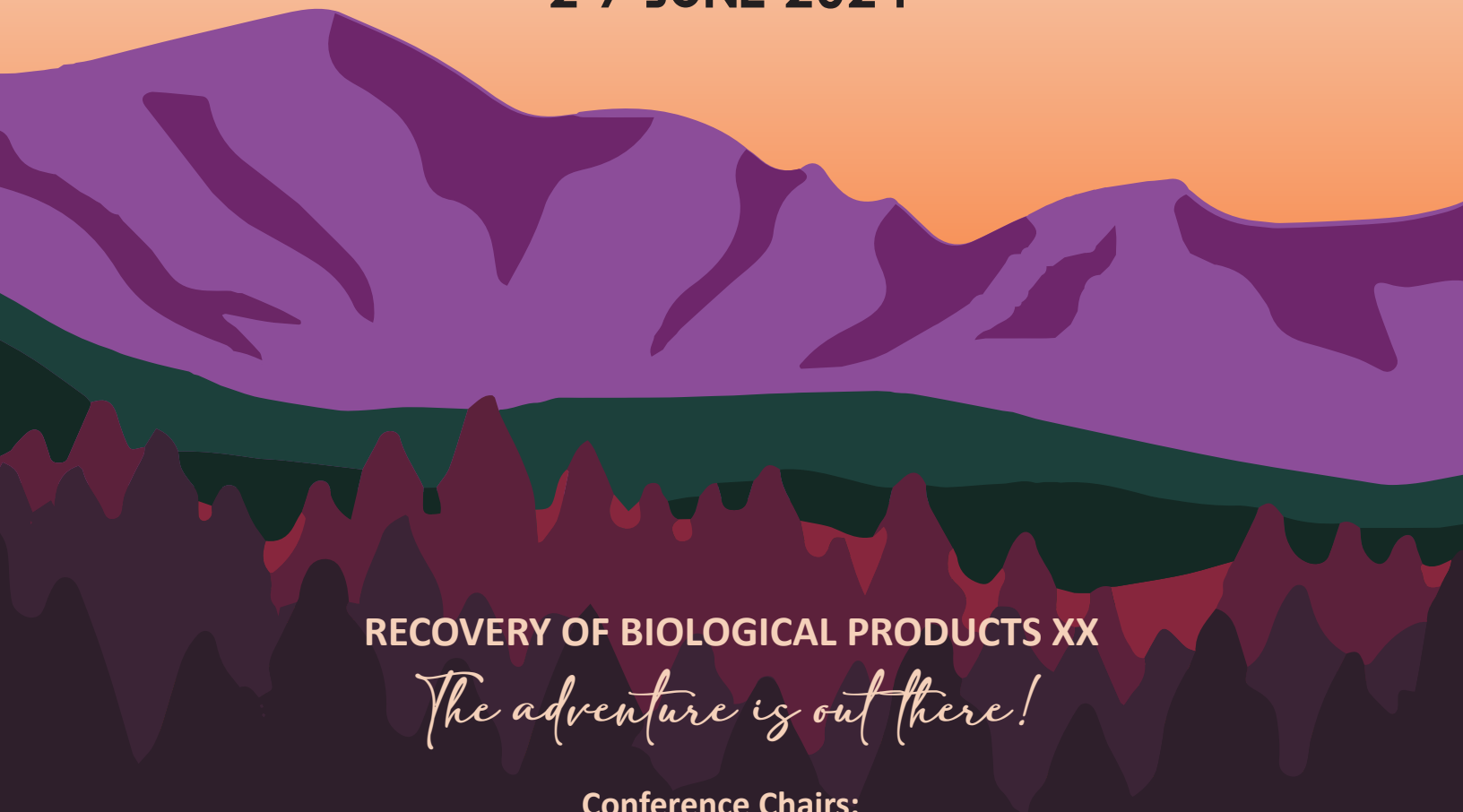
RECOVERY OF BIOLOGICAL PRODUCTS

SUNRIVER, OREGON, USA
2-7 JUNE 2024



RECOVERY OF BIOLOGICAL PRODUCTS XX

The adventure is out there!

Conference Chairs:

Jean Bender – Visterra, Inc.

