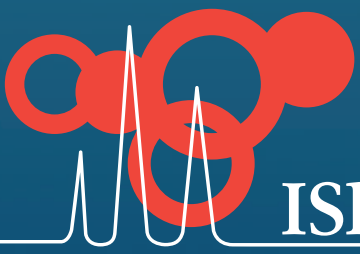
A B S T R A C T B O O K

NOVEMBER 7-10, 2021

40th International Symposium on the Separation of Proteins, Peptides & Polynucleotides



ISPPP
2021 PORTO

40th International Symposium on the Separation of Proteins, Peptides & Polynucleotides

acib GmbH / Krenngasse 37 / 8010 Graz / www.acib.at

Welcome to ISPPP 2021

I would like to welcome you all very warmly to the 40th International Symposium on the Separation of Proteins, Peptides and Polynucleotides, which is held in the beautiful city of Porto. This is an exciting time, not only because we now have the opportunity to run the conference face-to-face after postponing it due to the COVID-19 pandemic, but also because we are celebrating our 40th anniversary event.
Never, in the past, the bioseparation world faced so many challenges in a short period of time, as in present years. Challenges that brought about promising developments on several aspects of separation and characterization of biologically important molecules, and that will be highlighted during the different conference sessions:

- Analytical detection technologies
- Preparative separation technologies
- Fundamentals and Modelling
- Continuous Processing
- Biopharmaceuticals
- New Products

For 3 days, the program provides a unique opportunity to meet, listen, discuss, share information and plan for the future. I´m confident that this conference will be interactive and successful, bringing together all participants in an enjoyable atmosphere. I sincerely hope for your active participation to engage our presenters and attendees in the various presentation formats (Key notes, Lectures, Flash presentations and Posters). As always, the Organizing Committee is extremely interested in feedback on the ISPPP Symposium, so please do not hesitate to submit suggestions for future meetings.
I cannot finish without thanking the authors who have contributed to the high scientific level of the program and I´m also grateful to the sponsors and exhibitors and to all those who, through their dedicated efforts, have been of assistance to this event.

Enjoy your participation in the ISPPP2021 and a memorable time visiting Porto.

Cristina Dias-Cabral, PhD

Conference Chair

Content

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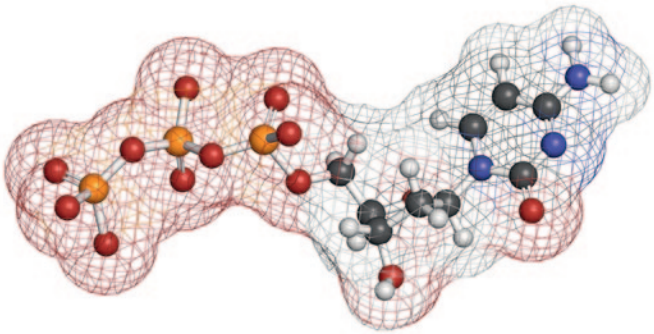
PRE-CONFERENCE WORKSHOPS

Sunday, November 7

9:00	START OF REGISTRATION	
9:30	**WORKSHOP 1** **Cristina Peixoto** (Instituto de Biologia Experimental e Tecnológica – iBET)	New insights in virus-based biopharmaceuticals purification
11:15	**WORKSHOP 2** **Alois Jungbauer** (BOKU Vienna) **Cristina Cabral** (Univ. of Beira Interior)	Mechanistic understanding of biomolecules adsorption: theory and applications
13:30	**WORKSHOP 3** **Sophia Hober** (KTH) **Michel Eppink** (Byondis BV)	Surface plasmon resonance in biotechnical development
15:15	**WORKSHOP 4** **Sonja Berensmeier** (TU Munich) **Egbert Müller** (Tosoh Bioscience) **Michel Eppink** (Byondis BV)	Nucleotide separation in biophamaceutical processing and their quality requirements

CONFERENCE PROGRAMME



Sunday, November 7

17:00	**Welcoming Remarks by Cristina Dias-Cabral**	

KEY NOTE LECTURE
CHAIR: CRISTINA DIAS-CABRAL

17:30	**Giorgio Carta** (Univ. of Virginia)	**KN1:** Chromatography, Adsorption Kinetics, and Purification of Conformationally-Flexible Multidomain Proteins
18:20	WELCOME RECEPTION	

Monday, November 8

KEY NOTE LECTURE
CHAIR: SOPHIA HOBER

08:30	**Ruedi Aebersold** (ETH Zürich)	**KN2:** Extracting new molecular biology knowledge from proteins and peptides

SESSION 1: ANALYTICAL DETECTION TECHNOLOGIES I
CHAIR: SOPHIA HOBER

09:20	**Rainer Bischoff** (Univ. of Groningen)	**OP1:** Affinity LC-MS for high-sensitivity analysis of proteins in complex biological samples
09:40	**Baubek Spanov** (Univ. of Groningen)	**OP2:** pH gradient-based cation exchange chromatography for the separation of charge variants of monoclonal antibodies after forced degradation study
09:55	**Christoph Gstöttner** (Leiden Univ. Medical Center)	**OP3:** Affinity sheathless-CE-MS as a new tool for the functional assessment of antibody - Fc receptor interactions
10:10	**Elena Dominguez-Vega** (Leiden Univ. Medical Center)	**OP4:** Unravelling the structural and functional heterogeneity of recombinant SARS-CoV-2 RBD domains
10:30	REFRESHMENT BREAK	

SESSION 2: ANALYTICAL DETECTION TECHNOLOGIES II
CHAIR: MICHEL EPPINK

11:00	**Clara Pérez Peinado** (Janssen Vaccines and Prevention)	**OP5:** RT-MALS: Real-time analysis of product attributes in downstream processing
11:20	**Jelle De Vos** (Vrije Universiteit Brussel)	**OP6:** Advances in microfluidic technology for spatial three-dimensional liquid chromato-graphy
11:40	**Juliane Diehm** (Karlsruhe Institute of Technology)	**OP7:** Development of a 3D Printed Micro Simulated Moving Bed Device as Process Analytical Tool
11:55	**Robin Schiemer** (Karlsruhe Institute of Technology)	**OP8:** Advanced machine-learning methods for the analysis of spectroscopic data in biopharmaceutical processes

FLASH SESSION 1 CHAIR: ANA AZEVEDO		
12:10	**Mariachiara Conti** (Univ. of Edinburgh)	**FP1:** Development of glycidyl methacrylate-based plaftorm material for 3D printing stationary phases
12:15	**Gregory Dutra** (BOKU Vienna)	**FP2:** 3D Printed convective media for the separation of small and large biomolecules
12:18	**Benjamin Summers** (Purolite)	**FP3:** Single Step Protein Purification And Immobilisation By New Chromalite M Chemistries
12:21	**Jorge Barroca-Ferreira** (Univ. of Beira Interior)	**FP4:** Separation of Histidine-Tagged Six-Trans-membrane Epithelial Antigen of the Prostate 1 from Detergent-Solubilized *Komagataella pastoris* Extracts
12:24	**Francisca Silva** (Univ. of Aveiro)	**FP5:** Human serum pre-treatment: removal of abundant proteins aiming the concentration of lung cancer biomarkers
12:27	Discussion	

FLASH SESSION 2 CHAIR: ANA AZEVEDO		
12:36	**Ana Margarida Gonçalves** (Health Sciences Research Centre)	**FP6:** Enhancing membrane-bound catechol-O-methyltransferase activity through an ionic liquid storage formulation
12:39	**Mafalda R. Almeida** (Univ. of Aveiro)	**FP7:** Purification of antileukemic drugs through silica-based supported ionic liquids
12:42	**Augusto Pedro** (Univ. of Aveiro)	**FP8:** Purification of interferon alpha 2b-based biopharmaceuticals using ionic liquid-based technologies
12:45	**Leonor Castro** (Univ. of Aveiro)	**FP9:** Sustainable liquids support as a media for biocatalytic reactions using aqueous biphasic systems
12:48	**Christina Wegner** (Karlsruhe Institute of Technology)	**FP10:** UV/Vis spectroscopy and Multi-Way Chemometrics for Precipitation and Crystallization Process development from Complex Media
12:51	Discussion	
13:00	LUNCH BREAK	

SESSION 3: PREPARATIVE SEPARATION TECHNOLOGIES CHAIR: ALEŠ PODGORNIK		
14.30	**Nico Lingg** (acib GmbH)	**OP9:** Affinity tag-based development and manufacturing platform for non-platform proteins
14:50	**Michel Eppink** (Byondis BV)	**OP10:** Nanofibers as an alternative for fast affinity separations
15:10	**Alexei Voloshin** (3M)	**OP11:** Enabling Large Particle Chromatographic Separations Using Advanced Materials
15:30	**Arne Staby** (Novo Nordisk)	**OP12:** Complex polypeptides: Regulatory and physical/chemical considerations related to manufacturing processes of follow- on products
15:50	**Clement Bassens** (Cytiva)	**Sponsored talk by Cytiva:** **OP13:** Making tomorrow's therapies a reality, what to consider when developing a chromatography toolbox approach.
16:20	REFRESHMENT BREAK	

FLASH SESSION 3 CHAIR: ALOIS JUNGBAUER		
16:50	**Michael Schobesberger** (TU Munich)	**FP11:** Natural magnetite ore as separation agent for valuable microalgal products
16:55	**Eva Krolitzki** (TU Munich)	**FP12:** Magnetic bioseparation with bare iron oxide nanoparticles on pilot scale
16:58	**Tatiana Aguiar** (CEB - Centre of Biological Engineering, Univ. of Minho)	**FP13:** Affinity purification/immobilization of His-tagged proteins using unmodified silica
17:01	**Jorge João** (IST, Univ. of Lisbon)	**FP14:** Chromatographic purification of small heat shock protein nanocages
17:04	**Tae Keun Kim** (Politecnico di Milano)	**FP15:** Experimental Design of the Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) Unit for the Separation of PEGylated Proteins
17:07	Discussion	

FLASH SESSION 4 CHAIR: ALOIS JUNGBAUER		
17:16	**Daniel Komuczki** (BOKU Vienna)	**FP16:** Media & Buffer On-Demand: Continuous Reconstitution Directly From Solids
17:19	**Lisa-Marie Herlevi** (Jacobs Univ. Bremen)	**FP17:** Continuous Fluidized Bed Riser System for Bioproduct Recovery employing a Double Network Hydrogel Cation Exchange Adsorbent
17:22	**Yijia Guo** (Jacobs Univ. Bremen)	**FP18:** Moving Adsorption Belt System for Continuous Bioproduct Recovery
17:25	**Tiago Castanheira Silva** (TU Delft)	**FP19:** Miniaturization of chromatographic process evelopment for fast results at minimal costs
17:28	**Tommaso De Santis** (acib GmbH)	**FP20:** Economic and ecological evaluation of new processing schemes for the industrial scale production of plastic-degrading enzymes
17:31	Discussion	

FLASH SESSION 5 CHAIR: ALOIS JUNGBAUER		
17:40	**Ignacio Montes Serrano** (acib GmbH)	**FP21:** Experimental and simulated determination of the volumetric power input in microtiter plates as a scale-up strategy for the development of downstream operations
17:43	**Touraj Eslami** (BOKU Vienna)	**FP22:** Capture step optimization in liquid chromatography
17:46	**Bettina Motycka** (BOKU Vienna)	**FP23:** Determination of the conformation of cellobiose dehydrogenase by small angle X-ray scattering
17:49	**Arménio Barbosa** (Nova Univ. of Lisbon)	**FP24:** Molecular Modeling on the Discovery of synthetic affinity-adsorbents
17:52	Discussion	

POSTER SESSION		
18:00	Poster session	
18:45	NETWORKING RECEPTION	

Tuesday, November 9

KEY NOTE LECTURE CHAIR: SONJA BERENSMEIER		
08:30	**Steve Cramer** (Rensselaer Polytechnic Institute)	**KN3:** Evaluation of Preferred Binding Orientations of Protein Therapeutics in Multimodal Chromatographic Systems using Biophysics and Simulations

SESSION 4: PRODUCTS – BIOPHARMACEUTICALS I CHAIR: SONJA BERENSMEIER		
09:20	**Hans Johansson** (Purolite)	**OP14:** A new Protein A resin allowing quantitative elution of human IgG at pH 5
09:40	**Julia Scheffel** (KTH Royal Institute of Technology)	**OP15:** A calcium-dependent Protein A-derived ligand for mild purification of antibodies
09:55	**Malin Jönsson** (KTH Royal Institute of Technology)	**OP16:** A metal-dependent protein domain derived from Streptococcal Protein G enabling gentle elution of mIgG1 and human Fab
10:10	**Egbert Müller** (Tosoh Bioscience)	**OP17:** Purification of IgY by Mixed Mode Chromatography
10:30	REFRESHMENT BREAK	

SESSION 5: PRODUCTS – BIOPHARMACEUTICALS II CHAIR: RAQUEL AIRES BARROS		
11:00	**Sandra C. Bernardo** (CICS-UBI)	**OP18:** Design of specific ligands for pre-miRNA purification
11:20	**Sara Sousa Rosa** (IBB - Institute for Bioengineering and Biosciences)	**OP19:** A chromatography alternative for mRNA manufacturing
11:35	**Ana Rita Santos** (iBB - Institute for Bioengineering and Biosciences)	**OP20:** DNA-origami nanostructures: exploring chromatography for the purification of single stranded DNA scaffolds
11:50	**Lucía Abarca Cabrera** (TU Munich)	**OP21:** Design of specific ligands for pre-miRNA purification
12:05	**Ana Cecília Roque** (UCIBIO, FCT-NOVA	**OP22:** Affinity magnetic precipitation for antibody purification

FLASH SESSION 6 CHAIR: ANA AZEVEDO		
12:25	**Jonas Wege** (Tosoh Bioscience)	**FP25:** Characterization of AAV5 Viral Vector by Size Exclusion
12:30	**Sónia Mendes** (Instituto de Biologia Experimental e Tecnológica – iBET)	**FP26:** Production of high-quality SARS-CoV-2 antigens: Impact of bioprocess and storage on glycosylation, biophysical attributes, and ELISA serologic tests performance
12:33	**Johanna Tawe** (Bio-Works)	**FP27:** Increased lifetime of RPC resins in insulin production by clean-up using a cation exchange step
12:36	**Cecilia Unoson** (Bio-Works)	**FP28:** Oligonucleotide purifications using anion exchange chromatography
12:39	**Manasi Gaikwad** (Univ. Medical Center Hamburg Eppendorf)	**FP29:** Fast quantification of proteoforms in therapeutic protein using flow injection analy
12:42	Discussion	
13:00	LUNCH BREAK	

SESSION 6: NEW PRODUCTS CHAIR: CRISTINA PEIXOTO		
14:30	**Friederike Eilts** (Univ. of Applied Sciences Mittelhessen)	**OP23:** Time- and charge-dependent purification of nanoparticles with the steric exclusion chromatography
14:45	**Keven Lothert** (Univ. of Applied Sciences Mittelhessen)	**OP24:** Development of a scalable downstream purification process for Parapoxvirus Ovis (ORFV) purification for human and veterinary application
15:00	**Ana Sofia Moreira** (Instituto de Biologia Experimental e Tecnológica – iBET)	**OP25:** Improving processes and exploiting new tools for Lentiviral vectors purification
15:15	**Viktoria Mayer** (acib GmbH)	**OP26:** Purification of enveloped Virus-Like Particles displaying different membrane proteins by Heparin affinity chromatography
15:30	**Angela Valentic** (Karlsruhe Institute of Technology)	**OP27:** Effects of bound nucleic acids on the phase behavior and purification process of HBcAg virus-like particles for gene therapy
15:45	**Nils Hillebrandt** (Karlsruhe Institute of Technology)	**OP28:** Process Analytical Technology for Diafiltration-based Virus-like Particle Disassembly
16:00	REFRESHMENT BREAK	

SESSION 7: CONTINUOUS PROCESSING CHAIR: MARCEL OTTENS		
16:30	**Amin Javidanbardan** (Instituto Superior Técnico)	**OP29:** Development of Next-generation Microchromatographic System for Miniaturization of Bioprocess Development with Some Insights from Philosophy of Science
16:45	**Narges Lali** (acib GmbH)	**OP30:** Residence Time Distribution of Chromatography Unit Operation in Continuous Processing
17:00	**Diogo Faria** (Instituto Superior Técnico)	**OP31:** Oscillatory flow reactor: a solution for continuous biomanufacturing
17:15	**Mattia Sponchioni** (Politecnico di Milano)	**OP32:** Design and Application of Twin-Column Countercurrent Solvent Gradient Chromatography (MCSGP) to the Purification of Oligonucleotides
17:35	**Mariana Neves Sao Pedro** (TU Delft)	**OP33:** Development of a Fluorescent Dye-Based Miniaturized Sensor for Aggregate Detection in Integrated Continuous Processing
17:50	**Maria del Carme Pons Royo** (acib GmbH)	**OP34:** How to design continuous devices to improve tangential flow filtration and product quality in protein precipitation
18:05	End of session	
20:00	CONFERENCE DINNER	

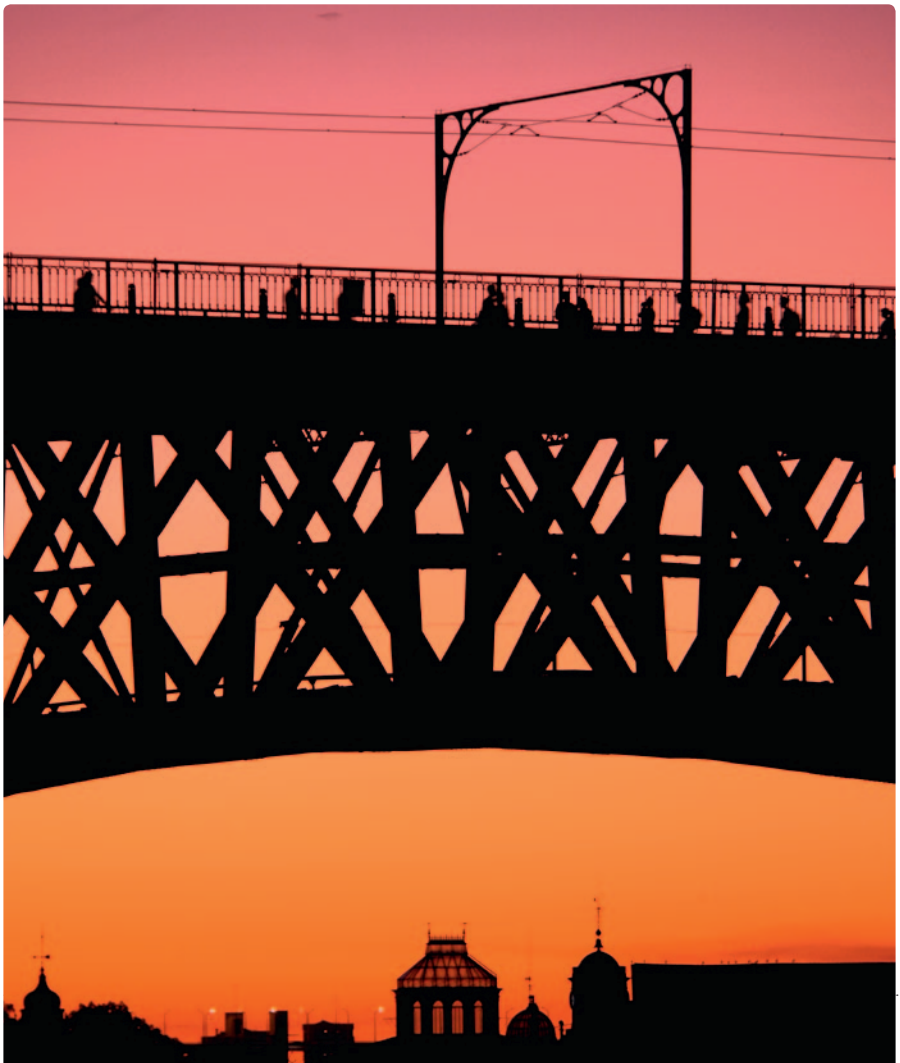
Wednesday, November 10

KEY NOTE LECTURE – CHAIR RAQUEL AIRES BARROS		
08:30	**Arlindo Oliveira** (Instituto Superior Técnico)	**KN4:** Deep Learning Applications in Molecular and Cellular Biology

