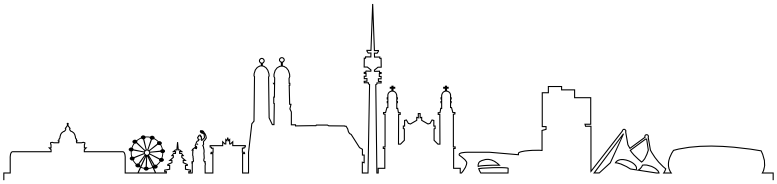
ABSTRACT BOOK

NOVEMBER 9-12, 2025

44th International Symposium on the Separation of Proteins, Peptides & Polynucleotides

ISPPP
2025 MUNICH

ISPPP 2025 MUNICH



44th International Symposium on the Purification of Proteins, Peptides & Polynucleotides

MUNICH (CAMPUS GARCHING), GERMANY
NOVEMBER 9 - 12, 2025

acib austrian centre of industrial biotechnology

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WELCOME TO ISPPP 2025

It is my great pleasure to welcome you to the 44th International Symposium on the Separation of Proteins, Peptides and Polynucleotides (ISPPP 2025), held this year at the Research Campus of the Technical University of Munich in Garching, Germany.

For more than four decades, ISPPP has provided an outstanding international forum for scientists and professionals from academia and industry to share their latest research, exchange ideas, and foster collaborations in the field of separation science. This year, we continue this tradition by bringing together experts and young researchers alike to discuss both fundamental advances and innovative applications in protein, peptide, and polynucleotide separation.

The program reflects the breadth and depth of our community: from chromatography and membrane technologies to bioseparations for new modalities, digital and process intensification approaches, and the emerging role of data-driven and AI-supported methods. Disruptive technologies such as magnetic separation and potential-controlled separation are now part of the scientific dialogue, while separation techniques for plasmid DNA and mRNA are gaining increasing importance in view of the rapid development of new therapeutics.

A special highlight this year is the dedicated session for young spin-offs and those preparing to found new ventures in the field. By hosting ISPPP at the TUM Research Campus in Garching – home to cutting-edge science, strong industrial collaborations, and the vibrant entrepreneurial ecosystem of TUM – we also underline our commitment to making the spirit of fresh ideas more visible and to accelerating the transfer of innovations into industry.

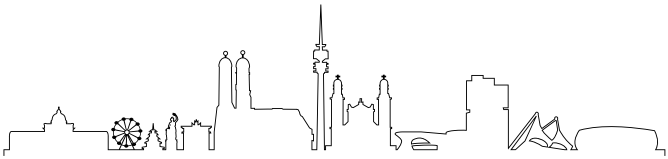
On behalf of the organizing committee, I would like to warmly thank all speakers, authors, sponsors, and participants who contribute to making ISPPP 2025 a vibrant and memorable event. Your engagement and passion are what drive the continued success of this symposium.

We wish you an inspiring conference, fruitful discussions, and an enjoyable time in Garching and Munich.

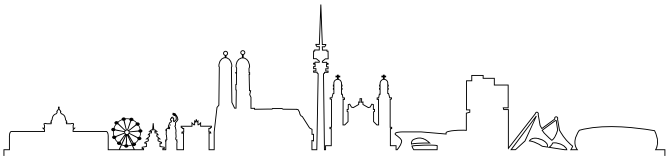
Sincerely,

Sonja Berensmeier

CONFERENCE CHAIR



CONTENT



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PRE-CONFERENCE WORKSHOPS

SUNDAY, NOVEMBER 9, 2025

10:00	**START OF REGISTRATION**	
11:00	**WORKSHOP 1** **Michel Eppink** TU Delft, Byondis B.V. **Egbert Müller,** Tosoh Bioscience GmbH	Oligonucleotide production, purification and application in biopharmaceutical processing and their quality requirements
12:15	**WORKSHOP 2** **Michel Eppink** TU Delft, Byondis B.V. **Sonja Berensmeier** TU Munich	Insights in Polynucleotide Production: Overcoming Purification Challenges in mRNA and Plasmid Manufacturing
13:30	**WORKSHOP 3** **Cristina Cabral** Univ. of Beira Interior	Microcalorimetry as a Tool in Preparative Chromatography: Versatility and Power
14:45	**WORKSHOP 4** **Alois Jungbauer** acib GmbH & BOKU University	Purification and characterization of viral cell and gene therapy vectors

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CONFERENCE PROGRAMME

SUNDAY, NOVEMBER 9, 2025

17:00	**WELCOMING REMARKS BY SONJA BERENSMEIER**	
KEY NOTE LECTURE CHAIR: SONJA BERENSMEIER		
17:20	**Scott Wheelwright** BioChromatographix International Pte. Ltd.	**KN1:** Purification of viruses for gene therapy and vaccines: A comparison of convective and diffusive chromatography
18:00	**WELCOMING RECEPTION**	

Picture: SOINI ASSET IMMOBILIEN

MONDAY, NOVEMBER 10, 2025

KEY NOTE LECTURE
CHAIR: ALOIS JUNGBAUER

08:30	**Alois Jungbauer** acib GmbH & BOKU University	Key Note & Session Introduction
08:35	**Stefano Menegatti** North Carolina State University	**KN2:** Stimuli-responsive peptides: A kaleidoscope of applications

SESSION 1: ADVANCES IN SEPARATION PROCESSES
CHAIR: ALOIS JUNGBAUER

09:15	**Aleš Podgornik** University of Ljubljana	**OP1:** Effect of support microstructure on compression and pressure drop during flow-through applications
09:35	**Fernando De Mathia** BOKU University	**OP2:** Purification and characterization of a recombinant neuraminidase influenza virus vaccine candidate
09:55	**Robert Klausser** Technical University Vienna	**OP3:** Protein denaturation maps – Charting a course for the solubilization and refolding of bacterial inclusion bodies
10:15	**REFRESHMENT BREAK**	

SESSION 2: HYBRID PROCESSING & NEW TECHNOLOGIES
CHAIR: EGBERT MÜLLER

10:35	**Egbert Müller** Tosoh Bioscience GmbH	Session Introduction
10:40	**Pedro Ferreira** University of Beira Interior	**OP4:** A reusable nanodiamond-based platform for selective purification of RNA
11:00	**Michał Kołodziej** Rzeszów University of Technology	**OP5:** Isolation and purification of monoclonal antibodies using combined precipitation and crystallization process

11:20	**Lisa Meier** Technical University of Munich	**OP6:** Electrochemically modulated purification of plasmid DNA using graphite electrodes in static and flow systems
11:40	**Peter Mayrhofer** Technical University of Munich	**OP7:** Light-controlled protein purification using a short photo-switchable affinity tag
12:00	**LUNCH BREAK**	

Old Town Munich

Picture: travelwithzhuk (Pixabay)

SESSION 3: FLASH & SPIN-OFF TALKS
CHAIR: MICHEL EPPINK

13:30	**Michel Eppink** TU Delft & Byondis B.V.	Session Introduction
13:35	**FLASH TALKS**	
	Yannick Krauke Knauer Wissenschaftliche Geräte GmbH	**FP1:** Large scale purification and quality control of therapeutic oligonucleotides
	Edina Császár Biomay AG	**FP2:** mRNA manufacturing: from template DNA to lipid nanoparticles – An integrated platform approach
	Djuro Josić Juraj Dobrila University of Pula	**FP3:** Direct application of undiluted human plasma and other complex biological fluids to polymethacrylate-based monoliths and subsequent isolation of biologically active therapeutic proteins and other biopolymers
	Jonas Wege Tosoh Bioscience GmbH	**FP4:** Towards seamless mAb purification: Dual-Step Multi-Column Chromatography
	Marina Linova Technical University of Denmark	**FP5:** Advancing *K. phaffii* bioprocesses: Evaluation of continuous perfusion processes and suitable purification strategies
14:10	**SPIN-OFF TALKS**	
	Nils Brechmann MAGic BioProcessing	**SP1:** Comparative economic modeling of magnetic bead-based processing as an alternative to legacy mAb manufacturing
	Robin Karl Technical University of Munich	**SP2:** Holistic pilot-scale magnetic separation platform development
	Eike Theel Technical University of Munich	**SP3:** Potential-controlled affinity membrane chromatography (pcMAC) - Redefining gentle biomolecule purification

	Andreas Reichert Technical University of Munich	**SP4:** Light-controlled antibody purification via a photoswitchable protein A platform
	Simone Dimartino University of Edinburgh	**SP5:** Planet Crafting Labs: Empowering the biotech industry to drive sustainable innovation
15:15	**REFRESHMENT BREAK**	

SESSION 4: CONTINUOUS & INTENSIFIED PROCESSING
CHAIR: MIRJANA MINCEVA

15:45	**Mirjana Minceva** Technical University of Munich	Session Introduction
15:50	**Mark Dürkop** Novasign GmbH	**OP8:** Process modeling as key to intensify continuous bioprocess development
16:10	**Sabrina Leigheb** BOKU University	**OP9:** Continuous flow ultracentrifugation enables efficient capture of adeno-associated viruses from clarified lysates
16:30	**Julian Galbusera** Technical University of Munich	**OP10:** Development and economic evaluation of an intensified magnetic nanoparticle-based purification process for microbial proteins
16:50	**Markus Berg** enGenes Biotech GmbH	**OP11:** Continuous production of plasmid DNA: Advances in integrated separation and purification
17:10	**Jonas Arnecke** Technical University of Applied Sciences Mannheim	**OP12:** Process intensification for protein purification: Continuous multi-column isolation of napin and cruciferin
17:30	**SHORT BREAK**	
17:40	**GUIDED LAB TOURS**	
18:45	**POSTER PARTY INCL. SNACKS**	
20:15	**END OF DAY 2**	

TUESDAY, NOVEMBER 11, 2025

KEY NOTE LECTURE CHAIR: ELENA DOMINGUEZ VEGA		
08:30	**Elena Dominguez Vega** Leiden University Medical Centre	Key Note & Session Introduction
08:35	**Charlotte Uetrecht** CSSB / DESY / University of Lübeck	**KN3**: Flying viruses – mass spectrometry meets X-rays
SESSION 5: ANALYTICS CHAIR: DOMINGUEZ VEGA		
09:15	**Christoph Gstöttner** Roche Diagnostics GmbH	**OP13**: Analytical techniques for rAAV genome integrity and identity assessment
09:35	**Katharina Dietmann** Ludwigs-Maximilians-Universität München	**OP14**: In-line infrared spectroscopic detection of chromatographic protein separation for medical diagnostics
09:55	**FLASH TALKS**	
	Roland Drexel Postnova Analytics GmbH	**FP6**: Multi-detector Field-Flow Fractionation for the assessment of critical quality attributes of AAVs
	Tomas Mesurado acib GmbH	**FP7**: Novel analytical HPLC method for characterization and quantification of VLPs
10:10	**Balasubrahmanyam Addepalli** Waters Corporation	**OP15**: Critical quality attribute analysis of RNA therapeutics by novel ribonuclease specificities
10:30	**Cláudia Paiva** iBET	**OP16**: Real-time monitoring in ultra- and diafiltration of adeno-associated virus and lentiviral vector using Raman Spectroscopy
10:50	**REFRESHMENT BREAK**	
11:00	**POSTER SESSION**	
12:30	**LUNCH BREAK**	

SESSION 6:
SEPARATION & PURIFICATION OF BIONANOPARTICLES
CHAIR: NICO LINGG

13:30	**Nico Lingg** acib GmbH & BOKU University	Session Introduction
13:35	**Patricia Pereira Aguilar** acib GmbH	**OP17:** Functionalized non-woven fibers for the harvest, clarification and purification of bionanoparticles
13:55	**Ricardo M. Silva** Instituto Superior Tecnico	**OP18:** Single-step extracellular vesicles isolation strategy using steric exclusion chromatography
14:15	**Mauri Belasko** Isolere Bio	**OP19:** Scalable purification of adherently-produced lentiviral vectors using IsoTag™ LV reagent
14:35	**FLASH TALKS**	
	Ronaldo Moraes Preto Instituto Butantan	**FP8**: Tangential flow filtration and multimodal chromatography as promising strategies for purification of outer membrane vesicles (OMVs) from *Neisseria lactamica*
	Ana Carolina Moreno Pássaro Instituto Butantan	**FP9:** Protein purification of soluble and insoluble Rhizavidin- fused potential *Schistosoma mansoni* antigen: a comparison of chromatographic performance
	Pragya Prakash Indian Institute of Technology, Delhi	**FP10:** Comparative analysis of chromatography-based approaches in the downstream processing of virus like particles
14:55	**REFRESHMENT BREAK**	

SESSION 7: OLIGO- & POLYNUCLEOTIDES
CHAIR: ALEŠ PODGORNIK

15:25	**Aleš Podgornik** University of Ljubljana	Session Introduction
15:30	**Natalia Vereszki** RotaChrom Tecnologies PLC	**OP20**: Isolation of oligonucleotides by scalable IEX-centrifugal partition chromatography
15:50	**Ana Rita da Silva Santos** Instituto Superior Técnico	**OP21**: 3D-printed matrices for steric exclusion chromatography of plasmid DNA
16:10	**Mikael Andersson Schönn** Bio-Works AB	**OP22**: Ion exchange as a sustainable alternative to reversed phase chromatography for the purification of TIDES molecules
16:30	**Francisco Marques** Instituto Superior Técnico	**OP23**: shRNA precipitation strategies for sustainable RNA-based biopesticides
16:50	**Sara Sousa Rosa** University College London	**OP24**: Simplifying mRNA manufacturing using alternative separation techniques
17:10	**END OF SESSION**	
19:30	**CONFERENCE DINNER**	

WEDNESDAY, NOVEMBER 12, 2025

SESSION 8: AFFINITY-BASED TECHNOLOGIES
CHAIR: JÜRGEN HUBBUCH

09:00	**Jürgen Hubbuch** Karlsruhe Institute of Technology	Session Introduction
09:05	**Ana Cecilia Roque** NOVA University	**OP25**: Improving the design and production of mixed-mode and affinity ligands
09:25	**Timon Kalchmayr** BOKU University	**OP26**: Designing for sustainability: Modelling the impact of buffer choice in chromatography
09:45	**Vanessa Kohl** Merck Life Science KGaA	**OP27**: Exploring the Nanofitin® Affinity Ligand Platform: Showcasing case studies from novel modalities to diverse protein classes
10:05	**FLASH TALKS**	
	Yasmin Kaveh Baghbaderani Technical University of Munich	**FP11**: Optimizing antibody-binding stoichiometry via the linker-region
	Cristina Dias-Cabral University of Beira Interior	**FP12***: In situ* analysis of monoclonal antibody fragment adsorption on phenylboronic acid chromatography media
	Gregor Stitz BOKU University	**FP13**: Cationic polymers reduce host cell protein burden for subsequent chromatographic mAb purification
10:25	**REFRESHMENT BREAK**	

SESSION 9: FUNDAMENTALS & MODELLING
CHAIR: CRISTINA CABRAL

10:45	**Cristina Cabral** University of Beira Interior	Session Introduction
10:50	**SPONSORED TALK**	
	Martin Sichting Cytiva Europe GmbH	**OP28**: DoE, mechanistic modelling and artificial intelligence in chromatography
11:10	**Nitika Nitika** Indian Institute of Technology Delhi	**OP29**: Application of machine learning for sustained verification of chromatography unit performance.
11:30	**Dorota Antos** Rzeszów University of Technology	**OP30**: Bulk crystallization for protein processing: new concepts, challenges and opportunities
11:50	**Eric von Lieres** Forschunsgzentrum Jülich	**OP31**: High-definition simulation of packed-bed chromatography in laterally unconfined compartments
12:10	**Marcel Ottens** Delft University of Technology	**OP32**: Host cell proteins profiling and characterization for model-based DSP design
12:30	**PRESENTATION OF POSTER AWARDS & CONCLUDING REMARKS** BY SONJA BERENSMEIER	
13:00	**END OF CONFERENCE**	

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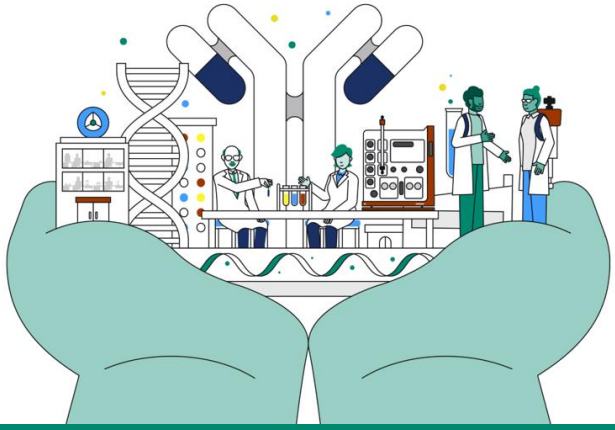

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POSTERS

presented on Monday, Nov. 10
18:45 – 20:15

All posters will be displayed throughout the conference. We ask presenters of the following posters to be at their poster for questions and discussions on Monday, Nov. 10, 18:45 – 20:15.

Fundamentals and Modelling

P1	**Giorgio Carta** University of Virginia	Advances in Understanding and Modeling pH Transients in Protein A Columns and their Effects on Elution of IgG
P3	**Jürgen Beck** BOKU University	Measuring Adsorption Equilibria: The determination of the maximum binding capacity depends strongly on the method of resin preparation.
FP12	**Cristina Dias-Cabral** University of Beira Interior	*In situ* analysis of monoclonal antibody fragment adsorption on phenylboronic acid chromatography media

Advances in Separation Processes

P7	**Jonas Guth** Technical University of Applied Sciences Mannheim	Enhanced monomer-aggregate resolution on Protein A membrane chromatography
P9	**Michael Niemeyer** Navigo Proteins	Affinity based purification of Hyaluronidase by using Precision X® ligands
P11	**Felix Veitl** Technical University of Munich	Bioseparation Controlled by Light: Engineering of an IgG Fc-Specific Affitin for the Affinity Purification of Antibodies
P13	**Tonima Akter Khan** Technical University of Munich	Optimizing Antibody Immobilization for Affinity-Based Separation
P15	**Eike Theel** Technical University of Munich	Potential-Controlled Membrane Affinity Chromatography: A Sustainable Strategy for Antibody Capture and Elution
FP11	**Yasmin Kaveh Baghbaderani** Technical University of Munich	Optimizing Antibody-Binding Stoichiometry via the Linker-Region

P18	**Markus Mozgovicz** acib GmbH	HIV-1 gag virus-like particle purification: A scalable chromatographic approach
P20	**Tina Simčič** Sartorius BIA Separations d.o.o.	Modulating and Understanding Retention of Proteins on Chromatographic Support by Changing Cation-Exchanging Ligand
P22	**Amélie Terreaux** Numab Therapeutics AG	Novel protein A and L ligands for the purification of multispecific antibody-based therapeutics
P24	**Gregor Richter** Thermo Fisher Scientific	Affinity purification as a platform for improved plasma-derived IgG manufacturing
P25	**Mauri Belasko** Isolere Bio	Scalable purification of adherently-produced lentiviral vectors using IsoTag™ LV reagent
P27	**Peter Menstell** Merck Life Science	Intensifying AAV9 Capture: The Role of Nanofitin® Affinity Ligands for Streamlined Purification Processes
FP1	**Yannick Krauke** Knauer Wissenschaftliche Geräte GmbH	Large scale purification and quality control of therapeutic oligonucleotides
FP8	**Ronaldo Moraes Preto** Instituto Butantan	Tangential flow filtration and multimodal chromatography as promising strategies for purification of outer membrane vesicles (OMVs) from *Neisseria lactamica*
P30	**David J. Andlinger** Bio-Rad Laboratories	A scalable weak AEX-HIC Mixed-Mode chromatography resin for biologics purification

Products

P31	**David J. Andlinger** Bio-Rad Laboratories	Saving Lives - Innovative Ion Exchange Resin Therapeutic Applications
P33	**Friederike Eilts** Fraunhofer Institute for Interfacial Engineering and Biotechnology	Incorporating virus stability into an oncolytic HSV-1 purification strategy
P36	**Viviane Maimoni Goncalves** Instituto Butantan	Lysis conditions and comparison of anion exchange resins for purification of untagged recombinant pneumolysin

Process Intensification

P39	**Sebastian Thürmann** Tosoh Bioscience	Smart MCC Integration for a Sustainable Downstream Future
P41	**David Achauer** Technical University of Munich	Development of an Automated Tangential Flow Filtration for mRNA Using Model Based Control and Real-Time Monitoring
P43	**Sabrina Styblova** **Paul Jacoby** **Bhagyeshri Mantri** Technical University of Munich	Integrated Downstream for Valorization of Soy Side-Streams via Fungal Fermentation
FP5	**Marina Linova** Technical University of Denmark	Advancing *K. phaffii* Bioprocesses: Evaluation of Continuous Perfusion Processes and Suitable Purification Strategies

Analytical Detection Technologies

FP6	**Roland Drexel** Postnova Analytics GmbH	Multi-detector Field-Flow Fractionation for the assessment of critical quality attributes of AAVs

Spin-off Poster

SP1	**Nils Brechmann** MAGic BioProcessing	Comparative economic modeling of magnetic bead-based processing as an alternative to legacy mAb manufacturing
SP2	**Robin Karl** Technical University of Munich	Holistic pilot-scale magnetic separation platform development
SP4	**Andreas Reichert** Technical University of Munich	Light-controlled antibody purification via a photoswitchable protein A platform
SP5	**Simone Dimartino** University of Edinburgh	Planet Crafting Labs: Empowering the biotech industry to drive sustainable innovation

POSTERS

presented on Tuesday, Nov. 11
11:00 – 12:30

All posters will be displayed throughout the conference. We ask presenters of the following posters to be at their poster for questions and discussions on Tuesday, Nov. 11, 11:00 – 12:30.