Abraham Lenhoff – University of Delaware

John Pieracci – Biogen

Program, Abstracts & Conference Information

#RXX | SUNRIVER RESORT | SUNRIVER | OREGON | 2-7 JUNE 2024

SCHEDULE AT A GLANCE

Sun 2 June 2024	Mon 3 June 2024	Tues 4 June 2024	Weds 5 June 2024	Thurs 6 June 2024	Fri 7 June 2024
9:00 AM - 5:00 PM Registration Abbot, Sunriver Lodge	**7:00 AM - 8:00 AM** Welcome Breakfast Homestead	**7:00 AM - 8:00 AM** Breakfast Homestead	**7:00 AM - 8:00 AM** Breakfast Homestead	**7:00 AM - 8:00 AM** Breakfast Homestead	**7:00 AM - 8:00 AM** Breakfast Homestead
4:30 PM - 6:00 PM Cocktail Reception The Backyard, Sunriver Lodge	**8:00 AM - 9:45 AM** Oral Session 1: The Adventure Begins at Harvest Sage Springs Pavilion	**8:00 AM - 9:45 AM** Oral Session 3: Mission Impurity Characterization: Advances, Challenges, and Regulatory Insights Across Modalities Sage Springs Pavilion	**8:00 AM - 9:45 AM** Oral Session 6: Adsorptive Separations Across the Universe of Biologics Sage Springs Pavilion	**8:00 AM - 9:45 AM** Oral Session 8: Capers and Antics Across the Purification Process Sage Springs Pavilion	
7:00 PM - 8:00 PM Keynote The Backyard, Sunriver Lodge	**9:45 AM -10:15 AM** Mid-Morning Break Sage Springs Pavilion	**9:45 AM - 10:15 AM** Mid-Morning Break Homestead, Landmark or Abbot	**9:45 AM - 10:15 AM** Mid-Morning Break Inside of Sage Springs Pavilion	**9:45 AM - 10:15 AM** Mid-Morning Break Sage Springs Pavilion	
8:30 PM - 10:30 PM Poster Session 1 Sage Springs Pavilion	**10:15 AM - 12:00 PM** Oral Session 2: Advances in Structure-Function Understanding and Developability Assessment for Novel Biologics Sage Springs Pavilion	**10:15 AM - 12:00 PM** Roundtable #1: Abbot Roundtable #2: Homestead 1 Roundtable #3: Homestead 2 Roundtable #4: Homestead 3 Roundtable #5: Landmark	**10:15 AM - 12:00 PM** Oral Session 7: Separating Without Sticking - Adventures in Non-Adsorptive Separations Sage Springs Pavilion	**10:15 AM - 12:00 PM** Oral Session 9: Manufacturing Control PAT: Successful Implementation and Continuing Obstacles Sage Springs Pavilion	
10:30 PM Recovery Lounge Opens Owl's Nest, Sunriver Lodge	**12:00 PM - 1:15 PM** Lunch (Boxed) Sage Springs Pavilion	**12:00 PM - 1:15 PM** Lunch Besson Commons	**12:00 PM - 1:15 PM** Lunch (Boxed) Sage Springs Pavilion	**12:00 PM - 1:00 PM** Lunch Homestead	
	1:00 PM - 5:30 PM Networking Activities (Timing varies by activity) Besson Commons	**1:15 PM - 2:35 PM** Oral Session 4: Escapades of a Continuous Nature Sage Springs Pavilion	**1:00 PM - 5:30 PM** Networking Activities (Timing varies by activity) Besson Commons	**1:15 PM - 2:35 PM** Oral Session 10: Instagram vs. Reality: Challenges, Surprises, and Successes in Fitting Your Perfect Process into an Imperfect Manufacturing Facility Sage Springs Pavilion	
	6:00 PM - 8:00 PM Cocktail Reception & Dinner: "Recovery Rodeo" Mt. Bachelor Lawn	**2:35 PM - 3:05 PM** Afternoon Break Sage Springs Pavilion	**6:00 PM - 7:15 PM** Cocktail Reception & Dinner The Backyard, Sunriver Lodge	**2:35 PM - 3:05 PM** Afternoon Break Sage Springs Pavilion	
	8:30 PM - 10:30 PM Poster Session 2 Sage Springs Pavilion	**3:05 PM - 5:15 PM** Oral Session 5: Principles, Strategies and Highlights of Modelling and Data Analytics in Biomanufacturing Sage Springs Pavilion	**7:30 PM - 8:30 PM** Roundtable Readout Sage Springs Pavilion	**3:05 PM - 4:50 PM** Oral Session 11: Innovative Purification Technologies Sage Springs Pavilion	
	10:30 PM Recovery Lounge Opens Owl's Nest, Sunriver Lodge	**5:30 PM** Buses Depart Sunriver for Offsite Event Besson Commons	**8:30 PM - 10:30 PM** Poster Session 3 Sage Springs Pavilion	**4:50 PM - 5:15 PM** Closing Remarks Sage Springs Pavilion	
		5:30 PM - 10:00 PM Downtown Bend Walking Tour & Dinner Downtown Bend	**10:30 PM** Recovery Lounge Opens Owl's Nest, Sunriver Lodge	**6:00 PM - 10:00 PM** Closing Dinner: An Evening Under the Stars Great Hall	
		10:30 PM Recovery Lounge Opens Owl's Nest, Sunriver Lodge		**10:00 PM** Recovery Lounge Opens Owl's Nest, Sunriver Lodge	

HELPDESK HOURS
Abbot, Sunriver Lodge
Monday: 7:00 AM - 6:00 PM
Tuesday: 7:00 AM - 5:00 PM
Wednesday: 7:00 AM - 6:00 PM
Thursday: 7:00 AM - 6:00 PM
Friday: 7:00 AM - 10:00 AM

RECOVERY OF BIOLOGICAL PRODUCTS XX

SUNRIVER, OREGON
2-7 JUNE 2024

An International Conference

In Association with the American Chemical Society's Division of Biochemical Technology (BIOT)

Conference Management Provided by
Precision Meetings & Events, Inc.

