteins. While effective, heparin is an animal-based material. Animal-derived components are subject to strict quality controls and the validation of effective GMP implementation. Therefore, efficient alternatives to animal-based materials are extremely interesting to process developers.

A heparin-binding protein was produced in E. coli in a fed-batch cultivation. Different animal-component free purification methods were tested and compared with the heparin affinity chromatography. By design of experiments optimal conditions to bind and elute this protein in these various purification methods will be determined. The final aim is the establishment of an animal-component free purification method consisting of different chromatography techniques to purify heparin-binding proteins.

T13 SHUICHI YAMAMOTO

Tuning pore size of monolithic chromatography for large biomolecule separations

Podgornik A[2], Yamamoto S[1], Yoshimoto N[1]

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

Monolithic chromatography is known to be best suited for high-speed separation of large biomolecules as mass transfer due to pore diffusion does not exist. However, the pressure drop is sometimes the limiting factor for the separation process. In this study, effect of pore size on the performance of ion-exchange monolith tube chromatography of large biomolecules was investigated. Radial flow 1 mL polymer based monolith tubes of different pore sizes (1.5, 2 and 6 micron) were tested with model samples such as 20mer poly T DNA, basic proteins and acidic proteins (molecular weight 14000-670000). Pressure drop, pH transient, the number of binding site, dynamic binding capacity and peak width were examined. Pressure drop-flow rate curves and dynamic binding capacity values were well correlated with the nominal pore size. While duration of the pH transient curves depends on the pore size, it was found that pH duration normalized on estimated surface area was constant, indicating that the ligand density is the same. This was also confirmed by the constant number of binding site values being independent on pore size. The peak width values were similar to those for axial flow monolith chromatography. These results showed that it is easy to scale up axial flow monolith chromatography to radial flow monolith tube chromatography by choosing the right pore size in terms of the pressure drop and capacity.

[1] Reference Podgornik et al., Electrophoresis, 38, 2892-2899(2017)

T14 GÜNTHER ALLMAIER

Nano Bioparticle (Liposomes, VLPs and viruses) Separation by means of differential mobility analysis in combination with spectroscopy and mass spectrometry

Weiss V[1], Pittenauer E[1], Allmaier G[1]

[1] *TU Wien (Vienna University of Technology), Vienna, Austria*

The characterization of different kinds of liposomes, virus-like particles (VLP) and virus preparations plays a growing role due to the fact of the increasing use and development of complex biological drug delivery systems as well as direct applications of VLPs and viruses. The physico-chemical characterization (size and molecular mass) by means of nano electrospray linked to charge reduction by Po-210 or soft X-ray radiation in combination with differential mobility analysis (nano ES-DMA,) and subsequent size-fractionation followed by on-line mass spectrometry and spectroscopy is gaining growing interest. The focus is on size-dependent lipid and protein compositions and drug loads. In case of liposomes and viruses the present lipidome and the detected proteins are of extraordinary interest due the variation of composition due to wide range of sizes. The size covers single-digit nanometers up to a couple of hundreds nanometers in case of liposomes. Nano ES-DMA will allow the determination of the dry, i.e. diameter of such desiccated nanoparticles and their size distributions. Afterwards nES-DMA size-separated bionanoparticles were collected by means of an electrostatic nanoparticle collector at atmospheric pressure on high quality MALDI MS targets or other suitable surfaces (e.g. mica for AFM or grids for EM) and subsequently analyzed. This will be the first step in the structural characterization of such complex systems. It will be followed in case of the different detected lipid classes as well as drug compound(s) by MS/MS. Such size-correlated data might open up new ideas of optimization of nanobioparticle preparations.

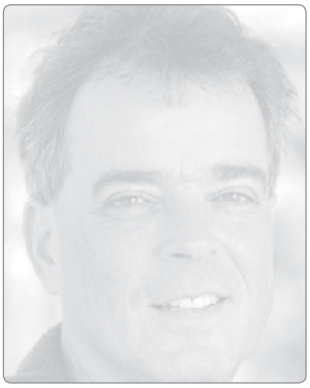
T15 HERBERT LINDNER

How does CE-MS compare with LC-MS in peptide and PTM analysis?

Lindner H.[1]

[1] *Medical University of Innsbruck, Division of Clinical Biochemistry*

Post translational modifications (PTMs) are important indicators of change in cells. Two of the top four most abundant PTMs are deamidation and phosphorylation. LC-MS methods struggle to identify and quantify Aspartate and iso-Aspartate isomers (associated with deamidation) as they have the same mass and similar fragmentation patterns, which often result in false positives. Location of the phosphorylation site is important in understanding the effect of this modification on the activity of proteins. Phosphorylated peptides are often polar and elute early in traditional LC analyses and positional isomers of phosphorylated peptides are identical in mass and have very similar fragmentation patterns, which makes their identification difficult by LC-MS alone. As both techniques are orthogonal, and CESI-MS can detect unique PTM sites, which were missed when only LC-MS was used, the combination of LC-MS and CE-MS provide essential more information on the sample composition. CE-MS can also provide the separation and detection of mono-phosphorylated peptide isomers, sites of citrullination and other challenging PTMS in a single analysis of biological samples. The advantages and disadvantages of both techniques, LC-MS and CE-MS, for the analysis of biological samples with a focus on challenging PTM analysis will be discussed.



T16 MICHEL EPPINK

Microfluidics as a tool in DSP for biopharmaceutical proteins

Eppink M[1]

[1] *Synthon Biopharmaceuticals BV, Nijmegen, Netherlands*

Production and development of biopharmaceutical proteins (e.g. monoclonal antibodies) is mainly performed in Chinese Hamster Ovary (CHO) cells. The process development of these biotherapeutic proteins includes Upstream and Downstream process development. During the Downstream Processing the protein of interest is purified according a chain of purification steps and exposed to different buffer, pH and light conditions as the purification process may take one/two weeks before purified product is obtained.

In this presentation a high-throughput microfluidics method will be explained which is able to detect modifications (e.g. deamidation, oxidation, glycosylation) in monoclonal antibodies. An imaging surface plasmon resonance (iSPR) platform is used in real-time and label-free.

T17 CÉDRIC DELVAUX

Combination of Capillary Electrophoresis and Ion mobility coupled to Mass Spectrometry and Theoretical Calculations for cysteine connectivity identification in peptides bearing two intramolecular disulfide bonds

Delvaux C[1], Massonnet P[1], Kune C[1], Upert G[2], Mourier G[2], Haler J[1], Gilles N[2], Quinton L[1], Far J[1], de Pauw E[1]

[1] *Mass Spectrometry Laboratory, University Of Liege, Liege, Belgium,*
[2] *Commissarait à L'Energie Atomique, DSV/iBiTec - S/SIMOPRO , Gif-sur-Yvette, France*

Disulfide bonds are post translational modification playing essential roles in the biological activity and stability of numerous peptides and proteins. Intra-molecular disulfide bonds are found in various natural-occurring peptides such as animal venoms. In such peptides, the appropriate cysteine connectivity provides the required conformation for efficient binding to their molecular targets, which ensures their bioactivity. The characterization of cysteine pairing is still a challenging issue in the analysis of peptides targeting pharmaceutical or pharmacological utilizations. Thus, the use of sensitive, robust and efficient characterization techniques to access the cysteine pairing is crucial.
In our workflow, the separation of the disulfide isomers of three peptides bearing two intra-molecular disulfide bonds but different cysteine connectivities have been tested using Capillary Zone Electrophoresis (CZE) and Ion Mobility (IM) coupled to Mass Spectrometry (MS). Results show that CZE-MS and IM-MS act as complementary techniques to unambiguously determine the cysteine connectivity of a given peptide. Indeed, the combination of the relative migration time to a reference peptide in CZE-MS, the drift time in IM-MS and the generation of fragments by Collision Induced Dissociation (CID) led to the attribution of the disulfide connectivities in all studied cases. Finally, theoretical calculations were performed to model the different structures in gas phase and solution, supporting the experimental observations on the basis of their predicted physicochemical properties.

T18 ANJA KRISTL

Influence of pressure on gradient separations of biomolecules by ion exchange chromatography

Kristl A[1], Pompe M[1], Podgornik A[1]

[1] *Faculty of Chemistry and Chemical Technology, Ljubljana, Slovenia*

Studies have shown that separation under high pressure alters the retention of biomolecules. For isocratic separations on reversed phased column the effect was estimated and explained using theoretical models. Such an attempt has not been made for applications of ion exchange chromatography, which is often utilized for separations of proteins and other macromolecules because of its excellent resolving power and biomolecules' stability during separations. In our study, we used a novel anion exchange column that can withstand UHPLC conditions to study the effect of pressure on the retention of biomolecules. To generate pressures of up to 550 bar we connected restrictor tubing to the column outlet and conducted experiments at a constant flow rate and temperature, while the composition of the mobile phase changed with a linear gradient of salt concentration at a constant pH value. Our results show an increase in retention of biomolecules with increasing pressure, observing greater difference for the separations of less rigid macromolecules. To account for the pressure effect on the ion exchange column, the ionic capacity of the stationary phase was characterised via pH transition profiles and no significant change was observed. Changes in the elution were also evaluated with a stoichiometric displacement model for linear salt gradient elution at a specific pressure. The results indicated a change in the molecular molar volume and in some cases also in the number of binding sites.

T19 FÁTIMA MILHANO SANTOS

Development of an artificial neural network for vitreous protein profiling by bidimensional electrophoresis

Milhano Santos F[1,2,3], Mesquita Gaspar L[1], Albuquerque T[1], M. L. Dias J[4], Paulo Castro e Sousa J[5], Paradela A[6], Teixeira Tomaz C[1], António Passarinha L[1,2,3]

[1] *CICS-UBI - Health Sciences Research Centre, University of Beira Interior , 6201-506, Covilhã, Portugal,*
[2] *Laboratory of Pharmacology and Toxicology - UBIMedical, University of Beira Interior, 6200-284, Covilhã, Portugal,*
[3] *UCIBIO@REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal,*
[4] *University of Cambridge, Cambridge System Biology Centre, Cambridge CB2 1GA, United Kingdom ,*
[5] *Hospital Center Leiria-Pombal, 3100-462 Pombal, Portugal,*
[6] *Unidad de Proteomica Centro Nacional de Biotecnología, CSIC, Madrid 28049, Spain*

Due to its pivotal localization, vitreous proteome has been studied to elucidate the pathogenesis of ocular diseases [1,2]. Bidimensional electrophoresis (2DE) has been applied for vitreous analysis but optimized experimental protocols may improve its coverage. Artificial neural networks (ANN) may allow to fully understand the complex interactions between multiple factors implicated in 2DE throughput. In this work, the recovery and detection of vitreous proteins by 2DE was enhanced using an ANN, which combines different solubilizing agents (CHAPS, Genapol, DTT, IPG buffer), temperature (15, 20, 25oC) and total volts-hour (35, 40, 45 kVh). The highest protein recovery (94.90%±4.49) was achieved using 4% CHAPS, 0.1% Genapol, 20 mM DTT, and 2% IPG buffer. The number of vitreous spots detected was modeled using an ANN model slightly biased with a slope and intercept of 0.91 and 20.49, respectively. Two iterations were required to achieve an optimized response (580 spots) using 4% CHAPS, 0.2% Genapol, 60 mM DTT, 0.5% IPG buffer at 35 kVh and 25ºC. The optimal conditions represent a 68%/20% improvement, respectively, over the standard and the best conditions of the experimental design. The analysis of depleted vitreous under optimal conditions resulted in a greater protein detection, with more than 600 spots. The high-throughput of this model provides an effective starting point for the improvement of sample preparation protocols for 2DE, and even to other proteomics techniques.

[1] FM Santos, et al., Int. J. Mol. Sci. 2018, 19, 1 – 22.
[2] LM Gaspar, et al., J. Chromatogr. B. 2017, 1061–1062, 334–341.

T20 BARRY BOYES

Protein Variant Separation Improvements using Superficially Porous Particles and Operating Condition Manipulations.

Boyes B[1], Libert B[1], Schuster S[1], Miles W[1], Lawhorn J[1], Destefano J[1]

[1] *Advanced Materials Technologies, Inc., Wilmington, United States*

High resolution analysis of larger proteins using very wide pore superficially porous particles (SPPs) of silica has now been well established. We have previously demonstrated that full advantage of the improved mass transfer properties of SPPs for separations of large biomolecules (>50,000 MW) require very large pore sizes. We have recently added several stationary phase options for reversed phase operation, including alkyl and aromatic silane surface bonded phases. Comparisons of short chain alkyl (C4), long chain alkyl (C18) and aromatic (diphenyl) bonded phases on 1000 Å pore size SPP demonstrate similarly high separations efficiencies, but varying the bonded phase enables tuning selectivity for separations of highly similar protein variants. Options to manipulate large molecule separations thus include the combination of varying bonded phase, acidic mobile phase additive selection, (FA, DFA, TFA), organic solvent selection, and column temperature. Systematic selection of these variables leads to improved resolution of highly similar protein variants. Examples of this approach include high resolution intact protein LC/MS analysis of difficult IgG mixtures, for example, the complex mixtures of lower abundance free sulfhydryl variants present in therapeutic mAbs. We describe resolution of these challenging bridge and free thiol variants, and outline a strategy to define the site-specific variant protein isoforms in therapeutic formulations of mAbs.