Fundamentals and Modelling

P2	**Hironobu Shirataki** Asahi Kasei	Reproduction calculation of pressure profile of tangential flow filtration for clarification of HEK cell culture with cell lysate using hollow fiber MF with asymmetric or symmetrical structur
P4	**Finja Probst** acib GmbH	Optimizing bionanoparticle purification: A CFD-driven design of flow distributors for monolithic columns
P5	**Sandeep Kaur** Technical University of Applied Sciences Mannheim	Electrically driven elution in digital Protein A membrane chromatography: An alternative to traditional low-pH elution

Advances in Separation Processes

P6	**Oussama Menouar** Technical University of Applied Sciences Mannheim	From Harsh to Mild: Redefining Protein A Elution with Excipient Modulation
P8	**Florian Settele** Navigo Proteins	Targeted purification of GLP-1 therapeutics: Precision X® ligands for Semaglutide and Tirzepatide
P10	**Andreas Pickl** Sartorius	Lab-Scale Tools for Advanced LV Downstream Processing
P12	**Nadja Kohn** Technical University of Munich	Expanded bed chromatography for the preparative age-specific separation of brewer's yeast
P14	**Robin Karl** Technical University of Munich	Push Button, Get Nanoparticles: Pilot-Scale, End-to-End Automation for Magnetic Nanoparticle Production

P16	**Alejandra Riera Hipp** Technical University of Munich	Continuous protein affinity chromatography using silica-binding peptides
P17	**Dieu Linh Nguyen** Fraunhofer Institute for Interfacial Engineering and Biotechnology	Exploring the Power of Membrane Chromatography for Effective HSV-1 Purification
P19	**Guilherme Costa** acib GmbH	Demonstration of Scale-down Model for Continuous-Flow Ultracentrifugation Using Sucrose Gradients for Particle Separation
P21	**Sara Rotar** Sartorius BIA Separations d.o.o.	Optimization of preparative-scale mRNA capture using small-scale Oligo dT affinity monoliths
P23	**Sebastian Wolniak** Numab Therapeutics AG	Modular high-throughput platform for the purification of scFvs and multispecific antibody-based therapeutics
P26	**Linus Laurin** Cytiva	Optimizing environmental sustainability in bispecific antibody manufacturing
P28	**Ulrike Krop** Knauer GmbH	Optimizing peptide purification: HPLC scale-up process for high purity and efficiency
P29	**Djuro Josić** Juraj Dobrila University	Immobilized recombinant FcγIIIA receptor and FcγIIIA receptor as a useful tool for characterization of human IgG antibodies
FP3	**Djuro Josić** Juraj Dobrila Univ. of Pula	Direct application of undiluted human plasma and other complex biological fluids to polymethacrylate-based monoliths and subsequent isolation of biologically active therapeutic proteins and other biopolymers

Products

P32	**Sonia Mendes** iBET and ITQB NOVA	Establishment of a Robust, Scalable and GMP-Compliant AAV Manufacturing Platform
P34	**Ewa Papiewska** University of Edinburgh	Additive Manufacturing Techniques for Immobilisation of T7 RNA Polymerase
P35	**Viktoria Mayer** acib GmbH	The importance of chromatin removal in bionanoparticle purification
P37	**Edina Császár** Biomay AG	Manufacturing of AAV and LV Starting Plasmids in GMP-Quality
P38	**Edina Császár** Biomay AG	Quality control of cGMP grade recombinant Cas9 nuclease for human therapeutic CRISPR/Cas9 genome editing applications

Process Intensification

FP2	**Edina Császár** Biomay AG	mRNA manufacturing: from template DNA to lipid nanoparticles – An integrated platform approach
P40	**Anja Trapp** Rentschler Biopharma SE	Continuous Multi-Column Chromatography – a Valuable Tool for Flexible CDMO Manufacturing?
P42	**Marko Tesanovic** Technical University of Munich	Integrated magnetic separation platform: from nanoparticle production to antibody capture with RS-HGMS
FP4	**Jonas Wege** Tosoh Bioscience	Towards seamless mAb purification: Dual-Step Multi-Column Chromatography
FP13	**Gregor Stitz** BOKU University	Cationic polymers reduce host cell protein burden for subsequent chromatographic mAb purification

Analytical Detection Technologies

P44	**Florian Christoph Sigloch** PolyQuant GmbH	CHOcoCAT standards: controlling high-risk CHO HCPs in drug products
FP7	**Tomas Mesurado** acib GmbH	Novel analytical HPLC method for characterization and quantification of VLPs

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Key Note Lecture Abstracts

Purification of viruses for gene therapy and vaccines: A comparison of convective and diffusive chromatography

Scott M Wheelwright [1], Alois Jungbauer [2]

[1] BioChromatographix International, Singapore
[2] Institute of Bioprocess Science and Engineering, BOKU University, Vienna, Austria

Viruses are increasingly used for modern therapeutics and prophylactics, especially in Advanced Medicinal Therapeutic Products (ATMPs) such as gene therapies and cell therapies, but also in vaccines. The recovery and purification and most biotherapeutics relies on chromatography and ATMPs are no exception. Historically, most biotherapeutics have relied on diffusive chromatography (such as beads packed in columns), however, new developments in convective chromatography (including monoliths) offer increased purity and better yields. In this presentation we review the history of virus purification and consider advances in the field of convective chromatography. We present data from various sources demonstrating the application of these materials to different viruses and suggest avenues for future development.

Stimuli-responsive peptides: a kaleidoscope of applications

Stefano Menegatti [1,2], Ashton Lavoie [1], Hunter Reese [1], Kevin Day [1], John Schneible [1], Raphael Prodromou [1], Brandyn Moore [1], Eduardo Barbieri [1], Ryan Kilgore [1], Carly Catella [1], Sobhana Sripada [1], Juliana O'Brien [1], Ka Zhang [1], Arjun Shastry [1], Yuxuan Wu [1], William Smith [1], Arianna Minzoni [1], Morgan Hurst [1], Taylor Pleines [1], Irfan Ismail [1], Keshav Raghuvanshi [1], Vladimir Podzin [1], Wenning Chu [1], Rajendra Shukla [1], Nathan Crook [1], Carol Hall [1], Scott Magness [1], and Michael Daniele [1].

[1] North Carolina State University
[2] ChromaGenix

Peptides and peptide mimetics are a continuously evolving language, used by scientists to communicate with life, quasi-life, and inert matter. The introduction of stimuli-responsive residues and moieties make them as exciting as the way of the future. Our team explores the use of structured (i.e., cyclic and hyperbranched) peptides hybridized with non-canonical groups to respond to external stimuli (e.g., electromagnetic and mechanical fields, temperature, and select cations) with technologically-relevant behaviors across the fields of biosensors, drug delivery, and biological separations. In my talk, I will present our latest results in using peptide-hybridized dendrimers with non-traditional intrinsic luminescence for gene delivery, photo-affinity chromatography for the purification of labile proteins, and micromachined sensors functionalized with seismo-tropic peptides for vibration-tuned biorecognition. This will be an interactive presentation: I look forward to discussing these technologies with the ISPPP conference attendees and identify future areas of collaborations.

Flying viruses – mass spectrometry meets X-rays

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Viruses affect basically all organisms on earth. Some are detrimental to human development as we experienced during the COVID-19 pandemic, whereas those targeting pathogenic bacteria or crop pathogens can be beneficial for us. An integral part of icosahedral viruses is the capsid protein shell protecting the genome. Many copies of the capsid protein often self-assemble into shells of defined size. Low binding affinity of individual subunits allows efficient assembly and gives rise to highly stable particles. These capsids can be studied by native mass spectrometry (MS), a single molecule like approach, in terms of stoichiometry, dynamics, assembly pathways and stability revealing coexisting states. However, the structural resolution provided is limited. Therefore, we built a prototype native mass spectrometer in the MS SPIDOC project to deliver select species to X-ray sources for gas phase SAXS and single particle imaging. First experiments reveal good performance of the MS setup.
Noroviruses are the main cause of viral gastroenteritis. Neither treatment nor vaccines are available. Initial host cell attachment in human noroviruses is mediated by glycans. Using MS, NMR and AFM, we reveal how size in norovirus particles is controlled and how glycan binding affects the dynamics in the viral particles.

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Oral Presentation Abstracts

Effect Of Support Microstructure On Compression And Pressure Drop During Flow-Through Applications

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Mechanical properties of a material play a pivotal role in its performance when such porous material is used in a flow-through mode. This study investigates the effect of porosity and microstructure on the compressibility of methacrylate polymer, focusing on two distinct microstructures: cauliflower and high internal phase emulsion. Samples of various porosities, yet identical chemical composition were prepared, and their Young's modulus was determined. Effect of porosity on Young's modulus was described by an exponential law model with the cauliflower microstructure exhibiting an exponent of 3.61, while the high internal phase emulsion of only 1.86. A mathematical analysis of the compression caused by a liquid flow unveiled significant disparities in the porosity threshold where minimal compression is observed, being around 0.45 for the cauliflower, while there is monotone decrease in compression with porosity increase for the high internal phase emulsion microstructure. Evaluating exponent integer values between 1 and 5 over the entire porosity range reveals that the porosity where the minimal compression occurs increases with a decrease in exponent value, being approximately 0.33 for n=5, 0.4 for n=4, 0.55 for n=3, 0.65 for n=2 while no minimum occurs for n=1. These findings indicate that lower exponent value results in lower compression under identical experimental conditions.

Purification And Characterization Of A Recombinant Neuraminidase Influenza Virus Vaccine Candidate

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Influenza viruses pose a significant public health threat, causing seasonal epidemics and occasional pandemics with substantial morbidity and mortality worldwide. The influenza virus displays two major glycoproteins on its surface: hemagglutinin (HA), which is immunodominant, and neuraminidase, which is immunosubdominant. Traditional influenza vaccines primarily target the viral surface glycoprotein HA to induce protective immunity. However, the high mutability of HA, particularly in response to selective pressure from immune responses, limits the durability and efficacy of current vaccines. The discordant evolution of HA and NA makes a recombinant neuraminidase (rNA) an attractive candidate, necessitating robust purification and characterization methods. Here we present scalable approaches for purifying rNA, expressed in Sf9 insect cells utilizing the baculovirus expression system. Initially, a purification process based on immobilized metal affinity chromatography (IMAC) and Tangential Flow Filtration (TFF) was developed for His-tagged rNA. The resulting product was characterized through a variety of analytical techniques, encompassing immunological assays, high-performance liquid chromatography (HPLC), isoelectric focusing gels (IEF), and mass spectrometry, which all confirmed the structural and functional integrity of the purified protein. The His-tagged rNA was successfully produced at manufacturing scale and is intended for phase I clinical trial. To further progress towards phase II clinical trials, a tag-less construct is desired. Knowledge gained from the characterization of the purified rNA guided the development of purification strategies for untagged rNA variants. A downstream process using conventional unit operations including cation exchange chromatography (CIEX) and hydrophobic interaction chromatography (HIC) was evaluated to achieve high purity and maintain structural and functional integrity without the use of an affinity tag. Scalability from laboratory to manufacturing scale ensured reproducibility, thus advancing rNA-based vaccines in later clinical phases of development.

Protein Denaturation Maps – Charting A Course For The Solubilization And Refolding Of Bacterial Inclusion Bodies

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Due to its fast growth kinetics and well-explored molecular biology the bacterium *Escherichia coli* is amongst the most popular production hosts for recombinant proteins. However, high production rates in this host often lead to the expression of the target protein as insoluble aggregates known as inclusion bodies. While inclusion bodies can be expressed rapidly and with high target protein purity, they have to be solubilized first and subsequently refolded in order to obtain the product. Optimization of these two process steps is still mainly done empirically, leading to long development times and a lack of transferability.
In the presented research, we examined the usage of protein denaturation curves as a knowledge-based approach for the development of inclusion body refolding processes. By measuring intrinsic tryptophane fluorescence as a response whilst varying the two most influential parameters in solubilization and refolding buffers, the denaturant concentration and the pH, we created two-dimensional denaturation curves. These denaturation curves were compared with the response surface of the refolding yield over a multivariate design space. The presented results show that the minimal denaturant concentrations required for the solubilization of inclusion bodies can be inferred from the denaturation map of the corresponding protein. Furthermore, a suitable range of refolding buffer pH and denaturant concentration could be derived from the generated maps. By exploring this approach rooted in the basics of protein science, we aim to contribute to more transferability and mechanistic understanding in the field of inclusion body refolding.

A Reusable Nanodiamond-Based Platform For Selective Purification Of RNA

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The purification of RNA is essential for a wide range of applications in diagnostics, therapeutics, and gene regulation studies. However, conventional methods, such as silica-based columns and organic solvent extractions, often fail to selectively isolate RNA from complex biological matrices, resulting in contamination, low yields, or RNA degradation.
This work presents a novel and improved platform using functionalized nanodiamonds (NDs) for fast, robust, and solvent-free RNA purification. Owing to their large specific surface area, chemical stability, and unique magnetic and optical properties, NDs are highly attractive for biomedical applications. Their surface reactivity further enables tailored functionalization to promote selective interactions with target biomolecules. In this study, a screening was conducted using hydrogenated, oxidized, and aminated NDs, alongside non-functionalized nanodiamonds and microdiamonds, to evaluate their performance in RNA adsorption and recovery. Adsorption capacity was thoroughly assessed and optimized using a factorial design approach. Maximum RNA adsorption ranged from 86.9 to 277.6 mg/g, with complete recovery achieved under mild conditions when using oxidized NDs. This method demonstrated robust reusability, maintaining recovery efficiency across five consecutive cycles without compromising RNA integrity. Selectivity was also demonstrated as no significant plasmid DNA contamination was detected in the recovered samples. Moreover, no significant protein adsorption was observed when using bacterial lysates, and the genomic DNA content was reduced by 86.3%. Importantly, the entire purification process was completed in under 15 minutes and consistently produced RNA samples free of organic solvent contamination, even from solvent-rich inputs.Overall, this nanodiamond-based purification strategy offers a highly selective, efficient, and scalable alternative for integration into high-throughput RNA workflows and RNA-based biomanufacturing processes.

Isolation And Purification Of Monoclonal Antibodies Using Combined Precipitation And Crystallization Process

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The global biotherapeutics market is growing at a very fast rate. The main group of biotherapeutics are monoclonal antibodies (mAbs). Despite increasing demand for bioactive mAbs, the high cost of their manufacturing limits their widespread use in medicine. Recent advances in upstream protein processing (USP) cause that downstream protein processing (DSP) has become the manufacturing bottleneck accounting for up to 70 percent of total production costs. Typical DSP procedure is dominated by chromatographic methods, which are the main cost driver. Recently new non-chromatographic methods, such as crystallization and precipitation are gaining increasing interest. PEG–aided precipitation has been intensively studied as a method for efficient capture of mAb from cell culture supernatants. The crystallization process is carried out in a similar way to precipitation, but the product is a crystalline phase which is characterized by higher activity, purity and stability compared to other formulation method. Protein crystallization is usually performed batchwise in stirred-tank crystallizers. However, this way of the process realization renders the supersaturation level and thus crystallization progress difficult for control. In this study, we have developed a holistic non-chromatography approach for mAb capture, purification and formulation. In this approach, precipitation was used for mAb capture and purification, and the obtained solution was subsequently subjected to forced convection crystallization (FCC) to obtain the final product in the crystalline form. Forced convection crystallization (FCC) is based on evaporation of water from the protein solution by forced convective air flow. The parameters of air stream are used to control the supersaturation level and temperature of the solution. The model protein was a therapeutic mAb (IgG1) that was derived from cell culture medium. IgG1 was precipitated and crystallized from aqueous solution of polyethylene glycol. In the first step IgG1 was isolated and purified from the cell culture medium using the two stage precipitation process coupled with solid-liquid extraction (SLE). Chromatographic purity in obtained solution was 99 %, whereas concentration of DNA and HCP were 2 and 8 ng mL-1 respectively. In the second step IgG1 was crystalized form this solution using FCC process with 99 % yield. A 30 % reduction in consumption of precipitating agent was achieved compared to batch crystallization.

Electrochemically Modulated Purification Of Plasmid DNA Using Graphite Electrodes In Static And Flow Systems

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Biotherapeutic production increasingly relies on the efficient separation of charged biomolecules like nucleic acids and proteins. Conventional purification methods, such as ion exchange or hydrophobic interaction chromatography, require high salt concentrations and offer limited resolution—particularly for structurally similar species. Although monolithic columns reduce pore diffusion issues for large biomolecules such as plasmid DNA (pDNA), they still face challenges in process intensification.

This study presents an electrochemically modulated purification method that eliminates salt gradients by using carbon-based electrodes as stable, low-cost stationary phases. Adsorption and desorption are reversibly controlled via electric double-layer (EDL) interactions under an applied potential—building on prior work (e.g., Trunzer et al., 2020). A static system with pencil graphite electrodes (±1.6 V) showed ~99 % pDNA depletion from bulk within 15 minutes. Voltage shut-off and a washing step enabled nearly complete desorption, confirming reversible, field-driven binding. Structural integrity and isoform distribution (~Δ5 %) were maintained, as confirmed by gel electrophoresis and HPLC, indicating minimal degradation.

For dynamic application, a modular flow-by cell was integrated into a standard liquid handling system. Field-controlled adsorption and salt-free desorption were achieved under continuous flow. Process optimization more than doubled eluate concentration, though increased sample loss was observed, likely due to stronger binding or incomplete release—highlighting both promise and areas for refinement.

To guide further scale-up and reduce experimental load, a 3D mechanistic model based on EDL theory is under development to simulate molecular transport under applied fields. Initial experiments suggest the method also works for other nucleotides like mRNA, with preserved integrity and reversible, field-induced interaction.

These results demonstrate the broad applicability and future potential of electrochemical purification as a salt-free, scalable approach for charged biomolecules.

Light-Controlled Protein Purification Using A Short Photo-Switchable Affinity Tag

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Affinity chromatography is a powerful technique for purifying proteins of interest from complex mixtures like whole cell extracts or culture supernatants. However, elution of the protein usually requires chemical additives or substantial changes in buffer conditions. To overcome these caveats, we developed a chromatography method solely controlled by light, termed Excitography. It utilizes a short N- or C-terminal peptide, the Azo-tag, as part of the recombinant protein. This affinity tag comprises a light-responsive non-canonical amino acid, p-(phenylazo)-L-phenylalanine (Pap), that can be switched from the trans ground state to the metastable cis state by irradiation with mild UV light of 355 nm. Due to the highly specific interaction of Pap in the trans configuration with α-cyclodextrin (α-CD), proteins carrying the Azo-tag are retained on an α-CD column when applied in daylight or in the dark, while endogenous proteins are quickly washed off. Switching Pap to cis upon illumination with UV light leads to the instant elution of the tagged protein in excellent purity. Since light is the determining factor, chromatography can be performed in a buffer of choice directly suitable for downstream applications. Furthermore, buffer changes or regeneration steps are not required before re-using the column for additional rounds of purification. Also, with the illumination devices relying on cheap LEDs, this method can be adapted to different formats, including the parallel purification of proteins in multi-well plates. This positions Excitography as a quick and robust one-step purification technique, especially for lab-scale and high-throughput applications.