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A WELCOME FROM THE CONFERENCE CO-CHAIRS

We are overjoyed to extend a warm invitation to an extraordinary event that promises to be a confluence of innovation, expertise, and camaraderie. We welcome not only first-time attendees but also our venerable colleagues, some of whom have been part of this since its beginning. We hope you will enjoy this unparalleled opportunity to meet **industry leaders** from around the world, representing both **eminent corporations** and **innovative startups**, as well as **renowned academic researchers**. As co-chairs, we are eager to share our enthusiasm for the upcoming adventures in Bioprocessing and to uphold the high standards set by previous conferences.

Together, we will explore **11 oral sessions** that tackle the pressing challenges of our field, showcase revolutionary technologies, and address manufacturing and purification obstacles. Here is a glimpse of the journey that awaits:

Oral session 1: The Adventure Begins at Harvest
Oral session 2: Advances in Structure-Function Understanding and Developability Assessment for Novel Biologics
Oral session 3: Mission Impurity Characterization: Advances, Challenges, and Regulatory Insights across Modalities
Oral session 4: Escapades of a Continuous Nature
Oral session 5: Principles, Strategies and Highlights of Modelling and Data Analytics in Biomanufacturing
Oral session 6: Adsorptive Separations Across the Universe of Biologics
Oral session 7: Separating without Sticking – Adventures in Non-Adsorptive Separations
Oral session 8: Capers and Antics Across the Purification Process
Oral session 9: Manufacturing Control and PAT: Successful Implementation and Continuing Obstacles
Oral session 10: Instagram vs. reality: Challenges, surprises, and successes in fitting your perfect process into an imperfect manufacturing facility
Oral session 11: Innovative Purification Technologies

Engage with peers and forge new connections during our **3 poster sessions**, which will be replete with case studies and innovative approaches to purification challenges.

We also invite you to broaden your horizons in our **5 Roundtables**, where we will delve into sustainable practices, pandemic preparedness, cost-effective therapeutics, modeling, and the unique challenges presented by new modalities.

The networking activities scheduled for Monday and Wednesday afternoons are a hallmark of our conference series, offering a blend of professional enrichment and personal enjoyment. We are thrilled to continue this tradition with a variety of activities that capture the spirit of Sunriver, Oregon. Join us for a leisurely stroll along the Deschutes River, or embrace the full array of outdoor adventures including biking, hiking, kayaking, canoeing, white water rafting, zip-lining, and cave exploring.

Welcome to Sunriver, where every path leads to a new discovery and every moment is an adventure waiting to unfold. At RXX, the Adventure is Out There!

Best regards,
Jean, Bramie and John
Co-Chairs, Recovery of Biological Products RXX Conference

From Left to Right: Jean Bender, Visterra, Inc., John Pieracci, Biogen, Abraham Lenhoff, University of Delaware

RECOVERY CONFERENCE SERIES

The Recovery of Biological Products Conference Series is the premier international forum for the presentation and discussion of recent advances in the operations used to recover biological products of therapeutic, diagnostic and industrial value to society. The biennial Conference Series provides a unique venue for networking with leaders in the field from both academia and industry to discuss the latest developments in downstream bioprocessing. The size, venue and schedule of the meeting are specifically designed to provide multiple opportunities for discussion and development of new areas and concepts relating to the separation, purification and efficient processing of biological products.

The Recovery of Biological Products Conference Series is associated with the American Chemical Society's Division of Biochemical Technology (BIOT). This relationship maintains the Not-for-Profit status of the Conference Series.

We are grateful to the ACS BIOT Division and to the Recovery Series Board for their support and encouragement.

ACS Chemistry for Life®

Recovery of Biological Products Conference Series

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RXX ORGANIZING COMMITTEE

Conference Chairs:

Jean Bender, Visterra, Inc., United States (Senior Chair)
Abraham Lenhoff, University of Delaware, United States
John Pieracci, Biogen, United States

Conference Management Team

Peggy Marilley, Precision Meetings & Events, Inc. | 703-625-1437
Cate Rock, Precision Meetings & Events, Inc. | 703-967-6506
Helana Hager, Precision Meetings & Events, Inc. | 843-343-9421

Recovery XX Logo Designed by Moira Lynch, Thermo Fisher Scientific, United States

Oral Session Chairs

Session 1: The Adventure Begins at Harvest
Arick Brown, Amgen, United States
Brandon Christensen, Visterra, United States

Session 2: Advances in Structure-Function Understanding and Developability Assessment for Novel Biologics
Chen Wang, AbbVie, United States
Peter Tessier, University of Michigan, United States

Session 3: Mission Impurity Characterization: Advances, Challenges, and Regulatory Insights Across Modalities
Kristin Valente, Merck & Co., United States
André Dumetz, GSK, United States