SESSION 8: FUNDAMENTALS AND MODELLING I CHAIR: EGBERT MÜLLER		
9:20	**Stefan Rauwolf** (TU Munich)	**OP35:** Interactions of Amino Acids and DNA at the Aqueous-Silica-Interface
9:35	**Leo Jakob** (BOKU Vienna)	**OP36:** Protein-protein interactions in HIC dual salt systems
9:50	**Jannette Kreusser** (TU Kaiserslautern)	**OP37:** Influence of Salts and pH Value on the Adsorption of Bovine Serum Albumin on a Mixed-Mode Resin
10:05	**Aleš Podgornik** (Faculty of Chemistry and Chemical Technology, Univ. of Ljubljana)	**OP38:** Fast non-invasive determination of ionizable functionalities via pH transient
10:25	REFRESHMENT BREAK	

SESSION 9: FUNDAMENTALS AND MODELLING II CHAIR: CRISTINA DIAS-CABRAL		
11:00	**Nikolaus Hammerschmidt** (Boehringer Ingelheim)	**OP39:** Centrifugal separation - A modeling approach considering feed and separator properties
11:20	**Maximilian Krippl** (BOKU Vienna)	**OP40:** Hybrid Modeling in Tangential Flow Filtration: Enabling digital solutions for fast process development and control
11:35	**Ana Rufino** (University of Aveiro)	**OP41:** Separation of monoclonal antibodies using ionic-liquid-based aqueous biphasic systems

11:50	**Jorge F. B. Pereira** (Univ. of Coimbra)	**OP42:** Purification of green, red and yellow fluorescent proteins using cholinium chloride-based aqueous biphasic systems
12:10	Presentation of Awards and Concluding Remarks	
12:30	END OF CONFERENCE	



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Poster Session - Overview

P1 **Effect of temperature on the operational efficiency of Protein A chromatography for the purification of a monoclonal antibody**
Armstrong A[1], Bracewell D[1], Farid S[1], Goldrick S[1]
[1]Advanced Centre for Biochemical Engineering, University College London, London, United Kingdom

P2 **Online optimization in protein purification**
Eslami T[1], Hirschmann G[2], Jungbauer A[1], Lingg N[1]
[1]BOKU University, Vienna, Austria,
[2]Evon Gmbh, Wollsdorf, Austria

P3 **An affinity approach for baculovirus clearance from viral-based bioprocesses**
Fernandes R[1,2], Fernandes R[1], Carvalho S[1,2], Bezemer S[3], Detmers F[3], Hermans P[3], Silva R[1], Alves P[1,2], Carrondo M[1], Peixoto C[1,2]
[1]IBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal,
[2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal,
[3]Thermo Fisher Scientific, Leiden, Holanda

P4 **One-step chromatography to produce anti-vp39 antibody for fast Baculovirus detection using Western blot analysis**
Frank A[1], Zollner A[2], Mayer V[1], Pereira Aguilar P[1], Jungbauer A[1]
[1]Acib Gmbh, Wien, Austria,
[2]Boku Vienna, Wien, Austria

P5 **Purification of immunoglobulin G antibodies using hybrid alginate-protein cryogel beads**
Freire M[1], Tavares A[1], Sharma M[1], Singh N[2], Mondal D[3], Prasad K[3]
[1]CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal,
[2]Natural Products & Green Chemistry Division, CSIR-Central Salt & Marine Chemicals Research Institute, Gujarat, India,
[3]Centre for Nano & Materials Sciences, Jain University, Jain Global Campus, Karnataka, India

P6 **Analysis and separation of proteoforms from a single protein via protein purification parameter screening and sample displacement chromatography**
Hidayah S[1], Gaikwad M[1], Schlüter H[1]
[1]University Medical Center Hamburg, Hamburg, Germany

P7 **FLASHDeconvQ: High-quality feature level label-free quantification algorithm for top-down proteomics mass spectrometry data**
Kim J[1], Jeong K[1], Gaikwad M[2], Babović M[3], Jensen O[3], Schlüter H[2], Kohlbacher O[1]
[1]University of Tübingen, Tübingen, Germany,
[2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,
[3]University of Southern Denmark, Odense, Denmark

P8 **Empty and Full Separation of Adeno-Associated Virus Vectors by Anion Exchange Membrane Chromatography**
Hejmowski A, Boenning K, Kavara A, Huato J, Schofield M, Figueiredo S
[1]Pall Corporation

P9 **Aqueous Biphasic Systems for tailoring the extraction and purification of antileukemic enzyme L-Asparaginase**

Magri A[1], Santos J[2], Coutinho J[2], Ventura S[2], Pereira J[3]
[1]School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquaraa, Brazil,
[2]CICECO, Institute of Materials, University of Aveiro, Aveiro, Portugal, 3Univ Coimbra, CIEPQPF, Department of Chemical Engineering, Coimbra, Portugal

P10 ***In Situ* Protein Recovery from Yeast Fermentations**

Pham D[1], Linova M[1], Carbonell R[2], Woodley J[1]
[1]The Technical University of Denmark, Kgs. Lyngby, Denmark, 2North Carolina State University, Raleigh, USA

P11 **Bioprocess monitoring of glycosylated protein concentration via In-process lectin polyHIPE chromatography**

Podgornik A[1,2], Stantič M[1], Mravljak R[1], Gunčar G[1]
[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia,
[2]COBIK, Ajdovščina, Slovenia

P12 **Single-step purification of ssDNA scaffolds using affinity magnetic beads for DNA-origami biomanufacturing**

Santos A[1], Oliveira-Silva R[1], R. Paulo P[2], F. Prazeres D[1]
[1]iBB - Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001, Lisbon, Portugal,
[2]Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 , Lisbon, Portugal

P13 **Immobilization of L-asparaginase towards surface-modified carbon nanotubes**

Tavares A[1], Almeida M[1], Cristóvão R[2], Barros M[2], Nunes J[1], Faria J[2], Neves M[1], Freire M[1], Santos-Ebinuma V[3], Silva C[2]
[1]CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal,
[2]Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal,
[3]Department of Engineering Bioprocess and Biotechnology, School of Pharmaceutical Sciences, UNESP-University Estadual Paulista, Araraquara, Brazil

P14 **Optimal State Estimation of an ADC Conjugation Reaction Using an Extended Kalman Filter**

Weggen J[1], Schiemer R[1], Schmitt K[1], Hubbuch J[1]
[1]Institute of Process Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

Key Note Lecture Abstracts

Kinetics of Binding of Conformationally-Flexible Multidomain Proteins on Porous Cation Exchange Resin

Carta G[1]

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

The search for more effective biotherapeutics is generating growing interest in new antibody formats that are able to interact with multiple targets simultaneously. The different domains in these molecules typically have diverse properties resulting in preferred binding orientations. In this work, we consider the binding behavior of bi-valent, bi-specific antibodies (BiSAbs) on cation exchangers. These molecules comprise scFv domains fused to a framework IgG molecule via flexible peptide linkers. BiSAbs are thus conformationally flexible. In prior work, we have shown that the kinetically-limited interconversion of different conformations results in multiple-peak behaviors during gradient elution that depend on residence time, temperature, and hold time. In this work, we explore how this surface-catalyzed interconversion affects adsorption kinetics by examining the transient patterns of bound protein within the particles using CLSM. While the framework IgG alone exhibits classical shrinking core behavior, BiSAbs show complex patterns of total bound protein within the particles that are influenced by ionic strength, pH, and temperature in unique ways. Sharp profiles are observed at greater binding strength conditions or when the protein is chemically crosslinked to restrict conformational flexibility. Temperature affects both diffusivity and interconversion kinetics. A mechanistic model indicates that the interplay of pore diffusion and adsorption with the kinetically-limited interconversion between binding forms is responsible for the experimental trends. The model predicts that the dynamic binding capacity will also be affected and suggests how experimental conditions can be chosen for improved performance. Lastly, we examine the molecular basis by means of interdomain docking simulations.

Extracting new molecular biology knowledge from proteins and peptides

Aebersold R.

Department of Biology, Institute of Molecular Systems Biology, ETH Zurich

The proteome is the ensemble of all proteins expressed in a cell or tissue. Human cells express a few billion protein molecules derived from the roughly 20.000 protein coding genes of the genome, spanning a cellular concentration range from a few molecules to tens of millions of molecules per cell. The analysis of the proteome, therefore, presents an enormous analytical challenge that is generally met by the sequential application of protein and peptide separation methods followed by the mass spectrometric analysis of separated analytes. The proteome is as biologically and clinically as important as it is complex. Essentially all biochemical processes of a living cell are catalyzed and controlled by proteins, proteins are the target of most drugs and aberrations of biochemical processes are the root cause of many diseases. The precise analysis of the proteome is therefore of broad interest.

In this presentation we will discuss the present state of proteome analysis and discuss new experimental and computational approaches to systematically analyze the proteome. At present proteomic analyses are largely focused on identifying and quantifying as many protein species in a sample. This has been exceptionally successful as now essentially all proteins of a sample can be measured and hundreds of replicates can be reproducibly measured. However, proteins present information that goes beyond determining their sequence and quantity. Of equal biological importance is knowledge of their interactions with other proteins and other biomolecules, their state of modification and the variability of protein species derived from a specific gene (proteoforms). We will discuss emerging strategies to explore these hitherto understudied aspects of the proteome and the analytical challenges they present.

Evaluation of Preferred Binding Orientations of Protein Therapeutics in Multimodal Chromatographic Systems using Biophysics and Simulations

Cramer S.M.

Rensselaer Polytechnic Institute, Troy, NY

The identification of preferred binding regions and specific residues on proteins that interact with ligands and ligand coated surfaces is important for understanding the molecular basis for the purification of biological products from product related impurities. In this presentation we will discuss a variety of biophysical and simulation tools that can provide insights into these interactions in multimodal chromatographic systems. NMR with labelled proteins and ligand coated nanoparticles is employed to identify preferred binding domains for the Fc domain of antibodies and to examine the impact of multimodal ligand chemistry and ligand densities on this interaction. Umbrella sampling molecular simulations are then used to provide information on the contributions of ligand clustering to these interactions. Protein surface footprinting using covalent labeling followed by LC/MS analysis is then employed to identify preferred binding domains and interacting residues of several new protein biotherapeutics such as bispecific mAbs in multimodal systems as a function of pH. Finally, this data set is examined using a variety of simulation tools including dewetting calculations of protein and chromatographic surfaces to shed light on the nature of selectivity in these complex bioseparation systems.

Deep Learning Applications in Molecular and Cellular Biology

Oliveira A.

Instituto Superior Tecnico, Lisbon, Portugal

Machine learning, a discipline that is more than 70 years old, is the driving force behind the most recent advances in Artificial Intelligence. The idea that computers can learn from experience, as humans and animals do, pioneered by Alan Turing in the end of the first half of the twentieth century, has made possible significant advances in planning, natural language processing, computer vision and machine perception.
On the other hand, advances in instrumentation technology, DNA sequencing, transcriptomics, genomics and proteomics, among many other fields, led to an explosion of biological data, challenging manual analyses existing automated methods. Existing technologies simply generate more data than can be analyzed by hand or with existing data analysis techniques.
Very likely, deep learning techniques, already prevalent in so may domains, will, in the near future, be more extensively used to process data obtained by molecular and cellular profiling, complementing and supplementing existing analysis methods. In this talk, I will describe the state of the art of deep learning technology and suggest possible applications of this technology in the field of molecular and cellular biology.

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Oral Presentation Abstracts

Affinity LC-MS for high-sensitivity analysis of proteins in complex biological samples

Bischoff R[1], Klont F[1,2], Wilffert D[1,5], Pouwels S[3], ten Hacken t[3], van de Merbel N[4]

[1]University of Groningen, Groningen, Netherlands,
[2]University of Geneva, Geneva, Switzerland,
[3]University Medical Center Groningen, Groningen, Netherlands,
[4]PRA Health Sciences, Assen, Netherlands, 5Ardena, Assen, Netherlands

Low-abundance protein bioanalysis relies primarily on ligand binding assays (LBAs) and notably on enzyme-linked immunosorbent assays (ELISAs). While highly sensitive, these assays suffer from the fact that the final readout is indirect (e.g. fluorescence, chemoluminescense) and does not provide direct chemical information about the actual analyte(s) that served to generate the signal. Recent years have seen the advent of a combination of affinity-capture liquid chromatography – mass spectrometry (LC-MS) assays that have the advantage of providing chemical information as well as allowing to extend the analysis to a range of proteins that can be quantified in a single assay (multiplexing).
In this presentation, I will show examples of biomarker analyses at the sub ng/mL level (pM level) after enrichment with antibodies (immunoaffinity), affimers or generic solid-phase extraction approaches. These affinity LC-MS assays were validated according to international guidelines for regulated, quantitative bioanalysis and applied to samples from preclinical and clinical studies. The presented work shows that protein bioanalysis must be considered in the context of which proteoforms are being measured and which recognition elements of the respective proteins are used for enrichment and detection. Omitting these important details from assay descriptions leads to varying results that may differ from one assay format to the other.

pH gradient-based cation exchange chromatography for the separation of charge variants of monoclonal antibodies after forced degradation study

Spanov B[1], Olaleye O[1], van de Merbel N[1], Lingg N[2], Jungbauer A[2], Bentlage A[3], Vidarsson G[3], Bischoff R[1]

[1]Department of Analytical Biochemistry, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands,
[2]Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria,
[3]Department of Experimental Immunohematology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Monoclonal antibodies (mAbs) are a class of biopharmaceuticals that have been used quite successfully in the treatment of various diseases such as cancer, asthma, and rheumatoid arthritis. mAbs are inherently heterogeneous. Heterogeneity may arise during manufacturing, storage, and in vivo because mAbs are subject to chemical modifications like asparagine deamidation, aspartic acid isomerization, and the oxidation of methionine and tryptophan residues. Such modifications lead to changes in physicochemical properties of mAbs, which may affect drug efficacy or induce immunogenicity. Potential „hotspots“ for modifications may be revealed under stressed conditions. Ion-exchange chromatography (IEX) and notably cation-exchange chromatography (CEX) is widely used to resolve charge heterogeneity of mAbs. In this study, we evaluated the application of pH gradient buffers for the separation of charge variants of trastuzumab and pertuzumab after a forced degradation study. The forced degradation study was performed under physiological conditions (PBS buffer pH 7.4 and 37 °C) by stressing up to 3 weeks. Charge variants were characterized by peptide mapping and middle-down mass spectrometry. Evaluation of the biological activity of stressed samples in terms of HER2 and Fcγ receptor binding was also performed. Initial results show that this approach can be combined with affinity enrichment of charge variants of trastuzumab and pertuzumab from patient plasma samples to study in vivo biotransformations.

Affinity sheathless-CE-MS as a new tool for the functional assessment of antibody - Fc receptor interactions

Gstöttner C[1], Wuhrer M[1], Dominguez-Vega E[1]

[1]Leiden University Medical Center, Leiden, Netherlands

Monoclonal antibodies (mAbs) consist of variable domain, for antigen binding, and a constant domain, necessary for an immune response via Fcγ receptors, and mAb recycle via the neonatal Fc receptor (FcRn). These interactions are influenced by structural features of the Fc domain and different posttranslational modifications (i.e different proteoforms) can impact their binding. Common approaches, such as SPR, provide an overall affinity response for all different mAb proteoforms instead for each specific proteoform.
In our lab, we have exploited for the first time the capabilities of Capillary Electrophoresis hyphenated with Mass Spectrometry (CE-MS) to study the binding affinity of antibodies and FcRs in a proteoform-resolved fashion. To this end, the FcRs were added to the background electrolyte whereas the mixture of antibody proteoforms were injected in the CE. We will show that we are able to determine the affinity of mAb proteoforms as a consequence of their different mobility shifts, using different amounts of FcR in the background electrolyte. Hyphenation to MS allowed us to simultaneously characterize the mAbs, the receptor and the complexes formed. Furthermore, as the receptor is free in the solution, higher-order structures can be formed reflecting the in vivo situation in contrast to immobilized receptors (e.g. affinity LC or SPR). Overall we believe that our approach will tremendously benefit the study of interactions between mAb proteoforms and FcRs. Understanding these interactions is essential for developing new drugs as well as defining (and redefining) critical quality attributes of biopharmaceuticals.

Unravelling the structural and functional heterogeneity of recombinant SARS-CoV-2 RBD domains

Dominguez-Vega E[1], Gstöttner C[1], Zhang T[1], Resemann A[2], Ruben S[3], Pengelley S[2], Suckau D[2], Welsink T[3], Wuhrer M[1]

[1]Leiden University Medical Center, Leiden, Netherlands,
[2]Bruker Daltonik GmbH, Bremen, Germany,
[3]InVivo BioTech Services GmbH, Hennigsdorf, Germany

Recombinant SARS-CoV-2 proteins - including the receptor binding domain (RBD) from the spike (S) protein - are essential instruments in the fight against COVID-19. RBD structural features such as glycosylation, are critical for binding to ACE2 receptor - yet not completely understood. We have performed an unprecedented structural and functional characterization of SARS-CoV-2 RBD domains produced in two different mammalian systems. We combined state-of-the-art mass spectrometric approaches at different protein levels (released glycans, glycopeptides and intact protein), permitting us to unravel the vast heterogeneity of RBDs in great detail. Our results showed distinct glycosylation and posttranslational modifications between CHO- and HEK293-RBDs. These features are also dependent on the expressed protein length (RBD, S1 subunit or S protein). We also demonstrated the presence of a single, fully-occupied, O-glycosylation site in the RBDs, and localized the previously unknown O-glycosylation site to T323. We studied the RBDs functionally by determining the binding to SARS-CoV-2 antibodies from positive COVID-19 patients as well as to the ACE2 receptor. Our data indicate that the previously suggested involvement of RBD glycosylation in ACE2 receptor binding originates from conformational stabilization of the spike protein rather than from a direct involvement in the binding. In summary, this work offers novel insights into RBD structural and functional features but also provides a workflow for the characterization of RBDs highly relevant for the integrated structural and functional characterization of RBDs and RBD-based vaccines.

RT-MALS: Real-time analysis of product attributes in downstream processing

Some D[1], Pérez-Peinado C[2], Apetri A[2]

[1]Wyatt Technology Corporation, Santa Barbara, United States,
[2]Janssen Vaccines & Prevention B.V., Leiden, The Netherlands

One of the key challenges during process development and production of biologics is the timely determination of essential product attributes. Rapid feedback of product-relevant characteristics simplifies and accelerates process development and scale-up, then helps ensure product yield and quality during full-scale manufacturing.
A promising process analytical technology for biologics such as viral vectors, and complex drugs such as lipid nanoparticles, is real-time multi-angle light scattering (RT-MALS). RT-MALS determines basic biophysical properties such as size, molar mass and concentration of virions or nanoparticles. MALS provides a non-destructive and essentially instantaneous measurement, making it suitable for in-line monitoring of rapidly changing analyte conditions.
For macromolecular applications such as protein purification, MALS can indicate the onset of aggregate elution and quantify the amount of aggregates present. For viral vector and nanoparticle PAT applications, MALS clearly distinguishes between the particles of interest and the much smaller impurities that may be present such as proteins or nucleic acids, in contrast with traditional process monitors such as UV absorbance. Hence RT-MALS is appropriate for automated pooling decisions that do not rely on timing and modeling associated with specific process conditions, and at the end of the process will even provide an estimate of the total number of particles in the pool and their concentration. We will review the principles and benefits of RT-MALS and present several examples including recent work demonstrating its use as PAT for adenovirus process development.

Advances in microfluidic technology for spatial three-dimensional liquid chromatography

De Vos J[1], Themelis T[1], Amini A[1], Eeltink S[1]

[1]Department of Chemical Engineering, Vrije Universiteit Brussel, Brussels, Belgium

High-performance liquid chromatography (HPLC) has emerged as a dominant technique in the field of analytical chemistry and is widely applied in many areas, including life-science research, clinical diagnostics, and the (bio)pharmaceutical sector. For the analysis of samples used in biomarker discovery studies, which are typically characterized by a large sample complexity and broad dynamic range, the current state-of-the-art HPLC technology does not allow to fully resolve the analytes of interest. Column-based multidimensional LC approaches, where the fractions originating from multiple columns are sequentially analyzed, have as drawback that the analysis takes a long time to complete. This makes the technology intrinsically unsuitable for high-throughput screening of biomolecules.