Process Modeling As Key To Intensify Continuous Bioprocess Development

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In this presentation, we demonstrate how machine learning, mechanistic modeling and intensified experimental workflows can substantially accelerate and intensify bioprocess development. Emphasizing the unit operations cultivation, filtration and chromatography, we illustrate how integrated modeling approaches reduce experimental load and streamlines decision making when developing fully continuous bioprocesses.
Within cultivation, we explore case studies, including microbial systems for enhanced process performance, monoclonal antibody (mAb) production, and gene therapy processes with a focus on critical quality attributes (CQAs). These predictive capabilities facilitate early risk assessment and support more confident, model-driven scale-up.
For filtration, we introduce an intensified, high-throughput workflow that accelerates identification of optimal product–membrane combinations for continuous ultrafiltration. This approach not only targets improved process performance but also incorporates impurity and quality considerations, streamlining development within a reduced experimental framework. We demonstrate how such intensified workflows support rapid design space definition, ensuring robustness and process efficiency.
In chromatography, we demonstrate how measuring critical process parameters such as conductivity through the process as well as column age are used to make critical decisions for controlling a multi-column chromatography system.
Finally, we present a fully integrated bioprocess case study spanning cultivation, filtration, and chromatography, unified by a comprehensive process model. This end-to-end modeling strategy enables synchronized optimization and control across all unit operations, driving consistent product quality and enhanced overall performance.
Overall, our work underscores the transformative role of modeling-based intensification strategies in modern bioprocessing, highlighting their potential to redefine biologics development through harmonized, data-driven workflows.

Continuous Flow Ultracentrifugation Enables Efficient Capture Of Adeno-Associated Viruses From Clarified Lysates

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Adeno-Associated Viruses (AAVs) are among the most widely utilized vectors in gene therapy due to their efficiency, safety, and versatility. Since 2012, seven AAV-based therapeutics have been approved by FDA or EMA. Still, downstream processing of AAVs remains a major challenge. Overall next generation processes have to be serotype agnostic, robust and scalable to enable efficient manufacturing with broad application. Common methods for AAV purification and polishing include density gradient ultracentrifugation using cesium chloride, sucrose, or iodixanol gradients. Alternately, affinity chromatography is used as AAV capture step but typically needs intense pre-purification of the lysates for efficient performance. Despite conventional ultracentrifuges not being scalable and having long process times, employing density gradient ultracentrifugation using a continuous flow ultracentrifuge overcomes these limitations.
Our findings show that continuous flow ultracentrifugation, using both iodixanol or sucrose density gradients, is an effective method for capturing AAVs directly from clarified lysate. The sucrose density gradient enabled the recovery of 72% full AAV particles but led to poor resolution between full and empty AAV particles and co-purified some host cell-derived impurities. Using iodixanol density gradient a recovery of 68% full AAV particles were obtained with the full AAV particles enriched in the higher density fractions. Moreover, the iodixanol density gradient ultracentrifugation successfully separated AAV particles from host cell-derived impurities, as 64% of HCPs and 70% of dsDNA were found in the flow through. In the enriched full-AAV fractions, the HCPs and dsDNA content was below the limit of quantification. Similar results were also obtained in the flow through deriving from the sucrose density gradient ultracentrifugation, where 57% of HCPs and 59% of dsDNA were quantified. In addition to that, in the enriched AAV fractions, less than 1% of HPCs and dsDNA were detected. In both cases, chromatin fragments were also captured in the density gradient, indicating a need for further improvements specifically targeting chromatin removal prior to ultracentrifugation.Continuous flow ultracentrifugation holds the potential for serotype-independent and scalable AAV capture directly from the clarified lysate without the need of elaborate and costly primary recovery steps upfront.

Development And Economic Evaluation Of An Intensified Magnetic Nanoparticle-Based Purification Process For Microbial Proteins

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Intensified bioprocessing drives the shift to sustainable, flexible, and cost-efficient protein production processes. Unlike process optimization, process intensification is defined by integrating novel technologies with a holistic view of the entire process, often with a focus on continuous processing. While process intensification is well established for mammalian cell culture processes, microbial-based processes currently often only rely on optimization of strains and media. To apply process intensification to microbial processing, we investigated extracellular protein production combined with protein purification using magnetic bare iron oxide nanoparticles (BION). The following economic evaluation of the developed process was central to determined the impact of process intensification on cost-efficient protein production and purification.
Initially, the iron oxide affinity (RH)4-tag was fused to the protein of interest (POI) and extracellularly produced using *Escherichia coli* fed-batch fermentations. The resulting fermentation broth was processed with or without a cell removal, thereby intensifying the process by omitting a full unit operation. We could bind up to 0.138 g g-1 of POI onto the BION with minimal amounts of HCP, independently of the pre-processing. This was achieved by the high affinity of the (RH)4-tag and a pH adjustment of the fermentation broth to the isoelectric point of the POI. The subsequent desorption accomplished high purities and recoveries (>95 %) using a buffer of phosphate and two amino acids. To investigate a process scale-up, we tested the adsorption and desorption process at BION concentrations up to 50 g L-1 and found no significant effects on relevant parameters. For scale-up, the fermentation broth and BION were processed in a fully automated set-up using a high-gradient magnetic separator linked to the bioreactor, enabling the handling of up to 30 g of BION per run. The data were used for economic evaluation using the SuperPro Designer software. The BION-based process is benchmarked against a traditional column-based affinity chromatography process. We identify remaining bottlenecks and optimization targets by comparing cost of goods, capital investment, and other relevant parameters. With the proposed strategy, we are confident that we can provide a new concept for microbial process intensification broadly applicable to various proteins, thus contributing to lower production costs of microbial proteins.

Continuous Production Of Plasmid DNA: Advances In Integrated Separation And Purification

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gene therapy vectors, mRNA vaccines, and DNA-based therapeutics. Within the iCAP project, a fully continuous and automated pilot system is being developed by enGenes, Qubicon, and Novasign, integrating upstream fermentation with continuous downstream purification. The downstream process employs continuous alkaline lysis directly coupled to continuous chromatography, enabling uninterrupted recovery and purification of pDNA with high yield and quality. Real-time monitoring and model-predictive control are implemented via a digital twin framework, facilitating advanced process stability and robustness. The use of genetically stabilized *E. coli* strains further supports sustained production under continuous conditions. This integrated platform is anticipated to significantly reduce production costs, improve process sustainability, and establish new standards for continuous polynucleotide separation at industrial scale.

Process Intensification For Protein Purification: Continuous Multi-Column Isolation Of Napin And Cruciferin

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Napin and Cruciferin, the major storage proteins of rapeseed, exhibit favorable techno-functional properties such as emulsification and gelation, making them attractive candidates for use as biocompatible and biodegradable carriers of bioactive compounds or drugs. Their GRAS status further supports their potential as natural alternatives to synthetic delivery matrices in food and pharmaceutical applications. However, scalable purification of these proteins with both high purity and yield remains a major challenge, as conventional batch chromatography methods are inadequate for industrial implementation.
To address this, we developed a chromatographic purification process based on ion exchange principles - cation exchange for Napin and anion exchange for Cruciferin - which was initially optimized under batch conditions to determine key process parameters. Particular attention was given to ensuring compatibility between the steps to allow seamless transfer to a continuous format.
Crucially, the optimized process was successfully transferred to an automated multi-column chromatography platform equipped with dynamic column switching and inline pH adjustment. This implementation enabled continuous, high-throughput protein purification with reduced buffer consumption and processing time. The resulting proteins exhibited high purity and retained their functional properties required for encapsulation applications. This work demonstrates the feasibility and advantages of transferring a batch-optimized purification strategy to an automated multi-column system, significantly advancing the scalable production of functional rapeseed proteins and supporting their broader application in bioactive delivery systems.

Analytical Techniques For rAAV Genome Integrity And Identity Assessment

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Recently, the development of therapies based on recombinant adeno-associated viruses (rAAV) has gained huge interest in the pharmaceutical industry. To ensure a safe and effective gene therapy product, a smart control strategy for the assessment of the rAAVv genome is of utmost importance. In this presentation, different analytical techniques for rAAV genome integrity and identity assessment will be discussed, highlighting their benefits and drawbacks.

In-Line Infrared Spectroscopic Detection Of Chromatographic Protein Separation For Medical Diagnostics

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To improve the earliest detection of pathophysiological conditions, such as cancer, it is crucial to develop simple, minimally invasive tools capable of profiling the complex molecular composition of human biofluids. One in vitro approach to detect disease-relevant multi-molecular changes of biofluids is infrared molecular fingerprinting (IMF) using optical infrared (IR) spectroscopy. Profiling native liquid blood plasma with IMF demonstrates high temporal stability and reproducibility, and could thus enhance the earliest medical probing of pathophysiological abnormalities [1].
IR fingerprinting can profile the whole repertoire of molecular species within a sample at once, but does not identify specific molecules [1]. In molecularly complex matrices however, IR signals from highly abundant molecules overshadow those from molecules present at lower concentrations. UV/Vis spectroscopy is commonly used to detect protein concentrations, with high sensitivity and a wide dynamic range. Nevertheless, this technique is limited in that it provides no structural information about the protein and it fails to detect co-eluting substances that do not absorb in the UV/Vis spectral range. Hence, our goal is to advance spectroscopic IR profiling by integrating it in-line with liquid chromatography (LC) and other pre-analytical strategies.
The proposed in-line integration of LC protein fractionation with IR spectroscopy could improve IR profiling capacities by increasing molecular sensitivity and enabling the detection of molecular sub-repertoires in bulk plasma previously undetectable due to their low abundance. Our findings reveal the technical feasibility of in-line LC-IR coupling, with a robust validation yet to be performed. Following this successful proof-of-concept setting with in-line frequency resolved IR spectroscopy (FTIR), the method will be tested for its feasibility when in combination with femtosecond laser-based field-resolved spectroscopy (FRS)[2].

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Critical Quality Attribute Analysis Of RNA Therapeutics By Novel Ribonuclease Specificities

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RNA therapeutics such as sgRNA and mRNA are important modalities for Gene Therapy applications to treat human diseases. Rapid development of these RNA molecules and their deployment require fast tracked development of new analytical methodologies. This methodology aims at determining the identity, purity, and modification(s) of therapeutic RNA through oligo mapping of its sequence by liquid chromatography coupled with mass spectrometry (LC-MS). MS-based sequencing involves direct detection of RNA fragments and localization of the nucleoside modifications in the sequence. Currently available enzymatic tools to produce RNA fragments yield incomplete or partial coverage with a very high ambiguity in sequence interpretation. The availability of enzyme tools with complementary cleavage specificity can overcome this problem and fill sequence gaps while streamlining data interpretation and increasing confidence. Th application note evaluates an optimized protocol for RapiZyme™ MC1 and RapiZyme Cusativin and explores each RNase's reproducibility and complementary specificity with a focus on increased sequence coverage during sgRNA and mRNA critical quality attributes. The new workflows include data processing software, including the MAP Sequence app within waters_connect™ and two supporting MicroApps. These provide a streamlined workflow to facilitate data analysis for routine RNA fragment identification, sequence coverage assessment including cap, poly(A) tail for mRNAs and quantification.

Real-Time Monitoring In Ultra- And Diafiltration Of Adeno-Associated Virus And Lentiviral Vector Using Raman Spectroscopy

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Process analytical technology (PAT) enhances product quality by monitoring and controlling critical quality attributes (CQA), offering better insights into process performance. Inside of the PAT strategies, Raman spectroscopy is revealing a promising tool for supporting control systems development for continuous and automated processes. In this study, Raman spectroscopy is used to monitor in real-time ultra- and diafiltration of adeno-associated virus (AAV) and lentiviral vector (LV), being applied into a tangential flow filtration (TFF) system. Operating the system in open loop, clarified bulks were concentrated 5 times, and then buffer was exchanged to PBS with 5 diafiltration volumes. The training set of experiments allowed the development of one model for each processing phase. The models showed good performance (relative errors lower than 20 % compared to conventional analytical methods) in predicting total particles (for LV), full and empty capsids concentration (for AAV), as well as impurities such as total protein and dsDNA. Furthermore, batch-to-batch variability was identified as the principal source of spectral variation and its impact on TFF operation was followed, fostering efficient operation time decisions. The study emphasizes the effectiveness of Raman spectroscopy as a PAT tool for monitoring downstream process unit operations. The results obtained support the development of integrated systems with closed-loop control feedback and will advance cell and gene therapy manufacturing by enabling more precise and controlled downstream processing operations.

Functionalized Non-Woven Fibers For The Harvest, Clarification And Purification Of Bionanoparticles

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Advanced biotherapeutics based on bionanoparticles (BNPs), including viruses, virus-like particles (VLPs), and extracellular vesicles (EVs), are demonstrating significant potential as both delivery systems and intrinsic therapeutic agents. These BNPs are being actively explored in clinical trials for a wide range of applications, from gene therapies and vaccines to regenerative medicine. However, in some cases, the transition of BNP-based therapeutics into late-stage clinical trials and eventual commercialization is hindered by the lack of scalable and efficient manufacturing processes. Current downstream processing methods for BNPs typically involve a combination of several ultracentrifugation, filtration, and/or chromatography steps. These approaches can suffer from poor scalability, limited selectivity, and suboptimal recovery yields. Among the most challenging impurities to remove is DNA, particularly in the form of chromatin, which can co-purify with BNPs and compromise product quality and safety.
Non-woven (NW) fibers represent a promising solution for the scalable and efficient manufacturing BNPs. These highly engineered materials feature an open-pore architecture that enables convective flow, overcoming the mass transfer limitations of conventional chromatography resins, especially for large molecules such as BNPs. NW fibers can be functionalized with ion exchange, affinity, or salt-tolerant ligands, offering versatility for impurity removal and product recovery. Additionally, NW fibers are easy to scale, compatible with steam sterilization, and suitable for integration into continuous processes.
Exemplified with EVs, our work demonstrates that NW fibers can effectively replace traditional clarification steps, such as differential centrifugation and sterile filtration, with a single chromatographic unit operation in BNP manufacturing. In measles virus production, the use of NW fibers resulted in a 7-fold improvement in productivity while maintaining product quality, even without an endonuclease digestion step. Our work with recombinant baculovirus revealed that NW fibers functionalized with a weak cation exchanger can be used in bind-and-elute mode to purify and recover infectious particles.
These findings highlight the potential of non-woven fibers to transform BNP manufacturing by enabling scalable, efficient, and high-yield purification processes, paving the way for broader clinical and commercial adoption of BNP-based therapeutics.

Single-Step Extracellular Vesicles Isolation Strategy Using Steric Exclusion Chromatography

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Over the last years, extracellular vesicles (EVs) have become a key area of interest. These cell-secreted lipidic nanoparticles can be characterised as apoptotic bodies, microvesicles and exosomes, depending on their size and biogenesis. Accumulating evidence reveals the potential of employing these vesicles as therapeutic agents and next-generation drug-delivery vehicles, with multiple clinical trials already underway. These advances have shifted the discussion from the potential use of EVs in clinical therapy to how they can be implemented. Challenges can be found throughout the entire biomanufacturing chain – from upstream to downstream. In downstream, challenges typically relate to the lack of scalable and reproducible purification platforms, capable of delivering highly purified products. For example, although differential ultracentrifugation (UC) is the most popular method, it has a number of bottlenecks, such as lack of scalability and cost-effectiveness, requiring specialised equipment and variable yields. Over time, commercially available kits have emerged facilitating purification at small laboratory scale, despite problems like the co-isolation of other contaminants and impurities such as albumin, which reduces the purity of the final product. More sophisticated approaches like size exclusion chromatography, immunoaffinity approaches and anion exchange chromatography emerged, bringing scalability, while increasing performance and isolation yield. In most cases, these EVs isolation strategies have been applied in a multi-step process designed to mitigate each method drawback. The perfect isolation strategy may never exist, but it should comprise a minimum number of operations to decrease complexity and increase process efficiency. In this work, we aim to follow these principles, by evaluating steric exclusion chromatography (SXC) for the single step purification of EVs intended to maximize recovery and impurities removal. To this end, EV-rich conditioned medium from HEK 293T cells was clarified and loaded into three different chromatographic resins. Performance was evaluated using different PEG sizes (3,350, 6,000 and 8,000) in a concentration range from 5% to 15%. The operating conditions evaluated show that increasing PEG size and concentration, increases particles recovery but also protein presence. In the end, almost 80% of initial particles were recovered, while more than 95% of proteins removed, in a single-isolation step.

Scalable Purification Of Adherently-Produced Lentiviral Vectors Using Isotag™ LV Reagent

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The use of Lentiviruses (LVs) as vectors has greatly increased in gene and cell therapies, due to their potency, safety, and large cargo capacity. Current manufacturing methods rely heavily on chromatography-based workflows, which are not optimal for the purification of large viral vectors. IsoTag™ LV reagent is a proprietary biopolymer that contains a specific binding domain for VSVG and is able to reversibly encapsulate VSVG-pseudotyped vectors, in the presence of heat and salt. This affinity based approach has the advantage of selectively separating LVs in a liquid-liquid environment, which in turn protects LVs from the forces that are involved in the purification procedure. Taking advantage of the unique properties of IsoTag™ LV reagent, the downstream processing workflow eradicates the use of chromatography and replaces it with a scalable Tangential Flow Filtration (TFF) process. The system has been validated for the purification of up to 3.3L of adherently-produced LV harvest material, where IsoTag™ LV technology selectively purifies LVs in shear conditions up to 6000s-1. Furthermore, the effects of clarification and nuclease treatment were explored using this process, with no evidence of a significant benefit to applying such treatments with this method. Lastly, the addition of IsoTag™ LV reagent prior to freezing was explored, with infectious LV recoveries as high as 78.9% with preincubated harvest material, and little to no aggregation in purified products by Dynamic Light Scattering (DLS). This approach to purification offers significant advantages in LV purification, with emphasis on LV purity and contaminant removal profiles, which meet or exceed current industry standards, as well as a significant reduction in processing time.

Isolation Of Oligonucleotides By Scalable IEX-Centrifugal Partition Chromatography

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Oligonucleotides are promising therapeutic molecules with growing applications in antisense therapy, mRNA stabilization, and cDNA synthesis. However, solid-phase synthesis often results in impurities, such as truncated sequences and deprotection by-products, which emphasizes the need to develop novel, efficient and cost-effective separation techniques. In this study, we present the purification of a 20-mer unmodified deoxythymidine strand (oligo(dT)) as a model sample, which specifically binds to the polyA tail of mRNA, making it essential for mRNA isolation and cDNA synthesis kits. Purification was carried out using ion-exchange centrifugal partition chromatography (CPC), a technique in which separation occurs between two immiscible liquid phases, so no solid stationary phase is needed. The retention of the compounds depends on their liquid-liquid partition coefficient (Kd), which is mostly determined by ion pair formation equilibrium processes. The IEX–CPC method provided promising results, in terms of both yield and selectivity. CPC technology is also advantageous in terms of industrial scalability, solvent efficiency, and lack of expensive resin stationary phases. In summary, our results suggest that CPC provides a competitive and scalable alternative to traditional IEX-HPLC-based purification strategies for oligonucleotide applications.

3D-Printed Matrices For Steric Exclusion Chromatography Of Plasmid DNA

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Currently, plasmid DNA (pDNA) holds great promise not only for gene therapy and DNA vaccination, but also as a raw material to produce mRNA and viral vaccines. However, achieving high-quality purification remains a significant challenge. Current purification methods, often involving multiple precipitation and chromatographic steps, can compromise the structural integrity and stability of the desired supercoiled (sc) pDNA isoform, which is crucial for effective gene transfer.
This work aims to explore the potential of steric exclusion chromatography (SXC) as a novel approach for capturing pDNA, immediately after cell lysis, and using 3D-printed chromatographic matrices. SXC is a size-based separation mode whereby retention is achieved through mutual steric exclusion of polyethylene glycol and a large target solute in a hydrophilic solid phase. SXC shares common mechanistic roots with PEG precipitation, with the main operational difference being that SXC employs a hydrophilic solid phase as a nucleation centre on which biomolecules accrete instead of forming precipitates. The experimental workflow included cultivation of *Escherichia coli* cells, harboring the model plasmids pUC18 (2,686 bp), pVAX-eGFP (3,685 bp), or pCEP4 (10,410) bp, alkaline lysis, and the use of SXC for pDNA capture and purification on 3D printed hydrophilic supports. The process concludes with concentration of the plasmid-containing fractions through precipitation with high concentrations of PEG. After resuspension, the fractions were analyzed by HIC-HPLC and the protein content was determined by BCA. Applying SXC on 0.70 mL 3D-printed monolithic columns successfully purified and isolated the desired sc pDNA isoform with high yield in a single chromatographic step. The SXC operation was tested with PEGs of different molecular weights (1500 to 8000 Da) and concentration (5 to 15%). Total pDNA retention was achieved when employing short PEG chains at high concentrations, or longer PEGs at lower concentrations. This had the spill over benefit of mitigating backpressures and shear stress effects. Most importantly, under the optimised conditions tested, the total purity of sc pDNA was consistently above 97%, with an 80% HCP reduction.