Session 4: Escapades of a Continuous Nature
Andrew Tustian, Regeneron Pharmaceuticals, United States
Daniel Bracewell, University College London, United Kingdom

Session 5: Principles, Strategies and Highlights of Modelling and Data Analytics in Biomanufacturing
Sophie Karkov, Novo Nordisk, Denmark
Eric von Lieres, Research Center Jülich, Germany

Session 6: Adsorptive Separations Across the Universe of Biologics
Stefano Menegatti, NC State University, United States
Kevin Brower, Sanofi, United States

Session 7: Separating Without Sticking – Adventures in Non-Adsorptive Separations
Elizabeth Goodrich, MilliporeSigma, United States
Caryn Heldt, Michigan Tech, United States

Session 8: Capers and Antics Across the Purification Process
Yinying Tao, Eli Lilly and Company, United States
Thomas von Hirschheydt, Roche, Germany

Session 9: Manufacturing Control and PAT: Successful Implementation and Continuing Obstacles
Astrid Dürauer, BOKU & Austrian Centre of Industrial Biotechnology, Austria
Emily Schirmer, Catalent Pharma Solutions, United States

Session 10: Instagram vs. Reality: Challenges, Surprises, and Successes in Fitting Your Perfect Process into an Imperfect Manufacturing Facility
Olga Paley, Takeda Pharmaceuticals, United States
Brad Stanley, Biogen, United States

Session 11: Innovative Purification Technologies
David Wood, Ohio State University, United States
Brenda Carrillo-Conde, Pfizer, United States

RXX ORGANIZING COMMITTEE (continued)

Poster Session Chairs

Ana Azevedo, Instituto Superior Tecnico, Portugal
Glen Bolton, Amgen, United States
Michaela Wendeler, AstraZeneca, United States

Roundtable Chairs

Recovery XX Roundtable 1: What Do We Do Today to Be Ready for the Next Pandemic? | Abbot

Sanchayita Ghose, BMS
Natarajan Ramasubramanyan, Thermo Fisher Scientific
Brian Kelley, Vir Biotechnology

Recovery XX Roundtable 2: Go Big or Go Home – Evolving Bioprocessing for New Modalities | Homestead 1

Alexei Voloshin, Solventum
Matthew Westoby, Resilience
Hans Johansson, Purolite

Recovery XX Roundtable 3: What is the Future of Modeling for Recovery of Biological Products? | Homestead 2:

Mariona Bertran, Novo Nordisk
Karol Lacki, Repligen
David Roush, Roush Biopharma Panacea – RBP

Recovery XX Roundtable 4: How Can We Drive Down Biopharma Costs to $1 Per Dose for Wider Access? | Homestead 3

Jörg Thömmes, JT Consulting
Lisa Connell-Crowley, Just – Evotec Biologics
Suzanne Farid, University College London

Recovery XX Roundtable 5: How Do We Implement Sustainable Production that is Compatible with Reduced Costs? | Landmark

Alois Jungbauer, BOKU
Kris Barnthouse, Johnson & Johnson
Jean Aucamp, Lonza

Conference Registration

Location: Sunriver Main Lodge, 2nd Floor Abbot
Saturday, 1 June **Sunday, 2 June**
12:00 PM – 5:00 PM **9:00 AM – 5:00 PM**
Please visit Conference Registration upon arrival to pick-up your conference badge and program. The Conference Staff will be there until Sunday evening to assist you.

Conference Help Desk

Location: Homestead
Monday, 3 June – Friday 7 June
The Conference Staff will be there to assist you with anything you need throughout the meeting. Please do not hesitate to contact a staff member if you have a question regarding the schedule, activities, attire or any other aspect of the program.

Wear Your Official RXX ID

Please wear your RXX ID (name badge) to all sessions, meals and other official events. Badges will be checked upon entrance to all technical sessions and social events.

Speaker Instructions

Each session has a designated time slot reserved to test your slides prior to your scheduled talk time. Please make every attempt to hand in your presentation by your scheduled time (as communicated to you by your session chair) so that it may be loaded onto the presentation computer in advance.

Poster Presentation Set Up

Please arrive at the Sage Springs Pavilion on Sunday 2 June between 2:00 PM – 5:00 PM to set up your poster display. It is the presenter's responsibility to arrive with the poster already printed.

Messages

There will be a message board located in Homestead. Messages will not be personally delivered, and technical sessions will not be interrupted.

Recording and Photography

Both audio and visual recording of any oral session during the Conference is strictly prohibited. Likewise, photographic documentation of posters is not permitted unless expressly permitted by the presenting author. Delegates should directly ask presenters if they wish to have copies of slides, posters, or other materials.

Networking Activities Information

If you have pre-registered for Networking Activities, your activity tickets will be included in your registration materials. Please be sure to bring your assigned ticket(s) with you to each activity. If you have not pre-registered or would like to make changes to your reservation, you will have the opportunity to do so at the registration desk in Homestead.

Attire - Casual Comfort (Layers and Walking Shoes!)