To advance the separation performance, and to overcome this bottleneck of sequential runs, the development of a microfluidic separation device for spatial 3D-LC was explored. In spatial 3D-LC chromatography components are separated inside the interconnected microchannels of the device with each peak being characterized by its X, Y, and Z coordinates in the separation body. We investigated different design aspects of a microfluidic device for comprehensive spatial 3D-LC. During the different developments, the analytes and therefore the flow should not convolute in other dimensions. To this end, we developed active flow confinement technology, which also allows to modulate the mobile-phase composition between the developments. Furthermore, approaches to integrate functionalized monolithic stationary phases at pre-defined locations in the microfluidic device have been realized. Novel engineering approaches have been explored (including high-resolution DLP 3D-printing) to realize an interface for hyphenation with MALDI-MS.

Development of a 3D Printed Micro Simulated Moving Bed Device as Process Analytical Tool

Diehm J[1], Ballweg T[1], Franzreb M[1]

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

Process Analytical Technology (PAT) is a core research topic in biopharmaceutical process engineering. Most applications in downstream processing of biopharmaceuticals reported so far use in-line spectroscopic measurements and on-line HPLC methods. Other established methods of off-line analytics during process development, such as mass spectrometry, are hardly used for PAT applications for manufacturing processes due to the required sample preparation and the associated complexity, although they would provide a great advantage for the on-line determination of critical quality attributes. Methods such as Simulated Moving Bed (SMB) chromatography allow for continuous sample preparation, but the required instruments are expensive, the handling is complex and the available devices are not suitable for analytical purposes.

Thus, we develop a micro SMB device, which can be used for analytical purposes. First, the factors limiting the down-scaling of SMB processes are examined. The ratio of the internal dead volume of the SMB system in relation to the single-column resolution of the desired separation problem as well as the variations in bed packing with the individual chromatography columns are identified as key aspects for the design of an analytical micro SMB.

In the next step a concept to overcome these limitations was developed, using 3D printing technologies to manufacture a compact SMB valving system to minimize the internal dead volume.

First promising results for continuous desalting of proteins with the 3D printed micro SMB system are presented, yielding desalting efficiencies above 95 % at feed flow rates as low as 15 µL/min.

Advanced machine-learning methods for the analysis of spectroscopic data in biopharmaceutical processes

Schiemer R[1], Ruedt M[1], Hubbuch J[1]

[1]Karlsruhe Institute Of Technology, Karlsruhe, Germany

In downstream processing of biopharmaceuticals, process analytical technology has been developed to monitor process parameters and quality attributes in real-time. With spectroscopy being the method of choice for most chromatography and filtration-based separation methods, the calibration of robust, accurate and interpretable multivariate regression models is essential. Although partial least squares regression presents a simple and fast method to analyze high-dimensional spectroscopic data, it lacks the functionality to model non-linearities and requires the user to decide on optimal preprocessing strategies.

Artificial intelligence and machine learning (ML) provide a plethora of tools for the analysis of non-linear data. In this work, we present an approach to model spectroscopic data by advanced ML methods such as convolutional neural networks and Gaussian process regression with regards to the prediction of concentration of multicomponent mixtures. By the use of deep learning or Bayesian regression modeling, targeted preprocessing and complex correlations can be automatically learned from the data, improving overall prediction accuracy. Having multiple models in place offers a high degree of flexibility to the user to find a tailored solution for a given regression problem. As advanced ML methods can be increasingly complex, they require large sets of data or robust methods for data generation. To this end, strategies were implemented to augment experimental data sets with in silico generated data, identify potential weak points and provide better interpretability. The advantages and trade-offs of the suggested modeling approaches over conventional chemometrics are exemplified in selected case studies from downstream process operations.

Affinity tag-based development and manufacturing platform for non-platform proteins

Lingg N[1,2], Fischer A[1], Striedner G[1,2], Oostenbrink C[1,2], Schneider R[1,3], Berkemeyer M[4], Jungbauer A[1,2]

[1]Austrian Centre Of Industrial Biotechnology, Vienna, Austria,
[2]University of Natural Resources and Life Sciences, Vienna, Vienna, Austria,
[3]University of Innsbruck, Inssbruck, Austria,
[4]Boehringer Ingelheim RCV, Vienna, Austria

Downstream process development for microbial products is a time-consuming task, that delays the market introduction of new biological entities. Affinity tag purification would be a perfect technology for non-platform proteins, such as new formats which do not contain a protein A binding region. These processes are only established in laboratory scale due to the lack of suitable proteases which remove the affinity tag in way that an authentic N-terminus of the target protein is generated. An affinity chromatography-based process using removable tags would enable a platform process, akin to that used for mAbs. Here, we present a novel protease that overcomes the present limitations. This protease is based on human caspase enzymes and allows the cleavage of affinity tags regardless of the target protein's N-terminus in minutes to hours. Moreover, this protease is easy to manufacture, stable and resistant to commonly used chemicals. Example processes for the production of human fibroblast growth factor 2 and other proteins are presented. The platform DSP consists of affinity capture, tag removal and subtractive affinity chromatography. Generation of authentic N-terminus as confirmed by mass spectrometry and Edman sequencing. The platform process can be applied in lab scale early stage research all the way to full scale manufacturing. This seamless approach shortens the process development and the time to market.

Nanofibers as an alternative for fast affinity separations

Eppink M[1], Kamps B[1], van Wijk D[1], Joosten - Stoffels L[1]

[1]Byondis 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.
Currently, the main purification step remains the capture step which is mostly a Protein A affinity step. One of the bottlenecks in this unit operation is the capacity and throughput of the resin for monoclonal antibodies. Processing needs to be performed in a fast and efficient way omitting product degradation. In the light of these challenges the new Fibro nanofibers might be a new approach for the efficient and fast processing of the monoclonal antibody product as capture step. In this presentation results will be discussed and some calculations performed if the nanofibers are a good alternative for the resin based protein A chromatographic purification step.

Enabling Large Particle Chromatographic Separations Using Advanced Materials

Voloshin A[1]

[1]3m Company, St. Paul, United States

Chromatography continues to form the core principle of high fidelity purification technologies in bioprocessing systems. Combined with various ligand chemistries, it enables highly reproducible high resolution separations that are required to meet the safety and quality of biopharmaceutical products today and in the future.

Until very recently, the industrially relevant chromatographic systems utilized porous supports, such as functional beads and membranes. Such approaches, while being very effective at separation of small biological molecules, such as simple proteins, are unable to effectively handle larger and more complex particles, such as DNA, aggregates of endotoxin, vesicles, viral particles, and other large assemblies. These larger entities appear more and more as key interfering contaminants in the bioprocesses, as well as emerging biotherapeutic modalities and delivery vehicles. As the range of the size of particles of interest increases, current systems dictate significant compromises between pore diffusion dynamics that affects system stability and robustness, and system capture capacity.

Here we present a novel chromatographic technology platform that is able to overcome limitations of present chromatography systems with respect to particle size range. We utilize advanced functionalized fibrous materials that eliminate diffusional limitation over a very wide size range, while preserving high binding capacity. These systems enable realization of the advantages of chromatographic separations in every part of the process, from clarification, to polishing, to nano-filter protection. We present applications of such technologies and demonstrate bioprocess simplification and intensification in academic and industrial spheres.

Complex polypeptides: Regulatory and physical/chemical considerations related to manufacturing processes of follow-on products

Staby A[1], Steensgaard D[1], Haselmann K[1], Marino J[1], Bartholdy C[1], Videbæk N[1], Schelde O[1], Bosch-Traberg H[1], Spang L[1], Asgreen D[1]

[1]Novo Nordisk, Bagsværd, Denmark

Manufacturing processes for polypeptide/protein drugs are designed to ensure robust quality, efficacy and safety. Follow-on products are developed to promote competition and pricing competitiveness, and regulatory requirements are working to reduce costly clinical testing for follow-on products. Process differences introduced by follow-on manufacturers may, however, result in changes in quality and potentially, clinical outcomes. This study investigated the impact of production methods on the stability, process- and product-related impurities of liraglutide and semaglutide drug substance and drug product, and the potential impact on drug quality, efficacy and safety. The presentation will thus discuss the impact of manufacturing processes on higher order structure of polypeptides, potential for aggregation & fibrillation, impurity profiles, trace impurities, potential for immunogenic responses, scale-up and stability considerations on regulatory assessment for various follow-on polypeptides.

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Making tomorrow's therapies a reality, what to consider when developing a chromatography toolbox approach

Bassens C

[1]*Cytiva, Amersham Place, United Kingdom*

Looking at the current pipeline, the last few years have seen an increase in molecular diversity with a promise of transformative outcomes for patients. In order to make these therapies affordable, there is an urgent need to overcome current productivity challenges. The presentation will focus on Cytiva's chromatography approach, looking at the need to develop a toolbox to accommodate the various molecules where a standard platform approach would have limitations.

A new Protein A resin allowing quantitative elution of human IgG at pH 5

Johansson H[1]

[1]Purolite, Uppsala, Sweden

The first Protein A resin designed for large scale purification of monoclonal antibodies were launched more than thirty years ago. The resin was based on the wild type of Protein A expressed by a pathogenic Staphylococcus aureus strain. Since then, manufacturing of monoclonal antibodies has grown tremendously and are now the most important group of molecules in the pharma industry. Along with this development, Protein A resins have been significantly improved both with respect to capacity, productivity, and alkaline stability, resulting in a dramatic enhancement of process performance. Current trends in antibody-based therapeutics include the development of a variety of different atypical IgG constructs that in some cases are prone to aggregation, are expressed at very high titer, and contain several product variants. There is thus an increasing interest in resins capable of resolving product variants at the Protein A step and eluting IgG at milder pH conditions compared to current Protein A resins on the market. This paper will present data from a new Protein A resin that allows elution of IgG, including IgG of the VH3 family at significantly higher pH compared to regular Protein A resins.

A calcium-dependent Protein A-derived ligand for mild purification of antibodies

Scheffel J[1], Kanje S[1], Gomis-Fons J[2], Isaksson M[2], Nilsson B[2], Hober S[1]

[1]KTH Royal Institute of Technology, Stockholm, Sweden, 2Lund University, Lund, Sweden

In the purification of antibodies through Protein A affinity chromatography, the acidic conditions required for elution can be detrimental to some antibodies and Fc-fusion proteins. To solve this problem, we have developed a Protein A-derived domain with a calcium-dependent binding to the Fc region of IgG. This domain, ZCa, allows for considerably milder elution of the captured antibody through the depletion of calcium. Calcium can be depleted from ZCa merely by adding sodium chloride at low concentrations, resulting in the efficient release of IgG1 at pH 6 and antibodies of subclasses IgG2 and IgG4 at neutral pH. This mild elution has been shown to eliminate the formation of antibody aggregates in the capture step, in contrast to elution at low pH using a commercial Protein A resin which resulted in aggregation of more than a third of the same antibody. Further, a tetrameric version of ZCa has demonstrated a binding capacity comparable to this commonly used Protein A resin, including exceptional selectivity and recovery. The tetrameric ZCa chromatography resin has been applied in a continuous antibody capture step where it proved to be highly robust by processing high antibody titers for a great number of cycles. This data was reproduced in a scaled up continuous antibody production and purification process at pilot scale, rendering high yields. The mild and efficient purification strategy based on ZCa has the potential to enable the development of a broader range of antibodies, which cannot tolerate the current acidic conditions used in antibody capture.

A metal-dependent protein domain derived from Streptococcal Protein G enabling gentle elution of mIgG1 and human Fab

Jönsson M[1], Parks L[1], Löfblom J[1], Hober S[1]

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

The monoclonal antibody market is ever-expanding with a need for functional pure antibodies to be used as therapeutics, for diagnostics and as common affinity reagents in research laboratories across the globe. The gold standard for purification of antibodies has long been Protein A and Protein G – with inherent affinities for the constant domain of Fc and Fab – during a process which allows for binding of the antibody at near neutral pH followed by elution at a very low pH of 2.7 for Protein G. However, such an acidic pH could cause detrimental structural changes making the antibodies prone to aggregate and resulting in a lower recovery of functional antibodies in the final sample. A revolutionizing improvement in this purification strategy would be the enabling of milder antibody purification by operating the entire procedure at near physiological pH. Inspired by nature where many protein-protein interactions have evolved to be dependent on metal ions we made an engineering attempt to introduce a calcium-binding loop into a domain of Protein G. Through a combination of protein design and randomization, selection by E. coli display, and FACS sorting it was possible to isolate several domains able to interact with mIgG1 and human Fab fragments only in the presence of calcium. Once depleted of calcium, the domains' target affinity is lost rendering it a promising advancement towards the dream of gentler protein purification. Here we will present the development, from the design of the library to its use as a purification ligand.

Purification of IgY by Mixed Mode Chromatography

Müller E[1], Endres P, Miehle A

[1]Tosoh Bioscience GmbH, Griesheim, Deutschland

IgY is an immunglobuline occuring in birds and reptiles as an essential protein of their adaptive immune system. The molecular mass of IgY is approximately 167 kDa.
IgY can be found in high concentration in hen egg yolks. On average 125 mg of IgY can be isolated from one chicken egg.
IgY does not bind to Protein A , Protein G nor to Fc receptors. It is thus more difficult to purify them by classical pseudo-affinity chromatography, compared to the mammalian antibodies. Alternative “Protein A-free” purification methods are necessary. We have investigated a two step purification procedure with precipitation and chromatographic separation.
For this, IgY was isolated from commercial available hen eggs by using polyethylene glycol and ammonium sulfate precipitation. In a second step cation exchanger resins, anion exchanger resins, resins for hydrophobic interaction chromatography and mixed mode resins were screened for their ability to further purify the precipitate, monitoring the IgY recovery and purity. Best candidates in this screening were the multimodal type TOYOPEARL NH2-750F and TOYOPEARL MX-TRP-650M resins. The conditions were further optimized by different gradient shapes and salt types, resulting in a final purity of 94.4% after purification with TOYOPEARL NH2-750F loaded to 80 % of it’s dynamic binding capacity.
It seems to be that multimodal type resins are superior for IgY purification in a “Protein A-free” process than the single mode resins.

Design of specific ligands for pre-miRNA purification

Bernardo S[1], Carapito A[1], Riscado M[1], Neves M[2], Freire M[2], Sousa F[1]

[1]CICS-UBI Health Sciences Research Centre of University of Beira Interior, Covilhã, Portugal,
[2]CICECO - Aveiro Institute of Materials , University of Aveiro, Aveiro, Portugal

RNA-based therapies have been emerging in the last years but incredible enthusiasm and interest were recently verified with the success of mRNA vaccines to tackle the COVID-19 pandemic. These nucleic acids hold the extraordinary potential to treat various concerning diseases. Recent studies showed the relevance of microRNA-29 in Alzheimer's disease (AD). Considering that the precursor form of microRNAs (pre-miR-29) is double stranded, with a hairpin loop structure, it presents higher stability during purification and further handling. Thus, pre-miR-29 has been gaining attention has a potential biomarker and therapeutic product in our research group. Rhodovulum sulfidophilum has been used as an alternative expression system for recombinant production of pre-miRNAs, since this bacteria has the advantage of secreting the produced RNAs to the extracellular medium, which greatly simplifies the purification, reduces protein contamination, and enhances the RNA stability.
This work describes the establishment of a simple, reproducible and effective purification strategy to obtain pure and stable pre-miRNAs targeting AD. For this, several ionic liquids were used to functionalize silica supports as novel chromatographic ligands (SILs), since in previous works these ILs demonstrated their ability to successfully stabilize the structure and activity of RNAs. Herein, the 1-(3-aminopropyl)-methylimidazole and dimethylethylenediamine were used to modify the silicapropylchloride and these novel SILs were used for further purification of pre-miR-29, with very promising results. Noteworthy is that some of the components of the extraction process were removed in the flowthrought and only the RNA is further recovered.
Authors acknowledges FCT for funding the project POCI-01-0145-FEDER-029496 (PUREmiR-SILs).

A chromatography alternative for mRNA manufacturing

S. Rosa S[1], C. Marques M[2], F. Prazeres D[1], Azevedo A[1]

[1]iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal,
[2]Department of Biochemical Engineering, University College London, London, United Kingdom

mRNA is an emerging technology in the vaccine field. This technology has demonstrated its potential as it was one of the first to reach commercial phase during the Covid-19 pandemic. It presents several advantages over more traditional vaccines owing to its safe profile, specificity, and immune response, as it promotes both humoral and cellular immune response and induces the innate immune system.

mRNA manufacturing is based on cell-free system, that uses an enzyme, such as T7 RNA polymerase, to produce mRNA from a DNA template, and using NTPs as substrate. This is simple and fast process that can be easily scaled up. However, to achieve a highly pure mRNA, it requires the implementation of a multi-step purification process. Traditional lab scale purification methods are based on DNA removal by enzymatic digestion with a DNAse followed by lithium chloride precipitation. These methods, although simple and well-established, do not remove impurities that can critically affect the mRNA performance.

In this work we explore the use of chromatography for the purification of mRNA vaccines. Affinity chromatography that uses a single-stranded sequence of deoxythymidine (dT) is implemented and optimised for the mRNA purification, and two different matrices, traditional resin and monolith, are compared. Additionally, new chromatographic operation modes that explore multimodal interactions are evaluated. Chromatography performance is assessed not only by quantitating recovery but also by analysing the final quality. Chromatography is widely used in pharmaceutical industry, and when well-established it can be a flexible and cost-effective step for the manufacturing of mRNA vaccines.

DNA-origami nanostructures: exploring chromatography for the purification of single stranded DNA scaffolds

Santos A[1], F. Prazeres D[1]

[1]iBB - Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001, Lisbon, Portugal

DNA-origami biomanufacturing relies on the use of asymmetric PCR to generate 500-3500 base pair (bp), object-specific, single-stranded DNA (ssDNA) scaffolds using the DNA of the M13 phage as template. Each scaffold is purified by agarose gel extraction, a technique that is laborious, limited, not scalable, presents low recovery yields and a low-quality product. We present a chromatography-based method to purify ssDNA scaffolds from asymmetric PCR mixtures, which can be used in DNA-origami techniques.

Asymmetric PCR was performed to generate 449 and 1000 nt-long single and double-stranded DNA (dsDNA) from M13mp18 genome. In order to isolate the target ssDNA from dsDNA and other PCR impurities, anion-exchange (Q-ligand) and multimodal chromatography (CaptoTM adhere ImpRes) were explored using a stepwise gradient with increasing NaCl concentrations. Unused primers and oligonucleotides were washed-out in the flowthrough due to their low charge density. In anion exchange chromatography, the less-charged ssDNA was eluted before the dsDNA. In multimodal chromatography, however, the elution pattern was reversed, highlighting the importance played by hydrophobicity. Gel electrophoresis revealed that ssDNA-containing fractions are homogeneous and impurity free.

Finally, the recovered 1000-nt ssDNA was used to assemble 63-bp edge length tetrahedrons using site-specific short oligonucleotides (staples), thermal annealing and high magnesium concentrations. Agarose gel electrophoresis showed a high assembly yield and purity.

In conclusion, chromatography was successfully used to purify 4.8 ug of 449-nt and 8.4 ug of 1000-nt ssDNA fragments per 10 PCR reactions, confirming that it can be used to produce high quality ssDNA at large scale for DNA-origami biomanufacturing.

Separation of the main macromolecules from model and biotechnological mixtures using iron oxide nanoparticles

Abarca Cabrera L[1], Fraga García P[1], Berensmeier S[1]

[1]Bioseparation Engineering Group, Department of Mechanical Engineering, TUM, München, Germany

Magnetic nanoparticles have demonstrated the ability to adsorb different molecules, attribute that can be exploited to use them as a potential bioseparation tool. The major challenge to apply this technology in the downstream processing area is to control the selective adsorption of a target molecule or a group of molecules from complex environments such as lysates, where there is a mosaic of several biomolecules and ions. Few studies have been done in mixture solutions, which focus on physiological media due to the interest in biomedical applications. Thus, binding studies of competition among proteins, lipids, carbohydrates, and nucleic acids are yet to be developed given that in the literature principally one-type molecule studies appear. With the objective of having a controlled reference, we establish model mixtures by including substances that represent each type of macromolecule. Such models assure saturation conditions where the molecules compete for a binding site. Sodium oleate, BSA, dextran and plasmid DNA are selected to portray the four main macromolecules, while a microalgae lysate is used as a biotechnological mixture reference. In this research, we carry out studies to determine the dynamics of the different biomolecules when binding to iron oxide nanoparticles, revealing that proteins are not the only group that spontaneously binds: lipids remain as stable layer over time and are able to displace other molecules from the surface.
We develop strategies to control the adsorption from model multi-component systems to biotechnological mixtures, aiming to utilize the whole biomass through fractionation and to design sustainable downstream processes.