Ion Exchange As A Sustainable Alternative To Reversed Phase Chromatography For The Purification Of TIDES Molecules

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Reverse phase chromatography (RPC) or ion pair chromatography (IPC) are frequently used separation techniques for peptides and oligonucleotides, respectively, since they can provide relatively high yields and purities in a single step. These techniques require very high concentrations of organic solvents and ion paring agents which are not environmentally friendly. The awareness of sustainability has led to a growing demand and desire to find alternatives. A common technique that is gaining more use, especially at production scale, is ion exchange chromatography (IEX), which can either replace RPC/IPC or complement the process by acting as an orthogonal step to a hydrophobic separation step. IEX is water-based and employ more environmentally friendly buffer systems.
Here, we discuss the advantage from a sustainability point of view of using IEX as a purification step for so called TIDES molecules.
The study consists of a quantitative comparison between IPC and IEX for preparative purification of oligonucleotides. IEX demonstrated much higher productivity than IPC for all specified purities, as well as significantly lower solvent consumption.

shRNA Precipitation Strategies For Sustainable RNA-Based Biopesticides

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Global food production needs to double by 2050 to feed a population projected to rise to 9.1 billion (UN). Achieving optimal yields in a scenario of intensified agriculture will require protecting crops from pests and diseases. One of the objectives of the EU Green Deal is to reduce by 50% the use of chemical pesticides. Spray-induced gene silencing (SIGS) is a highly efficient and selective strategy to control various plant viruses, fungi and even pests [1], and a strong contender to replace chemical pesticides. One obstacle facing RNA biopesticides is the cost of RNAi mass production. Current synthesis methods include chemical synthesis, microbial production, and in vitro transcription (IVT), with IVT being an attractive alternative to chemical synthesis and in vivo synthesis. Most purification strategies for this family of molecules are based on chromatography, as it offers scalability and it is able to meet purity requirements. However, the cost of this technology makes it unfeasible for the production of biopesticides, which need to compete with chemical alternatives, price wise. Precipitation of nucleic acids has been done for decades, but no comprehensive study of optimization for short hairpin RNA (shRNA) has been done. This study aims to investigate the more important factors in the precipitation of this small, double-stranded molecule of RNA. The length of the shRNA is 49 nucleotides (nt) when single-stranded. The loop is 9 bps long and the double stranded length is 20 bps. The factors to be studied are salt type, salt concentration, organic solvent, organic solvent volume, incubation time, incubation temperature and centrifugation parameters (speed and time). Different salts - sodium chloride, sodium acetate, ammonium acetate and lithium chloride - were compared using a standard, non-optimized precipitation protocol, in order to choose the best for further optimization. Precipitates were analysed using HPLC. Ammonium acetate was found to be the ideal salt, as it achieved highest recovery - 53% compared to 52% (sodium chloride) and 49% (sodium acetate) - and highest purification factor- 3.24 compared to 3.19 (sodium chloride) and 2.93 (sodium acetate).

Simplifying mRNA Manufacturing Using Alternative Separation Techniques

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RNA technology has emerged as a promising alternative to conventional vaccines after it played a crucial role during the COVID-19 pandemic. Since then, RNA technology has broadened applications beyond prophylactic treatments, to cancer, metabolic and genetic diseases. Traditionally, mRNA is produced in an in vitro transcription reaction, catalysed by an RNA polymerase, using NTPs and DNA as substrates. Optimised reaction conditions and sequences can achieve high yield at a high speed, (14 gRNA.L-1 in a 1h). The downstream unit operations (e.g filtration and chromatography) lack the versatility to rapidly adapt to different production scales and construct sizes. Additionally, with ever increasing mRNA titres and RNA modalities, the formation of product- (e.g. dsRNA) and process- related (e.g DNA) impurities will increase. Thus, enabling mRNA technology to reach its full potential requires the establishment of cost-effective and flexible downstream process.

To achieve this, we have explored different chromatography modalities to deliver high-quality mRNA. mRNA is traditionally purified by affinity chromatography. We have enhanced the performance the affinity ligand using AI-based approaches to increase dynamic binding capacity (DBC) by optimising retention time, NaCl and mRNA concentrations. An 8-fold increase was achieved; however, it is not sufficient to cope with high yield IVT reactions, and most importantly, purity is still lacking. Therefore, we explored one-step purification methods that exploit the physicochemical differences between mRNA and the IVT impurities, namely its hydrophobicity. Multimodal chromatography allows the separation of mRNA from the impurities, namely DNA and dsRNA, in a single step, with recovery yields above 80%, and a purity of 88%. This was achieved by optimising NaCl concentration during binding, to flow through impurities, and increase the pH during elution to improve yields. Finally, to tackle process costs, separation techniques based on steric exclusion were explored owing to its potential to cheaply isolate mRNA. By optimising NaCl and PEG concentration, not only mRNA can be separated from DNA, but also high yields (<80%) can be achieved. The simplicity of these techniques, compared to the conventional purification platform will lead to a higher yield and better quality product, and will ultimately contribute to a more cost-effective and flexible process.

Improving The Design And Production Of Mixed-Mode And Affinity Ligands

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Affinity and mixed-mode ligands are important for capture steps in downstream processing. We will present distinct strategies for the development of tailor-made ligands using a vast chemical space for ligand discovery and optimised ligand production methods. A first examples deals with the virtual design of small synthetic ligands, using both small focused rationally designed libraries or virtual screening of ultralarge ligand libraries. Secondly, scaffold design will be addressed, namely the computational design of a new peptide scaffold and its use to discover nM binders for desired targets.
The availability of purified antigenic proteins is critical to develop agents to prevent, diagnose, or treat infectious diseases. We present the design and development of an adsorbent bearing a synthetic affinity ligand to capture the SARS-CoV-2 spike protein, used as a model antigenic protein. A 120-ligand combinatorial library was designed _in silico_ and then synthesised in solid phase, and both were computationally and experimentally screened for binding to the spike protein. One lead ligand was selected for yielding > 95 % binding, and 64–73 % recovery of original strain spike protein, its receptor-binding domain (RBD), and Omicron BA.5 variant spike protein. An enrichment factor of 15 was found when capturing the spike protein from a clarified supernatant sample.
We designed a synthetic protein scaffold library based on the WW prototype sequence in which the loops were extended and randomized while maintaining structural stability. Using in vitro evolution by phage display against human serum albumin (HSA), we found a lead candidate that was produced by biological and chemical means and further characterized using experimental and computational tools. As a potential application for the lead binder, it was immobilized on a matrix and used to capture the target HSA.
Overall, this work shows the versatility of WW domains as peptide scaffolds amenable for in vitro evolution against non-cognate targets.

Designing For Sustainability: Modelling The Impact Of Buffer Choice In Chromatography

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The environmental impact of chromatography in biopharmaceutical manufacturing is significantly influenced by the buffers used, including the choice of buffering system. Sustainability metrics that are solely mass based – like Product Mass Intensity (PMI) – do not consider the different impacts of buffer components, so this aspect remains largely unexplored. This study investigates the direct and indirect sustainability impacts of different buffering systems used in monoclonal antibody (mAb) purification, emphasizing their contributions to water consumption, CO2 footprint, and water scarcity. We propose a workflow for evaluating these impacts that is suitable for application during process development, facilitating early decision-making based on sustainability criteria. Our analysis reveals that indirect contributions from buffer substances can account for a substantial portion of environmental burdens, which can exceed the impact of the water used for buffer preparation even when taking WFI production into account. Comparisons between phosphate-buffered saline (PBS), citrate, acetate, and glycine buffers in Protein A and cation exchange chromatography (CEX) demonstrate that buffer selection can lead to significant environmental savings. For protein A switching from citrate to acetate reduces water consumption by 27%, CO2 footprint by 19% and water scarcity impact by 62% For CEX, savings can be even higher for those buffer compounds, as replacing citrate with acetate in CEX resulted in reductions of 47% in net freshwater consumption, 34% in CO2 footprint, and 83% in water scarcity impact. Experimental validation confirmed that these buffer substitutions maintained process efficiency and product yield. These findings highlight the need for holistic sustainability modeling in bioprocessing and provide a data-driven approach for selecting environmentally sustainable buffer systems. By integrating sustainability metrics into process development, the biopharmaceutical industry can move towards greener and more resource-efficient manufacturing practices.

Exploring The Nanofitin® Affinity Ligand Platform: Showcasing Case Studies From Novel Modalities To Diverse Protein Classes

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The growing complexity of therapeutic targets, including vaccines and novel drug modalities, necessitates customized production and purification solutions for optimal yield and efficiency. Unlike traditional tag-based affinity purification methods, Nanofitin® affinity ligand technology offers advanced affinity chromatography materials that enhance the capture of specific targets. This versatile technology can capture a wide range of molecules, from small peptides to large particles, through ribosome display selection, screening over 1e14 different ligands to identify optimal candidates for affinity chromatography setups. This study highlights the efficacy of Nanofitin® affinity ligands in purifying a range of targets, from small proteins to large viral and gene therapy entities like adeno-associated viruses (AAVs) and extracellular vesicles. The showcased results illustrate the platform's potential for rapid affinity ligand discovery, characterization towards process-relevant process conditions like specific elution conditions, and prototype development. Promising application testing outcomes, including recombinant protein purification and serotype-specific AAV capture, as well as novel case studies involving larger particles, demonstrate a highly effective purification solution that achieves overall excellent impurity reduction and recovery rates exceeding 80%. In one notable case study, a three-step purification process was streamlined into an improved single-step purification approach. The second part of the study focuses on technological advancements, presenting the latest findings on ligand leaching from chromatography columns and the low toxicity of Nanofitins®. In summary, the existing tools for engineering and characterizing Nanofitin® ligands facilitate the customization of key process attributes, establishing it as a robust platform for a variety of biopharmaceuticals.

SPONSORED TALK

DoE, mechanistic modeling and artificial intelligence in chromatography

Martin Sichting [1]

[1] Cytiva

You want to increase the efficiency of your process development? You want to add modeling into your work? This presentation will give you an overview of Design-of-experiments (DoE), Mechanistic modeling and Artificial Intelligence (AI). The scientific principles will be explained, and the workflows will be outlined. Join this presentation to get you started into smart PD.

Application Of Machine Learning For Sustained Verification Of Chromatography Unit Performance.

Nitika Nitika [1], A.S. Rathore [1]

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Technological innovations, especially in artificial intelligence (AI) and machine learning (ML), are accelerating the biopharmaceutical industry's transition toward Industry 4.0. In line with USFDA regulations, biopharmaceutical process validation comprises three stages: process design, process qualification, and continued process verification (CPV). This study outlines a method for implementing CPV within a cation exchange chromatography (CEX) unit operation, highlighting the growing necessity of such methodologies in the industry. Critical process parameters (CPPs)—such as pH, conductivity, UV absorbance, and column pressure—were tracked in real time using built-in sensors. Additionally, critical quality attributes (CQAs) like charge variant profiles (measured via Raman spectroscopy) and protein concentration (via near-infrared spectroscopy) were continuously monitored. Statistical process control (SPC) charts were generated from this data, and a Python-based tool was developed to analyze the charts and flag any process deviations. Deep neural network (DNN) models integrated with NIR and Raman spectroscopy yielded high predictive accuracy ($R^2 > 0.90$), underscoring the effectiveness of the proposed approach. The Raman-based control strategy for monitoring charge variants was validated through intentional disturbances to the CEX load. Despite the perturbations, the eluate maintained stable charge variant distributions—acidic ~20 ± 2%, main ~62 ± 2%, and basic ~18 ± 2%—consistent with control batches. The results demonstrate that combining the right analytical technologies, soft sensors, and advanced data analytics enables reliable CPV and supports the broader goal of Industry 4.0 implementation in biopharmaceutical production.

Bulk Crystallization For Protein Processing: New Concepts, Challenges And Opportunities

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Increasing global demand for high-quality recombinant proteins in biomedical diagnostics, therapeutic applications, as well as food supplements and alternative non-animal nutrients is a driving force for the development of new technological solutions, which enable mass production of proteins in an economically efficient and sustainable manner. Bulk protein crystallization for large-scale production provides the possibility of meeting these challenges. The advantages of this process are the higher activity, purity, and stability of the crystalline biomolecule compared to other formulation methods. Therefore, crystallization has the potential to replace all or some of the chromatography steps in DSP to meet quality requirements. The high cost of resins and limited throughput make chromatography a bottleneck and a cost driver for overall production. Protein crystallization is usually performed batchwise in stirred crystallizers, where the crystallizing agent is added to obtain a supersaturated solution and initiate crystallization. However, this manner of process realization renders the supersaturation level, and thus the crystallization progress, difficult to control. Imperfect mixing between the protein solution and crystallization agent can trigger precipitation of amorphous solids or formation of inactive aggregates, while rigorous stirring induces high shear stress inhibiting growth of the protein crystals. To overcome this issue, we developed the concept of forced convection crystallization (FCC). In this process, water is evaporated from the protein solution by forcing a convective flow of air, the parameters of which are used to control the supersaturation level and temperature of the solution. The desired crystallization condition is achieved by controlling the removal of water from the protein solution. Since continuous operations outperform batch processes in terms of yield and productivity, we extended the concept of FCC to continuous process, in which mechanical stirrers are replaced with an Archimedean screw, which allows simultaneously effective and gentle mixing of the protein solutions. The concept of batch and continuous FCC will be presented and exemplified along with the design strategy, including issues typical for protein crystallization such as determining the boundaries of the phase diagram, selecting operating hydrodynamic conditions, and issues specific for FCC.

High-Definition Simulation Of Packed-Bed Chromatography In Laterally Unconfined Compartments

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While reduced order models enable efficient simulations of packed-bed chromatography, they rely on extensive experimental calibration and do not provide mechanistic insights into how specific morphological features influence local hydrodynamic profiles and separation performance within the column. These morphological characteristics include particle size distribution, particle geometry, local packing irregularities, and wall effects. High-definition modeling addresses these limitations by resolving full spatial details, yet previous studies have been restricted to confined cylindrical geometries with very narrow columns. These constraints introduce substantial wall effects, altering the local hydrodynamic behavior, and limiting the applicability of the results to larger-scale applications.
This work extends high-definition chromatography simulation from laterally confined to unconfined compartments by establishing a validated workflow encompassing periodic packing generation, meshing, partitioning, CFD simulation and post-processing. Through systematic validation at each stage, we verify the correct implementation of periodic boundary conditions. The established computational workflow allows to simulate packings with up to 10,000 particles in unconfined compartments, facilitating the investigation of local hydrodynamics without wall effects in industrial-scale applications.

Host Cell Proteins Profiling And Characterization For Model-Based DSP Design

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The development of purification processes of biopharmaceutical products is normally experimental in nature. This requires long development times as well as large amounts of precious samples that are not necessarily available in the initial stages for the development of a new product. This process development might be accelerated by combining High Throughput Experimentation (HTE) and mathematical models based on sound physical-chemical phenomena captured in so called mechanistic chromatographic models. This allows for investigating lager process configurations and operation conditions that might lead to optimal and robust purification processes having minimal buffer and resin consumption at high purity and yield obtained via computer simulations.
A pre-requisite for using these mechanistic models is information on the absorption behavior of the product and impurities on the resins to be tested. An important class of process related impurities - next to host cell DNA, and e.g. endotoxins - are host cell proteins (HCPs), essential to be reduced to acceptable and safe levels but are difficult to remove from a protein based biopharmaceutical product. In order to apply a hybrid model based High Throughput Process Development (HTPD) approach, methods are needed to obtain accurate isotherm data for HCPs.
This presentation will show approaches for determination isotherm data of HCPs using high end analytics and its subsequent use in the development of processes for industrially relevant biopharmaceuticals.

Spinoff Presentation Abstracts

Comparative Economic Modeling Of Magnetic Bead-Based Processing As An Alternative To Legacy mAb Manufacturing

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In today's biologics manufacturing, particularly in the production of monoclonal antibodies (mAbs), processes must adapt to new levels of intensification. New upstream intensification can now achieve very high cell densities and elevated mAb concentrations, conditions for which the traditional Protein A platform process was not originally designed. Over time, this platform has been incrementally adjusted to accommodate upstream changes. However, a holistic approach to improving the entire manufacturing process has been lacking. Cell clarification steps, in particular, represent a significant bottleneck, contributing limited value beyond the removal of solids. Previously, we demonstrated that magnetic bead-based processing is advantageous replacing celclarification step during the mAb processing (Brechmann et al., 2019, Brechmann et al., 2021), effectively addressing these bottlenecks. However, understanding the impact of such a technology over an extended production campaign is essential for determining under which conditions various downstream processing (DSP) strategies should be applied.

In this study, we modeled two one-year-long manufacturing campaigns using magnetic separation for the antibody capture step, integrating cell clarification and target cpature into a single unit operation (Brechmann et al., 2024). The model incorporated key experimental findings from our previous publication (Brechmann et al., 2021) alongside literature-derived assumptions and data. The economic model outputs were analyzed with respect to cost of goods per gram (COGs/g) and annual process output, enabling a direct comparison of the two magnetic processes against a conventional legacy process. Both modeled magnetic-based processes demonstrated significant improvements relative to the legacy process, achieving up to a 38% reduction in COGs, a 17% decrease in fixed investment, and up to a 40% increase in production output. Notably, the magnetic-based processes maintained productivity levels consistent with reached upstream and sustained a consistently high overall process yield. In contrast, the legacy process showed a decline in productivity and a corresponding decrease in overall yield.

In conclusion, the modeled magnetic separation-based processes offer significant improvments over the legacy process. Particularly in scenarios where the USP dictates a single high cell density harvest, magnetic separation provides a highly valuable alternative for downstream processing.

Holistic Pilot-Scale Magnetic Separation Platform Development

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Downstream processing remains a key cost driver in biomanufacturing, accounting for up to 80 % of total production expenses for therapeutic antibodies. Chromatographic workflows, while effective, are resource-intensive, hard to scale, and increasingly unsuited for emerging paradigms like continuous or decentralized manufacturing. Conventional resin-based chromatography also struggles to purify complex modalities such as viral vectors (AAVs), exosomes, and other advanced bioparticles used in gene and cell therapies. In parallel, sectors like food and nutraceuticals lack scalable purification technologies, hindering the valorization of bioactives from industrial sidestreams. This shortage of cost-effective separation solutions limits innovation across bio-based industries.
We present a modular magnetic separation platform designed to address these challenges. At its core is a rotor-stator high-gradient magnetic separator (RS-HGMS), enabling scalable magnetic particle production and high-performance biomolecule separation. The platform combines tailored nanoparticle synthesis, surface functionalization, and model-based real-time optimization. Unlike commercial magnetic beads—often expensive and inflexible—our system uses engineered tags for selective binding of target proteins to low-cost, functionalized particles. All unit operations, from magnetic capture to elution, are modular, automatable, and optimized for efficient particle–process interaction.
The system was validated at pilot scale: (i) nanoparticle synthesis was scaled to 10 L, yielding 288 g of particles, and processed via RS-HGMS [1]; (ii) recovery of human IgG from CHO supernatant achieved 85 % yield and 97 % purity [2]; and (iii) lactoferrin was extracted from acidic whey with 60.5 % yield and a purification factor of 38 [3]. The system currently reaches TRL 5.
The RS-HGMS platform achieves yields and purities comparable to chromatography, at up to 10× faster throughput [4]. Several R&D projects are ongoing, with a spin-off targeted for early 2026. Commercialization will proceed via: (i) customized process development, (ii) subscription-based nanoparticle supply, and (iii) licensing of digital tools for model-based control.

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Potential-Controlled Affinity Membrane Chromatography (pcMAC) - Redefining Gentle Biomolecule Purification

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Affinity chromatography resins based on Protein A-coated beads are the current industry standard for large-scale antibody purification. While providing excellent selectivity, these processes rely on harsh acidic elution conditions (pH < 3.5), leading to possible ligand and product degradation. This poses significant challenges, especially for sensitive next-generation biomolecules such as IgM, mRNA, plasmids, and AAV vectors, which separation processes would also benefit from highly selective affinity interactions.

We founded a team to achieve the goal of finding an outside of the box solution for these current and future limitations, by using Targeted Unconventional Recovery via Triggered Liquid-phase Electroseparation, short TURTLE. We developed the pcMAC technology, a novel platform technology using potential-controlled affinity membrane chromatography. By integrating an electroseparation cell design with Protein A affinity membranes sandwiched between conductive membranes, we demonstrated efficient capture and elution of monoclonal antibodies (e.g., Trastuzumab) using mild electrochemical stimuli (2.5 V). Compared to traditional pH-shift elution, the potential-controlled approach enabled faster cycles, high elution yields (~90 %), and preserved antibody integrity, as confirmed by non-reducing gel electrophoresis.

Beyond monoclonal antibodies, we see broad applicability of pcMAC for the gentle and scalable purification of other sensitive biomolecules. By combining membrane-based mass transfer advantages with the elimination of harsh buffer exchange steps, pcMAC represents a more sustainable and economically attractive alternative to conventional affinity chromatography.

In the framework of the GO-Bio initial, a program from the German ministry to support start-up ideas, team TURTLE is advancing pcMAC towards proof of concept and exploring commercialization pathways. Our goal is to overcome key bottlenecks in downstream processing and provide the industry with a robust, product-friendly, and next-generation separation platform.