We encourage attendees to dress comfortably in casual attire, suitable for both indoor sessions and outdoor activities. Given the potential evening chill and the expansive layout of the hotel property, we suggest layering your attire for warmth and wearing comfortable walking shoes to navigate the grounds with ease.

Transportation:

Airport transportation will be available for those who have notified the Conference Organizers of their departure time. Please see the app and conference website to learn more.

HOTEL INFORMATION

Location: Sunriver Resort, 17600 Center Dr, Sunriver, OR 97707
T: (855) 420-8206

Hotel Check-In: 4:00 PM
Hotel Check-Out: 11:00 AM
Early check-ins and late check-outs are subject to availability and approval by front desk staff.

Hotel Check-in: Sunriver Main Lodge

Please check into your hotel room at the front desk, located in the Sunriver Lodge. Registration fees cover conference nights 2 June – 7 June. If you informed Recovery Organizers of an extended stay, the front desk will collect your credit card information upon arrival for the additional night(s) stay.

Payment:

Hotel accommodation from Sunday 2 June to Thursday 7 June is included in your registration fee. For those staying additional nights prior to and/or after the conference whose reservations were made through Precision Meetings & Events, Inc. (the RXX conference management company), please present your credit card to the front desk clerk upon arrival (please note that the charges for those additional nights will appear on your personal folio). Any personal expenses incurred at the hotel, such as bar bills, use of recreational facilities, and food (other than scheduled conference meals), are the responsibility of each attendee and/or their guest(s) and must be paid upon check-out.

Hotel Activity Offerings:

- 4 Award-Winning Championship Golf Courses
- Sage Springs Club & Spa
- Bike Barn with 45 Miles of Paved Bike Paths (reserve a bike here)
- Indoor Golf Simulator
- Pickleball
- Fitness Center
- The Cove Aquatic Center
- Sunriver Stables Horseback Riding

Meeting Locations

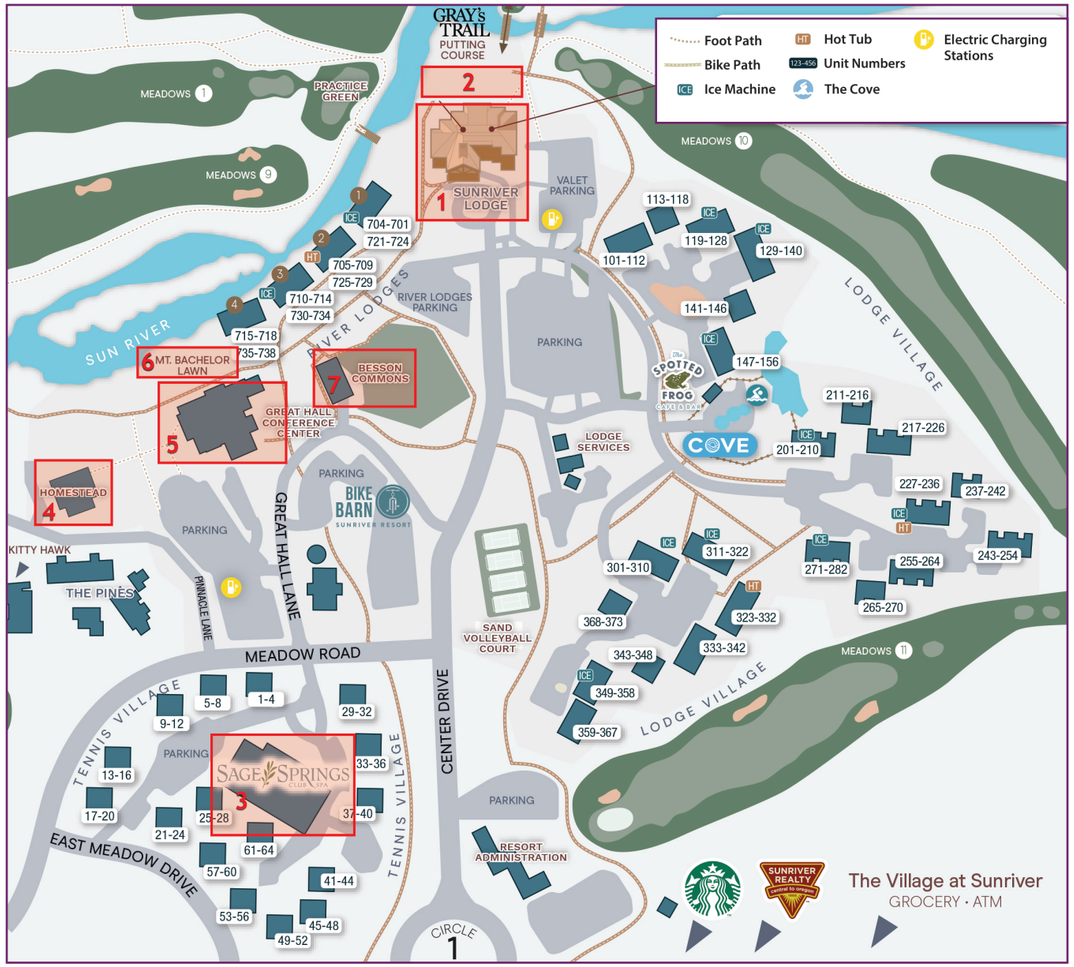
The Hotel map is located to the right of this page.