Affinity magnetic precipitation for antibody purification

Roque A[1], Santos R[1], Iria I[2], Manuel A[2], Leandro A[2], Madeira C[2], Goncalves J[2], Carvalho A[1]

[1]UCIBIO, Chemistry Department, NOVA School of Science and Technology, Caparica, Portugal,
[2]iMed- Research Institute of Medicines, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Precipitation and magnetic separation are two techniques already proven to accomplish protein purification from complex media alone, yet never used in synergy.
With the aim to capture antibodies directly from crude extracts, a new method combining precipitation and magnetic separation was developed and coined affinity magnetic precipitation. Precipitation screens based on the Hofmeister series and a commercial precipitation kit, were tested with magnetic particles for antibody capture from different sources. Two different antibodies sources were tested: (i) polyclonal antibodies from human serum plasma, and (ii) anti-TNFα mAb from clarified CHO cell supernatants. The study comprised the discovery of the best precipitant and precipitation conditions using magnetic particles functionalized with a synthetic ligand previously developed. The addition of particles was crucial to achieve higher antibody purity and recovery. Optimising precipitant and precipitation conditions also revealed to be essential to minimize antibody loss and maximize its purity. The elution was performed using PBS at pH 7.4, leading not only to antibody capture but also conditioning and concentration in one single step. The activity of the eluted mAb was assessed In terms of process performance, the hybrid system enabled 80% purity and 50% recovery of polyclonal antibodies from plasma, and 97% recovery yield and 99% purity from anti-TNFα mAb. The presence of aggregates and the biological activity of the eluted fractions were evaluated, showing homogeneous eluted samples with high biological activity.

Time- and charge-dependent purification of nanoparticles with the steric exclusion chromatography

Eilts F[1], Orbay S[1], Pagallies F[2], Amann R[2], Wolff M[1,3]

[1]Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen (THM), Giessen, Germany,
[2]Department of Immunology, University of Tuebingen, Tuebingen, Germany,
[3]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Giessen, Germany

Steric exclusion chromatography (SXC), a cost-effective purification method, has been applied in laboratory scale for different products like viral particles (VP) with recovery rates of > 90%. The method relies on crowding-out effects of target molecules using, e.g., polyethylene glycol (PEG), and is closely related to traditional PEG-precipitation. Like precipitation the process is mainly influenced by the PEG concentration, the VP's charge, and the salt type and concentration. Here, we investigated the influence of different salts following the Hofmeister series on the loading step of the SXC aiming to elucidate the mechanism of retention on the stationary phase as well as the impact of VP aggregates. For this investigation, the Orf virus (ORFV) was used as a model virus. The ORFV is a promising viral vector for gene therapy and vaccine application, which was successfully (recoveries > 90%) purified in the past via SXC.
Initially, salts and their respective concentrations were identified, which did not significantly reduce the ORFV's infectivity. Salts impairing the viral infectivity by ≤ 30% within 2 days were KCl, NaCl, and Na2SO4, with concentrations 20 to 200 mM. In the next step, those salt-concentration-combinations were applied in the SXC loading process. Aspects of interest were (1) the aggregation kinetics of the ORFV in presence of PEG and salt, (2) correlations of pressure increase and loading capacity of the column throughout loading, and (3) the recovery of the ORFV. All three aspects are expected to follow the Hofmeister series as well as the valence of the ions.

Development of a scalable downstream purification process for Parapoxvirus Ovis (ORFV) purification for human and veterinary application

Lothert K[1], Pagallies F[2], Eilts F[1], Sivanesapillai A[1], Feger T[2], Amann R[2], Wolff M[1]

[1]Technische Hochschule Mittelhessen - University Of Applied Sciences, Giessen, Germany, [2]University of Tuebingen, Tuebingen, Germany

The Orf virus has become a promising candidate for viral vector-based vaccines. In addition to an impressive safety profile, the virus offers a potent and effective immune response. However, currently no manufacturing process has been described that allows a continuous and scalable production of cell-culture derived ORF virus particles. In particular, there is currently no efficient downstream process in order to use these viral vectors therapeutically. Focus here is the development of such a process. Initially cell culture harvests were clarified by a scalable filtration scheme followed by a nuclease treatment. Overall, viral recovery for the primary (5 µm) and secondary (0.65 µm) filtration was about 90% and 70%, respectively. For the succeeding chromatographic steps, the following methods were characterized: ion exchange-, hydrophobic interaction- and pseudo-affinity membrane adsorbers as well as a membrane-based steric exclusion chromatography and the CaptoTM Core 700 resin. The most promising results, in terms of virus yield and purity was achieved, using the steric exclusion chromatography for virus capturing followed by a secondary purification step via CaptoTM Core 700. Utilizing clarified and nuclease treated culture broth, a chromatographic purification train comprising of steric exclusion- and CaptoTM Core 700 chromatography resulted in an overall virus recovery above 90% and a complete protein removal whereas 98% of DNA was depleted. Process robustness was confirmed using a different virus genotype with comparable recovery of infectious virus and purity. In conclusion, an efficient and scalable purification process was achieved allowing for an economic production of Orf viral vectors.

Improving processes and exploiting new tools for Lentiviral vectors purification

Moreira A[1], Faria T[1], Oliveira J[1], Kavara A[2], Schofield M[2], Sanderson T[1], Collins M[2], Gantier R[3], Peixoto C[1]

[1]Instituto de Biologia Experimental e Tecnológica – iBET, Oeiras, Portugal,
[2]Pall Corporation, Westborough, United States,
[3]Repligen Corporation, Waltham, United States

Lentiviral vectors (LVs) have been increasingly used as a tool for gene and cell therapies. The recent approval of LV-based therapies has awakened the industry's interest and demand for more efficient manufacturing processes. However, LVs bioprocessing involves several challenges, and improved bioprocesses are still required before LVs can be routinely used in preclinical and clinical research. We report an improved four-step LVs purification process and show the use of 3D cellulose chromatographic columns, as a replacement for traditional techniques. A scalable protocol was implemented for LVs purification that combines clarification, anion-exchange chromatography (AEX), and ultrafiltration membrane technologies towards the maximization of infectious virus recovery. Different clarification filters were evaluated, and Design of Experiments was used to develop a comprehensive analysis of the AEX step and determine the best-operating conditions. Overall, the process comprises only four steps and can be performed in less than 5 hours with a global recovery yield of 45%.
Aiming to extent the portfolio of chromatographic media available for LVs purification, we have also been exploring 3D cellulose printed columns functionalized with different ligands. The ability to design flow channels and tailored larger porous bed structures to the targeted product constitute an advantage for the purification of enveloped viruses. Overall, LVs were purified with a recovery yield of 57% transducing units, suggesting the power of 3D printing technologies on viral vector manufacturing. In the future, work under development can be further extended to redesign biomanufacturing strategies for other viral particles or other fragile macromolecules.

Purification of enveloped virus-like particles displaying different membrane proteins by Heparin affinity chromatography

Mayer V[1], Zollner A[2], Frank A[1], Jungbauer A[1,2], Pereira Aguilar P[1]

[1]Austrian Centre of Industrial Biotechnology (acib), Vienna, Austria,
[2]University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

Enveloped virus-like particles (eVLPs) can be produced in insect cells using the Baculovirus Expression Vector System (BEVS). The production results not only in the release of eVLPs, but also of different kinds of host cell-derived bionanoparticles like exosomes. Additionally, baculoviruses replicate in the cells and are also released to the cell culture broth. Since all these different bionanoparticles are formed by budding out of the cell membrane, their surface composition and properties are very similar. Hence, its separation is very challenging. Heparin-affinity chromatography has been successfully used for the separation of different bionanoparticles produced in mammalian cells. We tested the applicability of this type of chromatography to separate eVLPs not only from host cells bionanoparticles but also from baculovirus. Three different eVLP constructs with different surface properties were tested. All constructs had as base structural protein the GAG polyprotein from the Human Immunodeficiency Virus-1 (HIV-1). Two of the constructs had additionally an envelope protein from either the Influenza A Virus (Hemagglutinin, GAG-HA) or from the SARS-CoV-2 (Spike, GAG-S). Additionally, recombinant Baculovirus (rBV) that did not carry genes for eVLP production was used as a control. To remove heparin-binding proteins, a pre-processing step using flow-through chromatography (Capto™ Core 700) was used. This step was followed by Heparin-affinity chromatography (Capto™ Heparin) applying either a linear or a step salt gradient as elution strategy. Several biophysical and biochemical analytical methods were used to characterize and quantify the separated fractions. Separation of eVLPs from host cell-derived bionanoparticles and baculovirus was achieved for all constructs.

Effects of bound nucleic acids on the phase behavior and purification process of HBcAg virus-like particles for gene therapy

Valentic A[1], Hubbuch J[1]

[1]Karlsruhe Institute of Technology, Karlsruhe, Germany

Virus-like particles (VLPs) are macromolecular structures with great potential as vehicles for targeted administration of functional molecules. Loaded with nucleic acids, VLPs are a promising approach for nanocarriers needed for gene therapy.
The structure, loading, dispersity and phase behavior of cargo-loaded VLPs is of central importance for their therapeutic efficacy and thereby considerably affecting the manufacturing process. The disassembly and subsequent reassembly reactions are frequently used for purification of these molecules being strongly dependent on the capsid stability. To integrate the loading of VLPs with therapeutic nucleic acids into the downstream process, further general knowledge needs to be derived.
The presented study investigates the phase behavior and purification process of HBcAg VLPs in dependence on liquid phase conditions and presence of nucleic acids. HBcAg VLPs with different lengths of a nucleic acid binding site were produced in E. coli. To characterize VLP attributes such as size, charge and loading were evaluated by dynamic and electrophoretic light scattering, and UV-Vis spectroscopy, respectively. The effects of bound host cell nucleic acids were systematically investigated for different purification process steps including the capturing by precipitation, disassembly and reassembly. The purity and phase behavior of the VLPs were measured by capillary electrophoresis and size exclusion chromatography. In this study, effects of the presence of bound nucleic acids on the capsid stability were observed and a resulting correlation between this capsid stability and the purification process could be derived.

Process Analytical Technology for Diafiltration-based Virus-like Particle Disassembly

Hillebrandt N[1], Vormittag P[1], Dietrich A[1], Hubbuch J[1]

[1]Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

Virus-like particles (VLPs) are an emerging biopharmaceutical modality with great potential as a platform technology. VLPs can be applied as vaccines, vectors, or therapeutic vaccines. For non-enveloped VLPs, recombinant production of the protein subunits leads to intracellular particle self-assembly. The following purification includes VLP dis- and reassembly which aim at removing encapsulated impurities and improving particle morphology and immunogenicity. In VLP downstream processing, filtration-based separation and processing has proven successful but requires comparably large product quantities and laborious experiments in early development stages. Both challenges can be tackled by implementation of process analytical technology (PAT) which is a valuable tool to efficiently obtain (real-time) process information.
In this study, an existing PAT setup was extended to comprehensively monitor the diafiltration-based disassembly of Hepatitis B core antigen VLPs. Process-related signals (pressures, flow rates, pH, and conductivity) were monitored in-line, while product-related signals (UV/Vis spectra, static and dynamic light scattering) were monitored using an on-line loop. The applicability of the sensors for disassembly monitoring was evaluated for varying conditions (VLP concentration, liquid phase, and permeate flux). Partial least square regression (PLS) models were calibrated using VLP subunit concentrations and on-line UV data. The calibration approaches were evaluated with regards to subunit concentration prediction and applicability at manufacturing scale. Static light scattering intensity showed a strong inverse correlation with the disassembly state (Pearson correlation coefficient < -0.98) and PLS models accurately predicted subunit concentrations ($Q^2 \geq 0.94$). The presented PAT approach increases information obtained during VLP disassembly process development and reveals automation potential.

Development of Next-generation Microchromatographic System for Miniaturization of Bioprocess Development with Some Insights from Philosophy of Science

Javidanbardan A[1,2], Chu V[3], Conde J[2,3], Azevedo A[1,2]

[1]IBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal,
[2]Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal,
[3]Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologias (INESC MN) and IN – Institute of Nanoscience and Nanotechnology, Lisbon, Portugal

The current increase in the number of biosimilars has been pushing biopharmaceutical companies to reduce bioprocess development and production costs. The downstream process is the major bottleneck in the process development and production in which the cost-intensive chromatographic separation is the workhorse.
Miniaturization is one way to minimize material consumption and parallelize experimentations for cost-effective and accelerated process development. Nonetheless, the paradigm shift from macroscale models to micro/nanoscale models and the presence of current knowledge uncertainties in the micro/nanotechnology and bioprocess fields could lead to unexpected deviations in results, making predictions inaccurate. Some lessons from the philosophy of science for developing scale models and understanding the complexities in causation concepts could be helpful to create more rational methodologies to minimize uncertainties of the scale-up effect.
In this study, we have developed a microfluidic chromatographic system with more emphasis on model criteria of simplicity, transparency, theoretical tractability, and precision. For determining the initial dimension of the prototype, potential, influential factors in the scale-down process of chromatography were considered. For a cheap and accelerated microfabrication process, micromilling technology was used to evaluate and optimize the prototype design. Interdigitated planar microelectrode was also developed and integrated into the system as a label-free sensor. Currently, the microcolumn efficiency was evaluated based on mobile phase transition analysis with online monitoring of conductivity using LabVIEW software. With further development and characterization, an integrated microfluidic system consisting of more than one microcolumn will be constructed to mimic and optimize the whole purification process on a miniaturized scale.

Residence Time Distribution of Chromatography Unit Operation in Continuous Processing

Lali N[1], Satzer P[1], Jungbauer A[2]

[1]acib - austrian centre of industrial biotechnology, Graz, Austria,
[2]University of Natural Resources and Life Science, Vienna, Austria

Draft guideline of the FDA on continuous manufacturing requests a fundamental understanding of the mass flow of the integrated continuous manufacturing process. Residence time distribution (RTD) of integrated processes is a way to understand the mass flow and behaviour of the continuous process.
In this work, we investigated the RTD of capture chromatography, in both loading stream and elution stream. A commercial recombinant monoclonal antibody and MabSelect Sure resin from GE Healthcare were used. Experiments were performed on lab-scale using 1ml columns. To characterize the RTD, an inert tracer was needed, therefore, we labelled part of the monoclonal antibody with a fluorescent dye, removed the residual free dye, and used the labelled monoclonal antibody as the inert tracer.
The labelled product was injected during the loading phase, effectively generating a tracer pulse in the regular inlet. Then we traced and quantified the distribution of the tracer in the outlet. Meaning that we characterize the RTD of capture chromatography, in both batch mode and continuous mode. Periodic counter-current chromatography (PCC) was used as continuous capture chromatography, as it is the most popular chromatographic separation when a batch process is rendered into a continuous. The focus was to show how one part of loaded material will distribute through the elution peak to understand how disturbances in the feed propagate to the elution peak. An additional experiment was performed in a small column, in batch mode by using confocal laser scanning microscopy (CLSM) to visualize the process.

Oscillatory flow reactor: a solution for continuous biomanufacturing

Faria D[1], Santos M[1], Aires Barros R[1], Ferreira A[2], Azevedo A[1]

[1]IBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, University of Lisbon, Portugal,
[2]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Portugal

The usage of aqueous two-phase systems (ATPS) has been proven as an efficient operation for clarification and purification of biological products, but despite this fact ATPS have had limited use at large scale.
Oscillatory flow reactor (OFR) is a type of tubular reactor that has been used in multiple processes in chemical engineering. One of the most important features of this type of reactor is the uniform mixing that is provided by the combination of periodically spaced restrictions and the oscillatory motion of the fluid. This reactor could be the answer for a more cost-competitive aqueous two-phase extraction (ATPE).
In this work, the partition of two industrial enzymes – α-amylase from Aspergillus oryzae and protease from Bacillus licheniformis – in different polymer-salt ATPS was studied in batch and the systems presenting the highest partition coefficient were transferred to OFR, where different conditions for frequency and amplitude of oscillation, and total mass flow were evaluated.
In the case of pure α-amylase, using a system composed by 20% (w/w) PEG 1500 and 9% (w/w) potassium phosphate at pH 7.4, the yield of partition to the top phase in the batch systems was 86%, while in the continuous ATPE in the OFR these values were between 77% and 95% for three different frequencies (1, 3 and 5 Hz), amplitudes (1, 2 and 3 mm) and total mass flows (4, 32 and 60 g/min) showing OFR can be successfully used for continuous ATPE and thus opening a door to their widespread use by industry.

Design and Application of Twin-Column Countercurrent Solvent Gradient Chromatography (MCSGP) to the Purification of Oligonucleotides

Sponchioni M[1], Fioretti I[1], Müller-Späth T[2], Morbidelli M[1]

[1]Politecnico Di Milano, Milano, Italy, 2YMC ChromaCon, Zürich, Switzerland

Oligonucleotides are coming to the forefront of the biopharmaceutical market, due to their action already at the pre-translational level and to the possibility of developing personalized therapies. This expansion was even accelerated by the COVID-19 pandemic, as some of the most effective vaccines distributed to the population are based on mRNA chains.
While the production of oligonucleotides, mostly via solid-phase synthesis, was brought to significantly large scales, the bottleneck in their manufacturing is the purification. In fact, the separation of the target product from shortmers (n-1; n-2; etc.) and longmers (n+1; n+2; etc.) is extremely challenging, due to the molecular similarity of the impurities to the target. Single-column, batch chromatography is currently the solution of choice to achieve this separation. However, this suffers from an intrinsic purity-yield tradeoff, meaning that the product recovery could be improved only by broadening the product collection window, which means including more impurities and compromising the lot purity, and vice versa.
In this work, we demonstrate the potential of twin-column countercurrent chromatography based on solvent gradient (MCSGP) for the purification of oligonucleotides. It is shown how the internal recycle of the product/impurities overlapping regions that would be discarded as out-of-specification fractions in single-column chromatography enables to achieve higher product recovery, at a fixed purity. In addition, the role of column geometry and resin is investigated in the transition from batch to MCSGP.
Overall, MCSGP greatly improves the product recovery and thanks to the process automation is a valuable option towards the intensification of oligonucleotide manufacturing.

Development of a Fluorescent Dye-Based Miniaturized Sensor for Aggregate Detection in Integrated Continuous Processing

Sao Pedro M[1], Santos M[1], Eppink M[2,3], Ottens M[1]

[1]TU Delft, Delft, Netherlands,
[2]Byondis B.V., Nijmegen, Netherlands,
[3]Wageningen University, Wageningen, Netherlands

The transition to continuous biomanufacturing is considered the next step to reduce costs and improve process robustness in the biopharmaceutical industry. The biomanufacturing process for monoclonal antibodies (mAbs) is eligible for this continuous processing due to patent expiration and subsequent need to lower manufacturing costs. One of the critical quality attributes of interest during mAb purification is aggregate formation, a phenomenon which can lead to an adverse immune response or a decrease in product efficacy. Several processing parameters and environmental factors are known to influence antibody aggregation (i.e. pH or agitation), making their appearance unavoidable. Therefore, a real-time measurement to monitor aggregate formation is crucial to have immediate feedback, process control and achieve continuous processing. Microfluidic biosensors placed after each step can be a powerful solution to speed up analytical measurements due to the characteristic short reaction time, while minimizing the sample volume.

In this work, the development of an integrated biosensing microfluidic chip for fast at-line PAT is described, using hydrophobicity sensitive fluorescent dyes to examine possible size differences of mAb species. The development and intricate design of a microfluidic structure capable to effectively mix the laminar flowing mAb sample with the fluorescent dye and subsequently collect real-time information on mAb aggregation will be presented. Firstly, the developed prototypes are validated using mAb samples with diverse levels of aggregation and two fluorescent dyes, Bis-ANS and Thioflavin T. The final prototype is then validated in a continuous chromatographic workstation on an ÄKTA™ Avant unit, operated by the control software Orbit.