Light-Controlled Antibody Purification Via A Photoswitchable Protein A Platform

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Affinity chromatography using Protein A remains the gold standard for monoclonal antibody (mAb) capture due to its high selectivity and productivity. However, elution from high-affinity matrices typically requires harsh conditions—such as low pH—that risk aggregation, denaturation, or product degradation, especially for next-generation antibody formats. To overcome these limitations, we developed a photoswitchable Protein A platform that enables light-triggered, tag-free antibody purification under mild, near-physiological conditions.

Our approach centers on a visible-light-responsive azobiaryl photoswitch (PS2), covalently inserted into engineered Protein A domains. Red-light illumination (λ = 635 nm) maintains PS2 in its compact cis state, supporting high-affinity binding. Blue-light exposure (λ = 465 nm) induces trans-isomerization, mechanically distorting the ligand and triggering antibody release. This reversible conformational shift functions as an optical lever for precise control of binding interactions.

Immobilization on a convective-flow monolithic matrix enabled rapid IgG capture from clarified CHO supernatant, followed by >90% recovery in buffer of choice under blue light. The matrix maintained its binding capacity (>70 mg/run) over multiple purification cycles. Because the elution occurs without buffer switching, the system enables very fast process cycles and can directly feed into subsequent downstream steps such as ion-exchange chromatography without intermediate adjustment. High flow rates further accelerate processing, combining speed, efficiency, and product protection in a single platform.

This technology establishes light-controlled affinity chromatography as an orthogonal alternative to conventional pH-based elution. Its modularity allows adaptation to other ligands (e.g., Protein G, L), enabling gentle, high-throughput purification workflows for diverse biologics.

Planet Crafting Labs: Empowering The Biotech Industry With Bespoke Workshops To Drive Sustainable Innovation And Achieve Impactful Environmental Solutions.

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Dr. Simone Dimartino is a Senior Lecturer in Bioprocess Engineering at the University of Edinburgh, where he leads pioneering research in 3D printing of porous materials and architectures for the downstream processing industry.
Beyond academia, Dr. Dimartino is a certified workshop designer and facilitator, applying innovative, hands-on methodologies to foster creativity and collaboration. The first time he has integrated these techniques into Engineering was into the flagship course "Engineering Principles 1", engaging over 500 students in experiential learning.
Recognizing the power of structured group conversations in achieving solid outcomes, Dr. Dimartino founded Planet Crafting Labs. This venture merges his research experience in the Bioprocess sector, his passion for sustainability, and his facilitation expertise to design bespoke workshops that empower sustainability teams and representatives drive a responsible green transition.

Flash Presentation Abstracts

Large Scale Purification And Quality Control Of Therapeutic Oligonucleotides

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The field of oligonucleotide therapeutics has made remarkable progress in recent years leading to a growing need for purified oligonucleotides. One of the classic oligonucleotide production techniques is chemical synthesis followed by a chromatographic purification step. Especially for up scaling, ion exchange chromatography is the ideal method due to its high loading capacity combined with high resolution and yield. To assure the successful synthesis and purification, analytical quality control is mandatory. The quality control requires a confident confirmation of oligonucleotide mass as well as quantification of impurities. Expanding the HPLC-UV method for quality control with MS detection benefits in mass confirmation and impurity identification.
Due to the high similarity of impurities originating from the synthesis, purification and analysis can be challenging. Here, a larger scale purification of an oligonucleotide using anion exchange chromatography (AEX) is shown. After solid phase synthesis, HPLC analysis confirmed 89.1 % purity of the sample before the chromatographic purification. Ion exchange chromatography is an excellent method for the purification process of oligonucleotides due to its high separation efficiency, easy handling, process stability and scalability. A successful large scale ion exchange chromatography purification of an oligo(dT) with 95 % purity and 90 % yield was achieved.
During the purification process UV detection is sufficient but for a more sophisticated analysis during the quality control, another detection option is necessary. Aborted sequences are often not baseline separated, which complicates a reliable quantification. Other impurities that occur because of degradation, as for instance depurination, cannot be separated sufficiently from the main peak. HPLC coupled with negative ESI-MS provides a reliable method with unique capabilities for separation and identification. The described workflow only made use of the first quadrupole, but MS/MS or MRM experiments can be useful for the quantitative analysis of low-level oligonucleotide impurities and co-eluting substances.

mRNA Manufacturing: From Template DNA To Lipid Nanoparticles – An Integrated Platform Approach

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The manufacturing of mRNA has emerged as a pivotal technology in the development of novel therapeutics and vaccines. For mRNA production, Good Manufacturing Practice (GMP) is the essential quality standard to ensure product safety, consistency, and regulatory compliance. We provide a case study for the establishment of a GMP-compliant mRNA manufacturing platform integrating the whole process chain from template DNA, mRNA production, and lipid nanoparticle (LNP) formulation until final aseptic filling of drug product. The essential elements of GMP mRNA production are highlighted, emphasizing the key challenges to ensure the scalable, reproducible, compliant and economic manufacturing of mRNA-based therapeutics.

The GMP manufacturing process of the investigated mRNA products encoding CRISPR/Cas9 nuclease was carried out at full scale in certified clean rooms. Manufacturing of the linear DNA template consisted of construct generation, preparation of an *E. coli* master cell bank, fed-batch fermentation, alkaline cell lysis, chromatographic purification, and enzymatic linearization steps. The mRNA was synthesized by *in-vitro* transcription (IVT) and co-transcriptional addition of a 5´-cap analog. Subsequent mRNA purification was achieved by oligo dT affinity chromatography. The mRNA was encapsulated in lipid nanoparticles (LNP) by impingement jets mixing. The drug product was formulated by tangential-flow filtration and aseptically filled.

A comprehensive analytical platform conforming to GMP regulations has been set up, including raw material testing, in-process controls, Quality Control release and stability testing, as well as extended characterization of the mRNA. It was confirmed that all quality parameters (content, identity, purity, safety) were within pre-defined specifications at all stages. Bulk mRNA was highly homogenous (≥ 90 % by CE). Capping efficiency was ≥ 95 %, and poly(A) tail length was confirmed by LC/MS. The LNPs showed a high encapsulation efficiency (> 90 %) and a low polydispersity index (PDI). Satisfying stability was confirmed from both the bulk mRNA as well as from the formulated LNPs by performing stability studies at several conditions.

Functionality of the CRISPR/Cas9 mRNA was shown in a cellular assay by measuring Cas9-mediated recombination in HEK293 cells. As a conclusion, with the established mRNA platform, the parameters and requirements of yield, throughput time, GMP compliance and quality were successfully achieved.

Direct Application Of Undiluted Human Plasma And Other Complex Biological Fluids To Polymethacrylate-Based Monoliths And Subsequent Isolation Of Biologically Active Therapeutic Proteins And Other Biopolymers

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Use of polymethacrylate-based CIM monoliths as supports for processing of biological fluids like cell culture supernatants and human plasma is presented. Final products are therapeutic proteins like monoclonal and polyclonal antibodies, as well as other biologically active biopolymers and nanoparticles [1]. As first step, adsorption chromatography based on ion-exchange and hydrophobic-interaction, but also affinity and immunoaffinity chromatography based on sample displacement are used [2]. In the case of monoliths, flow rate as well as scaling up and scaling down is practically not a limiting factor, and it opened new possibilities for optimization and introduction of continuous chromatography, as well as a direct in process control. However, because of both high viscosity and high salt concentration of the starting material, a dilution step still has to be performed before sample application. Monolithic CIM supports with optimized channel diameter, surface chemistry and optimized ligands with tailored density open new ways for purification of different biologically active macromolecules and nanoparticles by direct application of undiluted samples on the column without previous dilution before application the chromatographic unit. Finally, an alternative process for isolation of therapeutic proteins and other biopolymers, as well as nanoparticles is presented, and future introduction of continuous chromatography based on use of CIM monoliths with parallel in process control is presented.

Towards Seamless mAb Purification: Dual-Step Multi-Column Chromatography

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Achieving true continuity in downstream processing remains a key challenge for monoclonal antibody (mAb) production. While Multi-Column Chromatography (MCC) is a high-performing operation modality for continuous capture because of increases in productivity and buffer savings, integration of subsequent steps is often limited by equipment or process constraints. The adapted multicolumn system used in this work enables two fully integrated chromatographic steps in a single 8-column setup, supporting uninterrupted operation and efficient resin use. Using a Protein A affinity resin, we demonstrate robust mAb capture and subsequent polishing with highly salt-tolerant resins under continuous conditions, advancing practical implementation of end-to-end continuous purification. In addition, a low-threshold pH step will be applied into this process integration to achieve inline virus inactivation. A high degree of automation has been achieved with this setup, while the footprint has been reduced both by reducing the size of the systems and by saving on buffers. As a result, this setup offers itself as an innovation to make downstream processing more sustainable.

Advancing *K. Phaffii* Bioprocesses: Evaluation Of Continuous Perfusion Processes And Suitable Purification Strategies

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Equitable access to medicines necessitates a significant reduction in biomanufacturing costs. Amongst others, the choice of production host, cultivation, and purification strategies significantly impacts the final cost of biopharmaceuticals. For example, transitioning from mammalian hosts to non-conventional cell factories, and from batch and fed-batch processes towards integrated continuous processes increases flexibility and simplicity, while reducing manufacturing costs. *Komagataella phaffii* (formerly *Pichia pastoris*), is a methylotrophic yeast which has become established as a promising alternative host. However, despite extensive research on perfusion processes as a continuous operational strategy for mammalian cell cultures, their application to *K. phaffii* has to date been limited.

Here, we demonstrate a continuous perfusion process for *K. phaffii* and investigate the optimal process duration with respect to cell-specific productivity. Our results show that the highest productivity is reached after 7 days of protein induction in perfusion mode and remains at its maximum for an additional 7 days. This is followed by a 10 to 30 % relative decrease in specific productivity over the final 6 days of operation.

This emerging mode of operation for *K. phaffii* requires the development of adequate downstream steps. With this in mind, we also investigated how multi-modal membrane chromatography and peptide-based adsorbents (PichiaGuardTM) can address the impurities found in the harvest (perfusate) stream of the perfusion system. Using proteomics, we have mapped the changes in host cell proteins (HCPs) and their removal efficiencies across the different purification steps. Combining the two purification strategies achieved over 95 % total reduction of HCPs. Additionally, the two-step process reduced unique HCP species by 79 %, including high-risk HCP species that can pose immunogenic risks. Our research highlights the unique downstream requirements of continuous *K. phaffii* perfusion systems and provides a perspective for the development of *K. phaffii*-tailored purification processes.

Multi-Detector Field-Flow Fractionation For The Assessment Of Critical Quality Attributes Of AAVs

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Viral vector-based systems have emerged as a promising tool in gene therapy. Among them, Adeno-associated viruses (AAV) have become the preferred choice for clinical gene therapy due to their minimal pathogenicity and their capability to establish long term gene expression in various tissues.[1]
To ensure their safe and efficient use, the determination of critical quality attributes (CQA) including e.g. particle size distribution, capsid molar mass, agglomerates and aggregates, impurities (e.g. host cell proteins) as well as viral titer is essential.[2,3] But, many of the current analytical methodologies used to determine these CQA show limitations such as e.g. large sample volume requirements, poorly understood measurement variability, and lack of comparability between methodologies.
Size exclusion chromatography coupled with multi-angle light scattering (SEC-MALS) has been used to characterize AAV capsids and to obtain detailed information with high accuracy and precision due to its simplicity, accuracy and robustness.[3,4] Alternatively, Field-Flow Fractionation (FFF) combines high size-resolution without the need for a stationary phase, and the ability of multi-parameter analysis in a single measurement. In contrast to SEC, FFF is much more flexible and enables fractionation of large molecules up to 1000 nm under minimal shear forces.[5]
We here present the application of asymmetrical flow FFF coupled with UV/vis, MALS, dynamic light scattering and refractive index detection (AF4-UV-MALS-DLS-RI) to derive a comprehensive set of CQAs for different AAV serotypes. In particular, quantitative information on size distribution profiles, viral titers as well as empty/full ratios was collected. The derived experimental data showcases the robustness of the presented methodology and highlights the applicability of multi-detector AF4 as a powerful tool to study gene therapy products.

Novel Analytical HPLC Method for Characterization and Quantification of VLPs

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The development of analytical methods for the quantification and characterization of enveloped VLPs remains a major challenge due to their overlap in size, buoyant density and similarity in membrane composition to other bionanoparticles such as host cell vesicles and chromatin. Especially in complex mixtures (e.g. cell culture supernatants), the presence of other bionanoparticles leads to inaccurate quantification of VLPs. The aim of the present work was to develop a method for the accurate quantification of VLPs even when other bionanoparticles are present in the mixture. Heparin affinity chromatography has been successfully used to separate different bionanoparticles populations on a preparative scale. In this work, we have developed an analytical HPLC method using preparative grade Capto™ Heparin affinity resin and incorporating multiple in-line detectors such as UV, MALS, DLS and RI. Consistent recoveries of 100% for pure VLPs samples and 88% for clarified cell culture supernatant were achieved through extensive flow rate screening, column hardware and filter evaluation and the addition of mobile phase additives. The developed method allows rapid and accurate quantification and characterization of VLPs, even in complex mixtures, improving the speed of downstream process development. In addition, the use of HPLC is non-destructive, allowing the collection and further analysis of high purity bionanoparticles.

Tangential Flow Filtration And Multimodal Chromatography As Promising Strategies For Purification Of Outer Membrane Vesicles (OMVs) From *Neisseria Lactamica*

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Outer membrane vesicles (OMVs) are spherical nanostructures spontaneously produced by Gram-negative bacteria through blebbing of the outer membrane. *Neisseria lactamica* is a non-pathogenic commensal bacterium that colonizes the upper respiratory tract of humans, especially neonates. OMVs released by this bacterium have emerged as an important platform for developing new vaccines. OMVs have adjuvant properties due to the presence of lipopolysaccharide (LPS) and other pathogen-associated molecular patterns (PAMPs) that activate the immune response. Despite their promising potential, challenges in OMV production and purification remain. Commonly used purification methods, such as ultracentrifugation (UC) and size-exclusion chromatography (SEC), have limitations regarding scalability, low yields, and the high costs of ultracentrifuges. This study evaluated alternative OMV obtention and purification methods, including tangential flow filtration (TFF), multimodal chromatography, and membrane chromatography, as substitutes for UC and SEC. TFF membranes with molecular weight cut-offs of 100, 300, and 500 kDa were evaluated. The TFF 300 kDa membrane showed a chromatographic profile similar to UC when analyzed by SEC, but with more than double the total protein recovery. Sartobind® Q membrane and multimodal chromatography Capto-adhere and Capto-Core 400 were evaluated as a second purification step. In the case of membrane chromatography, the highest recovery, under the conditions evaluated in this study, was 8.7%. On the other hand, multimodal chromatography using Capto-adhere achieved a recovery of 59%, while Capto-Core 400 presented the highest recovery among all chromatographic methods evaluated (72%). Thus, a TFF 300 kDa membrane combined with Capto-Core 400 for OMV purification enabled greater productivity and more efficient purification when analyzed by high-performance liquid chromatography (HPLC). The obtained OMV nanoparticles also had a low polydispersity index (0.14 ± 0.02) as assessed by dynamic light scattering (DLS). Given their high recovery rates and efficient purification, these methods offer a reassuring step forward in OMV research, providing a more scalable approach for OMV purification.

Protein Purification Of Soluble And Insoluble Rhizavidin- Fused Potential *Schistosoma Mansoni* Antigen: A Comparison Of Chromatographic Performance

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Schistosomiasis is a neglected tropical disease and one of the most important parasitosis in the world, responsible for over 11,000 deaths per year and high socioeconomic impact. For *Schistosoma mansoni,* prevalent parasite species in Brazil, SmTSP-2 antigen in fusion with rhizavidin stands out for the development of vaccine candidates based on multiple antigen presentation systems. Hence, suitable yield and purity are necessary to produce this antigen. In this sense, this work aimed to produce and purify rhizavidin-fused SmTSP-2 antigen (Rzv:SmTSP-2) out of *E. coli* culture and compare purification results of Rzv:SmTSP-2 obtained in both soluble and insoluble fractions. Rzv:SmTSP-2 was produced in BL21(DE3) *E. coli* cells cultivated in shaken flasks using Terrific Broth veggie medium with kanamycin. Cells were grown at 37°C up to stationary phase until induction with 0.6 mM IPTG for 5 h, when temperature was adjusted to 23°C. Cells were later centrifuged and disrupted in a high-pressure homogenizer. Both soluble and insoluble lysis fractions were submitted to immobilized metal affinity chromatography (IMAC-Sepharose FF charged with Ni2+ ions) and required imidazole concentrations for elution were determined through linear gradient. Purification of the insoluble fraction included 8 M urea in all buffers. Purity values were determined by SDS-PAGE and densitometry, and protein content was quantified using the Bradford method. Both insoluble (approximately 140 mM) and soluble (approximately 150 mM) fractions required similar imidazole concentrations for Rzv:SmTSP-2 elution. Nevertheless, purification of the soluble fraction resulted in poorer resolution than the insoluble fraction, since a contaminant peak could not be fully separated from the target-molecule peak. Moreover, purification of the insoluble fraction led to greater purity (approximately 78%) and purification factor (1.24-fold). Considering the soluble fraction had a greater initial concentration of contaminant host-cell proteins, co-elution of impurities led to inferior purity (about 43.5%) and no purification increase. The yield was also higher with the insoluble fraction than with the soluble one. Therefore, Rzv:SmTSP-2 purification from the insoluble fraction shows great potential to achieve higher purity, despite the need of pursuing protein refolding later. Ongoing studies will show if this soluble/insoluble protein behavior also happens with other chromatographic resins and metal ions.

Optimizing Antibody-Binding Stoichiometry Via The Linker-Region

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Affinity chromatography is still the workhorse for antibody production platform processes, with Protein A continuing to be the most frequently used affinity ligand. This ligand possesses five homologous IgG binding domains. However, despite the theoretical availability of five binding sites, steric hindrance limits the practical binding capacity to approximately two to three antibody molecules per Protein A ligand. Most attempts at ligand optimization have focused on modifying the binding region, such as through point mutations, to enhance binding capacity, lower the pH required for elution, or improve alkaline stability.
Despite these efforts, Protein A chromatography materials still exhibit relatively low binding capacities compared to other techniques, such as ion exchange chromatography. This limitation impacts both the productivity and the overall costs of the antibody production process.
Therefore, it is crucial to explore new routes for ligand improvement. The linker region holds untapped potential to enhance accessibility for IgG molecules. To investigate the impact of the linker region, distinct linker peptides – a rigid sequence and a flexible sequence – were inserted into the highly conserved wild-type linker sequence, and the so-modified B Domain of Protein A was polymerized eight times. The rigid ligand demonstrated over a 50% increase in dynamic binding capacity compared to polymerized B-domains with the wild-type-like or the flexible linker sequence. This finding highlights the linker region as a valuable approach to enhancing binding capacities.

In Situ Analysis Of Monoclonal Antibody Fragment Adsorption On Phenylboronic Acid Chromatography Media

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Therapies based on monoclonal antibodies (mAbs) is the most lucrative segment of the biopharmaceutical industry. However, recent efforts have increasingly focused on developing more efficient and cost-effective antibody formats. Among these, Fragment antigen-binding (Fab) antibodies are emerging as promising alternatives with already three products on the market: abciximab (ReoPro, Eli Lilly), ranibizumab (Lucentis, Genentech) and certolizumab pegol (Cimzia, UCB).
Unlike full-length mAbs, Fab fragments lack the Fc region, rendering them incompatible with Protein A affinity chromatography and thus limiting the development of standardized purification platforms. This study explores a novel purification strategy based on phenylboronate chromatography using Fabs obtained from digestion of IL-8 mAb and Adalinumab. Adsorption studies will be performed using both affinity-based and multimodal configurations of m-aminophenylboronic acid (m-APB) ligands, under varying pH conditions and in the presence of the mobile phase modulators that together selectively enhance or suppress specific interaction types. For this purpose, Flow Microcalorimetry (FMC) will be employed as a real-time, dynamic technique to measure instantaneous heat changes during adsorption and desorption events. This will allow for in-depth thermodynamic and mechanistic characterization of Fab–ligand interactions on m-APB supports.