T21 KIYOHITO SHIMURA

Immunoaffinity Capillary Isoelectric Focusing: Two facile micro analytical platforms for charge variants of protein pharmaceuticals

Shimura K[1]

[1] *Fukushima Medical University, Fukushima, Japan*

Protein pharmaceuticals often accompany charge variants. Capillary isoelectric focusing (CIEF) is a perfect choice for analysis of such charge variants. For a protein in a biological sample, however, sample pretreatment is necessary to exclude interference from a massive sample matrix. We have developed two micro platforms for charge-variant analysis of protein pharmaceuticals in biological samples, i.e., APCIEF and ACCIEF.
AP is an “affinity probe” that is an Fab fragment of antibody and is labeled with a fluorescent dye. It is added to a sample, and the formed antigen-AP complexes are separated with CIEF. Each variant can be quantified as different complex peaks. The detection of charge variants of erythropoietin using APCIEF will be demonstrated.
AC represents “affinity capture” or “affinity chromatography”. An AC column bearing immobilized antibody is directly connected to CIEF capillary. Captured proteins are eluted from the column by filling it with an acidic anode solution and subjected to separation in the connected capillary for CIEF. ACCIEF allows complete transfer of captured proteins from a sample of several-μL volume to CIEF separation and can reject matrix effect of even 50% serum.

The specific detection using AP and the micro purification with directly coupled AC column would expand the utility of CIEF for charge-variant analysis of protein pharmaceuticals from research and development to process monitoring in productions.

T22 GERHARD HEINZMANN

Electrical Asymmetrical Flow Field-Flow Fractionation coupled with Online Multi-Angle Light Scattering: Advanced Separation and Characterization of Charged and Polarizable Nanoparticles, Liposomes, Proteins, Antibodies and Viruses

Heinzmann G[1], Meier F[1], Klein T[1]

[1] *Postnova Analytics GmbH, Landsberg, Germany*

Field-Flow Fractionation (FFF) was invented in 1966 by Prof. Calvin Giddings, a scientist at the University of Utah in Salt Lake City, USA [1]. FFF is nowadays a reliable analytical tool for the separation and comprehensive characterization of Synthetic Polymers, Biopolymers, Nanoparticles and Microparticles [2,3]. Principle of the FFF Separation Technique is a separation field that is perpendicular to the laminar eluent flow in a separation channel or hollow fiber (Figure 1). The separation field can be a cross flow [4], a temperature gradient [5] or a gravitational field [6].
A new FFF-Technology is Electrical Asymmetrical Flow Field-Flow Fractionation; additional to a cross flow an electrical field is applied to the FFF separation channel.

In this presentation the technique of Electrical Asymmetrical Field-Flow Fractionation will be discussed. Several application examples will be presented that illustrate the performance of EAF4 coupled with online Multi-Angle Light Scattering (MALS) for the comprehensive characterization of charged Nanoparticles, Liposomes and Biological Macromolecules such as Proteins, Antibodies and Viruses.

T23 DIERK RÖSSNER

Sample injection with reduced pressure-drop for size exclusion chromatography for improved protein aggregate detection

Roessner D[1]

[1] *Wyatt Technology, Dernbach, Germany*

One solution for understanding and characterization of protein aggregation in biopharmaceuticals is Size Exclusion Chromatography (SEC). Monitoring the molecules eluting from SEC with online Multi Angle Light Scattering (MALS) adds additional information. MALS measures absolute molar masses and sizes, improving the qualitative characterization of monomers and aggregates, for example the composition of ADCs or glycosylated proteins.
The stationary phase of a SEC column is a porous solid. Common porous solids are silica or porous gels. The separation in SEC is based on the available pore volume for the eluting molecules, allowing large molecules to elute early, small molecules to elute late and very large molecules being excluded from separation. Protein aggregates elute in SEC due to their size early.

In my presentation I will show the effect of early eluting ghost peaks on the qualitative and quantitative characterization of protein aggregates. The ghost peaks are produced by the pressure drop during sample injection. I will show that the problem can be reduced or overcome by using an autosampler with pre-pressurize function for the sample loop.

In this presentation the technique of Electrical Asymmetrical Field-Flow Fractionation will be discussed. Several application examples will be presented that illustrate the performance of EAF4 coupled with online Multi-Angle Light Scattering (MALS) for the comprehensive characterization of charged Nanoparticles, Liposomes and Biological Macromolecules such as Proteins, Antibodies and Viruses.

T24 ALEŠ PODGORNIK

Analysis of adsorption phenomena and matrix based on pressure drop data

Podgornik A[1]

[1] *Faculty Of Chemistry And Chemical Technology, University Of Ljubljana, Ljubljana, Slovenia*

Measurement of pressure drop is extensively implemented in many areas, because of its importance in determination of thermodynamic equilibria and as a driving force for liquid flow. In chromatography it is mainly used to monitor performance of chromatographic columns. In addition to this information, pressure drop data can provide also a valuable insight into a structure of chromatographic support present in chromatographic column, becoming especially interesting due to increasing variety of microstructures encountered in convective based chromatographic supports.
In this work, we present methodology how pressure drop data can be used to obtain different type of information. Pressure drop can provide an insight into adsorption phenomena, more precisely, giving estimation about thickness of layer adsorbed on a matrix pore surface. Based on recently developed mathematical formalism we are able to estimate thickness of adsorbed plasmid DNA molecules of different size and different bacteriophages but also proteins. This approach can also be used for determination of thickness of grafted layer and good correlation between graft thickness estimated from pressure drop data and dynamic binding capacity was found. As recent methodology enables estimation of layer thickness without detailed information of monolith micro-topology, and based on the model accuracy of measurement can be estimated. This simple method can be used on any convective based chromatographic support and can also estimate effect of mobile phase on the matrix.

T25 NADIA GALEOTTI

Influence of Mixed Electrolytes on HIC-Adsorption and Prediction of Chromatographic Elution Profiles

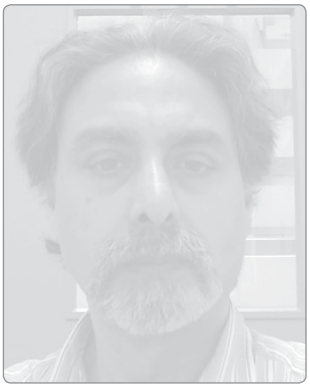
Galeotti N[1], Hackemann E[1], Hasse H[1]

[1] *Laboratory of Engineering Thermodynamics (LTD), University of Kaiserslautern, Kaiserslautern, Germany*

Proteins are often purified using chromatographic techniques in which the properties of the aqueous solvent are controlled by adding salts. The influence of the addition of single salts has been studied intensively, while much less is known on the effect of mixed salts. Therefore, a systematic study on the influence of single and mixed salts on protein adsorption was carried out using different model systems (see below). Unexpected synergetic behavior was observed.

The studied solutes are lysozyme, bovine serum albumin and poly(ethyleneglycol) (PEG). The salts are sodium chloride, ammonium sulfate, sodium sulfate, ammonium chloride and their mixtures. A 25 mM sodium phosphate buffer was used to adjust the pH value to 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 and elution profiles were measured at 298 K with an Äkta chromatographic system.

A mathematical model is developed, that describes the influence of the salts on the ad¬sorption. The model accounts for the influence of the different single ions and their cross-interactions. Using the model, adsorption isotherms can be predicted for different ionic strengths and different salts, including also salt mixtures. The model enables the identification of promising salt mixtures for chromatographic separations. Moreover, a simple equilibrium stage model was developed for the prediction of elution profiles. It enables the identification of suitable salt gradients for separations.



T26 AJOY VELAYUDHAN

Analysis of modulator effects in the polishing SMB of proteins

Wayne C[1], Velayudhan A[1]

[1] *University College London, London, United Kingdom*

Simulated moving bed (SMB) chromatography has now been well established for the capture chromatography of proteins. However, its use for polishing is less well understood. In recent work, we have demonstrated that accounting for the mobile phase modulator (MPM), such as the salt in ion exchange chromatography, as a bona fide component of the system produces novel and interesting effects. These effects, which do not arise if the MPM is simply treated as part of the background, can change the quality of a ternary protein polishing separation that uses steps or gradients of MPM to achieve the separation of a product protein from a ternary mixture. We show that, when the MPM is treated as another feed component, using the true moving-bed (TMB) approximation sometimes gives inaccurate results; thus only an SMB analysis will suffice to give accurate predictions. On this basis, a novel three-dimensional design space is proposed that is very useful for the rapid design of efficient ternary SMB separations. These efficient separations are near-optimal, and depend on the selection of four conditions in the form of the letter N; hence the approach is called N-theory. The accuracy of N-theory is demonstrated with several carefully chosen experiments using various ternary protein mixtures. It is also shown that this approach results in an efficient process for protein separations by SMB within the operationally simple framework of truly continuous flows of all species through all columns. This approach should dramatically simplify fault diagnosis and rectification in a manufacturing setting.

T27 TATJANA TURRINA

Potential-Controlled Chromatography - a Simple and Innovative Separation Technique for Charged Biomolecules

Turrina T[1], Fraga-García P[1], Berensmeier S[1]

[1] *Technical University of Munich, Garching, Germany*

Biomolecule capture and purification techniques can be an expensive part of downstream processing, reducing the profitability of the entire biotechnological process. A simple technique, called potential-controlled chromatography (PCC), has been developed which reduces costs and saves time. This technique combines electrochemical and chromatographic aspects and can be applied for all kinds of charged or polarized molecules, requiring only conductive resin material. Common disadvantages of established ion exchange chromatography processes can be avoided, in particular no salt elution step for column regeneration is required. By applying an electric potential to the stationary phase material, which functions as working electrode in the specific three-electrode setup, the resin surface charge is modified. Consequently, counter-charged molecules are retarded, while similar-charged molecules pass through. With a potential switch, the surface charge changes and electrosorbed molecules desorb. Using bare carbon-based resins, like flexible carbon nanotubes (CNT-K) or tough glassy carbon (Sigradur G), an innovative path for PCC is found. In current research, a multi-scale concept affording a high degree of interdisciplinarity is used to ascertain the different effects occurring at nano-, micro- and macro-scale levels. Thus, the packing quality is analysed in dependence on the mobile phase parameters, electrode setups and potential effects. To validate the experimental process, simulation tools, such as chromatography and fluid dynamic programs, are used. With the cross linkage of different disciplines, material properties and process characteristics can be confirmed and correlations compiled. Assembling the acquired knowledge, the fundament of an industrially applicable, simple yet multifunctional separation process, is hence extended.

T28 SONJA BERENSMEIER

New stationary phases as alternatives to conventional chromatography

Berensmeier S[1], Schwaminger S[1], Blank S[1], Turrina T[1], Fraga García P[1]

[1] *TU Munich, Bioseparation Engineering Group, Garching, Germany*

An expensive part in downstream processing arises from the often complex and sensitive surface functionalization of adsorption matrices or from the high consumption of ionic eluents, respectively. The talk shows the potential of two alternative stationary carrier materials; a) magnetic nanoparticles for capture and first purification steps by magnetic separation, and b) carbon nanotubes as conductive material in potential-controlled chromatography (PCC).

From the experience of the last years we have learned to develop processing forms appropriate for separating proteins or whole cells from fermentation broths using a platform of low cost magnetic nanoparticles. The biocompatible magnetic nanoparticles (iron oxide, MNP) are a promising material that has shown applicability in many areas. For defined surface properties MNP had been systematic synthesized and extensively characterized with regards to chemical composition, size distribution, shape, magnetization and surface properties. Our studies revealed an average size of 15 nm and high saturation magnetization of over 70 Am2kg-1.

Many new fields of application have also opened up for carbon nanotubes in the last years. We show their use in a simple potential-controlled chromatography technique, which reduces costs and saves time. This technique combines electrochemical and chromatographic aspects and can be applied for the separation and purification of molecules with charged or polarized character. In comparison to conventional chromatography, the PCC technique requires neither specific functionalized resin material nor particular salt elution steps for column regeneration. Its basic working principle is originated in electrosorption, which is driven by applying an electric potential to the stationary phase material.