How to design continuous devices to improve tangential flow filtration and product quality in protein precipitation

Pons Royo M[1,2], Beulay J[1], Valery E[1], Jungbauer A[2,3], Satzer P[2,3]

[1]Novasep, Pompey, France,
[2]University of Natural Resources and Life Sciences (BOKU), Vienna, Austria,
[3]Austrian Centre of Industrial Biotechnology (acib), Vienna, Austria

In recent years precipitation has gained interest as an alternative to the costly protein A chromatography for monoclonal antibody purification. Traditional precipitation processes are based on direct single time addition approach, where there is limited control on the co-precipitation of impurities and batch to batch variations are harder to consider. We propose a continuous dosage of polyethylene glycol to prevent co-precipitation and tightly control resulting floc size and improve purity. We demonstrate that the dosage time of PEG6000 and the final concentration significantly changes the PSD. We compared this approach to conventional PEG precipitation using batch addition demonstrating that sequential and continuous precipitant addition improves the quality attributes of the product such as yield and purity by a factor of 4.We studied the 3D structure of the precipitates by fractal dimension and showed that precipitates exhibited different compactness and density depending on the dosage time resulting in different filterability of the resulting precipitates in tangential flow filtration and depth filtration.
For a successful development and implementation of a bioprocess, acquiring all relevant data is essential. An interesting alternative in bioprocessing development are microfluidic or milli-fluidic devices. We developed milidevices for the precipitation of antibodies in continuous tubular reactors. The designed milidevices have a low volume and residence time, that enables to perform experiments with a couple of mL and in less than 5 minutes. We created a screening tool to compare different dosage times and to find the best precipitation conditions, accelerating considerably bioprocess development during early stage.

Interactions of Amino Acids and DNA at the Aqueous-Silica-Interface

Rauwolf S[1], Bag S[2], Schwaminger S[1], Wenzel W[2], Dias-Cabral C[3], Berensmeier S[1]

[1]Technical University Of Munich, Garching, Germany,
[2]Karslruhe Insitute of Technology, Karlsruhe, Germany,
[3]Universidade Beira Interior, Covilhã, Portugal

Silica in the form of silica gel is the most common material used in the history of liquid chromatography. The main advantages of silica gel are high solvolytic and mechanical stability, porous structure, and high surface area. Despite its widespread use, the interactions of biomolecules happening at the aqueous-silica interface are still not fully understood. We used chromatographic zonal elution and flow microcalorimetry (FMC) experiments combined with molecular dynamics simulation to shed light on the interaction of the 20 amino acids around neutral pH. Selected capped arginine and alanine were used to reveal the individual contribution of backbone and functional charged groups of the amino acids. Additionally, simulation and batch adsorption experiments explained DNA adsorption in the presence of different salts and amino acids. Experiments and simulations indicated that electrostatic interactions dominate the interaction of amino acids with silica. The mechanism is not classical ion exchange but ion-pairing, indicated by the FMC profile, which shows no desorption before the adsorption event. Adsorption of DNA to silica is dominated by the concentration and valency of positively charged ions in the solution highlighted by a cooperative adsorption model. The higher the concentration and valency, the higher the adsorbed amount of DNA. Our results demonstrate the importance of different charges on biomolecules and the surrounding environment for the complex biomolecule-silica interaction in aqueous solutions. We anticipate our findings to be applicable to other inorganic-biomolecule interactions in medicine and biotechnology to help understand the complex interactions between biomolecules and inorganic surfaces.

Protein-protein interactions in HIC dual salt systems

Jakob L[1], Beyer B[1], Janeiro Ferreira C[1], Lingg N[1], Jungbauer A[1], Tscheließnig R[1]

[1]Department of Biotechnology, BOKU, Vienna, Austria

In HIC, mixtures of two different salts can increase dynamic binding capacities (DBC). Although this phenomenon has been known for over a decade, the underlying mechanism is not understood. We have investigated whether ionic strength or surface tension of the load solution determines DBC in dual salt systems. The dual salt systems contained sodium citrate and a second sodium salt (either acetate, phosphate or sulfate) at pH 6. The stationary phase was Toyopearl Butyl-650M. Monoclonal antibody (adalimumab), lysozyme and GFP were used as model proteins. In order to analyze the intra- and intermolecular structure of the model protein in solution, we have performed small angle X-ray scattering (SAXS) experiments in said buffers without the stationary phase. Furthermore, we have analyzed the model proteins bound to the stationary phase with SAXS. For analysis of the data, we have fitted a self-avoiding random walk model and compared the resulting parameters of the loaded resin with the bare resin. Ultimately, we could show that the ionic strength of the buffering system is more decisive than the surface tension. Nevertheless, DBCs of the systems with comparable ionic strength still vary up to 30 %. The SAXS analysis revealed comparable internal structure for all investigated systems and protein-protein interactions for lysozyme and adalimumab. Systems exhibiting protein-protein interactions show non-Langmurian adsorption behavior which highlights the importance of lateral interactions in HIC. The SAXS analysis of the protein-stationary phase showed that the excluded volume of the self-avoiding random walk model inversely correlates with dynamic binding capacities.

Influence of Salts and pH Value on the Adsorption of Bovine Serum Albumin on a Mixed-Mode Resin

Kreusser J[1], Jirasek F[1], Hasse H[1]

[1]TU Kaiserslautern, Kaiserslautern, Germany

Proteins are often purified using chromatographic processes in which the properties of the solvent are controlled by adding salts. For single-mode techniques, like ion exchange chromatography (IEC) and hydrophobic interaction chromatography (HIC), the influence of different salts on the adsorption of proteins has been studied extensively. Recently, mixed-mode chromatography (MMC) resins as a hybrid of IEC and HIC resins have become promising alternatives for challenging separations due to the combination of multiple interaction mechanisms. However, comprehensive experimental studies of the adsorption of proteins on MMC resins and corresponding models are still rare to date. This impedes the conceptual design of MMC processes and their application in practice.

In this work, the influence of salts and pH value on the adsorption of bovine serum albumin (BSA) in MMC was studied in a systematic way. Adsorption isotherms of BSA on the mixed-mode resin Toyopearl MX-Trp-650M were measured at 25 °C in buffers containing sodium chloride, sodium sulfate, ammonium chloride, or ammonium sulfate with ionic strengths I up to 3000 mM at pH 4.0, 4.7, and 7.0. Interesting relations were found, e.g., an exponential decay of the adsorption with increasing I at low I and a linear increase at high I. Furthermore, a mathematical model is presented that quantitatively describes the influence of the studied salts on the adsorption of BSA. The model enables predicting adsorption isotherms for a wide range of ionic strengths of four technically important salts and can be transferred to other systems, as exemplified for the protein lysozyme.

Fast non-invasive determination of ionizable functionalities via pH transient

Podgornik A[1,3], Mravljak R[1], Bizjak O[1], Krajnc P[2], Paljevac M[2]

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia,
[2]PolyOrgLab, Faculty for Chemistry and Chemical Engineering, University of Maribor, Maribor, Slovenia,
[3]COBIK, Ajdovščina, Slovenia

Stepwise change between low and high salt concentration buffers of the same pH results in pH transition, the length of which was demonstrated to be proportional to the quantity of ion-exchange groups present on the matrix. In this work, we analyzed the effect of the ligand type, density, and buffer concentration on the pH transition shape for typical ion-exchange groups (QA, DEAE, SO3, and COOH) and ligands acting as metal-chelators, such as IDA, TAEA, and EDA. It was demonstrated that pH transition can occur either as a chromatographic or flat-top peak. pH transition peaks were evaluated by their length, height, and peak center parameters. While no parameter can describe the ligand density accurately with a single linear correlation for both peak types, all parameters can be used for the description of one peak type. Peak length and height exhibited the same accuracy, while their sensitivity depended on the pH transition shape: length being more sensitive for the flat-top peaks, while height for the chromatographic peaks. pH height can be obtained faster, at lower elution volume, and seems to be more suitable for the determination of low amounts of ligand, when typically chromatographic peak type pH transitions occur.

Centrifugal separation - A modeling approach considering feed and separator properties

Hammerschmidt N[1], Sailer C[1], Brocard C[1]

[1]Boehringer Ingelheim, Vienna, Austria

Centrifugal separation for inclusion-body (IB) harvest in microbial-based production processes poses unique challenges. IB harvest requires no classical solid-liquid separation, but rather a classification of solids, namely the separation of IBs from contaminating host cell debris. Varying centrifugation performances lead to differences in IB recovery and purity. These have major implications for subsequent downstream unit operations such as depth filtration or IB solubilization. Due to the lack of appropriate scale-down models, scale-up is currently performed empirically, which is often resource-intensive and error-prone.

We addressed the shortcomings of traditional practices and characterized the following aspects of centrifugal separation, the feed suspension and the centrifugal hardware. The feed suspension was characterized in terms of solids sedimentation velocity distribution. Separators of different types were characterized in terms of residence time distributions as means to assess the efficiency factors of individual instruments. Residence time distributions of solubilized tracers were measured and used to capture flow non-idealities, including dispersion and acceleration of the feed to solid-body rotation at the fluid entrance. Both distributions were used as input parameters to an extended model of equivalent sedimentation area.

Here we show the prediction capability of our model for two products. The established model was able to predict total solids and product recoveries in the concentrated slurry of the centrifugal IB wash step quantitatively, while the standard model failed to deliver meaningful results. In this work, we developed a workflow generally applicable to centrifugal separations and scale up thereof.

Hybrid Modeling in Tangential Flow Filtration: Enabling digital solutions for fast process development and control

Krippl M[1], Kargl T[1], Duerkop M[2], Dürauer A[1]

[1]Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria, 2Novasign GmbH, Vienna, Austria

Tangential flow filtration (TFF) is a powerful tool used in virtually all downstream processes of biopharmaceuticals. Multiple mechanisms such as fouling, concentration polarization and individual protein properties have significant impact on the process performance. Taking all these mechanisms into account using purely mechanistic models is cumbersome, requires extensive experimental effort and is often lacking the flexibility to act as adaptive process models. The combination of such mechanistic approaches with data-driven models, also known as hybrid-modelling, can reduce the experimental load and compensate for not fully understood phenomena during bioprocessing.

We present a hybrid modeling approach for ultra- and diafiltration TFF to predict various process parameters such as permeate flux across different filtration modes - batch, fed-batch and continuous processing. Due to the powerful interpolation capabilities of the hybrid models, the number of training experiments is reduced significantly, saving time and costs. This offers a promising modeling solution for ultra- and diafiltration processes that can operate at titers > 100 g/L were the number of experiments for process development and optimization is typically limited. Furthermore, the hybrid model can predict both - batch and continuous - filtration processes using the same training set. By this, the effort to transfer process models from batch to continuous can be significantly simplified. The presented hybrid models can be used as digital twins for process simulation to conduct virtual processes with varying input parameters. Digital twins are valuable tools for efficient process development or optimization and are the basis for advanced process control.

Separation of monoclonal antibodies using ionic-liquid-based aqueous biphasic systems

Rufino A[1], Capela E[1,2], Santiago A[1], Tavares A[1], Pereira M[1], Mohamadou A[3], Coutinho J[1], Barros M[2], Azevedo A[2], Freire M[1]

[1]CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Aveiro, Portugal,
[2]iBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal,
[3]ICMR – Institute of Molecular Chemistry of Reims, University of Reims ChampagneArdenne, CNRS UMR 7312, UFR des Sciences Exactes et Naturelles, Reims, France

Monoclonal antibodies (mAbs) have significant relevance for several therapeutic applications (e.g. treatment of pathologies such as oncologic diseases). The upstream processing of mAbs has been improved in recent years, while the downstream processing has not evolved at the same rhythm and requires alternatives to mainly replace the chromatography steps (one of them resorting to Protein A affinity chromatography). Aqueous biphasic systems (ABS), which are liquid-liquid systems, containing ionic liquids (ILs) can be considered as platforms due to the ILs designer solvents characteristic, and thus ability to improve selectivity and yield of mAbs. In this work, IL-based ABS formed by glycine-betaine analogues ILs and K_2HPO_4/KH_2PO_4 were studied for the purification of mAbs from cell culture supernatants. Recovery yields up to 100% with purification factors up to 1.6 were attained in a single-step for the IL-rich phase. However, by optimizing the IL concentration, the formation of a mAbs-rich precipitate occurs, improving the purification factor of mAbs, therefore resorting to the three-phase partitioning based on ABS. With a step of ultrafiltration, the purity can be increased up and mAbs can be stored in an appropriate final formulation. Furthermore, the IL can be recovered and reused with no significant loss of separation performance.

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Purification of green, red and yellow fluorescent proteins using cholinium chloride-based aqueous biphasic systems

Vieira dos Santos N[1], Martins M[2], Ventura S[2], Coutinho J[2], Santos-Ebinuma V[1], Pereira J[3]

[1]Department of Engineering Bioprocesses and Biotechnology, São Paulo State University, Araraquara, Brazil,
[2]CICECO, Institute of Materials, University of Aveiro, Aveiro, Portugal,
[3]Univ Coimbra, CIEPQPF, Department of Chemical Engineering, Coimbra, Portugal

Fluorescent proteins (FP) are biomolecules that can be used as biosensors and biomarkers for medical and biological applications. However, their high cost of production and purification limits their commercial use. The development of alternative platforms for their purification can potentially reduce production costs and democratize the access for medical biomolecules. Hence, in this study, three fluorescent proteins were used (Enhanced Green Fluorescent Protein, EGFP; Fezziwig Yellow Fluorescent Protein, YFP; and Red Fluorescent Protein, RFP) on the developing of cholinium chloride-based aqueous biphasic systems (ABS) for protein purification. It was possible to find a propylene glycol-400 (PPG-400)/cholinium chloride ([Ch]Cl) based-ABS capable of completely extracting all three FP to one of the phases of the system, while also increasing the purity of YFP and RFP and completely purifying EGFP in a single-step process. It was also possible to explain the partitioning behavior of the three FP with a hydrophilicity plot using Kyte-Doolittle scale. Additionally, the best system for EGFP was also integrated with an additional ultrafiltration step for the recycling of phase-forming agents and the polishing of EGFP. Hence, ABS are a promising platform for the purification of biomolecules, being able to extract and/or purify different biocompounds and be integrated with other downstream systems for the obtention of high purity biological products.

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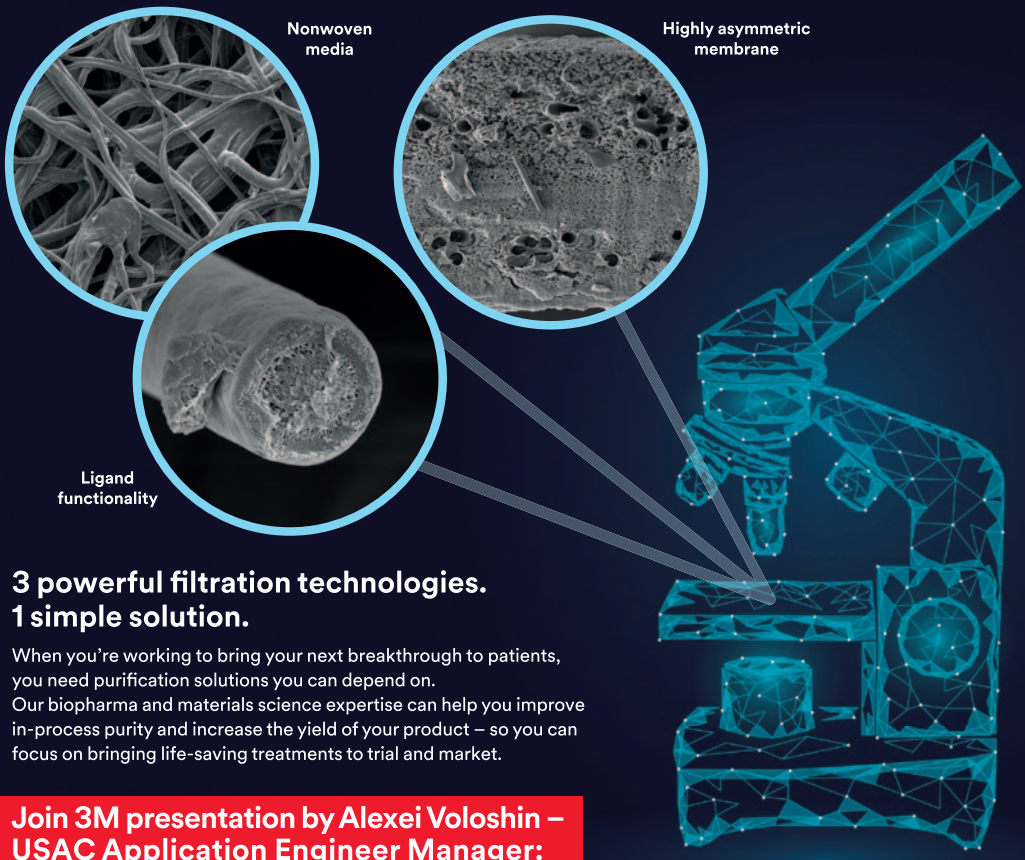

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Flash Presentation Abstracts

Development of glycidyl methacrylate-based plaftorm material for 3D printing stationary phases

Conti M[1], Dimartino S[1], Pullen J[2], Symington J[2]

[1]The University Of Edinburgh, Edinburgh, United Kingdom, 2FujiFilm Dionsynth Biotechnologies, Teesside, United Kingdom

Traditional chromatography columns are randomly packed with spherical beads. The resulting bed is heterogeneous and prone to flow irregularities which require stringent validation procedures. 3D printing can be applied to fabricate ordered stationary phases with reduced fluid dispersion and improved reproducibility over conventional columns. However, only few materials have been proposed for 3D printing of ordered chromatographic media, with their application mostly limited by printing resolution – currently around 500 µm.

Here, we introduce a novel material based on glycidyl methacrylate (GMA) that can be directly 3D printed using Digital Light Processing (DLP) at high resolution and with complex geometries such as triply period minimal surfaces (TPMS). The epoxy group of GMA can support different chromatographic derivatisations, making this material a flexible platform for 3D printed stationary phases for bioseparations.

Complex geometries were 3D printed using this new GMA-based platform material with resolution up to 200 µm. DLP printing with this new material proved to be reliable and fast, and produced mechanically robust TPMS models. The 3D printed structures were then functionalised for cation and anion exchange chromatography through substitution on the epoxy group, and the materials were characterised with FT-IR and SEM, and their flow and bind and elute properties were determined.

This study shows that DLP printing can fabricate structures with resolution of up to 200 µm and with diverse chromatography functionalities. Further efforts in increasing printing resolution and functionalisation chemistries will help 3D printed stationary phases mature and potentially revolutionise the world of bioseparations and downstream processing.

3D Printed convective media for the separation of small and large biomolecules

Dutra G[1], Jungbauer A[1], Satzer P[2]

[1]University of Natural Resources and life sciences, Boku, Vienna, Austria, 2Austrian Center of Industrial Biotechnology, Vienna, Austria

A recent explosion of 3D printing technology in conjunction with digital development tools like computational fluid dynamics rekindled the interest into fabrication of precisely ordered three-dimensional chromatographic applicability. In this work we were able to develop a novel material for chromatography generated by 3D printing intended for bioseparation of large and small biomolecules, including bionanoparticles like viruses and virus like particles. The generation by 3D printing allows for a strict control of the morphology and regularity of the resulting chromatography material. The resulting material can be strictly convective and therefore not mass transfer limited allowing the fast and efficient separation of large biomolecules with low diffusivities.

For a chromatographic base material, the resolutions need to be in the sub-micron range, and at the same time the generated object size must be at least a couple of milometers to be able to test the material on a chromatography system. Two-photon polymerization is a technology with a nominal resolution of 0.1-1 µm which can achieve the necessary resolutions for chromatographic base material. However, it has very long printing times of days or maybe even months for objects large enough for chromatography. Recently, this technology was advanced by UpNano with a 3D printer that can generate life size objects (up to 5x5x5 mm) with micro/sub-micro resolution in a matter of hours which was not possible before. This technology is suitable for the generation of chromatographic base material and additionally can print biocompatible materials like acrylates which are already in use for chromatographic separation.