Cationic Polymers Reduce Host Cell Protein Burden For Subsequent Chromatographic mAb Purification

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Monoclonal antibody (mAb) production processes are steadily improving due to increased demand, resulting in higher titers in mammalian cell cultures. These titers can be achieved through high cell density cell culture, usually associated with elevated levels of product- and process-related impurities, such as cell debris, host cell proteins (HCPs), and host cell DNA. In this study we aim to reduce (high-risk) HCP concentration, and complexity of mAb purification by addition of cationic polymers that support harvest clarification as well as impurity removal.
Certain cationic polymers have been shown to bind negatively charged impurities, such as host cell DNA and HCPs, thereby reducing impurity levels early in the downstream process (DSP) train. In this study, we examined the effect of such polymers on the subsequent purification process, focusing on the removal of HCP and potential simplification of the DSP. Cell culture was flocculated by the addition of cationic polymers and the supernatant was harvested by centrifugation and sterile filtration. Flocculated and non-flocculated material was purified using a conventional three-step process consisting of protein A capture, cation-exchange (CEX) chromatography in bind elute mode and anion-exchange (AEX) chromatography in flow-through mode. At each process step, we evaluated concentration (ELISA) and nature (mass spectrometry) of HCPs. Flocculation demonstrated encouraging results in decreasing DNA levels as well as the overall HCP load including a variety of high-risk HCPs.

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BIO-RAD

Poster Abstracts

Advances In Understanding And Modeling pH Transients In Protein A Columns And Their Effects On Elution Of IgG

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The evolution of pH and conductivity waves during elution of IgG from protein A columns has been studied for a range of commercial resins using glycine and acetate buffers as eluents [1]. For glycine buffers, conductivity and pH waves travel through the column at different speeds, with the conductivity wave emerging in the column void volume and the pH waves emerging much later. The corresponding IgG elution peaks exhibit fronting as a result of the pH behavior. Conversely, for acetate buffers, the effluent pH drops rapidly initially concurrent to the passage of the conductivity wave, but then only gradually approaches the eluent pH. As a result, the IgG elution peaks exhibit distinct tailing. In this work we have extended our measurements to citrate buffers and pH gradient elution and have extended our previously developed glycine model to predict elution with acetate and citrate buffers. Model calculations show that while for glycine elution the most critical factor is the exchange of glycine cations and sodium ions on the carboxyl groups associated with the protein A ligand, acetate and citrate elution are mostly affected by the exchange of protons and sodium ions. The model-predicted pH profiles are used to successful describe the elution of IgG at both low and high loadings (5-45 g/L) and provide the means to understand how buffer-ligand interactions affect the outcome of elution. The model can thus be used to design optimized buffers and predict the pH of the IgG elution pool.

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Reproduction Calculation Of Pressure Profile Of Tangential Flow Filtration For Clarification Of HEK Cell Culture With Cell Lysate Using Hollow Fiber MF With Asymmetric Or Symmetrical Structur

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Although AAV is the most promising vector for gene therapy, its purification process is not straightforward. In particular, the stable clarification and harvest of AAV from cell culture containing lysate with very low viability presents a challenge. Previous studies have reported on the effectiveness of reverse asymmetric hollow fiber membranes for harvesting AAV by tangential flow filtration (TFF), and the critical flux at different shear rates has been experimentally demonstrated.
This report presents a quantitative reproduction of the critical flux of TFF, which is important for stable harvesting, using an analytical equation based on mass balance. Furthermore, this analytical equation is used to reproduce the TFF filtration behavior of HEK cell culture with cell lysate using three types of hollow fibre MF with different structures. These three types of hollow fibre MF (BioOptimal MF-SL, UMP and UJP) have different average pore sizes (0.4 µm, 0.2 µm and 0.65 µm) and distinct structures: MF-SL is asymmetric and UMP and UJP are symmetrical. Therefore, when a HEK cell culture containing a lysate with viability of almost 0% is TFF filtered under a constant permeate flux condition, the filtration pressure behavior exhibits completely different profiles depending on the membrane used. Calculation results obtained using analytical equations based on mass balance can reproduce these pressure profiles by optimizing the filtration parameters included in the equations. The calculations particularly highlight the significant impact of increased membrane resistance (Rm) during filtration, which is dependent on membrane structure. Furthermore, modelling the structure of asymmetric membranes using a multilayer model and performing numerical calculations of filtration behavior reproduced the characteristics of the pressure profile and inner fouling.

Measuring Adsorption Equilibria: The Determination Of The Maximum Binding Capacity Depends Strongly On The Method Of Resin Preparation.

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Accurate determination of adsorption isotherms is essential for designing chromatographic separations and selecting resins based on maximum binding capacity. This study systematically evaluates different resin preparation methods for batch adsorption isotherm experiments, including the volumetric slurry-based method, weight-based methods using vacuum filtration and centrifugation, and a commercial PreDictor™ Isotherm plate. The binding capacities obtained were compared to reference values from column breakthrough curve (BTC) experiments using lysozyme on SP Sepharose FF and green fluorescent protein (GFP) on Toyopearl Gigacap Q650M as model systems. Our findings demonstrate that the centrifugation method, which involves weighing hydrated particles and determining their density, provides the most accurate and reproducible binding capacity values. These values closely match those obtained from BTC experiments, with deviations largely attributable to resin compression factors. In contrast, the slurry-based method and vacuum filtration exhibited limitations due to inaccuracies in void fraction estimation and uneven resin drying, respectively.
This study highlights the critical impact of resin preparation methods on the accuracy of adsorption isotherm measurements and recommends the centrifugation method for non-automated workflows. The results are consistent across different resin types, suggesting broad applicability for ion-exchange chromatography process development.

Optimizing Bionanoparticle Purification: A CFD-Driven Design Of Flow Distributors For Monolithic Columns

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Efficient purification of bionanoparticles (BNPs), including virus-like particles and adeno-associated viruses, is a central challenge in modern bioprocess engineering, due to their complex structures and the stringent purity requirements involved. The use of monolithic polymethacrylate-based columns is a great strategy for improving BNP purification. These columns are ideal for large biomolecules due to their ability to facilitate convective transport, which enhances mass transfer efficiency. A critical challenge with monoliths is their inherently low pressure drop which can result in uneven flow distribution and reduced purification efficiency. Thus, this study primarily focuses on designing and modeling flow distributors to achieve uniform fluid flow within the porous structure of the column.
Several distributor geometries will be evaluated using computational fluid dynamics (CFD) to assess their impact on pressure drop, residence time and distribution efficiency. These parameters are essential for evaluating uniform flow, leading to more effective purification. The optimized flow distributor will then be integrated into a 3D CFD model that incorporates a realistic monolith microstructure in order to simulate internal velocity fields and mass transport behavior. This design-centric approach offers a scalable solution to enhance BNP purification and can be used as a predictive framework for future convective chromatography applications.

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Electrically Driven Elution In Digital Protein A Membrane Chromatography: An Alternative To Traditional Low-pH Elution

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Digital Protein A membrane chromatography offers a novel approach to antibody purification by enabling gentle elution via electric fields rather than conventional low-pH buffers. This method eliminates the need for acidic elution conditions, thereby addressing concerns associated with low-pH-induced antibody degradation and aggregate formation. It is particularly well suited for the rapid capture and release of monoclonal antibodies and Fc-containing molecules, making it ideal for small-scale applications in research and process development.

In this study, we systematically investigated electrically driven elution in preparative Protein A membrane chromatography. By varying buffer composition, salt type and concentration, as well as applied voltage, we aimed to gain deeper insight into the mechanisms governing electrically driven elution. The findings of the present study demonstrate that both the ionic environment and electrical parameters exert a substantial influence on elution efficiency and reproducibility.

Ongoing work includes direct comparisons with commercial Protein A resins and membranes, as well as in-depth characterization of eluates with respect to host cell protein (HCP) clearance, antibody stability, and aggregate formation under varied process conditions. This emerging technology has great potential not only for rapid analytical applications in early-stage antibody development but also as a future alternative for primary capture in downstream bioprocessing.

From Harsh To Mild: Redefining Protein A Elution With Excipient Modulation

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Protein A affinity chromatography is regarded as the gold standard for the capture of monoclonal antibodies (mAbs). This method is valued for its high selectivity, robustness, and established platform status. However, a significant challenge associated with conventional Protein A ligands is the necessity of low pH elution (typically pH 3–4), which can compromise antibody stability, resulting in aggregation, structural deterioration, and loss of activity. Addressing these challenges and optimizing the capture process has become a key focus, with growing interest in developing strategies that allow for milder elution conditions without sacrificing performance.

The present study investigates the impact of a detergent utilized in viral inactivation processes on a recently developed recombinant Protein A (rProtein A) ligand that has been immobilized on a membrane chromatography platform. This novel ligand has been demonstrated to facilitate antibody elution at a higher baseline pH (~5.0) in comparison to conventional Protein A ligands. The utilization of the detergent resulted in a discernible shift in the elution pH towards a less acidic range (pH 5.5–6.5), more closely resembling physiological conditions. A systematic investigation was conducted to explore this phenomenon under various conditions, including the variation of excipient concentrations, elution modes, product loads, and the timing of additive addition. In order to assess the specificity of this effect, a screening of structurally related detergents was conducted. The findings of the present study demonstrate that the pH shift is unique to this additive, thus highlighting a specific interaction mechanism.

A comparative study with commercial Protein A columns and membranes demonstrated that this pH modulation is not restricted to the novel membrane system, but is observable across multiple platforms, suggesting a broader applicability. The findings of this study indicate that excipient-driven modulation is a promising strategy for optimizing mAb capture processes, achieving milder elution conditions, and integrating viral inactivation strategies directly into the chromatography step.

Enhanced Monomer-Aggregate Resolution On Protein A Membrane Chromatography

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Protein A affinity chromatography is widely used for antibody capture and purification. By optimizing this critical step, downstream purification steps can be minimized, significantly reducing overall production costs. The primary goal of the separation process is to achieve maximum product purity by effectively removing impurities, including antibody aggregates, which are highly regulated due to their potential negative impact on product quality. Recent research has focused on improving the separation of monomeric antibodies from aggregates during pH gradient elution.
It has been shown that the addition of salts such as sodium chloride or calcium chloride in combination with polyethylene glycol (PEG) can synergistically improve the resolution of monomers and aggregates. In addition, pH plays an important role in this separation, as acidic conditions are known to promote aggregation, thereby reducing product purity. Therefore, avoiding low pH conditions is essential to prevent aggregation and optimize the yield of monomeric antibodies.
In this study, we investigate the use of a prototype rProtein A ligand that exhibits antibody elution at pH 5, which facilitates the separation of aggregates under a controlled linear pH gradient. Our results show that increasing salt concentration, especially sodium chloride, shifts the elution pH to higher values. When using an aggregate-enriched antibody solution, the monomer-enriched peak shifts to higher pH values, while a secondary peak at more acidic conditions correlates with high aggregate content, as confirmed by size exclusion chromatography (SEC). Notably, the resolution between monomers and aggregates is directly proportional to the salt concentration. In contrast to previous studies that required additional additives to improve separation, we achieved monomer purities approaching 100% without the need for additional additives.

Targeted Purification Of GLP-1 Therapeutics: Precision X® Ligands For Semaglutide And Tirzepatide

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Incretin-based peptide therapeutics, particularly glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor agonists, have demonstrated remarkable efficacy in managing type 2 diabetes and obesity. As the only drug class approved for both indications, they have gained significant clinical traction due to their dual benefits of glycemic control and weight reduction. This therapeutic success has heightened demand for scalable, robust, and GMP-compliant production strategies, especially as these peptides are increasingly expressed in complex systems such as *E. coli* or yeast. This study highlights the early-stage development of a targeted purification strategy for two prominent incretin mimetics: Tirzepatide, a dual GIP/GLP-1 receptor agonist, and Semaglutide, a GLP-1 receptor agonist. Leveraging Navigo Proteins' proprietary Protein A-derived ligand libraries, we identified specific affinity ligands designed to selectively capture these therapeutic peptides. Ligand candidates were screened and evaluated in clarified *E. coli* lysates, with characterization focused on binding affinity, specificity, and enrichment performance.

Our results demonstrate the ligands' ability to selectively purify target peptides while minimizing non-specific interactions, establishing the foundation for efficient downstream processing. The ligand platform, a core component of Navigo Proteins' Precision Capturing® technology, expands the utility of Protein A affinity chromatography beyond antibodies to the purification of non-antibody biologics. Precision Capturing® enables simplified, scalable purification workflows with high yield and purity, offering enhanced process predictability and GMP compatibility.

This novel platform meets the growing industrial demand for robust purification of recombinant peptides, addressing the limitations of conventional methods in scalability and reproducibility. By enabling seamless integration into various expression systems and production scales, Precision Capturing® stands out as a powerful and adaptable solution for advancing peptide therapeutics from research to clinical and commercial manufacturing.

Affinity Based Purification Of Hyaluronidase By Using Precision X® Ligands

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The enzyme Hyaluronidase catalyses the degradation of hyaluronic acid (hyaluronan), a major component of the extracellular matrix. In medicine hyaluronidase is used in combination with other drugs to lower the viscosity of hyaluronic acid, to increase the tissue permeability, to speed drugs dispersion and delivery. In cosmetic medicine it's also used to dissolve hyaluronic acid dermal fillers. As market demand continues to rise there is an increasing need for manufacturing processes that are not only efficient but also robust and compatible with current GMP standards. This need is especially critical when proteins are expressed in complex biological systems.
In this study, we present our developments in ligand discovery aimed at the targeted purification of Hyaluronidase. Using Navigo Proteins' proprietary Precision X® ligand libraries, we identified a set of affinity ligands tailored for the selective capture of this enzyme. The ligands were evaluated in the context of CHO supernatant and were characterized in terms of their binding affinity, target specificity and overall capture performance.
Our findings demonstrate that the selected ligands effectively enrich the target molecule while minimizing non-specific interactions, establishing a foundation for streamlined purification workflows. The underlying ligand platform Precision X® developed by Navigo Proteins is designed to extend the advantages of Protein A affinity chromatography, traditionally applied to antibody purification, to the broader space of non-antibody biologics. Through our Precision Capturing® technology, we offer a novel and proprietary approach that enables simplified process architectures while maintaining high standards for yield and purity. This platform is particularly wellsuited for the purification of targets, where conventional methods often fall short in scalability, reproducibility, or regulatory compliance. By enabling a more predictable and scalable purification strategy, Precision Capturing® provides a powerful tool for bioprocess development teams aiming to meet the rigorous demands of clinical and commercial manufacturing. It offers not only a high degree of operational robustness but also flexibility in adapting to diverse expression systems and process scales. This positions our platform as a unique solution for next-generation biologics manufacturing, supporting the advancement of novel peptide-based therapeutics from research through to GMP-compliant production.

Lab-Scale Tools For Advanced LV Downstream Processing

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Lentiviral vectors (LVs) are widely used as a gene delivery platform for the ex vivo generation of chimeric antigen receptor (CAR)-T cells in cancer immunotherapy. With the increasing demand for LV production, there is a growing need for materials and process strategies specifically tailored to these fragile, enveloped viral vectors. Established downstream processing (DSP) approaches for proteins, such as monoclonal antibodies (mAbs), cannot be directly applied due to the distinct physicochemical properties and size of LVs, necessitating careful process optimization. The shift to suspension-based LV production impacts DSP, particularly the harvest and clarification. Purification remains a bottleneck, as traditional anion exchange (AEX) and affinity chromatography often reduce viral infectivity. Therefore, chromatographic supports specifically designed for LVs are crucial to enhancing process efficiency. Additionally, while ultrafiltration techniques are commonly employed, they must be carefully optimized to prevent loss of viral activity. This study presents a complete lab-scale DSP workflow for clarifying, purifying, concentrating LVs produced in suspension HEK cell culture.

Bioseparation Controlled By Light: Engineering Of An IgG Fc-Specific Affitin For The Affinity Purification Of Antibodies

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Protein A affinity chromatography is widely used for antibody purification; however, it requires an acidic elution step that may result in protein aggregation and deamidation. We present an alternative strategy utilizing an engineered affitin based on the archaebacterial Sac7d scaffold. A stable monomeric variant, dubbed C3A24, was developed on the basis of a published Ig-binding affitin by substituting a solvent-exposed Cys residue within the binding interface by Ala. Unexpectedly, this further enhanced the affinity towards human IgG1, resulting in KD = 76 nM. X-ray crystallographic analysis of the affitin/Fc complex revealed that C3A24 binds to the CH2/CH3 junction within the Fc region, thus overlapping with the well-known binding sites of protein A and protein G as well as the neonatal Fc receptor (FcRn). The interaction between the affitin and the Ig Fc is stabilized by an extensive hydrogen bond network, which also involves its C-terminal residue, contrasting with previously published biochemical data.

To enable mild elution of IgG1 antibodies during affinity chromatography, the affitin C3A24 was functionalized with the novel Azo-tag, which comprises the light-responsive non-canonical amino acid p-(phenylazo)-L-phenylalanine (Pap). By mixing the culture medium containing the recombinant antibody with the affitin adapter molecule, a complex between these binding partners is formed. Applying this mixture to an α-cyclodextrin (CD) affinity column allows the specific immobilization of the antibody complex mediated by the N-terminal Azo-tag of C3A24. However, when the column is illuminated with mild UV-A light (355 nm) using a strip of cheap LEDs, a trans -> cis configurational change of the Pap side chain is triggered. This more bulky configuration can no longer bind to α-CD, which effects the quick and selective elution of the antibody-affitin complex from the chromatography column – free from any impurities (in particular albumin) and in a buffer of choice. This approach avoids any pH shift and allows antibody elution in a buffer directly suitable for immunochemical or cell culture assays. Combined with the light-responsive Azo-tag, the tiny C3A24 adapter molecule opens new applications in bioseparation and antibody research.

Expanded Bed Chromatography For The Preparative Age-Specific Separation Of Brewer's Yeast

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Expanded Bed Chromatography (EBC) offers a highly promising platform for the preparative separation of whole cells, as it allows the direct processing of crude feeds while circumventing the void volume limitations of conventional packed-bed columns. This makes EBC particularly suitable for isolating viable yeast subpopulations based on replicative age.
Yeast like *Saccharomyces cerevisiae* proliferates asexually via budding, with mother cells retaining bud scars — reliable markers of replicative lifespan and thus cellular age. Due to asymmetric division and therefore the accumulation of cellular damage in mother cells, age-related physiological differences arise, often favoring younger daughter cells in fermentation performance. Although yeast is a widely used microorganism in the food, beverage, and pharmaceutical industries, the impact of viability and age distribution on cultivation performance has yet to be fully understood.
To enable the preparative separation of mother and daughter yeast cells, a bifunctional affinity ligand targeting the chitin-rich bud scars of mother cells and chromatographic matrices has been developed. Its successful immobilization on silica and ion-exchange (IEX) particles allows for its integration into EBC workflows. While spherical IEX particles are typically used in EBC, it was demonstrated that stable expanded beds can also be established using cost-effective, non-spherical silica particles. Together, these findings establish EBC as a scalable and efficient technology for isolating viable yeast subpopulations, providing new experimental access to age-dependent phenotypes and supporting applications in strain selection, metabolic profiling, and the development of high-value whole-cell bioproducts.

Optimizing Antibody Immobilization For Affinity-Based Separation

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Affinity-based separation techniques enable the depletion and identification of bioactive components together with their interaction partners from complex and natural samples. For this purpose, the antibody-based separation technique offers a highly specific and efficient approach for isolating target proteins. It offers advantages such as preservation of protein structure. Therefore, in this study, we applied a Design of Experiments approach to optimize the functionalization of resins with antibodies for protein capture. Three different resins, namely TOYOPEARL AF-Tresyl-650M (Tosoh), ProfinityTM Epoxide Resin (Bio-Rad), and NHS Activated SepharoseTM 4 Fast Flow (Cytiva) were tested for covalent immobilization of a model antibody (polyclonal human IgG). Antibodies can covalently couple to all three resins via the reactive functional groups and amino groups of the amino acid residues of the antibody. Therefore, we have investigated different buffer systems, pH, and different NaCl concentrations on the antibody-to-resin coupling ratio, and overall, the immobilization efficiency. Based on the optimized results, the most suitable resins will be selected and functionalized with the target antibody to capture specific proteins from the natural samples. This follows the principle of immunoprecipitation, where antibody-functionalized resins selectively pull down the protein of interest along with its interacting partners.

Push Button, Get Nanoparticles: Pilot-Scale, End-To-End Automation For Magnetic Nanoparticle Production

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Magnetic nanoparticles (MNPs) underpin magnetic separation as an emerging process-intensification strategy for biomolecule purification, enabling direct capture from crude harvests and thereby reducing the number of unit operations and the overall process costs. However, industrial bioprocesses demand large amounts of MNPs. For example, a 1,000 L bioreactor with a protein concentration of 4 g/L would require about 40 kg of MNPs assuming conservative binding capacities of ~0.1 gProtein gMPNs-1. Looking at current market prices for unfunctionalized magnetite nanoparticles, reveals costs ranging from 512 Euro per gram (Micromod) to 25,800 Euro per gram (Sigma-Aldrich). At the lower end of this range, supplying the required amount of ~40 kg would already account for nearly 20.5 million Euro, rendering large-scale application economically unfeasible. Production costs are further driven by labor, which contributes nearly 50%, mainly because synthesis and washing are still frequently performed manually.