T29 MILTON HEARN

Selectivity Modulation of Synthetic Peptides with Dynamic Hydrophobic Silica-based Adsorbents

Hearn M[1], Boysen R[1], Chowdhury J[1]

[1] *Monash University, Clayton, Australia*

As documented in recent investigations from this laboratory, the development of new types of stationary phases for the efficient purification of synthetic peptides, as well as recombinant proteins, under isocratic elution conditions can be based on alternative approaches for selectivity modulation, whereby the physical attributes of the stationary phase in response to an external stimulus rather than changes in chemical composition of the mobile phase are optimised. During the course of these investigations, surprising features of the interactive behaviour and structure-retention relationships of synthetic peptides related to the thrombin receptor-activating peptide (TRAP-1) with crystalline and isotropic states of comb-shaped n-octadecyl acryl polymer bonded silica and other classes of chemically modified n-alkylsilica stationary phases were discovered. These findings parallel other observations related to the structure function of these peptides. For example, serine proteases, such as thrombin, are known to activate protease-activated receptors (PARs) by cleaving their amino-terminal extra-cellular domains to reveal a new amino terminus that then functions as a tethered ligand. Peptides that conformationally mimic the agonist motif present within PAR-1 are capable of receptor activation in the absence of thrombin. The retention and interaction thermodynamics of various TRAP-1 related peptides with dynamic hydrophobic adsorbents replicate these structure-function properties, possibly due their ability to adopt analogous conformational states dominated by similar hydrophobic contacts of same amino acid side chains with these adsorbents as are responsible for receptor recognition. This presentation explores the origin of this phenomenon and its potential to holistically link structure-retention dependencies with structure-function relationships of bioactive peptides.

T30 CRISTINA DIAS CABRAL

The adsorption mechanism behind biomolecule adsorption onto a salt-tolerant anion exchanger: a calorimetric perspective.

Duarte J[2], Dutra G[1,2], Marques F[1,2], Dias-Cabral C[1,2]

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

It is well recognized that ion-exchange chromatography is a powerful technique for the separation/purification of biomolecules on large process scale. However, when starting with high conductivity feedstocks, additional steps are required before loading, which increases process time and costs. Thus, there is need of specifically designed resins for the direct processing of biological feedstocks. Toyopearl NH2-750F is an anion exchange resin that fits well with these expectations. It can maintain high DBC at conductivities greater than 30mS.cm-1 and works even at a pH similar to biomolecule isoelectric point. Nevertheless, its mechanism of interaction is still not fully understood.
Flow microcalorimetry is used to shed light on the mechanisms of interaction supported by Toyopearl NH2-750F, as it has been proved to provide an improved understanding of the driving forces involved. BSA is used as model protein, to elucidate how salt concentration, pH and the protein surface concentration affect its adsorption onto the up mentioned salt tolerant resin.
Obtained results demonstrated that driving forces of the adsorption process on salt tolerant supports are different from the ones present when using traditional IEX. Previously demonstrated, traditional IEX thermograms include an initial endothermic event, related to the desolvation process, followed by an exothermic event associated with the interaction between the protein and the support. In the case of the analyzed support, at pH 8.0 a single exothermic peak is observed conversely to pH 6.0 where two exothermic events are present. Possible explanations for the different profiles are discussed and supported by other parallel analysis.

T31 URSULA SIMON

3D-printed anion exchange monoliths for protein separations

Simon U[1], Dimartino S[1]

[1] *The University Of Edinburgh, Edinburgh, United Kingdom*

3D-printing, also known as additive manufacturing, allows to fabricate structures with high structural complexity and resolution down to micron scale according to digital models. The potential to 3D-print porous structures for chromatography is receiving increased attention. In fact, it can create highly ordered structures in contrast to traditional random stationary phases. The concept idea has been demonstrated recently, however, the application in protein separations has remained limited, mainly due to the lack of materials suitable for chromatography and compatible with 3D-printing techniques. In this work, a completely novel 3D-printable material for the printing of anion exchange monoliths has been developed. The novel material, composed of the crosslinker poly(ethylene glycol) diacrylate and the bifunctional monomer [2-(Acryloyloxy)ethyl]trimethylammonium chloride, is endowed with positively charged quaternary amine groups suitable for protein capture. The new material was optimised by studying the effect of ligand density on protein adsorption capacity and protein recovery. Exploiting the advantages of 3D-printing, different geometries for the stationary such as the Schoen Gyroid and Schwarz Diamond were analysed regarding flow distribution within the column.
Using this novel material, anion exchange monoliths with a porosity of 50 % and channel diameters below 500 µm were 3D-printed and their capability to separate protein mixtures was investigated. Batch adsorption as well as flow through experiments were carried out using both, model mixtures of proteins and cell crude extracts. The results revealed separation performances comparable to commercial chromatography materials, both in terms of protein adsorption and pressure drops characteristics.

T32 MATTHIAS FRANZREB

Temperature-controlled purification of monoclonal antibodies applying travelling heating zone chromatography

Ketterer B[1], Franzreb M[1]

[1] *Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany*

Bioprocess chromatography suffers from several problems that compromise its sustainability. Current 'adsorption – desorption' processes are expensive, suffer from low productivity (given that most involve batch-wise operation in fixed beds), and employ large volumes of buffer for equilibrating, washing, eluting and cleaning of columns. Recently we developed the principle of travelling heating zone chromatography (THZC), allowing quasi-continuous chromatography within a single column1). Ad-/desorption can be controlled by external temperature fields without changing the mobile phase composition. Therefore, elution can be decoupled from the mobile phase velocity, a feature enabling exact control of elution times and sharp peak profiles. In former studies, thermoresponsive ion-exchange resins have been used with the THZC principle for purification and concentration of lactoferrin. Recently a thermoresponsive Protein A resin has been launched by the company Nomadic Bioscience. By the combination of this resin and our THZC we could demonstrate a single column quasi-continuous capture of IgG with 90% yield and purities > 95%. Besides capture, the system can also help to make buffer exchange steps more economical, reducing original salt components to less than 0.1% while requiring only two sample volumes of new buffer.

[1] Cao, P.; Müller, T. K. H.; Ketterer, B.; Ewert, S.; Theodosiou, E.; Thomas, O. R. T.; Franzreb, M., Integrated system for temperature-controlled fast protein liquid chromatography. II. Optimized adsorbents and ‚single column continuous operation'. Journal of Chromatography A 2015, 1403, 118-131

T33 CHANTAL BRÄMER

Continuous purification of industrial relevant proteins with a self-established periodic counter-current chromatography device

Brämer C[1], Scheper T[1], Beutel S[1]

[1] *Institut für Technische Chemie Universität Hannover, Hannover, Germany*

Batch chromatography has several disadvantages, such as insufficient utilization of the resin capacity, high buffer consumption and discontinuity. Therefore and considering the high costs for downstream processing, a continuously working chromatography system was established. The basic principle of the setup is periodic counter-current chromatography (PCCC) which enables continuous operation by a sequential use of circularly arranged columns. To achieve maximum productivity, the system is operated with membrane adsorbers instead of commonly used bed columns because mass transport is mainly due to convection.

The PCCC-system consists of three membrane adsorber units (3MA-PCCC). It was tested and optimized with the model proteins BSA and lysozyme and was further used for capturing and purification of Candida antarctica lipase B (CalB) with anion exchange chromatography and patchoulol synthase with affinity chromatography directly from cell lysate in one single unit operation.

In the course of work, the system has been extended to provide full functionality and flexibility, as well as the option of integrating a 4th membrane adsorber unit. Compared to the batch process, the productivity increased by 40% and the buffer consumption was reduced by 20%. It could be found that the application of the PCCC-principle is advantageous especially for secreted products and affinity interaction.

Therefore and in order to evaluate the potential of the PCCC-device, it was focused on affinity chromatography of a monoclonal antibody from CHO supernatant in further experiments. Protein A chromatography with Sartobind® Protein A membrane adsorber was optimized and transferred to the PCCC-device for monoclonal antibody capturing.

T34 HANS BLOM

Productivity considerations for batch and continuous chromatography

Blom H[1]

[1] *GE Healthcare, Uppsala, Sweden*

Process intensification is driven by the need to reduce process time and cost, and can be accomplished, for example, by increasing the level of automation, implementation of single-use technologies, or by removal of intermediate hold steps. Moving from batch to continuous processing is another way to intensify your process. While continuous processing is well established in upstream applications, such as perfusion cell culture, implementation in downstream applications is still an evolving field. This presentation will focus on downstream purification of biomolecules in continuous bioprocesses using periodic counter-current chromatography (PCC). As a first step, the main process priority needs to be defined. Although productivity is commonly used as a measure of process efficiency, what ultimately drives process improvement is how this translates into process economy. To understand what can be achieved from a process, a fundamental understanding of the main process parameters and their relationships is of utmost importance. Improvement of one parameter often comes at the cost of another. For example, it is not possible to simultaneously achieve significant reductions in both resin volume and process time. To exemplify, different scenarios comparing batch chromatography with continuous chromatography, using ÄKTA™ pcc chromatography system in three and four column setups, will be discussed.

T35 GUIDO STRÖHLEIN

A stackable, single-use chromatography cassette enhancing continuous antibody capture

Stroehlein G[1]

[1] *JSR Life Sciences, Leuven, Belgium*

In downstream processing of biopharmaceuticals, increasing productivity and reducing footprint as well as cost of goods through continuous processing has been the subject of many presentations and discussions in the recent past. A stackable, single-use and pre-packed chromatography cassette with a supported bed (Chromassette) is a novel product concept in chromatography, combining the separation capability of conventional chromatography resins with the benefits of a stackable, pre-packed modular cassette format. These devices enable truly flexible scaling to meet capacity requirements and provide high convenience, even with a 5~6-cm bed height. A set of experimental data will be presented, showing how Chromassette enhances a continuous chromatography capture step, leveraging in particular the advantage of scalable short bed heights. Due to the supportive scaffold inside the cassette, this chromatography format allows using very high flow rates, thereby working synergistically with continuous manufacturing schemes.

T36 GIORGIO CARTA

Gradient Elution Behavior of Proteins in Hydrophobic Interaction Chromatography with U-Shaped Retention Factor Curves

Carta G[1]

[1] *University Of Virginia, Charlottesville, United States*

Protein retention in hydrophobic interaction chromatography is described by the solvophobic theory as a function of the kosmostropic salt concentration. In general, an increase in salt concentration drives protein partitioning to the hydrophobic surface while a decrease reduces it. In some cases, however, protein retention also increases at low salt concentrations resulting in a U-shaped retention factor curve. During gradient elution the salt concentration is gradually decreased from a high value thereby reducing the retention factor and increasing the protein chromatographic velocity. For these conditions, a steep gradient can overtake the protein in the column, causing it to rebind. A local equilibrium model is presented along with predictions based on the general rate model of chromatography. We show that the normalized gradient slope determines whether the protein elutes in the gradient, partially elutes, or is trapped in the column. Experimental results are presented for two different monoclonal antibodies and for lysozyme on Capto Phenyl (High Sub) resin. One of the mAbs and lysozyme exhibit U-shaped retention factor curves and for each, we determine the critical gradient slope beyond where 100% recovery is no longer possible. Elution with a reverse gradient is also demonstrated at low salt concentrations for these proteins. The elution behavior on other less hydrophobic stationary phases is also determined and compared. Understanding this behavior has important practical implications in the design of gradient elution since, in these cases, the gradient slope impacts protein recovery.

T37 DMYTRO IURASHEV

Computational fluid dynamics successfully predicts the scalability of chromatography columns

Iurashev D[1], Schweiger S[1], Dürauer A[1,2], Jungbauer A[1,2], Zanghellini J[1,2], Tscheließnig R[1,2]

[1] *Austrian Centre of Industrial Biotechnology, Vienna, Austria,*
[2] *Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria*

We analyse the peak broadening in chromatography columns using computational fluid dynamics (CFD). Peak broadening is influenced by extra column volume (ECV), mechanical dispersion, and in-beads diffusion. CFD permits to study the influence of these effects separately. Here we analyze the transport of three solutes – silica nanoparticles (SNP), acetone solution and lysozyme solution – in columns of two sizes under non-binding conditions. A mass transfer model (MTM) between mobile and stationary phases and a model of mechanical dispersion are implemented in the CFD environment STAR-CCM+. The implemented MTM combines the (Kataoka) model of external mass transfer with the (Glueckauf) model of pore diffusion. First, the employed MTM is validated versus experiments performed on the larger column. We find that ECV plays an important role in peak broadening of the SNP pulse in that column; it is less important for acetone and is weakly pronounced for lysozyme. Mass transfer is important for high flow rates of acetone and all studied flow rates of lysozyme. Then, peaks broadening is predicted in the smaller column with the packed bed parameters taken from the large column. The scalability of the prepacked columns is demonstrated for acetone and SNP by very good agreement with the experimental data. In contrast to the larger column, peak broadening is dominated by ECV in the smaller column all studied solutes, while the effect of mass transfer is less pronounced. We have shown that CFD successfully models peak broadening in chromatography columns. Our simulations underscore the importance of ECV.