Single Step Protein Purification And Immobilisation By New Chromalite M Chemistries

Summers B[1], Cruz-Izquierdo A[1], Basso A[1], Serban S[1]

[1]Purolite Ltd., Llantrisant, United Kingdom

Protein separation and isolation of specific protein fractions from fermentation broth or protein mixtures by using cost-effective, scalable, sterilisable resins offers a significant increase in value and productivity.

The Chromalite range contains methacrylate resins suitable for protein purification from such media: spherical beads with a mean pore diameter of ca. 1000 Å to ensure sufficient porosity for a range of protein binding applications. The resins are produced in three standard sizes, 40-90 µm using Purolite cutting-edge proprietary jetting technology and 75-125 µm and 100-300 µm made with traditional suspension polymerisation.

The new Chromalite MIDA has been specifically designed to selectively capture His-tagged proteins for purification and biocatalytic processes. The resins studied had iminodiacetic acid functional groups allowing metal loadings of 0.05-1 mmol metal/gram and extremely high affinity towards the His-tagged proteins.

The immobilisation of a His-tagged alcohol dehydrogenase was compared on various methacrylic resins that lead to different interactions, i.e.: affinity, covalent, ionic and hydrophobic. These experiments showed that the His-tag purification and hence immobilisation provides up to 20 times increased specific activity compared to standard protein capture, directly related to the selectivity of the purification carried out by the Chromalite MIDA resin. Recycling of the His-tagged alcohol dehydrogenase immobilised on metal loaded-IDA methacrylic resins showed excellent stability over 10 cycles.

This new range of IDA resins will increase the potential use of a wide range of His-tagged enzymes in the manufacture of key compounds while minimising the downstream processing cost by combining purification and immobilisation in one simple step.

Separation of Histidine-Tagged Six-Transmembrane Epithelial Antigen of the Prostate 1 from Detergent-Solubilized Komagataella pastoris Extracts

Barroca-Ferreira J[1,2], Santos Silva T[2], Maia C[1], Passarinha L[1,2]

[1]CICS-UBI – Health Sciences Research Centre, Faculty of Health Sciences of University of Beira Interior, Covilhã, Portugal,
[2]UCIBIO – Applied Molecular Biosciences Unit, NOVA School of Science and Technology, NOVA University Lisbon, Lisbon, Portugal

The Six-Transmembrane Epithelial Antigen of the Prostate 1 (STEAP1) is an integral membrane protein involved in cell communications (Esmaeili et al. 2018), in the stimulation of cell growth by increasing reactive oxygen species levels (Grunewald et al. 2012), and in the transmembrane-electron transport and reduction of extracellular metal-ion complexes (Oosterheert et al. 2020). The STEAP1 is particularly over-expressed in prostate, in contrast with non-tumoral tissues and vital organs, contributing to tumour development and aggressiveness (Barroca-Ferreira et al. 2018). The current reported studies lack experimental data on STEAP1, its putative structural, and chemical modifications. These facts highlight the relevance of exploring the biosynthesis of STEAP1 and its purification for further structural characterization and bio-interaction studies. In this work, recombinant histidine-tagged STEAP1 (hSTEAP1-His6) was expressed from Komagataella pastoris (K. pastoris) mini-bioreactor methanol-induced cultures and successfully solubilized with CYMAL-5 and n-decyl β-D-maltoside. Immobilized-metal affinity chromatography (Ni2+ and Co2+ charged column) provided the desired selectivity for hSTEAP1-His6 capture from solubilized K. pastoris extracts and allowed the recovery of the protein in a single fraction. After a polishing step using anionic-exchange chromatography (Q-Sepharose), a highly pure and immunoactive hSTEAP1-His6 was obtained. The strategy here established could be applied to obtain pure STEAP1, to gather additional insights concerning its putative structure, and both thermal and environmental stability conditions. Thereafter, the functional role and oncogenic behaviour of the protein in prostate cancer microenvironment might be deeply explored.

Human serum pre-treatment: removal of abundant proteins aiming the concentration of lung cancer biomarkers

Rosa M[1], Silva F[1], Coutinho J[1], Freire M[1]

[1]CICECO - Aveiro Institute of Materials, Aveiro , Portugal

Due to the long asymptomatic phase and lack of effective early-stage diagnosis, lung cancer is one of the deadliest types of cancer worldwide. To expedite disease diagnosis, the quantification of target biomarkers in human serum is a propitious approach. However, the low concentrations of biomarkers in human serum and their tendency to be masked by abundant proteins [immunoglobulin G (IgG) and human serum albumin (HSA)] may limit quantification accuracy. Thus, a sample pretreatment step to deplete abundant proteins, while enhancing biomarkers detection is often required. Here, three-phase partitioning (TPP) systems composed of polymers, phosphate buffer and ionic liquids as adjuvants are proposed as alternative serum pretreatment techniques. After adding human serum, these systems induced the formation of two aqueous phases, where biomarkers might be concentrated, and a solid interphase, where abundant proteins are depleted. Depletion studies using commercial human serum demonstrated that the most efficient TPP led to IgG and HSA depletion efficiencies exceeding 85%. Despite additional studies are ongoing, TPP systems show promise as cheaper and faster human serum pretreatment techniques.

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Enhancing membrane-bound catechol-O-methyltransferase activity through an ionic liquid storage formulation

Gonçalves A[1,2], Gallardo E[1,3], Sousa Â[1], Correia M[2], Romão M[2], Queiroz J[1], Passarinha L[1,2,3]

[1]CICS-UBI Health Sciences Research Centre of Uniersity of Beira Interior, Covilhã, Portugal,
[2]Applied Molecular Biosciences Unit, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Portugal,
[3]Laboratório de Fármaco-Toxicologia, UBI Medical, Universidade da Beira Interior, Covilhã, Portugal

Membrane bound catechol-O-methyltransferase (MBCOMT) as an integral membrane protein that catalysis the methylation of catechol molecules, has been linked to several medical conditions, in particular Parkinson's disease. As a therapeutic target, the research for new potent and nontoxic inhibitors is constant. To date, there is no MBCOMT resolve structure, and instability is a major drawback. MBCOMT is a highly thermolabile protein, especially its polymorphic form Val158Met. The protein presents approximately half of the original specific activity after 12h at 4 ºC. Ionic liquids, due to their virtually unlimited choices of cation/anion paring (leading to unique solvation features) are viewed as potential protein stabilizers to be used in storage buffers. Herein, we evaluate the effect of MBCOMT-IL interaction in the biological activity within distinct cation/anion ILs (L-glutamic acid monosodium salt monohydrate; choline Glu; choline DHP; choline Cl; 1- dodecyl-3methyimidazolium (C12Im-Cl); 1-butyl-3-methylimidazolium chloride (C4mim-Cl)) added to MBCOMT lysates, in a concentration range of 5 to 500 mM. Potential stabilizer effect was showed by L-glutamic acid monosodium salt monohydrate (500 mM) and choline DHP (10 and 50 mM). Moreover, to access the ideal storage conditions (temperature and time) a design of experiments (DOE) was implemented, and the predicted results, were validated in purified fractions of MBCOMT from arginine-affinity supports [1]. Thermal shifting temperature analyses was also performed to determine the variations between MBCOMT melting temperatures in the initial and final defined formulation.

1. Pedro, A.Q., et al., Purification of Membrane-Bound Catechol-O-Methyltransferase by Arginine-Affinity Chromatography. Chromatographia, 2015. 78(21): p. 1339-1348.

Purification of antileukemic drugs through silica-based supported ionic liquids

Almeida M[1], Albuquerque M[1], Carvalho C[1], Bento R[1], Nunes J[1], Neves M[1], Santos-Ebinuma V[2], Freire M[1], Tavares A[1]

[1]CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal,
[2]Department of Engineering Bioprocess and Biotechnology, School of Pharmaceutical Sciences, UNESP-University Estadual Paulista, Araraquara, Brazil

L-asparaginase (LA) is an enzyme used as a biopharmaceutical for the treatment of acute lymphoblastic leukemia. LA can be produced via fermentation and its purification usually comprises ion exchange chromatography, which is often preceded by precipitation with salts as a first pre-chromatographic step. However, this purification strategy result in low yields and purity, requires long processing times, while leading to a consequent increase of the process costs. Therefore, the demand for new cost-effective purification processes play now a priority role.
In this work silica-based supported ionic liquids (SILs) are investigated as an alternative technology to purify LA from the complex fermentation medium from Bacillus Subtillis. The concentration of the extract from the fermentation, material/ extract from fermentation ratio and contact time effects in the purity and yield of LA were optimized. With this strategy, process costs, energy consumed, and waste generated, may be significantly decreased, which may lead to this biopharmaceutical price decrease and wider application.

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Purification of interferon alpha 2b-based biopharmaceuticals using ionic liquid-based technologies

Pedro A[1], Castro L[1], Lobo G[1], Pereira P[2,3], Passarinha L[4,5,6], Neves M[1], Freire M[1]

[1]CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal,
[2]University of Coimbra, Centre for Mechanical Engineering, Materials and Processes, Department of Chemical Engineering, Coimbra, Portugal,
[3]IPN, Instituto Pedro Nunes, Associação para a Inovação e Desenvolvimento em Ciência e Tecnologia, Coimbra, Portugal,
[4]UCIBIO@REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal,
[5]Laboratory of Pharmacology and Toxicology – UBIMedical, University of Beira Interior, Covilhã, Portugal,
[6]CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal

Protein biopharmaceuticals, among which interferon alpha-2b (IFNα-2b) that can be used in the treatment of chronic hepatitis C, have become an indispensable product of current medicine. Aiming at finding new cost-effective, efficient and sustainable technologies for recombinant IFNα-2b purification, ionic liquids were investigated as adjuvants in polymer-polymer aqueous two-phase systems (ATPS) or as chromatographic ligands covalently attached in silica (Supported ionic liquids, SILs). The application of ionic liquids as adjuvants in ATPS composed of polyethylene glycol (PEG 600 g/mol) and polypropylene glycol (PPG 400 g/mol) enhanced the purity of IFNα-2b recovered in the PEG-rich phase. On the other hand, SILs exhibited promising results toward IFNα-2b purification both in conditions favouring ionic or hydrophobic interactions, reinforcing the multimodal character of these novel stationary phases. Also, the secondary structure of IFNα-2b is preserved with both purification processes, as appraised by circular dichroism and western-blot studies. Overall, our results demonstrate the high potential exhibited by ionic liquids toward the preparative purification of the recombinant IFNα-2b biopharmaceuticals.

Sustainable liquids support as a media for biocatalytic reactions using aqueous biphasic systems

Castro L[1], Ferreira A[1], Valente A[1], Pedro A[1], Coutinho J[1], Freire M[1], Tavares A[1]

[1]CICECO – Aveiro Institute of Materials, Department of Chemistry, Universidade de Aveiro, Aveiro, Portugal

Over the years a significant interest in the application of biocatalysts in several industries has emerged as an alternative to the commonly used chemical catalysts. Enzymes, such as laccases, allow the development of sustainable biocatalytic processes due to their high specificity to substrates and biodegradability [1]. Nevertheless, its application is still considered costly, thus enzyme reuse provides an excellent alternative [1]. Enzyme immobilization on solid supports are the most used methodology for enzyme reuse, however, a reduction of enzyme performance occurs. Therefore, aqueous biphasic systems (ABS) appear as a new approach to efficiently immobilize enzymes since they can be used as biocompatible liquid support [1], and additionally, the introduction of ionic liquids (ILs) in ABS can improve the biocatalyst performance. The objective of this work was to evaluate the use of ABS, composed of cholinium-based ILs and polypropylene glycol (PPG 400), as a liquid support for laccase immobilization and the laccase reuse capacity using the degradation of the textile dye Remazol Brilliant Blue R. The systems studied allow an easy recover and reuse of the biocatalyst for at least four reaction cycles, with no significant losses in the dye decolourization yield.

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UV/Vis spectroscopy and Multi-Way Chemometrics for Precipitation and Crystallization Process development from Complex Media

Wegner C[1], Hubbuch J[1]

[1]Karlsruhe Institute Of Technology, Karlsruhe, Germany

Spectroscopy and chemometrics have gained more interest as they can be coupled to selectively quantify different, overlaying species in bio-pharmaceutical processes. Due to the complexity of culture broth, protein precipitation and crystallization from culture broth are difficult to characterize, monitor and evaluate. Various impurities, present in the complex mixture, interfere with qualitative and quantitative analytics, calling for a calibration-free approach.
To overcome these limitations, ultraviolet-visible (UV/Vis) spectroscopy and the multi-way chemometric method parallel factor analysis (PARAFAC) can be combined to determine the protein spectra and relative protein concentrations in high-throughput batch measurements. These qualitative data are valuable for yield evaluation and early stage process development of protein precipitation or crystallization.
The specified methods shall be applied on two different data sets. First, one species of a defined ternary protein mixture was selectively crystallized in a high-throughput micro batch screening. The UV/Vis spectra of the supernatant of the different conditions was then recorded, preprocessed and analyzed with PARAFAC. This allowed fast, calibration free analysis of the relative supernatant compositions and, thus, finding optimal process conditions. Reference analytics and knowledge on the pure protein spectra validated the chemometric approach. Secondly, the developed workflow should be applied on the selective precipitation step of virus like particles (VLPs) in complex lysate. In micro-liter scale, the lysate was selectively precipitated with varying amount of ammonium sulfate. The supernatant shall be analyzed analogously to the first data set and offer an estimation of the precipitated target molecule amount.

Natural magnetite ore as separation agent for valuable microalgal products

Schobesberger M[1], Berensmeier S[1], Fraga-García P[1]

[1]Technical University Of Munich, Garching, Germany

Efficient cell separation is considered a critical bottleneck for exploiting microalgal biomass as a renewable source of valuable products. As the cell density after cultivation is usually <5% (bio dried mass), new methods for concentrating the biomass are intensively studied. A relatively young approach is magnetic separation. We characterize magnetic microparticles (MMPs) derived from iron ores and apply them as a harvesting agent for saline microalgae Microchloropsis salina (former Nannochloropsis salina). A harvesting efficiency of M. salina up to 98 % is reached at alkaline conditions by magnetic separation, while at acidic conditions the harvesting efficiency is <30 %.

The algal cells flocculate at alkaline pH around the MMPs due to precipitation of medium components (mainly calcium phosphate and magnesium hydroxide). We investigate the (surface-) properties of the MMPs, the microalgae and the medium precipitates. The interaction of these three components originates the harvesting effect that can potentially be used in a low-energy-consuming process for microalgae exploitation. Our results are the base for further research to understand the interaction of lysate components (proteins, pigments) released during cell disruption from biotechnological cultivation broths.

Magnetic Separation of Whey Proteins with Bare Iron Oxide Nanoparticles

Krolitzki E[1], Schwaminger S[1], Berensmeier S[1]

[1]Technical University of Munich, Garching, Germany

By-products from food and beverage production often still contain large amounts of valuable proteins. For their isolation and purification, established packed bed column chromatography setups are sometimes too costly. This is especially true for products in price-competitive markets, such as the dairy industry. Magnetic separation is a promising alternative approach to packed bed chromatography. It was already successfully tested for the selective purification of proteins from fermentation broth and dairy streams with magnetic microparticles on a pilot scale. We now introduce biocompatible low-cost iron oxide nanoparticles for the same task and thereby further facilitate the industrial application of high gradient magnetic separation technologies for the purification of proteins. The employed iron oxide nanoparticles are the product of an easy and scalable co-precipitation reaction. They have a crystal size of 5-20 nm with a specific surface area of approximately 98 m^2 g^{-1} and show superparamagnetic behavior. Because there are no mass-transfer limitations on their non-porous surface, maximum target protein loads can be achieved within minutes. The particles' achievable protein load and selectivity depend on the feed matrix composition, such as pH, ionic strength, and present biomolecule species. We introduce pathways for the set-up of suitable processing procedures for the selective purification of proteins from viscous feedstock including pH and salt-driven elution strategies from lab scale to pilot scale.

Affinity purification/immobilization of His-tagged proteins using unmodified silica

Freitas A[1], Domingues L[1], Aguiar T[1]

[1]CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal

The development of affinity-based purification/immobilization schemes relying on low-cost, earth-abundant and environment-friendly matrices is highly desirable. Silica fits well this purpose. Protein purification/immobilization schemes using unmodified silica matrices have been developed based on silica-binding affinity peptides such as the Si-tag, CotB1/CotB1p and Car9. These positively charged peptides (rich in R/K residues) bind strongly to anionic silica surfaces and unbind with L-arginine or L-lysine eluants. Histidine-rich silica-binding peptides are frequently isolated in biopanning experiments. Histidine seems to be important for the binding, as its aromatic imidazole ring can form hydrogen bonds with silanol and siloxide groups on silica surfaces. Thus, we hypothesized that the widely used polyhistidine-tag (His-tag) could function as silica-binding peptide.

To assess the viability of using bare silica as an alternative matrix for the purification/immobilization of His-tagged proteins, His6 and Car9 tags alone or in combination were fused to EGFP. Non-tagged EGFP was used as control. After production in Escherichia coli, His6-EGFP was shown to bind to unmodified silica particles of different size and porosity. Small-scale purification schemes were then developed using silica gel Davisil grade 643 and 646 as matrices and L-arginine as eluant. These supported high purities of His6-EGFP (~96%) and yields comparable to those obtained for EGFP-Car9 (~70%). The combination of tags improved the binding of His6-EGFP-Car9, reducing the recovery yields to 30-55%. Thus, the combination of these tags is advantageous for single-step purification and immobilization purposes.

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Chromatographic purification of small heat shock protein nanocages

João J[1], Sousa Rosa S[1], M. Azevedo A[1], Miguel F. Prazeres D[1]

[1]iBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal

Protein nanocages are versatile vehicles for drug delivery. These nanometer scale architectures feature an inner cavity that can be loaded with different drugs. The clinical development of protein nanocages requires large amounts of pure, well-folded assemblies. However, while the currently used purification approaches are suitable for proof-of-concept studies at lab scale, biomanufacturing at large scale will require more efficient bioprocess technologies to enable the use of protein nanocages for clinical applications. The main objective of this work was to develop scalable and cost-effective processes for the biomanufacturing of protein nanocages with particular emphasis in the downstream steps. The 16.5 kDa small heat shock protein from Methanococcus jannaschii (MjsHSP) was used as model. The in vivo assembly of 24 units of MjsHSP originates 12 nm nanocages with octahedral symmetry and demarcated exterior and interior surfaces. The purification strategy consisted of an intermediate purification followed by a polishing step to achieve a highly purified and formulated product. Different approaches of chromatography as well as traditional and novel chromatographic supports with distinct properties were tested and analysed. Characterization of the pure MjsHSP nanocages and impurities demonstrated that a downstream processing strategy based on two chromatography steps could be an efficient platform to obtain pure protein nanocages for pre-clinical/clinical applications.

Experimental Design of the Multicolumn Countercurrent Solvent Gradient Purification (MCSGP) Unit for the Separation of PEGylated Proteins

Kim T[1], Angelo J[2], Xu X[2], Ghose S[2], Li Z[2], Morbidelli M[1], Sponchioni M[1]

[1]Politecnico di Milano, Milano, Italy, 2Bristol Myers Squibb, Inc., Devens, USA

The rapid diffusion of polyethylene glycol (PEG)-protein conjugates on the biopharmaceutical market has increased the demand for robust downstream processing to assure the quality of drugs. In the current industrial production, most of the purification methods are performed through single-column batch chromatographic separations, ensuring the purity specification at the expenses of low yield. This limitation can be overcome by continuous countercurrent operations, such as the Multicolumn Countercurrent Solvent Gradient Purification (MCSGP). In fact, the low yield in the single-column batch chromatography is attributed to the discard of the out-of-specification product/impurities overlapping regions, which in the MCSGP are internally and automatically recycled, thus significantly improving the yield while satisfying the same purity specification. This advantage comes at the cost of additional process parameters for the MCSGP system, which complicates its design and optimization when compared to the batch. To solve the drawback, an ad hoc design procedure for the optimization of central-cut separations at a target purity using PEGylated lysozyme as model protein is studied. From the batch chromatography, the load, the elution gradient, and the collection intervals for optimal yield and productivity at fixed purity specifications are identified. The obtained optimal process parameters are transferred to the MCSGP unit, showing an improvement of 10.7% in the yield and 32.3% in the productivity compared to the batch. Furthermore, using Langmuir isotherm and the experimental data collected in diluted and overloaded conditions, a model for PEGylated lysozyme purification is developed and evaluated by comparison with experimental data.