To overcome this limitation, the development of a novel fully automated pilot-scale process for MNPs production was targeted. We established a synergy between the newly developed synthesis reactor and a rotor–stator magnetic separator (RSMS), creating a truly end-to-end automated production line for MNPs. Through full automation, manual laboratory effort was minimized, while process performance improved relative to manual particle production: the yield increased by 8.8% (288 g of MNPs at 10 L scale), and the overall production time was reduced by 41%, without compromising critical physicochemical properties like magnetization or hydrodynamic particle diameter.

The system follows a holistic design and, via state-of-the-art communication protocols (OPC UA), integrates seamlessly into existing plant infrastructures. In the next phase, it will be coupled to an upstream bioreactor to complete a fully automated, end-to-end magnetic separation platform. We will benchmark this platform by techno-economic analysis and life-cycle assessment to determine whether it outperforms classical chromatography-based separation processes on cost and environmental footprint.

Potential-Controlled Membrane Affinity Chromatography: A Sustainable Strategy for Antibody Capture and Elution

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Protein A resin-based chromatography remains the gold standard for monoclonal antibody capture in large-scale bioprocessing, valued for its high selectivity. Yet, its reliance on acidic elution (typically pH 3.5) often leads to ligand degradation, partial antibody denaturation, and high buffer consumption during elution and neutralization.

Here, we present potential-controlled membrane affinity chromatography (pcMAC) as an alternative capture concept that enables antibody recovery without harsh pH shifts. In our approach, a Protein A-functionalized membrane is integrated into an electroseparation cell positioned between two electrodes. At neutral pH, 1 mg of trastuzumab was loaded at 0.5 mL min^{-1} with >95% binding efficiency. Antibody release was then triggered by applying an electric potential of 2.5 V, yielding 92% recovery within 4 minutes after initializing the elution step. Under identical flow conditions, conventional acidic elution required 12 minutes to achieve 90% recovery.

Product quality was verified by non-reducing SDS-PAGE and ELISA, confirming intact structure and preserved binding activity. Notably, the electric-driven process maintained higher functionality of human IgG compared to acid elution, highlighting benefits for pH-sensitive molecules. Process evaluation further showed a 65% reduction in buffer consumption. Over multiple cycles, binding capacity and performance remained stable, underlining the robustness of the membrane system.

This study demonstrates that electro-modulated Protein A membranes can provide fast, mild, and resource-efficient antibody purification. By eliminating acid-based elution and buffer-exchange steps, pcMAC combines sustainability with high performance and offers a promising route for next-generation biomanufacturing.

Continuous Protein Affinity Chromatography Using Silica-Binding Peptides

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Due to its high mechanical strength and easy manufacturing, silica gel is often used as a stationary phase in different chromatographic processes but not as an affinity material. Its relatively low cost compared to other chromatographic resin materials makes it an attractive choice. Silica gel is 20- to 200-fold cheaper than Ni-NTA, one of the most used resin materials in popular Immobilized-Metal Affinity Chromatography (IMAC). To achieve high-purity protein capture, silica gels can be combined with selectively silica-binding amino acid tags, as the in-house developed octapeptide (RH)4 tag, namely the PosH-Tag. Due to electrostatic interactions of the deprotonated silanol groups with the tag's arginine groups, the PosH-Tag binds to bare silica gel by the mechanism of ion-pairing. Based on preliminary studies showing that the model protein PosH-tagged GFP can be purified with a yield of 90–95 % and a purity of 90–93 % from *E. coli* cell lysate in a single-step chromatography column, we show here the transferability to industrially relevant proteins of interest (POIs) and the implementation in a continuous multi-column chromatography. Analysis with the AI-based prediction tool AlphaFold 3 show that the N-terminal tag has no apparent influence on the conformation and isoelectric point of the studied POI, and that it can even lead to a stabilization of the protein.
Experiments of the single-column silica-based capture of the POI are supported in silico by mechanistic modelling using CADET process, which significantly reduces the overall workload. The obtained model-based and experimentally validated basic parameters are then used in the next step for the design of the continuous process. The latter is carried out according to the principle of periodic counter-current chromatography (PCC).

Exploring The Power Of Membrane Chromatography For Effective HSV-1 Purification

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Oncolytic viruses (OVs) represent a promising strategy for cancer treatment, selectively targeting and lysing tumor cells while stimulating strong anti-tumor immune responses. Among the various OVs, Herpes Simplex Virus 1 (HSV-1) vectors are well-recognized for their extensive research validation and large capacity to deliver therapeutic transgenes to diverse cell types. However, their large particle size poses significant challenges in downstream purification, particularly with resin-based chromatography, underscoring the need for alternative purification strategies.

To overcome this bottleneck, research has focused on convective chromatography media. Platforms such as membranes, nanofibers, monoliths, and superporous resins feature highly interconnected, large-pore structures that enable efficient mass transfer via convective rather than diffusive transport. In this study, we examine membrane chromatography (MC) as an underexplored strategy for HSV-1 purification. The flat, highly porous membrane architecture provides advantages, including rapid processing, reduced buffer consumption, a smaller system footprint, and compatibility with single-use processing workflows.

We systematically evaluated various commercially available membrane types, including six anion exchange (AEX), two cation exchange (CEX), and one pseudo-affinity type, for their suitability in HSV-1 purification. Process performance was assessed based on virus recovery (infectivity and genome copies) and contaminant removal (total protein and double-stranded DNA, dsDNA), aiming to maximize impurity clearance while preserving viral integrity.

Among the tested membrane adsorbers, Sartobind Convec D and Mustang Q demonstrated the most promising results, each yielding ~30% recovery of infectious virus. Sartobind Convec D achieved excellent protein clearance (94%) and moderate dsDNA reduction (63%), whereas Mustang Q removed 86% of dsDNA and 24% of total protein. For benchmarking, these membranes were compared to convective chromatographic monoliths previously applied to HSV-1 purification, namely CIMmultus QA and CIMmultus DEAE. In screening experiments, CIMmultus™ QA achieved a 27.3% yield of infectious virus, while CIMmultus™ DEAE yielded only 7.0%.

Our study identified membrane adsorbers particularly effective for HSV-1 purification. These finding supports the applicability of MC for this purpose and provides a solid foundation for future process development studies.

HIV-1 Gag Virus-Like Particle Purification: A Scalable Chromatographic Approach

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The development of bionanoparticle-based pharmaceuticals, such as virus-like particles (VLPs), offers great potential for addressing medical needs in gene therapy, gene editing, and vaccine development. These innovative therapeutics not only offer solutions for rare and currently incurable diseases but also enhance preparedness against emerging infectious diseases. However, the manufacturing of bionanoparticles remains a challenge due to limited understanding of how process conditions affect product recovery, economy and quality.
In this study, we present a scalable platform for the production and purification of HIV-1 gag VLPs using a combination of advanced chromatographic and filtration techniques. Initial small-scale experiments involved the treatment of harvested cell culture fluid with salt-active nuclease SAN to reduce chromatin and DNA impurities. The clarified material was further purified using Capto Core and Capto Heparin chromatography, followed by tangential flow filtration (TFF) for polishing and concentration. To demonstrate scalability, the purification process was successfully upscaled by increasing the bed volume of the chromatography columns and the filter area used for TFF.
Our results demonstrate the effectiveness of this integrated purification strategy in achieving substantial depletion of cell-derived impurities, including 98% DNA removal and 85% protein removal, resulting in high-purity HIV-1 gag VLPs while maintaining scalability. This work advances bionanoparticle manufacturing by enabling rapid, scalable production and strengthening biopharmaceutical capacities to address potential drug shortages and pandemic preparedness.

Demonstration Of Scale-Down Model For Continuous-Flow Ultracentrifugation Using Sucrose Gradients For Particle Separation

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Bionanoparticles (BNPs) such as virus-like particles and adeno-associated viral vectors (AAVs) are increasingly used in vaccination and gene therapy. Continuous-flow ultracentrifugation (CFUC) has long been used for large-scale vaccine production, enabling high-throughput processing and overcoming the scale-up limitations of tube-based ultracentrifugation. For academic and early-stage industrial laboratories, process development at pilot scale is impractical due to the large material requirements.
Manufacturer data from Alfa Wasserman indicate linear scalability across their CFUC systems using Iodixanol gradients. Recent studies [1,2] showed that CFUC can achieve recovery yields above 50% for AAVs in a single capture step at research (Promatix 1000TM) and pilot (PKII) scales using sucrose or Iodixanol gradients. Nonetheless, no study has tested whether a BNP purification strategy using sucrose gradients, developed at one scale, can be linearly transferred to another CFUC system and achieve equivalent purification.
This work replicates gradients used at pilot-scale and downscales them to research scale. The goal is to identify operational parameters that preserve gradient shape and particle banding at consistent densities across systems and to develop a scale-down model. Demonstrating this model using synthetic beads, such as polymethyl methacrylate, in sucrose gradients proves that scale-down studies using smaller volumes are feasible. This approach can be applied to BNP purifications using CFUC, thus facilitating early-stage process development.

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Modulating And Understanding Retention Of Proteins On Chromatographic Support By Changing Cation-Exchanging Ligand

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Ion-exchange chromatography is widely utilized for large biomolecules separation and purification both at analytical and preparative scale. In last decades, multimodal ion chromatography has emerged as a complementary approach, especially for difficult protein mixtures or cases where conventional ion exchange shows limited discrimination. The complexity of protein binding to ion exchangers stems from numerous parameters affecting retention, presenting a challenging yet intriguing area of study.
Our research investigates the effects of sulfonate (-SO3—) and multimodal cation-exchange (CEX) ligand on the chromatographic separation of two basic proteins: equine cytochrome c (Cyt) and chicken egg-white lysozyme (Lys). Chromatographic monoliths were used as a stationary phase in ascending salt gradient for protein elution. Despite some similarities in protein sizes and pI, the effect on the retention time, when replacing sulfonate ligand with multimodal CEX ligand, is more pronounced for Lys than for Cyt. The goal of the study is to describe the chromatographic differences by performing and interpreting thermodynamic analyses for Lys and Cyt adsorption at different binding conditions.

Optimization Of Preparative-Scale mRNA Capture Using Small-Scale Oligo dT Affinity Monoliths

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Sample availability during early process development is often limited and its production financially or time-consuming. Reducing sample consumption enables screening of a broader range of conditions needed to achieve an optimised process. In chromatography, the bed volume of the resin is inversely proportional to the number of realisable experiments with a fixed quantity of sample. CIMmic discs are chromatographic monoliths with 100 µL bed volume and properties comparable to the preparative CIMmultus line; therefore, they can be used for initial screening and developing preparative purification methods for a variety of large biomolecules. This column format can be operated with HPLC and FPLC systems, or even manually by connecting the column to a syringe.
Coupling oligo-deoxythymidilic acid (Oligo dT) probes to monolith surface yields affinity chromatographic columns, capable of selectively binding messenger RNA (mRNA) via hybridization between Oligo dT and mRNA's poly-adenylic acid (poly A) tail. Here we present the optimization of mRNA dynamic binding capacity (DBC) using CIMmic Oligo dT discs. With the help of CIMmic technology and down-scaling the experiments we were able to utilize a limited amount of mRNA to increase DBC from 3-4 mg/mL up to 8 mg/mL by optimizing the flow rate and buffer composition.

Novel Protein A And L Ligands For The Purification Of Multispecific Antibody-Based Therapeutics

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Affinity chromatography is a widely used technique for the purification of monoclonal and multispecific antibodies. However, the inherent complexity of multispecific antibodies could present challenges due to the presence of product-related impurities. In this study, the performance of the highly specific resins MabSelect VH3 and MabSelect VL were compared to MabSelect PrismA and Capto L using two multispecific antibody formats: IgG-scFv2 and scMATCH3. Resin performance was assessed through dynamic binding capacity and bind-and-elute experiments focusing on elution profiles, monomeric content and the separation of high and low molecular weight species. The impact of residence time on resin performance was also explored.

Modular High-Throughput Platform For The Purification Of scFvs And Multispecific Antibody-Based Therapeutics

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Rapid and efficient production of single-chain variable fragments (scFvs) and multispecific antibodies (msAbs) is essential in early drug discovery to screen hundreds of candidates for pharmacodynamic and biophysical profiling. Customized robotic systems capable of handling vast arrays of molecules are large and expensive and therefore make their use in drug discovery unattractive for many biotech companies and academic institutions. Additionally, many high-throughput methods using plate or tip column formats often fail to yield sufficient quantities with acceptable monomeric purity required for extended biophysical characterization and stability studies to evaluate developability and manufacturability. Here, we describe an ÄKTA system-based high-throughput platform using mostly commercially available devices and modules that can easily be implemented in academic or industrial R&D environments. The platform consists of (1) ASX-560 autosampler (AS) and ALIAS Bio PREP AS systems to automate injections, (2) multi-column purification protocols to automate and streamline manual handling steps and (3) novel membrane chromatography to reduce the method run time. As a result, these versatile and automated multi-column purification protocols enable the cost-effective production of hundreds of scFvs and msAbs per week, yielding large quantities with high monomeric purity.

Affinity Purification As A Platform For Improved Plasma-Derived IgG Manufacturing

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Conventional production of IgG from human plasma predominantly relies on ethanol-based fractionation techniques. Although well-established, these methods often involve compromises between yield and purity and have a high level of process complexity. This study presents a novel purification strategy based on affinity chromatography as an efficient and scalable alternative for IgG isolation from human plasma. In addition, we will explore optimization methods for an ion exchange polishing step using a high-performance anion exchange resin.

The suggested purification platform efficiently captures high-purity IgG from plasma, delivering yields that exceed those obtained through conventional fractionation. In addition, this method facilitates recovery from side fractions where IgG is typically lost during fractionation, resulting in a significant increase in overall yield.

The data summarized in this poster demonstrate that the affinity purification approach results in high recovery of functional IgG and effective reduction of key impurities, including IgA, IgM, and activated coagulation factors such as FXI/FXIa. Moreover, the subclass distribution of the purified IgG reflects that of native plasma. In addition to product quality improvements, the affinity resin shows robust reusability across multiple cycles, offering a cost-effective option for large-scale processing. When further impurity depletion is required, we show efficient polishing methods via anion exchange chromatography that can increase purity while optimizing yield and preserving subclass distribution.

This affinity-based platform provides a flexible and efficient alternative for plasma purification by eliminating the need for multi-step ethanol precipitation cascades.

Scalable Purification Of Adherently-Produced Lentiviral Vectors Using Isotag™ LV Reagent

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The use of Lentiviruses (LVs) as vectors has greatly increased in gene and cell therapies, due to their potency, safety, and large cargo capacity. Current manufacturing methods rely heavily on chromatography-based workflows, which are not optimal for the purification of large viral vectors. IsoTag™ LV reagent is a proprietary biopolymer that contains a specific binding domain for VSVG and is able to reversibly encapsulate VSVG-pseudotyped vectors, in the presence of heat and salt. This affinity based approach has the advantage of selectively separating LVs in a liquid-liquid environment, which in turn protects LVs from the forces that are involved in the purification procedure. Taking advantage of the unique properties of IsoTag™ LV reagent, the downstream processing workflow eradicates the use of chromatography and replaces it with a scalable Tangential Flow Filtration (TFF) process. The system has been validated for the purification of up to 3.3L of adherently-produced LV harvest material, where IsoTag™ LV technology selectively purifies LVs in shear conditions up to 6000s-1. Furthermore, the effects of clarification and nuclease treatment were explored using this process, with no evidence of a significant benefit to applying such treatments with this method. Lastly, the addition of IsoTag™ LV reagent prior to freezing was explored, with infectious LV recoveries as high as 78.9% with preincubated harvest material, and little to no aggregation in purified products by Dynamic Light Scattering (DLS). This approach to purification offers significant advantages in LV purification, with emphasis on LV purity and contaminant removal profiles, which meet or exceed current industry standards, as well as a significant reduction in processing time.

Optimizing Environmental Sustainability In Bispecific Antibody Manufacturing

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The environmental impact of manufacturing processes is becoming increasingly important in the biopharmaceutical industry. Here we present a comprehensive cradle-to-grave life cycle assessment (LCA) comparing the environmental impacts of legacy and modern protein L affinity chromatography resins used in the bispecific antibody (bsAb) capture process. The assessment covers the entire resin life cycle, including chromatography resin manufacturing, distribution, use phase, and end-of-life. We will highlight the importance of selecting high-capacity chromatography resins and optimizing various process parameters to enhance environmental sustainability in bsAb manufacturing. These findings provide valuable insights for biopharmaceutical companies aiming to reduce their environmental footprint while maintaining efficient production processes.

The significantly higher dynamic binding capacity of a modern protein L chromatography resin compared to a legacy protein L resin results in reduced buffer consumption and a smaller equipment footprint. When cleanrooms are operated with carbon-intensive electricity, the emissions related to cleanroom energy consumption become a significant hotspot. In such cases, transitioning to a high-capacity resin, which allows for a smaller cleanroom area, can lead to substantial carbon footprint reductions.

The study also emphasizes the importance of efficient cleanroom utilization especially in countries with high-carbon intensity electricity. Increasing the number of products and thereby batches per year in a multiproduct facility by using larger columns and running fewer cycles (shorter batch process time) can reduce the overall carbon footprint, even if the chromatography resin's lifetime is not fully utilized. Conversely, when renewable energy sources are used during the cleanroom operation phase, the resin manufacturing footprint may become the primary hotspot if the resin lifetime is underutilized.

Furthermore, process optimizations including the use of alternative buffers and storage solutions achieves a notable reduction in carbon footprint compared to the baseline process. Improved chromatography system sanitization methods, along with the use of disposable bags for elimination of cleaning and sanitization of stainless-steel tanks will further help reduce both carbon and water footprints. The environmental impact of distributing single-use disposables by intercontinental transport by either boat or air is also examined.

Intensifying AAV9 Capture: The Role Of Nanofitin® Affinity Ligands For Streamlined Purification Processes

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In this study, we demonstrate the potential of the innovative Nanofitin® affinity ligand technology for adeno-associated virus serotype 9 (AAV9) purification, representing a versatile, scalable and cost-effective affinity solution for bioprocesses. This research advances AAV-based therapies production by providing a reliable technology for the efficient purification of AAV9, thereby paving the way for enhanced therapeutic applications in gene therapy.

The purification of AAV is a significant challenge in the field of gene therapy, primarily due to low yields and limited chromatography resin reusability. Current chromatographic purification methodologies frequently exhibit inadequate binding capacities for AAV capsids and result in suboptimal recovery rates. Furthermore, reduced caustic stability adversely affects throughput, thereby escalating the overall cost of goods.

This study aimed to establish a novel affinity chromatography purification solution utilizing the proven Eshmuno® chromatography resin base-bead technology, incorporating a high-binding Nanofitin® affinity ligand specifically engineered to enhance performance in capturing AAV9. We executed a feed stream study with AAV9 derived from various expression systems, including HEK and insect cells, achieving a dynamic binding capacity exceeding 1E+14 vp/mL at a one-minute residence time. During cycling studies, we implemented a cleaning-in-place (CIP) procedure utilizing sodium hydroxide after each cycle, successfully completing 15 cycles while maintaining high yields and AAV9 binding capacity.

Optimizing Peptide Purification: HPLC Scale-Up Process For High Purity And Efficiency

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Peptides have emerged as promising therapeutic agents in recent years, offering high specificity and selectivity in treating various diseases. Effective peptide purification is essential to ensure drug efficacy and safety. Here, we present a systematic workflow for the linear scale-up of high performance liquid chromatography (HPLC) methods to purify synthetic peptides, using Angiotensin I, a peptide precursor in blood pressure regulation, as a model. The scale-up process from an analytical HPLC method involved gradient optimization, method adjustments to parameters such as particle size, flow rate, and column dimension, as well as overload studies to ensure the consistent purification of the crude peptide. The KNAUER HPLC Method Converter [1] was used to calculate the preparative scale method parameters.

The optimized preparative HPLC method resulted in a purity increase of Angiotensin I to over 99% with minimal sample loss. To further enhance throughput and flexibility, a preparative liquid handler could be integrated into the system allowing for injection of large volumes and quantities, as well as fraction collection and re-injection of target fractions using one instrument.

Our approach can serve as a guideline for efficient peptide purification workflows, optimizing both purity and productivity while maintaining chromatographic performance at a larger scale.