T38 YANNICK KRAUKE

Continuous purification of xylitol by simulated moving bed (SMB) process

Krauke Y[1], Benkhäuser C[1], Luebbert M.[1], Monks K[1]

[1] *Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany*

Simulated moving bed chromatography (SMB) is a continuous chromatography technique that enables binary or pseudo-binary mixtures to be separated into pure substances or fractions. Compared to traditional batch chromatography this process leads to higher yields of purified substances while consuming less eluent and packing material. In traditional chromatographic separations with one column only a small part of the column contributes in the separation, while the predominant part remains unused. This results in a significantly lower productivity compared to a SMB process where the whole column bed is efficiently used.
The SMB process was used to purify xylitol from fermentation mash of a fed-batch culture. Xylitol is the sugar alcohol of xylose which is mainly used in food industry as glucose replacement e.g. in chewing gums. It is classically produced by chemical conversion of xylose to xylitol.
Within the European Valor Plus research project an alternative, biological way of xylose conversion was investigated. Xylose from a hemicellulose hydrolysate was converted to xylitol by a *Candida* yeast strain. HPLC analysis of the fermentation mash revealed that the xylose to xylitol conversion was successful. The chromatogram showed a baseline separation of xylitol from all other compounds and the elution order allowed a potential purification of xylitol by SMB process. The polymer - based Eurokat Ca columns were chosen as solid phase for the purification as they are specialized for sugar separation.
In a next step, a SMB process was designed for xylitol purification using the new KNAUER AZURA SMB system. A set-up with eight Eurokat Ca columns was chosen. The fractions from the extract and raffinate outlets, were analyzed by HPLC. The results confirmed that xylitol was successfully separated. In one hour, 1.8 g of xylitol was purified with 99 % purity and 100 % recovery. In future, experiments the SMB process will be further optimized to increase the throughput, purity, and concentration of xylitol.

This project has received funding from the European Union‘s Seventh Framework Program for research, technological development and demonstration under grant agreement no FP7-KBBE-2013-7-613802.

changing diabetes®

novo nordisk®

Short Presentation Abstracts

SP1 IRSYAD KHAIRIL ANUAR

Bacterial superglue engineered for protein separation and plug-and-display nanoassembly

Khairil Anuar I[1], Howarth M[1]

[1] *University of Oxford, Oxford , United Kingdom*

Peptide tags are a key technology for protein purification, because they can minimize the change to protein function and a consistent process can be used for a wide range of proteins. However, following the affinity chromatography step, many peptide tags provide little value thereafter. By splitting and engineering the Streptococcus pyogenes CnaB2 domain, we previously designed a peptide, SpyTag, that is able to form a spontaneous isopeptide bond upon mixing with its protein partner SpyCatcher. Here we demonstrate evolution and computational design to develop new generations of SpyCatcher and SpyTag with faster interaction and are applicable to protein separation. We show application in bacterial and mammalian expression systems. We also demonstrate that the localization of SpyTag at the N- or C-terminus of the protein of interest does not impact the yield and efficiency of the methodology. Subsequently, the use of SpyTag provides a platform to extend the functionality of a protein of interest. This includes linking to various enzymes or fluorophores, or the generation of multi-specific binders to control cancer cell signaling. SpyTag also helps to produce a variety of protein architectures, with applications including hydrogel functionalization to probe extracellular matrix contacts, super-resolution fluorescent microscopy, and decoration of virus-like particles to accelerate vaccine generation.

SP2 LENNART KLEINFELDT

Structuring and functionalization of iron oxide particles for continuous automated *in situ* protein purification

Kleinfeldt L[1,2], Gädke J[2,3,4], Biedendieck R[2,4,5], Krull R[2,3,4], Garnweitner G[1,2]

[1] *Institute for Particle Technology, TU Braunschweig, Braunschweig, Germany,*
[2] *Center of Pharmaceutical Engineering (PVZ), Braunschweig, Germany,*
[3] *Institute of Biochemical Engineering, TU Braunschweig, Braunschweig, Germany,*
[4] *Braunschweig Integrated Centre of Systems Biology, Braunschweig, Germany,*
[5] *Institute of Microbiology, TU Braunschweig, Braunschweig, Germany*

Magnetic nano- and microparticles have become highly promising for separation and purification, including downstream processing in biotechnology, due to their highly selective manipulation. For these applications, the surface chemistry must be tailored to ensure the interaction with the targeted product.

We have established a multi-step synthesis process for biofunctionalized iron oxide nanoparticles, and studied their application for the purification of recombinant model proteins. The particle surface was modified with a ligand system capable of forming metal complexes with histidine. Purification experiments were performed, utilizing recombinant proteins with His_6-tags produced by genetically engineered Bacillus megaterium. In situ purification was studied by adding the particles to the cultivation broth in shaking flasks. In these experiments, the successful use of the nanoparticles for separation of model proteins with handheld magnets was demonstrated.

To improve the magnetic separation of the particles, the as-synthesized nanoparticles were structured in a spray drying process resulting in micrometer-sized aggregates. The functionalization method was directly applied to the aggregates. The functionalized aggregates were evaluated in a semi-automated in situ purification of the recombinant model protein directly from a growing cultivation of B. megaterium using a lab-scale stirred tank bioreactor with an external separation loop using handheld magnets. The set-up was further developed into an automated mode, and in situ purifications of the previously examined model protein and also an antibody fragment were performed. The approach allows facile and highly efficient selective purification of recombinant proteins and antibody fragments under repeated use, and appears promising for large-scale applications.

SP3 LUIS PASSARINHA

Soluble catechol-O-methyltransferase capture from a *Pichia pastoris* X33 lysate through a new batch method with gellan microspheres.

Passarinha L[1,2,3], Gonçalves C[2], Sousa A[2]

[1] *UCIBIO@requimte, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal,*
[2] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal,*
[3] *Laboratório de Fármaco-Toxicologia-UBIMedical, Universidade da Beira Interior, Covilhã, Portugal*

Nowadays, soluble catechol-O-methyltransferase (SCOMT) has become an interesting biopharmaceutical target due to its relationship in Parkinson disease (PD) [1]. Since 1960s human SCOMT has been extensively studied and subjected to numerous purification strategies [2]. Despite some of this procedures led to the successful resolving of SCOMT atomic structure, some presented as a drawback significant COMT activity and yield losses [2]. Monolithic chromatography has several advantages in nucleic acids purification, however, its application in SCOMT isolation remains a challenge. The present work consists in a new formulation of gellan microspheres by the water-in-oil technique to capture and clarify the SCOMT sample from a Pichia pastoris X33 lysate. Briefly, the cell lysates were obtained from mini-bioreactor Pichia pastoris X33 methanol-induced cultures. Applying an experimental design tool, the gellan microspheres was obtained with 1.27 % of gellan concentration, 750 rpm of stirring and 92.54 ºC of temperature by water-in-oil emulsion technique and reinforced with nickel ions. SCOMT was captured by the gellan spheres using two strategies. The first one consisted in manipulation of pH and ionic strength. SCOMT was retained with citric acid/sodium citrate buffer, pH 4.0, and eluted in a MES buffer, containing 500 mM NaCl, pH 6.2. The second strategy studied the affinity of the SCOMT histidine-tag by the divalent ions from the gellan spheres. SCOMT was almost captured with a Tris buffer, pH 7.5, and eluted with 250 mM NaCl and 500 mM imidazole.

[1] Bonifácio, M.J. et. al., 2007, 13, 352–379.
[2] Pedro, A.Q. et al., 2016, 91, 3035–3044.

SP4 IGOR TADEU LAZZAROTTO BRESOLIN

Purification of murine, rabbit and human IgG onto iminodiacetic acid immobilized onto monolithic disks

de Mello P[1], Lazzarotto Bresolin I[1], Rocha Antunes Pereira Bresolin I[1], Alves Bueno S[2]

[1] *Federal University of São Paulo (UNIFESP), Chemical Engineering Department, Diadema, Brazil,*
[2] *University of Campinas (UNICAMP), Chemical Engineering School, Campinas, Brazil*

Immunoglobulins G (IgG) are antibodies employed in diagnostic and therapeutic areas, requiring high purification levels, with purification being performed using selective techniques, such as adsorption-based chromatography. Among the chromatographic supports used, the monoliths, which are porous materials composed of interconnected channels, but with high mechanical resistance. The objective of this work was to use a monolithic disc column with the IMAC chelating ligand iminodiacetic acid (IDA), as ioniogenic group, for the purification of IgG from mouse, rabbit and human sera. The results showed that the column had good functioning with high percentages of recovery and purity for all sera studied according to SDS-PAGE analysis. The impact of different operating flow rates between 0.5 and 4.0 mL/min was analyzed, which indicated that the increase and/or reduction of this parameter did not alter the values of the eluted mass peaks nor the purity obtained. Reduction of the purification time was confirmed with the use of monoliths, where pressure drops on the column were not observed.

SP5 YASMIN KAVEH BAGHBADERANI

Magnetic Nanoparticles as New Stationary Phases for Antibody Affinity Purification

Kaveh Baghbaderani Y[1], Schwaminger S[1], Fraga-García P[1], Berensmeier S[1]

[1] *Bioseparation Engineering Group, Technical University of Munich, Garching, Germany*

Monoclonal antibodies (mAbs) are still the most important and successful segment of the global biopharmaceuticals market. Over several decades, the fermentation process during antibody production has been optimized in terms of high product titers (up to 10 g L^{-1} [1]). However, the capture of mAbs using affinity chromatography still represents a bottleneck and major cost factor of overall mAb production. This is because of the high costs of affinity resins and limitations regarding lifespan, column bed dimensions, flow rates, and dynamic binding capacities. For this reason, there are repeated calls for an end of the era of using Protein A for mAb capture. However non-affinity approaches to the downstream processing of mAbs are still underrepresented, since they are not as convenient as a generic platform process. Instead, we want to chart a different path by moving away from porous stationary phases for antibody affinity purification. We use Protein A-based ligands optimized by protein engineering [2] and immobilized on low-cost iron oxide magnetic nanoparticles (MNPs). They have high specific surface areas and provide a simple concentration as well as separation of mAbs by inducing magnetic fields. Due to the versatility of the MNPs, they offer the possibility of being used with different coatings, altering the inertness and selectivity of these materials.

[1] Y.-M. Huang, W. Hu, E. Rustandi, K. Chang, H. Yusuf-Makagiansar, T. Ryll, Biotechnol. Prog. 26, 1400 (2010).
[2] M. Freiherr von Roman, S. Berensmeier, J. Chromatogr. A 1347, 80 (2014).

SP6 JURE SENČAR

A narrow residence time incubation reactor for continuous virus inactivation based on packed beds

Senčar J[1], Hammerschmidt N[1], Lima Mantins D[1], Jungbauer A[1,2]

[1] *Austrian Centre for Industrial Biotechnology, Vienna, Austria,*
[2] *Department of Biotechnology, University of Natural Resources and Life Sciences, Austria*

In recent years, the biopharmaceutical industry is moving towards continuous process operation with the ultimate aim of a fully continuous integrated process. To achieve the latter all unit operations have to be operated in a fully continuous or semi continuous mode. One of the steps suitable for such fully continuous operation is the mandatory virus inactivation which is required in production of cell-culture derived biopharmaceuticals. Virus inactivation in process intermediates is typically performed by low pH inactivation or solvent-detergent treatment. Both methods require typically exposure times of at least 30 min in batch mode. Incubation time in batch becomes a time distribution in a fully continuous operating mode. It is highly desirable to keep the residence time distribution as narrow as possible in order to guarantee a certain minimum residence time while preventing excessive exposure of product fractions to harsh conditions. We developed a continuous virus inactivation step employing a column packed with non-porous inert beads to offer such a narrow residence time distribution across different scales and operating flow rates. The effect of column parameters and flow rate on the residence time distribution was studied using various laboratory scale columns packed with microbeads of different sizes. A larger 1.6 L column was packed to confirm the trend of narrowing residence time distribution with scale-up. Furthermore, fluorescent nanoparticles (30 nm and 200 nm) were used to simulate traveling of viruses through the packed bed reactor.