Media & Buffer On-Demand: Continuous Reconstitution Directly From Solids

Komuczki D[1], Satzer P[1], Jungbauer A[1]

[1]Institute of Bioprocess Sciences and Engineering (IBSE), University of Natural Resources and Life Sciences (BOKU), Vienna, Vienna, Austria

Process materials such as cell culture media, buffers, stock solutions, nutrients or additives are of utmost importance in the chemical, biotechnological or food industry. Therefore, companies optimize their supply chains, for instance by buying and storing compounds in a solid form for decreased storage space and increased shelf life, but with the necessity to reconstitute before use. Current trends in the biopharmaceutical industry indicate a move towards continuous production which lead to a constant demand of reconstituted process materials, but there is no continuous reconstitution from solids available and buffers and media are typically reconstituted batch-wise. This leads either to necessarily large vessels if buffers a constituted on a low frequency, or high personal costs if the reconstitution is done regularly, for instance every 8 hours or daily. We therefore developed a first prototype and demonstrated a proof of concept for a continuous reconstitution of media and buffers from solids in lab-scale to be directly connected to any upstream or downstream unit operation with continuous demand. We showed that this reconstitution of media is equivalent in terms of growth characteristics and product quality of a CHO cell culture to a batch-wise reconstituted media. This opens a new playground for development of strategies in upstream and downstream and several theoretical applications of this concept will be presented.

Continuous Fluidized Bed Riser System for Bioproduct Recovery employing a Double Network Hydrogel Cation Exchange Adsorbent

Herlevi L[1]

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

Fluidized bed technology allows simultaneous clarification and protein capture, and has been applied for continuous downstream processing by employing expanded bed adsorption (EBA) and liquid-solid circulating fluidized bed (LSCFB) technology. Although systems based on the aforementioned technologies were able to recover protein in a continuous manner, they were incapable of increasing the concentration of the targeted protein and due to mechanical constraints set by continuous processing the choice of resin was limited to polymeric based materials.

In this work, a continuous protein adsorption system utilising a fluidized bed riser and EBA technology is presented for recovery of lysozyme, as a model protein. The system consists of an adsorption column in which the feedstock and the adsorbent are continuously added from the bottom part of the column. Simultaneously, the adsorbent is removed from the top of the column and moved to the next stage. The subsequent wash, elution and regeneration stages are operated in counter-current mode by adding the adsorbent at the top of the column, resulting in a downward flow of the adsorbent particles. Additionally, the elution and regeneration columns have a modified inside structure to increase residence time and packing of the adsorbent which allows reduced column height. The adsorbent is re-circulated back to the adsorption stage from bottom part of the equilibration stage.

A double network hydrogel with cation exchange properties was developed for increased mechanical strength and improved binding capacity, and the system was able to double the target protein concentration.

Moving Adsorption Belt System for Continuous Bioproduct Recovery

Guo Y[1]

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

This presentation will propose that applying synthetic polymeric fibre (SPF) and various surfaces modification methods produce sulphopropyl (SP) functionalized composite fibrous adsorbents. Specifically, dextran was introduced to the surface of the SPF backbone. Furthermore, the conjugated dextran layer was functionalized to introduce binding properties to biomacromolecules in order to separate them from a feedstock. The developed fibrous adsorbent was implemented in a moving adsorption belt system in the form of endless belt. Basically, it was a belt conveyor consisting a 4-chamber tank, each chamber responsible for association, wash, desorption, and regeneration respectively and with its own buffer and liquid speed. Because of the flexible and bendable structure of belt it can pass through each chamber in sequence via rollers located at the bottom of each chamber and at the top of each chamber divider readily for continuous bioproduct recovery.

Miniaturization of chromatographic process development for fast results at minimal costs

Castanheira Silva T[1], Eppink M[2,3], Ottens M[1]

[1]Department of Biotechnology, Delft University of Technology, Delft, Netherlands, 2Downstream Processing, Byondis B.V., Nijmegen, Netherlands, 3Bioprocessing Engineering, Wageningen University, Wageningen, Netherlands

Biopharmaceutical processes greatly rely on chromatographic steps for the purification of their products due to its specificity and better performance. This comes, however, at the expense of this unit operation representing the majority of operating costs for the production of several products. In the search for optimal chromatographic processes, several parameters need to be estimated together with a thorough characterization of protein adsorption behavior, obtained from protein adsorption isotherms. High-Throughput Screening has aimed at reducing the costs for process development and optimization, targeting a reduction of materials and sample consumption as well as faster assays.

The most prominent technologies used are liquid-handling stations and microfluidics, the first offering very high levels of automation whilst the latter offers very high levels of miniaturization. Liquid-handling stations capitalize on the high degree of automation achieved by the equipment which allows to minimize handling errors. Microfluidics minimizes material and sample consumption, usually in the microliter to nanoliter scale. These two technologies represent different alternatives for process development, enabling fast process development at very low sample consumption.

In this work, a novel microfluidic chip design is presented for the determination of protein adsorption isotherms in batch uptake mode. The microchip allows for a 100-fold decrease in the amount of resin used, compared to liquid-handling stations. The data generated with the microfluidic device is compared to the data obtained using the liquid-handling station, to compare operability and implementability of both technologies.

Economic and ecological evaluation of new processing schemes for the industrial scale production of plastic-degrading enzymes.

De Santis T[1], Striedner G

[1]acib, Wien, Austria

Mankind produces 27.1 million tons per year of plastic waste and only 31.1 % can currently be recycled (PlasticsEurope, 2018). About two third of plastic waste are landfilled or incinerated. These huge amounts of waste come along with a CO_2 emission of 6.7 million tons per year. Not to forget about the economic impact: the management of plastic waste causes costs of more than 19.3 billion Euros per year (Enzycle consortium, 2020). Enzymatic plastic depolymerization has emerged as a new promising technology to target the plastic waste fractions (e.g. PET trays) which are currently not recycled. Despite the discovery of several plastic-degrading enzymes (Kawai et al., 2020) and the proof of concept at a pilot scale of the process (Tournier et al., 2020), multiple challenges are found ahead to make the enzymatic recycling of plastic a commercially competitive technology. One of the requirements is that enzymes will need to be produced as cheaply as possible at a scale of several tons per year. Continuous *in-situ* production is hypothesized to be an effective strategy to lower the final unit cost of the plastic-degrading enzymes. In this study, four different large scale enzyme production processes (continuous vs. fed-batch mode and intracellular vs. extracellular production) were modelled and used to generate a techno-economic analysis that might serve as a decision-making tool for the industry.

Experimental and simulated determination of the volumetric power input in microtiter plates as a scale-up strategy for the development of downstream operations.

Montes Serrano I[1], Dürauer A[1]

[1]Austrian Centre Of Biotechnology, Vienna, Austria

Microtiter plates have gained a high importance for the screening and optimization of process parameters in downstream processing. However, a strategy for the direct scale-up from microtiter plates to macroscale reactors has not been established yet. Different strategies such as conservation of stirrer tip velocity, mixing time or volumetric power input are used to scale-up stirred systems. Volumetric power input can be measured in microtiter plates with the increment of temperature in a fully insulated system. This was investigated in three microtiter plates, 96-, 24- and 6-wells, each one with different filling volumes, under different shaking frequencies and orbital diameters. The higher values of volumetric power input were obtained in the smaller wells, as shown at 600 rpm and 3 mm of orbital diameter with 31, 18 and 9 W/m3 for 96-, 24- and 6-well. Computational Fluid Dynamics were utilized to study the hydrodynamics in microtiter plates and observe how different working conditions affect the volumetric power input. Results have shown an increase of power input as the ratio of the contact surface to the overall volume increases. The results allow to mimic the volumetric power input of stirred tank reactors up to 20 L working volumes. A generalized model for the prediction of the volumetric power input in microtiter plates has been developed based on the motion of the system and characterized by two dimensionless numbers, Reynolds and Froude with the introduction of a hydrodynamic constant dependent on the properties of the fluid and the geometry of the wells.

Capture step optimization in liquid chromatography

Eslami T1, Satzer P[1], Jungbauer A1, Lingg N[1]

[1]Boku University, Wien, Austria

Long loading steps with a constant residence time are common in traditional chromatographic purification operations, especially in the early stages of the downstream process. The product‘s breakthrough behavior is generally well defined. This data can be utilized to enhance the loading phase by initiating with a low residence time and gradually increasing it to improve dynamic binding capacity. This improvement is due to a more favorable breakthrough curve at a lower residence time. The optimal residence time gradients were discovered by modeling the loading of lysozyme on a strong cation exchanger, Toyopearl SP-650C. In the time domain, these gradients result in a faster breakthrough, whereas in the volume domain, they have the same breakthrough as constant residence time loading. Simulations also revealed that by loading at a low residence time for a set of time, then increasing the residence time gradually boosts productivity even further. Several mathematical optimizations were employed and compared with simulations. the model used for adsorption was confirmed by monolocal antibody breakthrough in protein A affinity chromatography and lysozyme breakthrough in ion-exchange chromatography. Productivity is boosted by 70 and 130 percent using these optimized residence time gradients in affinity and ion-exchange chromatography sequentially, while the resin utilization is maintained at the highest level at the constant high residence time. This achieves similar productivity improvements as multicolumn chromatography while applying a straightforward process model that can be implemented on standard systems.

Determination of the conformation of cellobiose dehydrogenase by small angle X-ray scattering

Motycka B[1,2], Kracher D[1,3], Ludwig R[1], Tscheließnig R[2]

[1]Biocatalysis and Biosensing Laboratory, Department of Food Science and Technology, BOKU-University of Natural Resources and Life Sciences, Vienna, Austria,
[2]Department of Biotechnology, BOKU-University of Natural Resources and Life Sciences, Vienna, Austria,
[3]University of Technology, Graz, Austria

Cellobiose dehydrogenase (CDH) is an electron transferring enzyme present in the secretomes of biomass-degrading fungi. It consists of a catalytic FAD-containing dehydrogenase domain (DH) linked to a mobile heme b-containing cytochrome domain (CYT). It`s native function is the reductive activation of lytic polysaccharide monooxygenase (LPMO) via the CYT domain.1 While a closed conformation of CDH favors interdomain electron transfer to the heme b, the spatial separation of DH and CYT is required for the interaction with LPMO.1 By using SAXS, we elucidated the conformation of CDH in solution and the interaction of the DH and CYT domain. We determined the occurrence of the closed- and open-state conformation in regard to pH an ion concentration. Our results suggest a difference in the conformation by changing pH and the concentration of ions. However, currently we cannot prediction the conformation of the domains. To obtain a reference for the scattering signal of the closed-state, we performed protein engineering and introduced cysteines on both domains that can anchor a Maleimide-PEG cross-linker, purified by SEC, and forces the two domains into the closed-state. The scattering results validated by these reference states will be essential to optimize biomass hydrolysis, biofuel production and to develop LPMO-based biosensors for analytical applications.

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Molecular Modeling on the Discovery of synthetic affinity-adsorbents

Barbosa A[1], Matos M[1], Costa C[1], Costa H[1], Dias M[1], Pina A[1], Roque A[1]

[1]UCIBIO, Chemistry Department, NOVA School Of Science And Technology, NOVA University of Lisbon, Caparica, Portugal

Synthetic affinity-adsorbents have been gaining their share in biomolecular purification. The selective nature of molecular interactions between an affinity ligand-target provides the basis for efficient molecular capture and isolation. The application of molecular modeling techniques in this biotechnology field is still gaining traction, however their application in critical phases of synthetic affinity ligand pipeline delivers essential date for ligand development and binding mode elucidation. Following are described approaches used to develop synthetic ligands for purification of: antibodies, chicken IgY, and antigen-binding fragments; Human Serum Albumin (HSA); and Virus Like Particles (VLPs) pseudotyped with protein AMPHO4070A.

Computational techniques allowed to determine the putative binding sites of the synthetic ligand to target biomolecules (antibodies and HSA), using blind molecular docking calculations. The ligand B1Al2A2, for antibody molecules, revealed to bind the non-CDR regions of Fab and Nanobody structures, and the HSA adsorbent A6A5, was confirmed to prefer the binding site in HSA domain II. In VLP adsorbent, a combined theoretical (docking, Molecular Dynamics) and experimental protocol was employed to select the top hit ligands, of which two synthesized ligands, A5A10 and A10A11 successfully purified the VLPs.

Molecular modeling techniques reveal improve affinity-adsorbent discovery giving insights on the molecular interactions of affinity ligands to their targets and the understanding on how the binding and elution buffers affect these interactions in the affinity chromatography process.

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Characterization of AAV5 Viral Vector by Size Exclusion Chromatography-Multi-Angle Light Scattering Technology

Wege J[1], Vitrac H[2], Stewart J[2], Lu W[2], Evans W[2], Chakrabarti A[2], Endres P[1], Kervinen J[2], Müller E[1]

[1]Tosoh Bioscience Gmbh, Griesheim, Germany, 2Tosoh Bioscience LLC, King of Prussia, USA

Recombinant adeno-associated virus (AAV) vectors provide one of the major platforms for gene delivery in cell and gene therapy applications. AAVs (size 20-25 nm) are non-enveloped, single-stranded, DNA viruses that belong to the parvovirus family, and contain genomes up to 4.7 kilobases in length. Development of clinically desirable AAV capsids requires rapid, accurate, and robust analytical methods to confirm AAV purity, capsid titer, and DNA content. Size exclusion chromatography (SEC) coupled with multi-angle light scattering (MALS) detection offers a powerful analytical method for AAV characterization.

Herein, we provide a detailed and comprehensive methodology using SEC coupled with the LenS3 MALS and UV detection for the detailed analysis of AAV5 and its DNA content. Linearity and sensitivity were estimated, demonstrating that as low as 7 x 10^9 particles per mL are detectable by MALS. Size determination confirmed a diameter of ~22 nm for the AAV5, devoid of aggregated material. Absolute molar mass determination using the SECview™ software was first conducted on empty capsids, resulting in a MW of 3.7 MDa, in agreement with the theoretical value derived from primary sequences and the icosahedral structure of the virus. Further analysis of empty and full AAV5 capsids demonstrated the linear increase in MW with increasing full capsid content, enabling the correlation between MW and empty/full capsid composition in the mixture. In summary, the methodology described here for AAV5 provides a powerful and robust tool for the analysis of AAVs across various serotypes, both during production and quality control phases.

Production of high-quality SARS-CoV-2 antigens: Impact of bioprocess and storage on glycosylation, biophysical attributes, and ELISA serologic tests performance

Castro R[1], Nobre L[1], Eleutério R[1], Mendes S[1], Costa J[2], Bandeiras T[1,2], Gomes-Alves P[1,2], Demengeot J[3], Alves P[1,2]

[1]iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal, 2ITQB, Instituto de Tecnologia Química e Biológica, Oeiras, Portugal, 3IGC, Instituto Gulbenkian de Ciência, Oeiras, Portugal

Serological assays are valuable tools to study SARS-CoV-2 spread and to identify individuals that were infected and would be potentially immune to a virus reinfection. Structural studies of SARS-CoV-2 Spike protein and its receptor binding domain (RBD) are key to understand the molecular basis for Spike interaction with angiotensin converting enzyme 2 receptor. Thus, it is urgent that significant amounts of this protein became available at the highest quality. In this study, we produced Spike and RBD in HEK293-E6 and Expi293F™. We evaluated the impact of different and scalable bioprocessing approaches on Spike and RBD production yields and quality attributes. Using negative and positive sera collected from human donors, we show an excellent performance of the produced antigens, assessed in serologic enzyme-linked immunosorbent assay (ELISA) tests. We show robust Spike productions with final yields of approx. 2 mg/L of culture that were maintained independently of the production scale or cell culture strategy. To the best of our knowledge, the final yield of 90 mg/L of culture obtained for RBD production, was the highest reported to date. The antigen‘s oligomeric state, glycosylation profiles, and thermal stability during storage were studied. The correlation of these quality attributes with ELISA performance shows equivalent reactivity to SARS-CoV-2 positive serum. Overall, we provide straightforward protocols to produce high-quality Spike and RBD antigens and integrate, for the first time, studies on the impact of bioprocess with an in-depth characterization of these proteins, correlating antigen‘s glycosylation and biophysical attributes to performance of COVID-19 serologic tests.

Increased lifetime of RPC resins in insulin production by clean-up using a cation exchange step

Tawe J[1], Heijbel A[1], Unoson C[1]

[1]Bio-Works, Uppsala, Sweden

We have conducted a study with recombinant human insulin precursor expressed in Escherichia coli, in which a CIEX capture is the first step prior to RPC purifications. A standard purification process for this recombinant human insulin precursor involves two RPC steps using different buffer systems with different pH for complementary selectivity. By introducing a cation exchange chromatography (CIEX) step upstream of the high-performance silica-based RPC step, the burden from impurities is significantly reduced. When we included an upstream CIEX step we improved the selectivity even further, which provided the desired target peptide purity of more than 99%.

Oligonucleotide purifications using anion exchange chromatography

Unoson C[1], Andersson Schönn M[1], Heijbel A[1]

[1]Bio-Works, Uppsala, Sweden

Therapeutic RNA molecules have an absolute and stringent requirement for purity. This is often achieved using high resolution anion exchange chromatography (AIEX) which can separate full-length sequences from incomplete or erroneous sequences due to the negatively charged phosphate backbones. In this application we have studied how parameters such as residence time, temperature and type of resin affect loading capacities for a 45 nt-long RNA. We have also carried out purification of an RNA preparation where the correlation between purity and yield was analyzed. Optimal conditions for this RNA were obtained with an elevated temperature, a short residence time and using a resin with an optimized pore size. These conditions generated an RNA product with high yield and purity.

Fast quantification of proteoforms in therapeutic protein using flow injection analysis coupled to mass spectrometry

Gaikwad M[1]

[1]University Medical Center Hamburg Eppendorf, Hamburg, Germany

A recombinant therapeutic protein arising from a single gene of interest harbours multiple protein species comprising of sequence variations, splice isoforms, post-translational modification variants (now collectively termed as “proteoforms”). The excess abundance of certain proteoforms in a therapeutic protein sample can affect the total safety and efficacy of the protein drug. Thus, not only qualitative but also quantitative analysis of proteoforms in therapeutic protein sample is crucial for the biopharmaceutical industry. Additionally, to adapt to the ‘quality by design’ principle, there is a need of a fast but sensitive technique for the required quantitative analysis of proteoforms. Currently, the go-to analytical technique for studying intact therapeutic protein sample with multiple proteoforms is ‘reverse-phase liquid chromatography-MS’ (RPLC-MS). However, RPLC-MS is associated with the risk of intact proteoforms irreversible binding to the RP column, giving biased quantitation only for the RP column recovered proteoforms. Thereby, flow injection analysis with MS detection (FIA-MS) is proposed herein as a fast, alternative method for proteoform quantification in the therapeutic protein sample. The proposed FIA-MS setup provides relative quantification of the intact therapeutic protein along with its associated low abundant proteoforms in merely 3min/sample. This fast FIA-MS method can be thus applied for evaluating and optimizing purification process parameters in process design as well as the fast screening of the various production batches of therapeutic proteins.

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Poster Abstracts

Effect of temperature on the operational efficiency of Protein A chromatography for the purification of a monoclonal antibody

Armstrong A[1], Bracewell D[1], Farid S[1], Goldrick S[1]

[1]Advanced Centre for Biochemical Engineering, University College London, London, United Kingdom

A key aspect of bioprocess chromatography process design is maximizing product quality attributes by optimizing process parameters and performance attributes. Process parameters include but are not limited to bed height, elution pH, and eluate cut points. An under characterized chromatography process parameter for protein A chromatography is process temperature. Here, we present the effects of controlling temperature (10, 20, and 30°C) on the purification of a monoclonal antibody (mAb) from a perfusion bioreactor using MabSelect SuRe (Cytiva) chromatography resin. A self-designed 3D-printed heating jacket controlled the process temperature during the loading, wash, elution, and cleaning in place (CIP) steps. The results at 10°C demonstrated a 20% increase in binding capacity and a 35% reduction in mAb loss during the loading and wash phases, respectively. Furthermore, eluting at 30°C resulted in the formation of a second early eluting peak which contained 60% of the total aggregates and dimers and only 7% of the total mAb bound to the column. Removing this early eluting impurity-rich peak offers the potential to simplify further polishing steps by reducing the impurity content in the pooled product. The addition of temperature control to protein A chromatography purification provides a potentially novel method to enhance yields and purity whilst reducing resin volume required.