Immobilized Recombinant FcγIIIa Receptor And FcγIIIa Receptor As A Useful Tool For Characterization Of Human IgG Antibodies

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Glycosylation is one of main sources heterogeneity of therapeutic preparations based on monoclonal antibodies as well as intravenous polyclonal human immunoglobulin G concentrates. Posttranslational modifications of monoclonal antibodies can significantly impact their therapeutic mechanism of action, and also some undesired side effects. As a consequence, monitoring of glycosylation of therapeutic antibody concentrates is a critical part of development as well as quality control of these important biopharmaceuticals. The main reason for observed changes related to IgG activity is the impact on antibody capability to bind receptors such as FcγRs or C1q important for carrying out the function. Monitoring of the glycosylation of polyclonal immunoglobulin G concentrates can give additional information about quality and origin of these therapeutic preparations. Present work opens a new way for parallel determination of glycosylation changes of IgG antibodies by use of affinity columns with immobilized FcγIIIA receptor and newly developed affinity columns with immobilized neonatal Fc receptor (FcRn). In order to demonstrate differences in their specificity these two columns were parallelly tested with two commercial concentrates of human IVIG. Future use of these methods for diagnosis and prognosis of and possible disease treatment was discussed.

A Scalable Weak AEX-HIC Mixed-Mode Chromatography Resin For Biologics Purification

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Chromatographic purification is a significant step in the biomanufacturing of biomolecules. Traditionally, bioprocess manufacturing involves two to four orthogonal steps using anion exchange, cation exchange, or hydrophobic interaction chromatography after the initial capture step. However, achieving high purity and yield with single-mode ion-exchange chromatography has often been challenging. To address these limitations, the implementation of multimodal chromatography has significantly increased, reducing the number of steps and improving overall process economics while achieving sustainability goals.
A new anion exchange (AEX) hydrophobic (HIC) multimodal resin, Nuvia wPrime 2A Media, with modulable selectivity, was employed to design a mAb purification process in a flow-through mode. This process demonstrated a tenfold reduction in high molecular weight species while achieving high mAb monomer recovery.
The ability to adjust the resin's charge provides a broad design space for the purification of monoclonal antibodies and other molecules like bispecific antibodies (bsAb), proteins, and virus-based therapies including adeno-associated virus (AAV) therapies. It may also be used for diverse new constructs in development, including those that lack affinity handles and/or have an isoelectric point (pI) that is close to the pI of an unwanted molecule.
In conclusion, utilizing multimodal chromatography resins such as Nuvia wPrime 2A Media provides a versatile and efficient approach to the purification of biologics, enhancing the downstream process and ensuring high-quality product recovery.

Saving Lives - Innovative Ion Exchange Resin Therapeutic Applications

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Chromatography resins are commonly used in biomolecule purification and analysis for therapeutic purposes because they can produce high-yield, high-purity end products. Recently, it has been found that these resins, beyond their analytical and manufacturing roles, also have unique properties that make them suitable for use in therapies administered directly to patients. Bio-Rad Aminex Resins, made from a styrene divinylbenzene copolymer with sulfonic acid functional groups, are strong cation exchangers and have been used in microsphere-based therapies for clinical applications. This discussion highlights the significant potential of these resins in treating human diseases, including cancer. The versatility and effectiveness of these resins make them a valuable tool in the development of innovative medical treatments.

Establishment Of A Robust, Scalable And GMP-Compliant AAV Manufacturing Platform

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The increasing demand for AAV-based gene therapies requires robust and scalable manufacturing platforms. To meet this need, we developed and implemented a streamlined, easily adaptable (to various AAV serotypes) workflow for production (in HEK293T cells and stirred-tank bioreactors), purification (using a two-step chromatographic scheme), and characterization (using an array of biophysical and biochemical methods) of AAVs. Validated at 2 L and 50 L scales, the platform achieved high AAV recovery yields, full capsid enrichment, and low impurity levels. Overall, this platform offers a robust and efficient alternative to current AAV manufacturing, supporting broader gene therapy applications.

Incorporating Virus Stability Into An Oncolytic HSV-1 Purification Strategy

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The development of downstream processing strategies for therapeutic viruses remains a volatile field with few standards. The reasons for this are obvious: The structural variety and complexity of the viral assets and their sensitivity to infectivity loss call for individual adaptation. In this project, we aim to develop an efficient downstream processing strategy for an oncolytic Herpes Simplex Virus type 1 (HSV-1) based on its structural and stability characteristics.
HSV-1 is among the most commonly employed viruses for viral oncotherapy applications. In our group, the TheraVision platform for personalized virus-immunotherapy was developed based on a modified oncolytic HSV-1. The TheraVision HSV-1 was genetically engineered to combine targeting to tumor cells for viral-mediated oncolysis and immune-cell-mediated therapy in a single viral vector, showing efficacy, e.g., in an animal model for lung carcinoma.
Concerning the manufacturing of HSV-1, little data is published about suitable downstream processing strategies. However, as shown, the big dsDNA virus (~160 nm) is prone to aggregation and loss of infectivity. Thus, we conducted studies on the virus stability and its physicochemical characteristics to incorporate it into the downstream processing strategy. Degrading factors were expected to be thermal, chemical, and mechanical. Additionally, to stabilize processing, sugar addition, depending on the processing state, was considered. Overall, elevated temperatures and phase transition during freeze-thawing were found to be significant problems throughout processing. The effect gained weight with increasing purity of HSV-1. Sugars and sugar alcohols, like sorbitol, were able to limit loss of infectivity.
With this knowledge, we propose a processing train focusing on in-process addition of stabilizers, e.g., sugars, and reduced processing and holding times.

Additive Manufacturing Techniques For Immobilisation Of T7 RNA Polymerase

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mRNA vaccines have been used as the main technology to fight the recent COVID-19 pandemic, which proved the importance of RNA-based therapeutics [1]. The sudden growth in mRNA vaccines caused an immediate demand for cost-effective methods for large-scale mRNA production.
Enzymatic processes have been progressively adopted as green and sustainable methods for chemical manufacturing. Enzyme immobilisation is a proven technique that facilitates enzyme handling and their separation from the product [2]. However, the method has not been extensively applied for the manufacturing of mRNA vaccines.
Additive manufacturing (AM) is a versatile fabrication technique that enables to achieve structures with fully customizable design. In the context of protein synthesis, 3D-printed supports for enzyme immobilisation have shown significant potential to lower production costs, enhance process sustainability, and accelerate manufacturing timelines [3].
This work is concerned with combining the aforementioned techniques to develop an enzyme-immobilised 3D-printed bioreactor prototype for large-scale mRNA production. Preliminary data showing the effectiveness of purification and immobilisation of T7 RNA Polymerase will be presented.

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The Importance Of Chromatin Removal In Bionanoparticle Purification

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In biopharmaceutical manufacturing of gene therapies and viral vaccines, chromatin-bound DNA presents a significant challenge due to its complex structure and charge variability. This form of DNA is often overlooked by standard detection methods, such as the PicoGreen assay, and is co-purified with bionanoparticles (BNPs). Regulatory guidelines from the FDA require residual DNA levels to be below 10 ng per dose, with DNA fragments shorter than 200 base pairs. However, conventional detection methods frequently underestimate chromatin-bound DNA, and enzymatic degradation using endonucleases like Benzonase is often ineffective.
Salt-active nucleases address this limitation by maintaining high enzymatic activity in high-salt environments. These nucleases retain specificity for nucleic acids even under physiological or chromatin-decondensing salt conditions, overcoming the charge-shielding effects that impair traditional nucleases. For enveloped BNPs, such as HIV-1 Gag virus-like particles (VLPs) and measles virus, the integration of salt-active nucleases into downstream purification workflows improves chromatographic performance. This is achieved by reducing DNA-driven column fouling and enhancing product yield through optimized impurity clearance.
Our findings highlight the critical importance of targeted chromatin detection and removal in bionanoparticle manufacturing. The application of salt-active nucleases is particularly impactful for viral vectors (e.g., adeno-associated viruses, AAVs) and vaccines, where residual DNA poses a risk to product safety and regulatory compliance.

Lysis Conditions And Comparison Of Anion Exchange Resins For Purification Of Untagged Recombinant Pneumolysin

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Streptococcus pneumoniae is a Gram-positive bacterium responsible for respiratory tract and other life-threatening diseases. The current vaccines based on capsular polysaccharides have low serotype coverage, leading to a selective pressure and serotype replacement that mitigates the benefits of vaccination with time. Therefore, novel protein-based vaccines have been investigated to provide serotype-independent protection. This work aimed to evaluate different anion exchange resins to purify the recombinant genetically detoxified pneumolysin (PdT), a promising vaccine protein antigen. Moreover, due to environmental issues related to Triton X-100, different detergents were evaluated to replace it for cell lysis. PdT was cloned into a pET28a vector and produced in fed-batch culture of *E. coli* BL21(DE3). Three detergents were evaluated to replace it for cell lysis: Tergitol 15-S-9, Virodex TXR1, and Virodex TXR2. Cells were disrupted using a high-pressure homogenizer at 70/700 bar, and optical density and protein release were monitored up to 4 cycles. After centrifugation, the clarified sample was concentrated and diafiltrated in a 10 kDa membrane. The following resins were evaluated for the capture step: Capto-DEAE, DEAE-Sepharose FF, Praesto Q45, Praesto Q65, and Fractogel TMAE, all commercially available in packed columns of 4.7 mL and 10 cm in height. Virodex TXR1 showed the best lysis results and the highest amount of PdT was released after the third passage. The main challenge faced during purification was the presence of a host-cell protein (HCP) with the same molecular mass as the target, which leads to overestimation of PdT in the raw material. Most of this HCP was eluted before PdT in a linear gradient from 0 to 1 M NaCl at pH 7. The comparison between Capto-DEAE and DEAE-Sepharose FF showed that the latter exhibited better performance, reaching a purification factor of 1.6-fold, whereas Capto-DEAE reached 1.2-fold. Despite greater nominal binding capacity, Capto-DEAE showed lower PdT recovery than DEAE Sepharose FF, emphasizing the importance of selecting an adequate resin. The ongoing investigation will indicate which factor is more important for PdT separation: the homogeneity of Praesto beads or the surface extenders present in Capto and Fractogel resins, compared to the traditional non-grafted Sepharose.

Manufacturing Of AAV And LV Starting Plasmids In GMP-Quality

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Vectors based on the adeno-associated virus (AAV) and on lentiviruses (LV) have emerged as the most preferred concept in gene therapy. Manufacturing of the respective starting plasmids in high quality and quantity has become a bottleneck in the supply chain of viral vector-based gene therapies, posing a considerable challenge for plasmid manufacturers. Biomay has established a proprietary manufacturing platform for AAV and LV starting plasmids under the quality standards of Good Manufacturing Practice. For manufacturing, *E. coli* Master Cell Banks were generated, which were then used in a high cell-density fed-batch fermentation process to produce the plasmids. After alkaline lysis of the cell mass, the plasmids were purified in a two-step chromatography process and formulated by ultra-/diafiltration followed by a final aseptic filling.

Manufacturing has been optimized to cover the most relevant AAV serotypes and large adenovirus helper plasmids as well as the packaging, envelope and transgene plasmids of the second and third generation of recombinant LV plasmids. Our results prove that the process is well scalable (from milligrams over multiple grams up to ~100 g) and can accommodate different *E. coli* host strains with comparable outcomes. Plasmid quality was characterized by a comprehensive set of analytical methods. Particular attention was paid to confirming the identity and integrity of repetitive DNA elements (ITR). Our data show high plasmid homogeneity (>90% of supercoiled conformation), low host-cell derived impurity and high batch-to-batch consistency across all AAV and LV plasmids manufactured.

In addition to custom manufacturing, a concept has been established to provide ready-to-use AAV and LV starting plasmids in GMP quality on an off-the-shelf basis. The set of Rep/Cap plasmids include the major AAV serotypes used in current clinical studies (AAV1/ 2/ 3/ 5/ 6/ 7/ 8/ 9 and rh10). An optimized Ad helper plasmid with minimized size was designed, constructed and manufactured. Suitability of the starting plasmids for AAV and LV virus particle manufacturing in HEK293 cells was confirmed by quantifying the vector genome titer by qPCR, and the capsid titer by ELISA. The functionality of the virus particles carrying a GFP reporter transgene was shown by evaluating the transduction efficiency in HT1080 cells.

With our established platform, more than 20 different AAV and 6 LV starting plasmids have been manufactured under GMP conditions to date.

Quality Control Of cGMP Grade Recombinant Cas9 Nuclease For Human Therapeutic CRISPR/Cas9 Genome Editing Applications

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Genome editing with the CRISPR/Cas technology has revolutionized molecular-biological methods and enabled novel therapeutic approaches. Here we report manufacturing and quality control of the nuclease Cas9 compliant with current Good Manufacturing Practice (cGMP) standards, which serves as an essential component of a recently market-approved ex-vivo gene-editing therapy against sickle cell disease (SCD) and transfusion-dependent beta-thalassemia (TDT).

The Cas9 endonuclease for human therapeutic use must meet stringent, pre-defined quality standards for protein characteristics and batch-to-batch consistency to ensure safety and efficacy. Accordingly, we developed and validated a panel of analytical assays for routine in-process control and final quality control, establishing specifications for critical quality attributes (CQAs) including homogeneity, aggregate content, enzymatic activity, protein concentration, and residual host cell impurities.

Extended biochemical characterization confirmed the Cas9 amino acid sequence and structural integrity, and quantified post-translational modifications. Long-term stability studies showed that Cas9 retains its integrity and functional activity over multiple years under various storage conditions, with no degradation observed. Forced degradation studies under extreme physico-chemical conditions were performed to evaluate the resilience of Cas9 in terms of activity and integrity.

Gene editing activity was analyzed in a cell-based assay: delivery of the Cas9-guide RNA ribonucleoprotein complex into HEK293 cells generated targeted insertion/deletion mutations (indels) in at least 60% of alleles, demonstrating robust on-target efficiency.

In total more than twenty GMP compliant batches of Cas9 including final bulk drug substance and sterile filled product were manufactured, each consistently meeting all pre-defined criteria for CQAs and stability. A robust and high-yield manufacturing process for Cas9 was developed using relevant in-process control analytics, enabling a continuous supply of high quality Cas9.

This comprehensive overview addresses the key concepts, challenges, and results of the late-stage clinical / pre-market analytical characterization of GMP-grade recombinant Cas9 for therapeutic use.

Smart MCC Integration For A Sustainable Downstream Future

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This work presents the integration of multi-column chromatography (MCC) using the Octave BIO system into a continuous biomanufacturing platform. The complete, bacteria-based continuous manufacturing system was developed through a six-partner collaboration within the ECOnti project. We will highlight the software interface, command architecture, and the MCC's responsiveness to titer fluctuations. Furthermore, we will explore the broader potential of this approach for seamless integration into end-to-end processes, simplifying the adoption of MCC in continuous downstream processing.

Continuous Multi-Column Chromatography – A Valuable Tool For Flexible CDMO Manufacturing?

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The poster explores the potential of continuous multi-column chromatography as a tool for flexible CDMO manufacturing. It compares two approaches: a fast high-cycling affinity capture using conventional batch mode, and a periodic-counter-current (PCC) and multi-column chromatography (MCC) system for continuous capture. The evaluation includes productivity, buffer consumption, and total cost of goods across different harvest titer scenarios. Interestingly, the PCC approach does not offer benefits for all scenarios, and even the advantages of the MCC approach are limited. Our high-cycling STORY (space-time-optimized resin & yield) approach remains the most favorable for early phase clinical supply. The key advantage of MCC approaches lies in their high degree of automation. Utilizing innovative orthogonal materials (resins, functionalized filters, etc.) that allow for high automation or intensification will be more valuable for us as a flexible CDMO with a broad and diverse product pipeline.

Development Of An Automated Tangential Flow Filtration For mRNA Using Model Based Control And Real-Time Monitoring

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Efficient downstream purification of mRNA vaccines is essential for ensuring product quality and process scalability. Traditional methods rely heavily on chromatography, which while effective, significantly increases production costs. To address these challenges, we propose an alternative strategy that exclusively relies on mRNA purification via filtration under dynamic buffer conditions, utilizing varying membranes. This approach aims to provide a scalable, cost-effective solution for impurity removal.

The success of this strategy depends on precise control of process parameters and real-time monitoring of product attributes. To achieve this, we are developing an advanced tangential flow filtration (TFF) platform that integrates multiple layers of process intelligence. The system combines hard sensors—such as pressure, conductivity, and mass flow—with model-based soft sensing. Future integration of infrared-based spectroscopy will enable online monitoring of product-related attributes, such as mRNA integrity and residual impurities.

Designed for autonomous operation, the platform will leverage a hybrid control model that merges feedback and predictive control strategies. This allows real-time adjustments to operating conditions, ensuring the process remains within predefined design spaces. By embedding Quality by Design (QbD) principles, the system will continuously monitor and control Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs), reducing variability and enhancing robustness. Ultimately, this integrated approach aims to lower production costs, improve scalability, and deliver consistent product quality, thereby accelerating the development and manufacturing of mRNA-based therapeutics and vaccines.

Integrated Magnetic Separation Platform: From Nanoparticle Production To Antibody Capture With RS-HGMS

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Efficient, scalable, and integrated separation processes are essential for modern biomanufacturing. We present a dual-use rotor–stator high-gradient magnetic separation (RS-HGMS) platform that unifies two critical steps: (i) synthesizing and functionalizing magnetic nanoparticles (MNPs) and (ii) capturing monoclonal antibodies (mAbs) directly from cell culture. This integration reduces total footprint and CAPEX compared to traditional column-based affinity capture.

We demonstrate two ligand strategies: a MNP-selective tagged Protein A variant enabling rapid, economical capture and a covalently immobilized, calcium-dependent cysteine-tagged Protein A variant (ZCa) enabling milder elution. In experimental validation, MNP@B8 enabled antibody capture with 85% yield and 97% purity, while MNP@ZCa achieved recoveries of up to 95% under mild elution conditions. In both cases, total capture time remained below 3 hours when processing clarified CHO supernatants. Parallel automated nanoparticle production shows reproducible particle quality and yields, further shrinking development timelines.

Supporting this, we built a digital twin to model ferrohydrodynamics and adsorption, and performed Bayesian optimization. In silico we observed productivity & recovery gains of up to 5%. Real-time monitoring and soft sensors allow adaptive control, enhancing process robustness and regulatory transparency.

This platform highlights magnetic separation not simply as an alternative to affinity chromatography, but as a basis for end-to-end purification, marrying synthesis, capture, and digital process control. It holds promise for sustainable, cost-efficient purification in bioprocessing, particularly when scale, flexibility, and resource use are critical.

Integrated Downstream For Valorization Of Soy Side-Streams Via Fungal Fermentation

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Under-utilized side-streams from soy processing are abundant, low-cost input. We present an integrated, modular downstream process that converts fungal fermentations of these heterogeneous residues into two purified product classes via three equally weighted operations: clarification/concentration by dynamic crossflow filtration (DCF), chromatographic concentration capture and enrichment of volatile aroma compounds from the clarified permeate, and lectin purification from the retentate.
The in-line DCF unit retains and concentrates protein- and polysaccharide-rich biomass while delivering a clarified permeate for chromatography. The filtration plant is fully digitalized, and a digital shadow of the process is already implemented. In the next step, a hybrid mechanistic/machine-learning model is being developed to enhance real-time process control and sustain the continuous process.
Aroma compounds in the permeate are captured by a low toxic and sustainable ethanol–water solvent using a solid-phase extraction, achieving up to 90% overall recovery and 136-fold enrichment of key bioaromas (HPLC-based quantification).
The retentate is processed by gentle cell disruption, clarification, and a biocompatible chromatographic purification followed by a polishing step. The resulting fractions exhibit hemagglutinating and glycoprotein-binding activity.
Together, these separation steps build a scalable cascade for the valorization of industrial soy side-streams, providing quantitative handles on retention, recovery, purity, productivity, and cleanability that support scale-up and technology transfer.

Chococat Standards: Controlling High-Risk CHO HCPs In Drug Products

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Mass spectrometry is getting more and more important as an orthogonal technique to traditional ELISA-based HCP approaches. MS-based techniques enable ultra-sensitive and unbiased detection of HCPs, but their quantitative accuracy is limited without proper quantification standards. These limitations can be overcome by using our internal stable-isotope labelled (SIL) CHOcoCAT standards.
Here, we present our newly developed QconCATTM standards for 28 known CHO high-risk proteins (Jones et al., 2021), e.g. PLBL2, LPL and Clusterin. For each of the target HCPs, we chose three quantitative peptides that are commonly identified in public CHO datasets. The peptides were concatenated into two artificial QconCATTM proteins. Quality control measurements showed extremely high labelling efficiency and high purity of the standards, as well as high solubility in H20 or ABC.
Our standards are compatible with many sample preparation techniques and can be combined both with untargeted (DDA, DIA) or targeted measurement techniques (SIM, PRM/MRM, SureQuant). Here, we showcase their introduction into an untargeted DIA approach, enabling both exact quantification of the covered high-risk HCPs as well as identification and quantification of other HCPs. QconCAT approaches can be easily adapted to other host organisms, like *E. coli*, or even uncommon production hosts.

separations

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