Value through Innovation

For more than 130 years we have demonstrated our strong research spirit in the interest of future generations.

www.boehringer-ingelheim.at

Boehringer Ingelheim

Boehringer Ingelheim RCV GmbH & Co KG, Dr. Boehringer-Gasse 5-11, 1121 Vienna, phone 01/801 05-0, fax 01/804 08 23

Poster Presentation Abstracts

P1

Protein adsorption behavior in multimodal cation exchange resin

Adamíková J[1], Očková M[1], Antošová M[1], Polakovič M[1]

[1] *Faculty of Chemical and Food Technology STU in Bratislava, Bratislava, Slovakia*

This work studied interactions between multimodal adsorbent Capto MMC and five different proteins depending on the concentration of NaCl in the adsorption buffer varied from 0 to 2.2 mol/L. Four proteins, BSA, HSA, lysozyme and cytoglobin had similar adsorption capacities at pH 5 regardless their different sizes, isoelectric points and glycosylation patterns. The reason of this phenomenon is that multimodal adsorbents offer multiple binding interactions. Ovalbumin was the only protein that was not able to bind to Capto MMC at given conditions. Electrostatic repulsion between negatively charged ovalbumin and adsorbent was stronger than potential hydrophobic bonds. To confirm the reversibility of complex multimodal adsorption, batch equilibrium desorption with fresh adsorption buffer was conducted. Desorbed amounts of various proteins varied from around 5% of adsorbed protein amount at low NaCl concentration to 40% at the highest concentration. Increasing desorbed amount with concentration of NaCl predicts decreasing slope of isotherms. Protein adsorption and elution were conducted also in chromatographic column mode. Isocratic elution experiments showed that desorption was very slow. Therefore, the measured protein concentration dropped to undectable values almost instateneously after the switch from adsorption mode to elution mode. The conclusion that the rate of desorption is significantly lower than the rate of adsorption was also supported by the results of batch experiments.

Acknowledgments

This work was supported by the grant from the Slovak Research and Development Agency (Grant number: APVV-14-0474).

P2

Effect of process conditions on recombinant human erythropoietin separation by multimodal chromatography

Molnar T[1], Antosova M[1], Adamíková J[1], Polakovic M[1]

[1] *Faculty of Chemical and Food Technology STU in Bratislava, Bratislava, Slovakia*

In this work, application of the multimodal resin Capto MMC in separation of hormone erythropoietin (EPO) from concentrate produced by HEK 293 cells has been investigated. The optimal binding and elution conditions in terms of pH and salt concentration were specified during batch experiments. In the adsorption phase, binding capacities of EPO and total proteins showed different patterns depending on buffer pH and salt concentration. Adsorption in citrate phosphate buffer with pH 6 containing 300 mM NaCl was optimal for maximal EPO retention while the total amount of bound proteins was half of the maximum. Desorption of the target protein was complicated as simultaneous increase of pH and ionic strength did not provide an adequate recovery yield. Effective elution was achieved with 1M arginine in Tris-HCl buffer at pH 7 with the EPO recovery yield of about 90%. Batch experiments results were verified by separation in a chromatography column when 75 % of impurities were removed and above 85 % of EPO was recovered. Using the simple bind-elute mode column chromatography, the EPO content increased from 14 % of total proteins in the feed solution to 75 % in the product fractions.

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

P3

Making Hydrophobic Interaction Chromatography compatible with MS by 2D-LC for the analysis of mAbs and ADCs

Arendt M[1]

[1] *Agilent Technologies, Waldbronn, Germany*

The need for novel analyzing methods to adapt to the ever-growing variety of biopharmaceuticals demands the development of new approaches and the combination of previously incompatible methods. Two of those methods are Hydrophobic Interaction Chromatography (HIC) and mass spectrometry.
HIC is an interesting alternative to reversed phase chromatography as it allows for the hydrophobicity-based separation of polar molecules without the denaturing conditions of reversed phase separations and with often orthogonal selectivity. It is gaining popularity for the characterization of mAb variants and especially antibody-drug conjugates (ADCs). The combination with mass spectroscopy would be beneficial as more information about the analytes could be obtained, but the high salt conditions of HIC are often incompatible with mass detection and especially with ESI.
In this work, we show the use of HIC in 2D-LC with multiple heart cutting in combination with Q-TOF. After the hydrophobic separation in the first dimension, the analytes get desalted and further separated by reversed-phase chromatography in the second dimension. A diverter valve detours the excess of salt to waste before reaching the Q-TOF. With this method formerly incompatible LC techniques could be made compatible to MS. We show this method using brentuximab vedotin as example for a ADC model substance analyzing the antibody-drug ratio, but it could be also applied to other characterizations.

P4

Opportunities and challenges of nanoparticle based magnetic separation for biomolecule recovery

Fraga-García P[1], Schwaminger S[1], Blank-Shim S[1], Brammen M[1], Berensmeier S[1]

[1] *Bioseparation Engineering Group, Technical University of Munich, Garching, Germany*

Downstream processing lacks the revolutionary advances which other fields, such as nanotechnology, have undergone in recent decades. No new processes are currently being applied: instead a long sequence of cost and time intensive processing steps is still necessary. Magnetic separation, a technique which has long been used in several fields as wastewater treatment, promises to reduce the number of isolation steps. This fast and robust separation technology can replace the capture and first purification steps of the target biomaterial from the cell suspension or fermentation broth. Based on experience from the last few years, we have learned to develop appropriate processing forms for separating small molecules, proteins as well as whole cells using a platform of magnetic nanoparticles as adsorbents. Several examples demonstrate the benefits this technique has for downstream processing and provide valuable insights for (1) understanding the mechanisms at the bio-nano interface, (2) recognising the leading driving forces involved in the interaction of the biomolecules with the surface, (3) evaluating the effect of environmental parameters and finally (4) identifying the current challenges for advance in magnetic biorecovery applications. Our experimental results demonstrate that magnetic separation can enhance the productivity and sustainability of downstream processes, although for high performance separation further plant development is needed.

P5

Internal structure of chromatographic membranes analyzed by microscopic techniques

Gramblicka M[1], Kurak T[1], Polakovic M[1]

[1] *Slovak University of Technology, Bratislava, Slovakia*

The family of ion-exchanging Sartobind membranes (Sartorius, Göttingen, Germany) is widely popular in the field of protein purification. In this work, we demonstrate the usefulness of optical and transmission electron microscopy techniques as methods for studying the internal porous structure of these membranes. The sample preparation for transmission electron microscopy was adopted from histology, which enabled preservation of wetted and swollen structures, such as the surface-grafted layer. Selective staining of adsorbed proteins was used to analyze the thickness of the grafted layer, its structure, and accessibility of variously-sized proteins into this layer. The relatively large dimensions of the pore network (pore diameters > 2 μm) enabled inspection of ultra-thin cuts by an optical microscope. The images revealed a highly heterogeneous and anisotropic structure with respect to all axes. Apart from the pore network, such imaging enabled also inspection of positions of the reinforcing fibers (diameter of 14 μm) in the membrane cross-section. Various staining methods were investigated for selective contrast enhancement of different substructures. If applicable, the porosimetric data acquired from the image processing were compared to results of mercury porosimetry and membrane permeability data.

P6

FFF-MALS: A Powerful Technology for Separation and Quantification of Proteins, Antibodies, Viruses and High Molar Mass Aggregates

Heinzmann G[1], Meier F[1], Klein T[1]

[1] *Postnova Analytics GmbH, Landsberg, Germany*

Field-Flow Fractionation (FFF) coupled to Multi-Angle-Light-Scattering (MALS) is an established, reliable and accurate analysis technique today. It is used in many pharmaceutical companies and research institutes as a supplemental method to SEC-MALS.
Major advantages of FFF-MALS vs. SEC-MALS is the highly extended molar mass and size range of proteins, antibodies, viruses and aggregates that can be separated and characterized.
In FFF the sample is separated inside an open flow channel without the presence of any packing or stationary phase inside.
Inside the channel a laminar flow with a parabolic stream profile is formed. The different used force fields, such as liquid flow, centrifugal force, temperature gradient or gravity, are applied perpendicular to the main flow which transports the sample through the channel. Under the influence of these force fields and the counteracting diffusion of the particles, different equilibrium layer heights are formed by the different particle size fractions. By coupling the FFF systems to suitable Light-Scattering Detectors such as Multi-Angle-Light-Scattering Detectors and concentration detectors such as UV- and Refractive-Index Detectors the molar mass of biological samples can be measured. Due to the lack of a stationary phase even the presence of very large protein and/or antibody aggregates can be determined.
In this presentation the application of Asymmetric Flow FFF coupled to Multi-Angle-Light-Scattering Detectors will be demonstrated regarding the separation and characterization of proteins, antibodies, viruses and aggregates. Several application examples will be shown to illustrate the wide range of results that can be achieved when using the powerful FFF-MALS technology.

P7

A novel range of monodisperse agarose beads designed for large-scale manufacturing

Johansson H[1], Gilbert P[1]

[1] *Purolite, Llantrissant, UK*

The work horse of today's commercial processes is still porous resins based on styrenic, acrylic, or agarose chemistry, produced by the same batch emulsification methodology since the mid-half of the last century. This technology produces resin that have a very wide particle size distribution and requires extensive screening to achieve the column performance demanded in modern processes. In contrast, by utilising a scalable continuous emulsification technology termed "jetting", beads with a very narrow particle distribution have been manufactured, omitting the need for sieving, and resulting in almost quantitative yield. In this paper, performance data of a new range of chromatography resins, manufactured by the jetting technology will be presented. Included are Protein A resins with very high capacity, >80 mg IgG/ml, and different types of IEX resins. Interesting, the particle size distribution, also has been shown to have a significant impact on resistance to fouling. Results of comparing batch emulsified beads with jetted beads of the same mean particle size, will also be presented and discussed.

P8

Development of monolithic silica capillary columns for high resolution separation of intact proteins

Kobayashi H[1], Wada H[1]

[1] *Shinwa Chemical Industries, Ltd., Kyoto, Japan*

In the field of liquid chromatography, there are important subjects for the high resolution separation of highly complicated bio-macromolecular mixtures. To achieve high resolution separation of bio-macromolecules in liquid chromatography, two characteristics are required as a separation medium. One is a highly ordered packed bed with thin adsorption layer around sub-micron, and the other is real high plate number [1]. A monolithic silica capillary column is reported as one of the separation medium having sub-micron adsorption layer with highly ordered packed bed. And it showed relatively high permeability, resulting real high plate number [2].
Previously, we have reported the development of a low-density octadecylsilylated (ODS) monolithic silica capillary column (0.1 mm I.D., 250 mm and 700 mm length) targeting for peptide separation, which showed over 35,000 and 100,000 plate number for small molecules [3]. In addition to the ODS column, we have developed the C4, Phenyl, C8 modified monolithic silica capillary columns targeting for intact protein separation.
Here, we discuss on the challenges for high resolution separation of the complex peptide mixture and protein mixture utilizing C18, C8, phenyl and C4 modified monolithic silica capillary columns.

[1] Gilar, M., Daly, A. E., Kele, M., Neue, U. D., Gebler, J. C. (2004) J. Chromatogr. A, 1061, 183-192.
[2] Tanaka, N., Kobayashi, H., Ishizuka, N., Minakuchi, H., Nakanishi, K., Hosoya, K., Ikegami, T. (2002) J. Chromatogr. A., 965, 35-49.
[3] Kobayashi, H. Sukegawa, M., Fujimura, K., Kubo, T., Otsuka, K. (2016) CHROMATOGRAPHY, 3, 133-139.

P9

Development of laser-induced fluorescence detector for analysis of prostate specific antigen by capillary electrophoresis-based immuno-fluorescence assay

Krenkova J[1], Prikryl J[1], Vaclavek T[1], Foret F[1]

[1] *Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*

Over the last decades there has been increasing interest in using capillary electrophoresis (CE) as a tool for performing immunoassays, a technique in which antibodies or antibody-related substances are used as selective binding agents for chemical detection. Due to the high sensitivity laser-induced fluorescence (LIF) is the most commonly used detection in CE-based immunoassays.
In this work we present development of a laboratory-built fluorescence detector for CE-based immunoassays intended to use in the commercial CE instrument. The fluorescence detector was assembled using commercially available optical components, whereas mechanical parts were fabricated by CNC milling. Detector is based on a Y-shaped bundle of seven optical fibers. The central fiber guides excitation light of a 488-nm semiconductor laser towards the fused silica capillary and the remaining six fibers surrounding the central one collect emitted fluorescence. The detector operates in a simple fluorimeter regime using long-pass and band-pass filters. Emitted fluorescence is detected by a photodiode with the embedded preamplifier.
The developed detector was tested in terms of a fiber position to the fused silica capillary and the optimized position was used to fabricate a space-saving holder usable inside the capillary cassette of the Agilent CE instrument. The performance of the detector was evaluated by analysis of a fluorescein dye and under optimized conditions the limit of detection of 52 pmol/L was determined. Finally, the CE-LIF system was applied for detection of prostate specific antigen by immunofluorescence assay.

P10

Simplify your purification with AZURA FPLC. Examples for automated two step purification.