Daily General Sessions: Sage Springs Pavilion
Poster Sessions: Sage Springs Pavilion
Roundtable Sessions: Homestead/Landscape/Abbot
Daily Breakfast Buffet: Homestead

Other Meals and Events

Consult the Daily Conference Program in this document to find the locations of all remaining meals and events. Lunch and dinner locations vary, so please do check your schedule for locations. There will also be staff present to direct you.

HOTEL MAP:



1. Sunriver Lodge
Hotel Check-In
Saturday/Sunday Conference Registration
Roundtable 1: Pandemic
Friday Airport Shuttle Pick Up

2. The Backyard of Sunriver Lodge
Sunday Dinner
Wednesday Dinner

3. Sage Springs Pavilions
Oral Sessions
Poster Sessions
Monday/Wednesday Boxed Lunch

4. Homestead
Monday-Friday Conference Help Desk
Breakfast Buffet
Tuesday/Thursday Lunch Buffet
Roundtable 2: New Modalities
Roundtable 3: Modeling
Roundtable 4: $1 per Dose

5. Great Hall Conference Center
Roundtable 5: Sustainability
Thursday Dinner

6. Mt. Bachelor Lawn
Monday Dinner

7. Besson Commons
Activities Meeting Point
Tuesday Lunch

Sunday 2 June 2024: Opening Dinner & Keynote

Location: The Backyard Sunriver Lodge
16:30 PM – 18:00 PM Cocktail Reception
18:00 PM – 19:00 PM Dinner
19:00 PM – 20:00 PM Keynote

Set against the backdrop of Sunriver's breathtaking landscape, take the opportunity to kick off RXX by connecting with colleagues new and old. We will gather for dinner while immersed in the natural beauty of Sunriver's surroundings. The highlight of the dinner will be a special keynote, setting the tone for the days ahead. Dress comfortably and bring layers as temperatures drop in the evenings.

Monday 3 June 2024: Recovery Rodeo!

Location: Mt. Bachelor Lawn
18:00 PM – 20:00 PM

Saddle up for our rodeo-themed dinner! Embrace the spirit of the West with your attire, opting for:

- Jeans for a classic cowboy look
- Cowboy boots
- Plaid or denim shirts for a rustic flare
- Gingham or bandana prints for a touch of western charm

Tuesday 4 June 2024: History Walking Tour of Bend and Dinner

Departure Time: 17:30 PM
Location: Besson Commons
You must arrive at Besson Commons by 5:30pm to ride the buses!

The story of historical Bend begins along the life-giving source of the Deschutes River. This 45-minute journey on foot will begin at Farewell Bend Ranch and end at Bend Brewing Company located on the Deschutes River. We'll cover the indigenous people to today's thriving city all while taking in the natural and cultural history of Bend.

NOTE: If you are unable to walk approximately 2.3 miles, please notify the Precision team at the Conference Help Desk and we can make special arrangements. Walking shoes recommended.

Dinner will be hosted at Bend Brewing Company, the second oldest brewery in Bend.

Featured at the event will be an exclusive "Recovery Artisan Market" composed of local merchants (including custom leather goods, glass blown sculptures, and art). Recovery's intention is to highlight the talents of local Bend artisans to provide attendees with the opportunity to acquire unique pieces of Oregon craftsmanship as lasting mementos of their visit while promoting Bend's local charm & heritage.

To read about each featured artisan, please go to page 22 (where the artisan page is located).

Wednesday 5 June 2024: Buffet Dinner

Location: The Backyard, Sunriver Lodge
18:00 PM – 20:00 PM

Enjoy a casual dinner before the Poster Session begins! Dress comfortably and bring layers as temperatures drop in the evenings.

Thursday 6 June 2024: Closing Dinner: An Evening Under the Stars

Location: The Great Hall
18:00 PM – 22:00 PM

Prepare for an enchanting conclusion to our conference with "An Evening Under the Stars"! As we bid farewell, we invite you to join us for a celestial celebration with dinner, music and the opportunity for our astronomers to guide you on a journey through the cosmos.

Attire: Elevated yet relaxed dress code. We encourage you to dress up a little more than your average day, as this is a special final evening!

RXX Networking Activities will occur during the afternoon of Monday (3 June) and Wednesday (5 June).
The timing indicated is when you should be ready to load the bus.
Meeting Location: Besson Commons

Activity Tickets:

If you have pre-registered for Networking Activities, your activity tickets will be included in your registration materials. Please be sure to bring your assigned ticket(s) with you to each activity. If you have not pre-registered or want to make changes to your reservation, you can do so at the registration desk.