Online optimization in protein purification

Eslami T[1], Hirschmann G[2], jungbauer A[1], Lingg N[1]

[1]BOKU University, Vienna, Austria, 2Evon Gmbh, Wollsdorf, Austria

In downstream processing, chromatography is the most important unit operation for the purification of therapeutic proteins [1]. The dynamic binding capacity governs the process economics due to its impact on the whole process [2]. therefore, traditional purification operations consist of long loading steps at a constant flow rate to achieve high resin utilization. However, this will result in low productivity which will increase the operation cost at the same time. Therefore, a balance between all the economic factors is necessary. Model Predictive Controller (MPC), is one of only a few advanced control methods that are used successfully in industrial control applications. MPC uses the model to predict the process and employs this estimation in the mathematical optimization procedure to find the best possible candidate to optimize the system. Additionally, different constraints are applied to ensure the optimality of the solution. In this study, aim is to maximize productivity while the resin utilization is maintained at the highest level.

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An affinity approach for baculovirus clearance from viral-based bioprocesses

Fernandes R[1,2], Fernandes R[1], Carvalho S[1,2], Bezemer S[3], Detmers F[3], Hermans P[3], Silva R[1], Alves P[1,2], Carrondo M[1], Peixoto C[1,2]

[1]IBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal,
[2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal,
[3]Thermo Fisher Scientific, Leiden, Holanda

The use of virus-based biotherapeutics in the pharmaceutical industry have been progressed considerably in the last years, with applications in vaccination, gene therapy, and oncolytic therapy. However, given their biological complexity, there are still unmet challenges in the bioprocessing of these particles, particularly on the downstream processes. Insect-cell biopharmaceuticals produced using the baculovirus expression vector system (BEVS) have an additional purity-related concern due to the co-production of baculovirus particles. Their rod-shaped form and similar envelope to virus-like particles (VLPs) lead to a purification bottleneck, given the lack of efficiency in discriminating both particles.
In the present work, a purification strategy based on an impurity-focused affinity chromatography approach for baculovirus removal from VLPs-based vaccine candidates manufacturing was developed. Several affinity ligands were discovered by phage display towards baculovirus particles and the most promising candidates were selected and immobilized in chromatographic support. The chromatographic performance and dynamic binding capacity were assessed for those resins. The impact of operational parameters, such as residence time was studied and their influence on the purification performance was described. The best candidates showed baculovirus removals above 70% and VLPs' recoveries above 60%. As a proof-of-concept, these ligands were also evaluated using two different biopharmaceuticals produced by BEVS: hepatitis C VLPs and adeno-associated virus. Furthermore, these resins showed to be robust to the process and cleaning conditions, maintaining the purification performance over 20 cycles. Overall, by selecting the optimal operation setpoints, it was possible to develop a powerful chromatographic tool for baculovirus removal from biopharmaceuticals produced using BEVS.

One-step chromatography to produce anti-vp39 antibody for fast Baculovirus detection using Western blot analysis

Frank A[1], Zollner A[2], Mayer V[1], Pereira Aguilar P[1], Jungbauer A[1]

[1]acib Gmbh, Wien, Austria,
[2]Boku Vienna, Wien, Austria

Virus-like particles (VLPs) mimic the structure of native viruses and can induce high immune responses but are not able to replicate. Therefore, VLPs play an important role in vaccine development. The production of different types of VLPs in the Baculovirus-insect cell expression system is well established. The depletion and discrimination of numerous process related impurities, which are very similar in structure and/or size such as, exosomes, chromatin and Baculovirus is very challenging. A fast and easy tool to distinguish Baculovirus from VLPs, omitting time consuming infectivity assays, is crucial for efficient process development. We developed a Western blot analysis to detect the major capsid structure protein (vp39) of Baculovirus. The Baculovirus specific antibody was purified from rabbit serum by affinity chromatography using protein G as a ligand. The specific binding of the purified antibody to detect only Baculovirus in Western blot analysis was proven by using a Baculovirus standard as positive control, non-infected insect cell culture supernatant as negative control and process samples obtained from a preparative chromatographic separation of VLPs and Baculovirus. Different antibody concentrations ranging from 0.6 mg/mL to 0.6 µg/mL were tested to detect a clear band around 39 kD, which is the molecular weight of vp39. At an antibody concentration of 5.6 µg/mL vp39 was clearly detected in positive control and process samples, whereas no signal was detected for the negative control. The Western blot analysis, enabling discrimination of Baculovirus and VLPs is an extremely valuable tool to support fast process development.

Purification of immunoglobulin G antibodies using hybrid alginate-protein cryogel beads

Freire M[1], Tavares A[1], Sharma M[1], Singh N[2], Mondal D[3], Prasad K[3]

[1]CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal,
[2]Natural Products & Green Chemistry Division, CSIR-Central Salt & Marine Chemicals Research Institute, Gujarat, India,
[3]Centre for Nano & Materials Sciences, Jain University, Jain Global Campus, Karnataka, India

Despite the relevance of immunoglobulin G antibodies for therapeutic purposes, there are still some challenges to overcome their current high-cost, including the development of simpler and cost-effective techniques. Herein, bio-based and low-cost hybrid alginate-protein cryogel beads were prepared, characterized, and investigated to act as novel and efficient adsorbent materials for the purification of IgG from human serum. Several operating conditions were optimized to improve the recovery yield and purity of IgG. The developed hybrid materials are more efficient than the respective alginate beads since the presence of proteins increases the materials selectivity for IgG. Adsorption isotherms were determined to infer the adsorption mechanism of IgG onto the cryogel beads and to determine their adsorption capacity (175 mg of IgG per g of cryogel beads). At the optimized conditions, IgG can be recovered from the hybrid materials using buffered aqueous solutions, with high purity and recovery yield. The re-generation and reuse of the cryogel beads was evaluated, with no losses on the IgG adsorption performance and stability. Although significant efforts have been placed on the development of novel affinity ligands to replace the standard chromatographic methods to purify IgG, this works demonstrates the potential of bio-based and low-cost hybrid materials as promising alternatives, in which proteins can be used to improve the materials selectivity.

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Analysis and separation of proteoforms from a single protein via protein purification parameter screening and sample displacement chromatography

Hidayah S[1], Gaikwad M[1], Schlüter H[1]

[1]University Medical Center Hamburg, Hamburg, Germany

Liquid chromatography (LC) of proteoforms is one of the most challenging problems in analytical chemistry and in down-stream-processing because proteoforms are coded by the same gene which makes most of them physically and chemically very similar. Moreover, many proteoforms are often present in a low abundance. The displacement mode (DC) in LC is advantageous with respect to its separation efficacy compared to the gradient mode. DC has been tested for the separation of monoclonal antibody charge variants in cation exchange chromatography column. However, there are several limitations regarding displacer and inefficient use of chromatography columns. We present here sample displacement chromatography in a batch mode (SDBC) for the separation of proteoforms, which is a simple and fast alternative to conventional DC. Mass spectrometry (MS) of intact proteins (top-down MS) was used for detection and quantification. To ensure the success of SDBC, systematic screening of chromatographic parameters (PPS) was performed. We screened different ion exchange chromatography resins, pH values, buffers concentrations, different addition of sodium chloride in the sample application buffers, and different elution buffers to achieve the most optimal condition for the purification of proteoforms. Using the parameters from PPS, separation of proteoforms coded by the same gene and enrichment of low abundant species were achieved. With different pH values in sample application buffer, the enrichment of different group of proteoforms of interest was achieved. Furthermore, we demonstrated the successful of SDBC for the separation and purification of proteoforms.

Keywords: proteoforms purification, sample displacement chromatography

FLASHDeconvQ: High-quality feature level label-free quantification algorithm for top-down proteomics mass spectrometry data

Kim J[1], Jeong K[1], Gaikwad M[2], Babović M[3], Jensen O[3], Schlüter H[2], Kohlbacher O[1]

[1]University of Tübingen, Tübingen, Germany,
[2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany,
[3]University of Southern Denmark, Odense, Denmark

Top-down proteomics (TDP) has drawn a lot of attention for proteoform-level functional studies. Accurate quantification of proteoforms is crucial for such studies, and label-free quantification (LFQ) has several advantages over labeled approaches such as easy sample preparation and no extra cost for labeling. Most freely available software tools for the analysis of TDP LFQ data have not been designed for quantification, but for spectrum deconvolution and thus do not provide very accurate quantification results. Here we present FLASHDeconvQ, a highly accurate and sensitive quantification algorithm for TDP mass spectrometry data (https://openms.de/FLASHDeconvQ).

FLASHDeconvQ comprises three main stages: mass trace detection, deconvolution, and proteoform abundance calculation. In the first stage, mass traces (individual ion chromatograms) are constructed from signals in centroided spectra using a self-consistent kernel density estimator (Kenar et al., 2014). Next, the mass traces within a retention time (RT) window are projected along the RT direction into a spectrum. By performing deconvolution for the generated spectrum, mass traces are assembled into a feature group, that is the features from the same analyte having multiple charge states. Lastly, proteoform quantity is achieved by the summed full width at half maximum area of mass traces in the feature group, approximating the abundance of the proteoform.

To test FLASHDeconvQ, simple and complex datasets were prepared; the simple dataset is a two-fold dilution series (from 1000 ng to 31 ng) of filgrastim (RPLC, Q Exactive Hybrid MS), and the complex dataset is based on an E. coli lysate (nano-RPLC, Orbitrap Eclipse).

Empty and Full Separation of Adeno-Associated Virus Vectors by Anion Exchange Membrane Chromatography

Hejmowski A, Boenning K, Kavara A, Huato J, Schofield M, Figueiredo S

[1]Pall Corporation

The approval of adeno-associated virus (AAV) gene therapies has led to increased research into AAV production and the burgeoning of promising clinical trials. However, significant challenges persist in AAV purification as AAV harvests typically contain a majority population of empty capsids that generate an immune response without delivering the therapeutic payload. In addition, subtle differences between therapies restrict platformability.

Here we assess the performance of anion exchange (AEX) membrane chromatography as the polishing stage of an AAV platform process following affinity purification. Utilizing a novel 1 mS/cm step gradient approach, we are able to demonstrate separation of empty and full AAV capsids of serotypes 5, 8, and 9 with the Mustang® Q XT membrane. This process maximizes the high flow rate benefits of membrane chromatography relative to traditional column chromatography, while providing improved separation. Distinct populations in the UV 260/280 chromatogram, analytical trends with PCR and ELISA tests, and capsid standards prepared by ultracentrifugation reaffirm separation. This technique is scalable between the 0.86 mL Mustang Q XT Acrodisc® unit and 5 mL Mustang Q XT capsule, and effectively clears residual host cell protein and DNA contaminants.

Aqueous Biphasic Systems for tailoring t he extraction and purification of antileukemic enzyme L-Asparaginase

Magri A[1], Santos J[2], Coutinho J[2], Ventura S[2], Pereira J[3]

[1]School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquaraa, Brazil,
[2]CICECO, Institute of Materials, University of Aveiro, Aveiro, Portugal,
[3]Univ Coimbra, CIEPQPF, Department of Chemical Engineering, Coimbra, Portugal

L-Asparaginase (ASNase) is an important biopharmaceutical for the treatment of acute lymphoblastic leukemia (ALL); however, with some restrictions due to its high manufacturing costs, particularly from the complex and expensive downstream purification processes. Aqueous biphasic systems (ABS) were evaluated as more economical preparative separation platforms, also aiming a full understanding of the mechanisms behind the ASNase. Polymer/salt-based ABS with different driving-forces (salting-out and hydrophilicity/hydrophobicity effects) were herein applied to control the partition of commercial ASNase as well as to extract it from a E. coli lysate supernatant.[1] Depending on ABS, the ASNase was partitioned to the salt- or polymer-rich phase (extraction efficiencies higher than 95%). Cholinium-salts-based ABS were able to promote a preferential ASNase partition to the polymer-rich phase using PEG-600 and to the salt-rich phase using a more hydrophobic PPG-400 polymer. PEG-2000 + potassium phosphate buffer ABS was the most efficient to separate the ASNase from the main contaminant proteins (purification factor = 2.4±0.2), while it was able to maintain the enzyme activity for posterior application as part of a therapeutic. ABS can be used to control the partition of ASNase and adjust its purification yields, which clearly shows the potential of this type of liquid-liquid extraction systems as cost-effective platform for the selective recovery of therapeutic enzymes from complex broths.

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Reference:
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In Situ Protein Recovery from Yeast Fermentations

Pham D1, Linova M1, Carbonell R2, Woodley J1

[1]The Technical University of Denmark, Kgs. Lyngby, Denmark, 2North Carolina State University, Raleigh, USA

The biopharmaceutical market has grown tremendously in recent years, and especially therapeutic proteins have had a huge impact with its treatment of a broad spectrum of diseases. This growth is expected to grow even further, which is reflected by the ever-increasing demand of therapeutic proteins. The application of gene-modified organisms in bioreactor fermentations to produce therapeutic proteins is a very costly process. Yet, this might not be the greatest concern, since heterologous proteins as a macromolecule might denature due to instability in the fermentation media, or they can be toxic towards the production host, hence limiting the overall yield. *In situ* product recovery (ISPR) is a process, which removes the fermentation product simultaneously to its production by applying a capture device, which selectively binds the target protein. The integration of ISPR could potentially circumvent some of the production issues and lead the way for a more continuous fermentation process. Still, several questions remain to be investigated such as what the most efficient experimental ISPR model design should look like, what is the longitude of an ISPR fermentation process, which capture technologies are available and how are the cells behaving during an ISPR fermentation. These are just a few questions, which still need more research data.

Bioprocess monitoring of glycosylated protein concentration via In-process lectin polyHIPE chromatography

Podgornik A[1,2], Stantič M[1], Mravljak R[1], Gunčar G[1]

[1]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia, [2]COBIK, Ajdovščina, Slovenia

In-process monitoring of glycosylated protein concentration becomes very important with the introduction of perfusion bioprocesses. Affinity chromatography based on lectins allows selective monitoring when carbohydrates are accessible on the protein surface. In this work, we immobilized lectin on polyHIPE type of monoliths and implemented it for bioprocess monitoring. A spacer was introduced to lectin, which increased binding kinetics toward Fc-fusion protein, demonstrated by bio-layer interferometry. Furthermore, complete desorption using 0.25 M galactose was shown. Affinity column exhibited linearity in the range between 0.5 and 8 mg/ml and flow-unaffected binding for the flow-rates between 0.5 and 8 ml/min. Long-term stability over at least four months period was demonstrated. No unspecific binding of culture media components, including host cell proteins and DNA, was detected. Results obtained by affinity column matched concentration values obtained by a reference method.

Single-step purification of ssDNA scaffolds using affinity magnetic beads for DNA-origami biomanufacturing

Santos A[1], Oliveira-Silva R[1], R. Paulo P[2], F. Prazeres D[1]

[1]iBB - Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001, Lisbon, Portugal, [2]Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001, Lisbon, Portugal

DNA nanotechnology encompasses the self-assembly of nucleic acids into complex nanostructures by exploring Watson–Crick base pairing. Usually, asymmetric PCR (aPCR) is used to generate 500-3500 nucleotide (nt) long, object-specific, single-stranded DNA (ssDNA) scaffolds using the DNA of the M13 phage as template. Scaffolds are purified by agarose gel extraction, a technique that is laborious, limited, not scalable, presents low recovery yields and a low-quality product. We present an affinity-based method using magnetic particles to purify ssDNA scaffolds from asymmetric PCR mixtures, which can be used in DNA-origami techniques.

aPCR, using the genome of the M13mp18 phage as template, generated 449 and 1000 nt-long single and double-stranded DNA (dsDNA). Carboxyl-modified magnetic particles were functionalized with a 20-nt oligonucleotide complementary to the 3' terminal of the 449 and 1000 nt-long ssDNA scaffolds. Hybridization between the ssDNA scaffolds in the aPCR mixture and the affinity beads was promoted at high LiCl concentrations. The dsDNA did not hybridize and could thus be separated from the magnetic beads. Following washing, magnetic beads were heated up to denaturation temperatures and ssDNA were recovered in the solution by magnetic separation.

The recovered ssDNA were used to assemble 31 and 63-bp edge length tetrahedrons using site-specific short oligonucleotides (staples), thermal annealing and high magnesium concentrations. Agarose gel electrophoresis and photochemical studies showed high assembly yield and purity.

In conclusion, affinity-based purification was successfully used to purify 550 ng of 449-nt and 880 ng of 1000-nt ssDNA fragments per aPCR reaction, which were subsequently folded into DNA-origami nanostructures.

Immobilization of L-asparaginase towards surface-modified carbon nanotubes

Tavares A[1], Almeida M[1], Cristóvão R[2], Barros M[2], Nunes J1, Faria J2, Neves M1, Freire M1, Santos-Ebinuma V3, Silva C2

[1]CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal,
[2]Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal,
[3]Department of Engineering Bioprocess and Biotechnology, School of Pharmaceutical Sciences, UNESP-University Estadual Paulista, Araraquara, Brazil

L-asparaginase (LA) is an enzyme that catalyzes L-asparagine hydrolysis into L-aspartic acid and ammonia and is mainly applied in pharmaceutical and food industries. The LA currently commercialized for pharmaceutical purposes is produced from two main bacterial sources: recombinant Escherichia coli and Erwinia chrysanthemi. However, some disadvantages are associated with its free form, such as the shorter half-life. Immobilization of LA has been proposed as an efficient approach to overcome this limitation. In this work, a straightforward method, including the functionalization of multi-walled carbon nanotubes (MWCNTs) through a hydrothermal oxidation treatment and the immobilization of LA by adsorption over pristine and modified MWCNTs was investigated. Different operation conditions, pH, contact time, ASNase/MWCNT mass ratio, and operational stability of immobilized LA, were evaluated. The characterization of the LA-MWCNT bioconjugate was addressed using different techniques, namely TEM, TGA, and Raman spectroscopy. Functionalized MWCNTs showed promising results, with an immobilization yield and a relative recovered activity of commercial LA above 95% (pH 8, 60 min of contact, 1.5×10^{-3} g/mL LA). The LA-MWCNT bioconjugate also showed improved enzyme operational stability (6 consecutive reaction cycles) proving its suitability for application in industrial processes.

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Optimal State Estimation of an ADC Conjugation Reaction Using an Extended Kalman Filter

Weggen J[1], Schiemer R[1], Schmitt K[1], Hubbuch J[1]

[1]Institute of Process Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

Antibody-drug conjugates (ADCs) are among the most promising new formats in the bio- pharmaceutical industry. A central step during ADC production is the conjugation reaction which links a cytotoxic drug to a monoclonal antibody (mAb) via a linker. Recently, UV/Vis spectroscopy in combination with partial least square regression (PLS) has been established as a Process Analytical Technology (PAT) to monitor the progress of the conjugation reaction on-line for a DAR2 conjugation. In addition, an ADC kinetic model was developed to determine the reaction mechanism and predict the time-course of conjugated ADC species. Nevertheless, both models come with statistical uncertainties due to the calibration with experimental data and different model assumptions. For the deployment in process control and automation, robust models are required.

In this work, we investigated an optimal state estimation algorithm using an Extended Kalman Filter (EKF) for fusion of both PLS and kinetic model. Based on limited amount of experiments conducted with different mAb-concentrations and drug excesses, we calibrated the two models and were able to estimate initial states and process/measurement noise. We then applied the EKF scheme in which we calculated the time-varying process noise via a linearized method. Through a comparison to offline data, it could be shown that the filter successfully allows the estimation of the conjugation stage and even species concentrations which are non-determinable by the PLS model itself. Finally, the EKF was applied to other experimental conditions to further validate its performance and advantage over both separate models.

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