Krop U[1], Krauke Y[1], Monks K[1]

[1] *Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany*

Protein purification is the key for many research applications. For a highly pure product, often a sequence of different methods is used. The transition from one to another step generally involves manual interaction and thus is time consuming. The quick and automated linkage of multiple chromatographic purification steps into one method eliminates manual sample handling and minimizes time spent between steps. Two purification setups are discussed.

The first approach was the purification of mouse antibodies. Antibodies have significant importance in the biotechnology and pharmaceutical industry where quality and purity of the antibodies is crucial. The most widely used technique for antibody purification is protein A affinity chromatography. This efficient capture step delivers highly clean protein. The antibodies are eluted under acidic conditions requiring an additional buffer exchange step. Our example highlights the combination of a protein A with a size exclusion step without manual interaction.

The second example deals with affinity chromatography by His-Tag which is one of the most widespread purification techniques for recombinant proteins requiring in most cases an additional cleaning step. 6xHis-tagged GFP was purified by an automated two step protocol combining an affinity chromatography method to capture 6xHis-tagged GFP following a size exclusion chromatography step to change the buffer of the purified protein to a suitable storage buffer.

In both cases proteins could be successfully purified. The two examples show the flexibility of automation and adaptability of the system to a variety of protein purification protocols to save time and resources.

P11

Unconventional purification of His-tagged proteins using TiO_2 Nanotubes Decorated by Fe_3O_4 Nanoparticles

Kupcik R[1], Macak J[2], Rehulka P[3], Sopha H[2], Bilkova Z[1]

[1] *Department of Biological and Biochemical Sciences, University of Pardubice, Pardubice, Czech Republic,*
[2] *Center of Materials and Nanotechnologies, University of Pardubice, Pardubice, Czech Republic,*
[3] *Department of Molecular Pathology and Biology, University of Defence, Hradec Kralove, Czech Republic*

Synthesis of recombinant proteins is important in many fields in the life sciences. The efficient purification is crucial for recombinant biomolecules as they come contaminated with host cell proteins. Chromatographic step, especially affinity chromatography, is commonly employed for this purpose. One of most utilized techniques, Immobilized-Metal Affinity Chromatography (IMAC), exploits the affinity of metal ions with His-tagged proteins. Unfortunately, purity and applicability of such purified proteins is limited due to low selectivity of IMAC and toxicity of released ions. The aim of our work is to demonstrate the unique characteristics of TiO_2 nanotubes decorated by Fe_3O_4 nanoparticles with higher selectivity for His-tagged proteins in comparison to commercial IMAC particles. Cell lysate containing His-tagged protein was used for both materials and obtained fractions were analyzed with SDS-PAGE.

The presented TiO_2 nanotubes decorated with Fe_3O_4 nanoparticles showed an enhanced selectivity for His-tagged recombinant proteins [1]. As compared to the IMAC system, specific isolation of selected His-tagged proteins on behalf of other proteins was improved and the presence of contaminants was significantly reduced. Magnetic properties of material provide simple and quantitative separation of material from analyte. The testing of the toxicity and mutagenicity of TiO_2 nanotubes confirmed the applicability of this material for applications with high demands on biocompatibility. Higher selectivity and magnetic properties allow thus obtaining highly pure recombinant proteins.

[1] R. Kupcik, P. Rehulka, Z. Bilkova, H. Sopha, J.M. Macak, ACS Appl. Mater. Interfaces 9 (2017) 28233.

Acknowledgements
This work was supported by OP RDE project with acronym NANOBIO, reg. n. CZ.02.1.01/0.0/0.0/17_048/0007421.

P12

Continuous virus inactivation process using a novel packed-bed reactor

Martins D[1,2], Sencar J[1,2], Hammerschmidt N[1,2], Tille B[3], Kindermann J[3], Kreil T[3], Jungbauer A[1,2]

[1] *Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Vienna, Austria,*
[2] *Austria Center for Industrial Biotechnology, Vienna, Austria,*
[3] *Global Pathogen Safety, Shire, Vienna, Austria*

The biopharmaceutical industry is moving fast towards continuous manufacturing due to the advantages associated with it. Some unit operations were adapted to continuous or semi-continuous operation, such as periodic counter current chromatography or single-pass tangential flow filtration. However, other unit operations have been overlooked. One such unit operation is the virus inactivation, mandatory in any plasma- or animal cell-derived processes. A packed-bed continuous virus inactivation reactor (CVIR, Figure 1) has significant advantages over other continuous processing approaches, namely scalability, ease of operation and being truly continuous. Two industry-relevant virus models (X-MuLV and BVDV) were used in this study to investigate the effectiveness of the CVIR for solvent/detergent treatment (S/D) unit operation. The CVIR achieved the same virus clearance performance as the traditional batch operation. To our knowledge, this is the first report where equivalent performance was shown using virus models – a requirement for regulatory acceptance. An extensive array of controls proved that the observed virus inactivation was due to the S/D inactivation and not induced by the system. The S/D critical process parameters were subject of independent confirmation. Comparison against batch data showed that the virus inactivation capacity of the solvent detergent step using the packed-bed CVIR is as effective as batch operation and delivered comparable logarithmic reduction values (LRV). The CVIR fitted with a 10-L column can process a stream of 85 L/24h.

P13

A fast and scalable purification technique for vaccines and viral vectors: Purification of parapoxvirus particles using steric exclusion chromatography

Lothert K[1], Pagallies F[2], Feger T[2], Amann R[2], Wolff M[1,3]

[1] *Institute of Bioprocess Engineering and Pharmaceutical Technology - Technische Hochschule Mittelhessen, Gießen, Germany,*
[2] *Department of Imnunology - University of Tuebingen, Tuebingen, Germany,*
[3] *Bioprocess Engineering - Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany*

For the purification of proteins and viruses, membrane based steric exclusion chromatography (SXC) has proven an alternative to common chromatographic applications. An important benefit of the method is the fast and simple procedure at mild chromatography conditions. Moreover, SXC is highly suitable as a platform technology as selective retention of the product depends mainly on its size. Samples are initially mixed with a polyethylene glycol (PEG) containing buffer of choice. Steric exclusion of the target macromolecules from the PEG and the stationary phase allows retention. By increasing the polymer concentration in the buffer or the PEGs' molecular weight, smaller process contaminants, i.e. host cell proteins and DNA, are washed out, in contrast to the targeted larger virus particles. These are subsequently eluted by a polymer free mobile phase.
We describe the application of cellulose membranes to purify parapoxvirus particles, which might be applied for vaccination and viral vectors (e.g. cancer therapy). After mammalian cell culture-based amplification, virus harvests were clarified by centrifugation and directly used for the SXC without further pretreatment. In an initial proof of concept study virus yields exceeded 60% with a closed material balance of infective particles over the process. Infectivity was confirmed by a FACS-based titration. Currently critical process parameters are determined by a design-of-experiment approach and the process is subsequently optimized in terms of product yield and contaminant depletion.
Based on literature and after successful optimization, the SXC can improve the productivity and economics of downstream processes for the purification of cell culture derived parapoxviruses.

P14

Formulation development of Biosimilar drugs: A multitechnique analytical approach using DLS, DSC and SEC for in-depth stability profiling

Hiteshri Makwana[1], John D. Stenson[1], Amy Ross[1], Mark R. Pothecary[2], Natalia Markova[1]

[1] *Malvern Panalytical Ltd, Malvern, Worcestershire, UK.*
[2] *Malvern Panalytical Ltd. Westborough, MA 01581, USA*

With some of the most popular biological drug entities coming off patent there is an opportunity to produce biosimilar products with the equivalent function as the innovator at significantly cheaper costs. This study highlights the complex challenges encountered during the formulation development process of biosimilar drugs and the importance of employing orthogonal analytical techniques to ensure (a) robust stability profile of the innovator drug is generated and (b) biosimilarity is demonstrated by the generic drug molecule when compared.

Here, stressed Denosumab; Prolia innovator and biosimilar samples were analyzed using dynamic light scattering (DLS) as an efficient, initial screening tool for the generation of a sizing profiles. Size distribution data between the two samples were comparable indicating similar colloidal stability of the biosimilar. To confirm this differential scanning colorimetry (DSC) was applied, where variations through in Tonset were noted, suggestive of differences in conformational state and thus colloidal stability (not previously identified with DLS). Multi-detection size exclusion chromatography (SEC) further confirmed these differences by demonstrating the presence of two different aggregation profiles.

Results from this study highlight the strong requirement for complementary techniques during the development of biosimilar drugs. Thorough characterization is imperative to ensuring the biosimilar product delivered has the same response in the clinic as the marketed product.

P15

Investigation of Biosimilars and Innovator Monoclonal Antibodies Using Multi-Detection SEC

Marenchino M[1], Stenson J[2], Ross A[2], Pothecary M[3]

[1] *Malvern Panalytical GmbH, Kassel, Germany,*
[2] *Malvern Panalytical Limited, Malvern, United Kingdom,*
[3] *Malvern Panalytical Inc., Westborough, USA*

Biologicals are useful for the treatment of a wide range of illnesses. Development of these materials is a very expensive process which must be funded by passing on the costs to the consumer. With some of the most popular biologicals coming off patent this has opened the opportunity for the production of biosimilars which have the same function as the innovator but are significantly cheaper. Biosimilars have shortened licensing pathways when compared to innovator materials as long as they can be shown to be biologically similar with already licensed products. For a product to be considered as biosimilar it must undergo analytical studies demonstrating that it is highly similar to the innovator and include information to show that it is expected to produce the same clinical response as the innovator in any given patient. A successful biosimilar program has comparative analytical data as its foundation. As part of the analytical study the FDA requires the use state-of-the-art technology to compare the innovators' and biosimilars' higher order structures, including aggregation, in addition to any formulation effects on purity, stability, product and process related impurities.
A key tool in demonstrating biosimilarity is multi-detection SEC. This poster highlights the value of multi-detection SEC in assessing the biosimilarity of biologics using a series of results obtained for innovator and biosmimilar candidates.

P16

HILIC separation of positively charged compounds

Moravcová D[1], Křenková J[1], Foret F[1]

[1] *Institute of Analytical Chemistry of the CAS, Brno, Czech Republic*

Cationic labels with the permanent positive charge such as (Hydrazinocarbonylmethyl)trimethylammonium chloride (Girard's reagent T) and 2-aminoethyltrimethyl-ammonium chloride (AETMA) have been used for labeling of glycans before separation by CE or HPLC. Furthermore, these labels allow the sensitive MS detection in the positive ion mode. However, the pre-treatment and pre-concentration of the sample before the analysis is often required. Cation exchangers and HILIC sorbents are suitable for these purposes. Cation exchangers require a high concentration of salts used in the elution step and HILIC utilizes organic-rich mobile phases containing a low concentration of additives such as acetic acid, formic acid, and ammonium acetate or ammonium formate buffers. Thus, this separation mode is well compatible with MS detection. However, the HILIC separation of cationic compounds is challenging due to their positive charge significantly affecting their retention.
In the presented study a benzaldehyde and its hydroxy derivatives labeled by Girard's reagent T and AETMA were used as analytes to investigate analyte-stationary phase interactions by HILIC-UV. Amide and two zwitterionic stationary phases (a sulfobetaine- and a phosphorylcholine-type) were evaluated and the effects of the composition of the mobile phase were investigated. The sulfobetaine stationary phase showed the highest retention of labeled benzaldehydes due to the electrostatic interactions between the analyte and sulfo groups of the stationary phase.

Acknowledgements
This contribution has been supported by the Czech Science Foundation (Project No. GA18-00062S) and by the Czech Academy of Sciences (institutional support RVO:68081715).

P17

Process integration and optimization for food enzyme production including large scale purification

Jeroen den Hollander[1], Ruud van Beckhoven[1], Emile van de Sandt[1], Catarina Neves[1]

[1] *DSM Biotechnology Center, PO Box 1, 2600 MA Delft, The Netherlands*

One of the trends in food industry is the application of high quality and rather pure enzyme products. Especially for non-GMO production hosts, side activities cannot always be reduced by optimized cultivation conditions. The undesired side activities need to be removed by other technologies such as pH shock, temperature shock or preparative purification methods such as chromatography.
The well known modes of chromatography, such as hydrophobic interaction and ion exchange require conditions (pH, activity, ionic strength) are not always aligned with the economically optimized conditions for downstream processing of industrially produced food enzymes. In order to commercially produce food enzymes, a number of criteria need to be met. Among others, these criteria are high yield, low cost price, fast processing and minimization of waste streams. In order to achieve such a process, the recovery unit operations cannot be fine tuned individually but process integration is needed to achieve the overall most cost-effective process.