Suggestions for Most Outdoor Events:
Sunscreen, Hat, Sunglasses, Comfortable Closed-Toed Walking Shoes.

Activities and Timing:

Prohibition Cave Exploration

Time: 13:15 PM – 17:30 PM
Attire: Long pants and closed toed walking shoes – keep in mind you might want to wear clothes you don't mind getting dusty. Jacket for 45°F weather.

Kayaking/Canoeing the High Cascade Lakes

Time: 13:15 PM – 17:30 PM
Attire: Bathing suit if you would like to swim, towel, jacket, or layer for warmth based on weather, sun protection, shoes that you don't mind getting wet.

Lava Lands + River Stroll

Time: 13:15 PM – 17:30 PM
Attire: Hiking clothes, walking shoes, jacket, or layer for warmth based on weather, sun protection.

White Water Rafting

Time: 13:15 PM – 16:00 PM
Attire: On warmer days, bathing suits, t-shirts, and shorts are great options. On cooler days, light layers of clothes made of technical and synthetic fabrics, common to workout gear, or wool will help wick moisture and keep you warmer. Try to avoid cotton clothing if possible – cotton does not dry very well. Footwear options like river sandals, water socks, or athletic shoes that can get wet. Flip flops are not allowed. Shoes are available for purchase in the retail shop as well as complimentary splash gear to block wind and big splashes. Sun protection, water, towel, change of dry clothing.

Brews & Cruise

Time: 13:00 PM – 15:00 PM
Location: Meet at Bike Barn
Attire: Comfortable clothing, walking shoes, layer for warmth based on weather, sun protection.

Mt. Bachelor Zipline Tour

Time: 13:15 PM – 16:00 PM
Attire: Pants, capris, leggings, or long shorts, comfortable shirt, closed-toed shoes, sun protection, jacket.

Guided Mountain Bike Ride

Time: 13:15 PM – 17:00 PM
Attire: athletic wear or moisture-wicking clothing, shorts or pants, layer depending on weather, closed toed athletic shoes, socks, water bottle, sun protection, eyewear to protect your eyes from dust, debris, and UV rays.

High Desert Museum (Self-Guided)

Time: 13:15 PM to 16:30 PM
Attire: comfortable shoes for walking, casual clothing, layers depending on weather, avoid large bags

SUNDAY 2 JUNE 2024

09:00 - 17:00 Registration | Abbot

16:30 - 17:30 Cocktail Reception | The Backyard

17:30 - 18:00 Opening Remarks | The Backyard

19:00 - 20:00 Keynote | The Backyard

20:30 - 22:30 Poster Session 1 | Sage Springs Pavilion

MONDAY 3 JUNE 2024

07:00 - 08:00 Welcome Breakfast | Homestead

Oral Session 1: The Adventure Begins at Harvest | Sage Springs Pavilion
Arick Brown, Amgen, United States
Brandon Christensen, Visterra, United States

08:05 - 08:30 Single-use continuous centrifugation harvest for high density cell culture
Oliver Kaltenbrunner, Amgen, United States

08:30 - 08:55 Efficient clarification strategies for high solid content cell culture fluids
Haikuan Liu, WuXi Biologics, China

08:55 - 09:20 5-in-1 application of an off-the shelf charged fibrous device allows for hyper-intensified production of recombinant Adeno-Associated Viral vectors
Daniel Hurwit, Bristol Myers Squibb, United States

09:20 - 09:45 A new multifaced player in the field of AAV harvest recovery and viral clearance
Yulia Ivanova, Pfizer, United States

09:45 - 10:15 Mid-Morning Break | Inside of Sage Springs Pavilion

Oral Session 2: Advances in Structure-Function Understanding and Developability Assessment for Novel Biologics | Sage Springs Pavilion
Chen Wang, AbbVie, United States
Peter Tessier, University of Michigan, United States

10:20 - 10:45 Developability Evaluation of Discovery Biologic Candidates by Screening Physicochemical and Accelerated Stability Properties
Rajeeva Singh, Abbvie, United States

10:45 - 11:10 Evaluation of descriptors and machine learning strategies for monoclonal antibody chromatography process developability prediction
Andrew Maier, Genentech, United States

11:10 - 11:35 Modeling the chromatography behavior of monoclonal antibodies in hydrophobic interaction chromatography
Douglas Nolan, Takeda Pharmaceutical Company, United States

11:35 - 12:00 Assessing the developability of fractionated monoclonal antibody proteoforms: Impact of charge, hydrophobicity, and glycans on aggregation susceptibility
Solomon Isu, MilliporeSigma, United States

12:00 - 13:15 Lunch (Boxed) | Sage Springs Pavilion

13:30 - 17:30 Networking Activities | Offsite Participants will Meet at Besson Commons for Bus Pick Up

18:00 - 19:30 Dinner (Recovery Rodeo) | Mt. Bachelor Lawn

20:30 - 22:30 Poster Session 2 | Sage Springs Pavilion

TUESDAY 4 JUNE 2024

07:00 - 08:00 Breakfast | Homestead

Oral Session 3: Mission Impurity Characterization: Advances, Challenges, and Regulatory Insights across Modalities | Sage Springs Pavilion
Kristin Valente, Merck and Co., Inc., United States
Andre Dumetz, GSK, United States