P18

Enhancing Subunit-Level Profiling of mAbs and ADCs with MS-Quality Difluoroacetic Acid

Nguyen J[1], Smith J[3], Friese O[3], Rouse J[4], Walsh D[2], Lauber M[2]

[1] *University Of Copenhagen, Frederiksberg, Denmark,*
[2] *Waters Corporation, Milford, USA,*
[3] *Biotherapeutics Pharm. Sci., Pfizer WRD, St. Louis, USA, 4Biotherapeutics Pharm. Sci., Pfizer WRD, Andover, USA*

Protein reversed phase chromatography, while preferred for LC-MS, is heavily dependent on the conditions under which it is performed. Methods employing polymeric columns and trifluoroacetic acid (TFA) have been preferred by chromatographers but are inherently restricted to low pressure, low throughput analyses and compromised MS detection.

Our investigations show that it is possible to achieve higher resolution separations when difluoroacetic acid (DFA) is used in place of TFA. For separations of reduced, IdeS digested NIST mAb, up to 40% gains in peak capacity are observed. Alongside a column technology based on an optimized superficially porous particle and novel phenyl surface chemistry, it has been possible to boost resolution and to accelerate analyses using high flow rates.

Additionally, DFA also confers notable gains in MS sensitivity versus TFA. A 4-fold increase in MS signal has been observed when 0.1% DFA is used in place of 0.1% TFA. Nevertheless, the use of DFA has presented a surprising challenge since a reagent of purity suitable for MS work is not commercially available. We have addressed this issue by purifying DFA to a quality appropriate for MS analyses.

Coupling the phenyl-based column with DFA can also grant exceptional levels of protein recovery, resolution, and MS sensitivity for ADCs. Recovery of subunits bearing multiple drug payloads, like Fd'(+3 payloads), can be greatly improved alongside the enhanced resolution of protein variants. Ultimately, we can envision a new platform LC-MS method where unforeseen levels of detail can be observed with high fidelity using higher throughput LC-MS runs.

P19

µSEC-MALS adds Multi-Angle Light Scattering to UHP-SEC for Measurement of Absolute Molecular Weight and Size

Roessner D[1]

[1] *Wyatt Technology, Dernbach, Germany*

We describe recent advances in multi-angle light scattering (MALS) instrumentation that bring absolute molar mass and size measurements to microliter-scale ultra-high-performance size-exclusion chromatography (UHP-SEC).
µSEC-MALS achieves the highest resolution and sensitivity of any online light scattering system. The novel design combines dynamic light scattering (DLS) with MALS in the same flow cell.
The National Institute of Standards and Technology (NIST) monoclonal antibody standard reference material SRM 8671 is the benchmark molecule against which many biomolecular analysis techniques are assessed and evaluated. We present µSEC-MALS measurements of SRM 8671 as a demonstration of the unique capability of this system to carry out all the usual SEC-MALS-DLS characterization tasks, while preserving the superb resolution and rapid separations of UHP-SEC. These abilities will have far-reaching impacts at every stage of the development and production of biologics and bio-therapeutic formulations.

P20

Affinity-free purification process for a single-chain variable fragment developed using DoE and proteomics approach

Sakhnini L[1,2], K. Pedersen A[1], R. León I[1], J. Greisen P[1], Hansen J[1], B. Vester-Christensen M[1], Bülow L[2], Dainiak M[1]

[1] *Novo Nordisk A/S, Måloev, Denmark, 2Lund University, Lund, Sweden*

Fragments of monoclonal antibodies (mAbs), such as the single-chain variable fragment (scFv), are becoming widely used as therapeutic drugs, diagnostic probes and affinity ligands in industry. The advantages of scFv over mAb include improvement in capacity, reduction of steric hindrance and enablement of multivalent formatting. However, from a purification point of view, it can be challenging to obtain high yields and purity of scFv preparations unless using either expensive Protein L-based affinity adsorbents or affinity tags, which can be immunogenic if not removed. In this work, an affinity-free purification process for a scFv using mixed-mode chromatography (MMC) has been developed by design of experiments (DoE). It was hypothesized that the presence of large hydrophobic patches on the surface of scFv, observed by the spatial aggregation propensity map, can be exploited in MMC in order to improve selectivity. Capture of scFv from HEK293 feedstock was performed by hydrophobic charge induction chromatography (MEP HyperCel). This was followed by anion hydrophobic MMC (Capto Adhere) developed by fractional screening design and full factorial optimisation design. Host cells proteins (HCPs) were identified and quantified by a proteomics approach using nano LC-MS/MS. Optimum yield and HCP reduction factor were determined to 98% and 14, respectively. In addition, it resulted in the removal of 258 HCPs, which corresponded to 84% of HCPs identified in the load. Taken together, MMC is a highly selective technique enabling new selectivities based on surface property characteristics of the protein in question and the chromatographic conditions, which can be optimised by DoE.

P21

Charge Variant Analysis of Therapeutic Proteins Using a Novel Weak Cation Exchange Stationary Phase

Schwahn A[1], Lin S[2], Bechler S[2], Baek J[2], Liu X[2]

[1] *Thermo Fisher Scientific, Reinach, Switzerland,*
[2] *Thermo Fisher Scientific, Sunnyvale, USA*

Protein therapeutics, especially monoclonal antibody (mAb)-based products, continue to grow as a major class of treatment for various sever diseases.
The cellular production processes used to manufacture mAbs may result in post-translation modifications (PTMs), e.g. glycosylation and additional modifications, such as oxidations, may happen during downstream processing and storage. Together, these modifications result in product heterogeneity that can adversely affect the efficacy and safety of the drug. Therefore, manufacturers are required to verify the fidelity of their products by characterizing the nature and quantity of structural variants.
Charge variants, such as sialylated glycans, alter the overall charge of the mAb. Ion exchange chromatography is a technique commonly employed to separate these variants based on their accessible surface charge. Cation exchange chromatography is the standard separation approach since the isoelectric point (pI; pH at which the protein charge is neutral) of most mAbs is typically between 6-10. When the buffer pH is less than the protein pI, the mAb will be cationic and readily bind to anionic stationary phase of cation exchange columns. A gradient from low to high salt concentration is then used to disrupt the ionic interactions of the protein with the solid phase, resulting in protein desorption and elution from the column.
Here, we report the use of a novel weak cation exchange (WCX) column for the evaluation of pharmaceutically relevant mAbs, biosimilars, and their associated charge variants. Parameters such as temperature, loading capacity, carryover, and salt gradient versus pH gradient separations are considered and compared.

P22

Charge Variant Method Design for Analysis of Monoclonal Antibodies

Schwahn A[1], Lin S[2], Baek J[2], Bechler S[2]

[1] *Thermo Fisher Scientific, Reinach, Switzerland,*
[2] *Thermo Fisher Scientific, Sunnyvale, USA*

Chromatographic methods are commonly employed to separate and characterize the structural heterogeneity that is inherent to modern biotherapeutics. Specifically, cation exchange chromatography has become a method of choice for separating charge-related heterogeneity, such as variants formed due to deamidation and lysine truncation.
The diverse structure of monoclonal antibody and monoclonal antibody derived therapeutics (such as ADC and bi-specifics) present a challenge in designing a cation exchange chemistry which can accommodate a wide range of analysis. Here, we report the development of a 5 µm weak cation exchange (WCX) stationary phase which is designed for high-resolution charge variant separation of a wide range of therapeutic proteins. This 5 µm divinylbenzene-based particle is coated with an hydrophilic polymer, followed by grafting of poly(acrylic acid) to provide WCX functionality.
We evaluated this weak cation exchange stationary phase using multiple therapeutic IgG1, IgG2, and IgG4. In the case of eight IgG1 (rituximab, trastuzumab, infliximab, bevacizumab, secukinumab, pertuzumab, golimumab, and vedolizumab) and one IgG4 (nivolumab), all acidic and basic charge variants are base line separated using either salt gradient or linear pH gradient method. IgG2 charge variant analysis is known to be difficult due to its complex inter-disulfide bond structure. We examined the charge variant analysis of two IgG2 (panitumumab and denosumab). In both cases, acidic and basic variants are significantly separated from the main peak to allow reliable quantitation. These examples demonstrate that the new WCX stationary phase provides unique selectivity and high resolution for charge variants separation of a wide range of biomolecules.

P23

New concepts for protein purification: magnetic separation with bare iron oxide nanoparticles

Schwaminger S[1], Blank-Shim S[1], Fraga-García P[1], Rauwolf S[1], Zanker A[1], Berensmeier S[1]

[1] *Bioseparation Engineering Group, Garching, Germany*

Recombinant proteins are often fused with affinity tags for purification with affinity chromatography. Popular purification systems, such as His-Tags, are based on expensive, merely process-stable chromatography materials which are not economically viable for industrial use. Therefore, superparamagnetic bare iron oxide nanoparticles (BIONs) are chosen which facilitate high specific surface areas at low synthesis costs and new magnetic processing possibilities. For a purification process, the target protein selectively adsorbs via new designed tags on the BION, is magnetically separated, washed and eluted from the adsorbent which can be recycled. Proteins can be bound to the BION surface selectively through affinity tags designed for the chosen buffer system.

Nanoscale magnetite is produced by an optimized co-precipitation synthesis as BION adsorbent material with high specific surface (~100 m^2/g) and a high saturation magnetization (>80 Am^2/kg). The amphiphilic properties of the particles´ surface can be adjusted to different protein/peptide systems by changing the pH and the buffer.

To find an affinity tag for the BIONs, the binding of different amino acids is observed and analyzed. The trend to the complexation of negatively charged amino acids (NegPeps) is further investigated by short peptide sequences allowed to bind BIONs. This trend can be confirmed with peptide arrays and the NegPep is used as affinity tag for the purification of a model protein from E. coli cell lysate. Here, a purity of 74% of the model protein can be achieved with a liter scale magnetic separation process in one step.

P24

Miniaturized and simple gradient liquid chromatography system for capillary columns

Šesták J[1], Kahle V[1]

[1] *Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*

Miniaturization of liquid chromatography requires development of suitable pumping and injection systems such that low flow rates and low sample volumes are introduced into the capillary separation column. Miniaturization of standard gradient HPLC system scheme based on binary two piston pumps was already done by majority of HPLC system manufacturers and robust but complex instruments for capillary LC were already put on the market.

Our poster presentation describes an alternative and very simple gradient HPLC scheme based on single piston pump and selector valve. Pump is connected with the selector valve via capillary which serves as a gradient generator. Selector valve allows automated flow path change such that solvents and sample are sucked by the pump and consequently pumped on column. Gradient of solvent composition is created by successive sucking of individual solvent mixtures into the gradient loop where the boundaries between mixtures are dispersed due to parabolic flow profile. Taylor-Aris dispersion theory is used for prediction of gradient shape. Repeatable separation of BSA peptides is presented as a proof of proper system operation.

P25

Intensification of mAb downstream processes using modular innovative purification technologies

Trapp A[1], Kössler V[1], Faude A[1], Schubert S[1]

[1] *Rentschler Biopharma SE, Laupheim, Germany*

New innovative strategies improving performance and flexibility of monoclonal antibody (mAb) purification processes are necessary for further bioprocess intensification. Two modular technologies to reduce column-based purification steps are presented:
(1) Caprylic acid (CA)-induced impurity precipitation to enhance the low pH viral inactivation step: By adding CA for impurity precipitation after protein A chromatography, host cell protein (HCP) log10 reduction values (LRVs) of 1-2.5 LRVs can be achieved. Thus, HCPs are successfully removed to final specifications already after subsequent particle removal by depth filtration. Moreover, CA enables precipitation of mAb aggregates and efficient virus inactivation of ≥ 5 LRVs. The CA-induced precipitation step constitutes a simple and economic step, which can be implemented as an excellent alternative to column chromatography.
(2) Replacement of a column chromatography polishing step by a static batch chromatography: Using a cation exchange resin in overload mode enables efficient aggregate removal of > 1 % at high mAb load of 400 g L-1resin and a yield of 90 %. This single-use approach can be performed in a simple mixing bag followed by resin separation by body feed filters. Key process advantages include elimination of column packing and cleaning, reduced process time, disposability and suitability for continuous processing.

P26

Micro Pillar Array Columns: A novel robust chromatography platform for deep and reproducible proteome coverage

van Ling R[1], Op de Beeck J[1], Van Mol K[1], Claerebout B[1], Van Landuyt N[1], De Malsche W[2], Desmet G[2], Jacobs P[1]

[1] *PharmaFluidics, Gent, Belgium, 2Free University Brussels (VUB), Brussels, Belgium*

Bottom-up proteomics relies on the use of 50 cm long packed columns, coupled to mass spectrometry to analyze digested protein samples. However, gradient times above 240 minutes using packed column have resulted only in marginal gains in identification, with robustness and reproducibility not yet fulfilling the promise of routine use of such workflows.
micro Pillar Array Column or µPAC™ technology utilizes a unique approach, taking a chromatographic support structure that builds upon micromachining chromatographic separation beds into silicon. This results in exceptional properties in terms of chromatographic performance, repeatability and reproducibility, flexibility and robustness.
Chromatographic performance of the µPAC™ is demonstrated in nanoflow (300 nL/min) and capillary flow (1 µL/min), using short and longer gradients with commercially available retention standards.
Column efficiencies are demonstrated using 1 µl direct injection runs from a dilution series of HeLa-digests (ranging from 0.01 to 1 µg/µL), coupled to high resolution mass spectrometry.
Long term stability and repeatability is illustrated using a 6 months continuous column evaluation, running sequences of Cytochrome C standard, HeLa-digest and blanks (1 hour runs, 3526 injections in total, 1000 HeLa-digest injections) showing less than 1% retention time variation for the Cytochrome C peptides over the whole period.
Further robustness is demonstrated using three sets of contaminated samples, utilizing detergents routinely used in the sample preparation (NP-40; Triton X-114) or contain precipitating compounds. Such samples can be quite problematic for packed columns, retention time deterioration or column clogging, the µPAC™ columns show stable pressure profiles and retention time variation below 2%.

P27

Accelerated downstream process development: Mechanistic modeling of LMW, HMW and HCP separation from product on a multimodal resin

Wittkopp F[1], Kraus J[1], Isik E[1], von Hirschheydt T[1], Schaubmar A[1]

[1] *Roche Diagnostics GmbH, Pharma Research and Early Development (pRED), Large Molecule Research (LMR), Roche Innovation Center Munich, Penzberg, Germany*

Early stage purification process development is predominantly achieved by applying experience based platform processes containing an affinity step with subsequent ionic or multimodal chromatography steps. However, complex molecule formats and the need of deeper process understanding lead to a great number of empiric wet-lab experiments requiring significant resources. Mechanistic modeling can be a complementary evaluation method supporting the process definition phase as well as later challenges like up-scaling or robustness investigations.
In this poster we present the mechanistic modeling of a multimodal anion exchange resin operated in a dual gradient (pH + salt). The separation of the antibody monomer from impurities like low-molecular-weight species (LMWs), high-molecular-weight species (HMWs) as well as different host-cell proteins (HCPs) is described. A specific challenge of the project was the low number of experiments available for the calibration of the model. Nevertheless, the model deepened process understanding and helped to identify critical process parameters to design pH/salt step elution.

P28

Exploring a 2μm non-porous ODS column for proteins and peptides separation

Yazawa I[1]

[1] *Imtakt Corp., Kyoto, Japan*

Life science research requires the separation of many different proteins and peptides. For example, monoclonal antibody (mAb), pegylated protein and proteomics. To meet these demanding separations, we developed a novel 2um non-porous ODS column. We conducted a study on the utility value of using this non-porous ODS column on protein and peptide separations, and would like to share the results.

We used 250x4.6mm high-resolution column packed with 2um non-porous ODS packings.. Analytes for this study were commercially available mAb, IgG, and other proteins. Peptides were prepared by tryptic digestion of alpha-casein. HPLC was usually performed with TFA or formic acid in water and acetonitrile gradient elution. Detection was done with UV 220 nm or ELSD.

We found that our non-porous ODS column:

Generates a much larger number of peptide peaks compared to porous ODS columns.

Offers excellent separation performance for mAb with shallow gradient elution at high temperature. Using a gradient slope was very important for molecular recognition of the protein.

Generated 1200 peak numbers with a 300 minute run time for IgG in human serum. This result demonstrates that, despite a low surface area, our non-porous ODS column has excellent molecular recognition performance.

Identified around 700 peaks of hemocyanin and hemoglobin. Pegylated protein consists of a fixed MW protein and various MW of PEG. This column separated several peaks of different MW of pegylated protein.

The non-porous 2um ODS column improves peak resolution and recovery due to the absence of pores. It is useful for protein and peptide separation.

P29

A proteome wide approach to understand chromatography behavior on hydrophobic adsorbents

Yelemane V[1], Ali N[1], Fernández-Lahore M[1]

[1] *Jacobs University Bremen Ggmbh, Bremen, Germany*

The separation behavior of the Saccharomyces cerevisiae cell proteome was explored by hydrophobic interaction chromatography (HIC). Several beaded adsorbents were studied. A first group of HIC adsorbents (n=3) harbored the same ligand moiety (i.e., Phenyl) but presented differences in the chemical nature of the matrix backbone. On the other hand, a second group of adsorbents (n=3) presented the same chemical structure (i.e. based on polymethacrylate; Toyopearl / TP) but differed in the chemical nature of the HIC ligands. Chromatography runs were performed under typical conditions employing ammonium sulfate / phosphate buffer (pH=7.5) as a mobile phase. Chromatography fractions were collected and further analyzed by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE); selected spots were identified by MALDI-ToF-MS and database search.

A comparative analysis of protein separation behavior is presented as a function of the adsorbent type. As expected, TP-Hexyl and TP-Butyl showed increased protein retention in comparison with TP-Ether. Moreover, an influence of protein size and isoelectric point (pI) was revealed. Larger and/or neutral proteins showed increased retension, while acidic (pI < 6) and basic (pI >8) proteins depicted decreased or increased retention, depending on the adsorbent type. Protein characteristics, such as average surface hydrophobicity, average hydrophobicity, average polarity, average bulkiness and average flexibility were tabulated for the identified spots and correlated with chromatography behavior. This work studied the separation behavior of a real and complex cell proteome with available HIC adsorbents. Results will favor the understanding and the application of HIC, a method of choice in many industrial bioseparation schemes.

P30

Identification of High-Affinity Peptide Scaffold from *In vitro* and *in silico* Selection Process

Yun S[1], Lee S[1], Choo J[1], Lee E[1]

[1] *Hanyang University, Ansan-si, South Korea*

A short peptide scaffold, which could bind to a target protein with high affinity, can replace an original antibody for 'antibody mimetics'. In this study, we identified the single-chain peptide scaffold that can replace anti-HER2 antibody by a combined process of in vitro phage display/biopanning for Fab region and in silico selection for Protein A-binding Fc region. We present two case studies: (1) Affinity maturated peptides that bind to human epidermal growth factor receptor 2 (HER2/neu), and (2) a truncated peptide scaffold for IgG Fc region that can bind to the Z-domain of Protein A. In Case (1), we screened two 12-mer peptides (HLTTTHPEPPYG, YSHTLKIPAPDF) that could potentially replace the anti-HER2 antibody. For the affinity maturation, we made homo- and heterodimer using a hairpin structured linker peptide. In Case (2), a in silico selection process was used to identify three key fragments for Z-domain binding: Fragment 1; (KPK)DTLMISRTPE, Fragment 2; VLHQNWLDGK, and Fragment 3; EALHNHYTQ. The distances between each fragment were estimated and suitable peptide linkers were inserted for a single-chain scaffold. Their secondary structures were predicted by using PEP-FOLD S/W and the binding site was simulated by using a protein-protein docking simulation program(ZDOCK). The two optimal scaffold sequences were identified: "(KPK)DTLMISRTPE-GGGPG(or GGPGG)-VLHQNWLDGK-GGGGG-EALHNHYTQ". Each peptide motif was analyzed by SPR for its binding affinity to the target proteins. They are integrated into a single-chain peptide scaffold for ‚artificial antibody scaffold'. It is expected to be used for various applications such as cancer diagnosis and antibody drug conjugates for oncology therapeutics.

P31

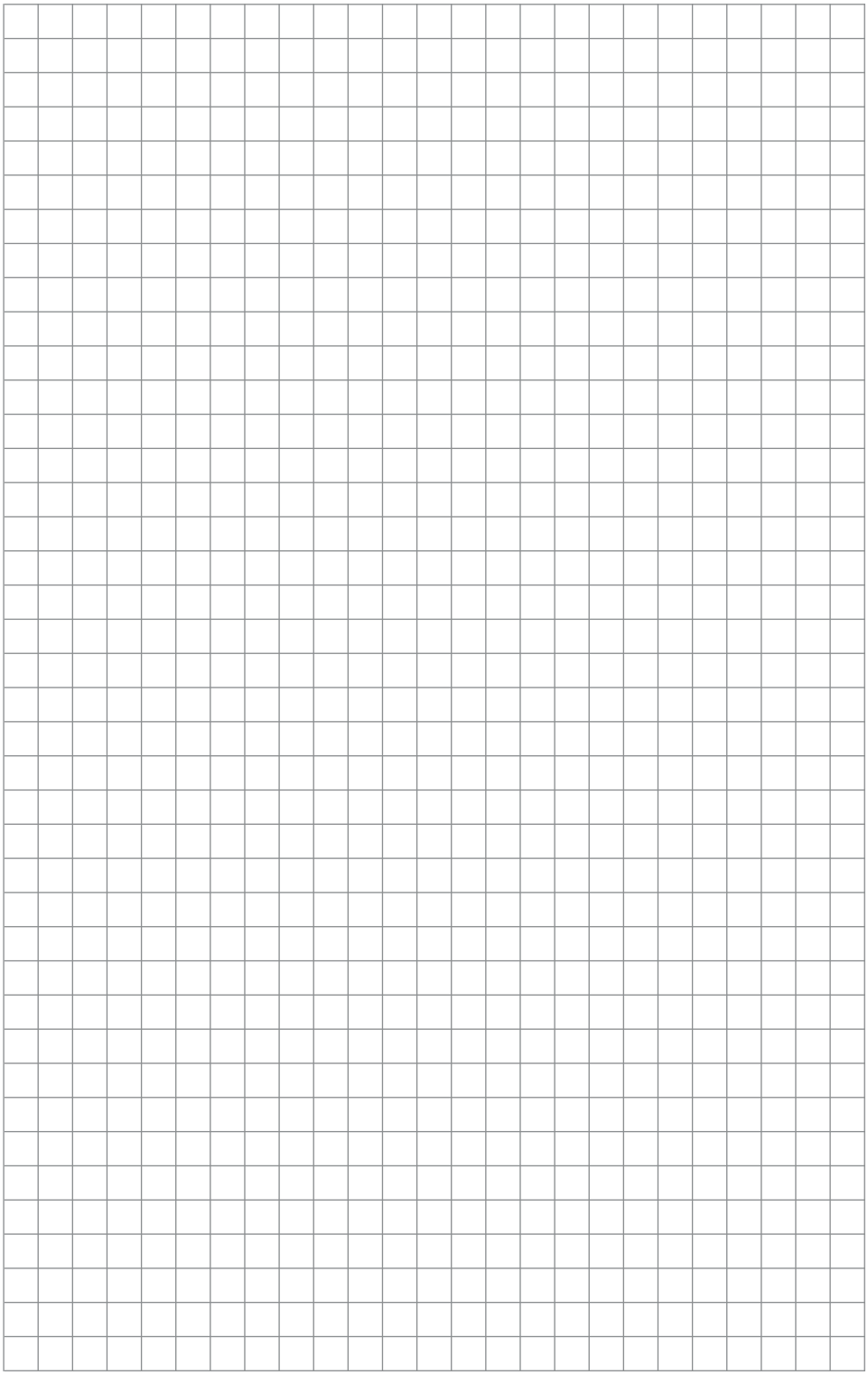
Potential Application of Taiwan Actinomycetes Broth in Treatment of Alzheimer's Disease

Hui-Ming Yu[1], Yu-Hsuan Chu1, Kuo-Ching Lin[1]

[1] *Genomics Research Center, Academia Sinica, Taipei, Taiwan*

Alzheimer's disease (AD), characterized by a loss of cognitive ability, abnormal behaviors, and ultimately death, is a progressive neurodegenerative disorder. Imbalance between the production and the removal of β-amyloid peptide (Aβ) may result in self-assembly aggregation in AD brain and is now considered a possible contributor to the onset of AD. Recent studies suggested that neprilysin (NEP) and insulin-degrading enzyme (IDE), two major enzymes for Aβ degrading in the brain, may become a promising AD treatment through improving their expression. In the current study, 269 Taiwan Actinomycetes broth were screened and a quenched fluorogenic peptide substrate was synthesized for ADE (NEP and/or IDE) activity assay in human SH-SY5Y neuroblastoma cell. The cytotoxicity of screened samples was determined by MTS assay. The results show that there are 4 broths exhibited great ADE activity (>200%). Among them, sample B79 (activity 223.4± 0.9%) had better fermentation stability. Based on these findings, sample B79 was thus selected for further extraction, separation and screen by ADE activity assay. The result revealed that the HPLC fraction 1, 2 and 5 of B79 EA extracts could enhance ADE activity by more than 150% with concentration 5ug/ml and fraction 1 has highest ADE activity (>250%).

NOTES



INNOVATIONS FROM NATURE

acib austrian centre of industrial biotechnology

www.acib.at

Printed on recycled paper