08:05 - 08:30 Quality By Design for Control of Polysorbate Degrading Host Cell Protein in Biologicals
John Mattila, Regeneron Pharmaceuticals, Inc, United States

08:30 - 08:55 Tailoring Polishing Steps for Effective Removal of Polysorbate-Degrading Host Cell Proteins in Monoclonal Antibody Purification
Melanie Maier, Boehringer Ingelheim, Germany

08:55 - 09:20 5-in-1 Progress and challenges towards characterization and control of impurities in antibody-drug conjugates
Michaela Wendeler, AstraZeneca, United States

09:20 - 09:45 Advances in the Purification of Antisense Oligonucleotides
Robert Gronke, Biogen, United States

09:45 - 10:15 Mid-Morning Break | Homestead Landmark Abbot

10:15 - 12:00 Roundtables

Roundtable #1: What do we do today to be ready for the next pandemic? | Abbot
Chairs: Sanchayita Ghose (BMS), Natarajan Ramasubramanyan (Thermo Fisher Scientific), Brian Kelley (Vir Biotechnology)

Roundtable #2: Go big or go home – evolving bioprocessing for new modalities. | Homestead 1
Chairs: Alexei Voloshin (Solventum), Matthew Westoby (Resilience), Hans Johansson (Purolite)

Roundtable #3: What is the future of modeling for Recovery of Biological Products? | Homestead 2
Chairs: Mariona Bertran (Novo Nordisk), Karol Lacki (Repligen), David Roush (Roush Biopharma Panacea – RBP)

Roundtable #4: How can we drive down biopharma costs to $1 per dose for wider access? | Homestead 3
Chairs: Jörg Thömmes (JT Consulting), Lisa Connell-Crowley (Just – Evotec Biologics)
Suzanne Farid (University College London)

Roundtable #5: How do we implement sustainable production that is compatible with reduced costs? | Landmark
Chairs: Alois Jungbauer (BOKU), Kris Barnthouse (Johnson & Johnson), Jean Aucamp (Lonza)

12:00 - 13:15 Lunch | Besson Commons

Oral Session 4: Escapades of a Continuous Nature | Sage Springs Pavilion
Andrew Tustian, Regeneron Pharmaceuticals, United States
Daniel Bracewell, University College London, United Kingdom

13:20 - 13:45 Integrated and continuous purification: the journey from hybrid to fully continuous, and from vision to reality
Jason Walther, Sanofi, United States

13:45 - 14:10 Turning the crank using a hybrid continuous purification platform
Michelle Najera, Just-Evotec Biologics, United States

14:10 - 14:35 Truly continuous purification platform – beyond the PoC
Irina Ramos, AstraZeneca, United States

14:35 - 15:05 Afternoon Break | Inside of Sage Springs Pavilion

TUESDAY 4 JUNE 2024 (CONTINUED)

Oral Session 5: Principles, Strategies and Highlights of Modelling and Data Analytics in Biomanufacturing | Sage Springs Pavilion
Sophie Karkov, Novo Nordisk, Denmark
Eric von Lieres, Research Center Jülich, Germany

15:10 - 15:35 Process development using an autonomous process optimizer
Cornelia Walther, Boehringer-Ingelheim RCV, Austria

15:35 - 16:00 Automated generation of digital twins and their use in real-time monitoring of process chromatography
Daniel Espinoza, Lund University, Sweden

16:00 - 16:25 Universal Hybrid Chromatography Modeling Framework for Optimization of Multi-column Chromatography Systems
Brandon Corbett, Sartorius, Canada

16:25 - 16:50 pH Transients and Elution Profiles in Protein A Affinity Chromatography: Experimental Observations, Modeling, and Approaches to Elution Buffer Engineering
Rainer Hahn, BOKU Vienna, Austria
Giorgio Carta, University of Virginia, United States

16:50 - 17:15 Implementation of Mechanistic Model-Informed Chromatography Process Development and Validation: Successes and Challenges
Connor Thompson, Genentech, United States

17:30 - 22:30 Offsite Evening Event | Bend Brewing Company

WEDNESDAY 5 JUNE 2024

07:00 - 08:00 Breakfast | Homestead

Oral Session 6: Adsorptive Separations Across the Universe of Biologics | Sage Springs Pavilion
Stefano Menegatti, North Carolina State University, United States
Kevin Brower, Sanofi, United States

08:05 - 08:30 Cleavable affinity tags can revolutionize biologics manufacturing – but how do we convince the FDA?
David Wood, Ohio State University, United States

08:30 - 08:55 3D-printed matrices for the purification of plasmid DNA via steric exclusion chromatography
Ana Rita Santos, iBB - Institute for Bioengineering and Biosciences, Portugal

08:55 - 09:20 Lentiviral Vector Determinants of Anion- Exchange Chromatography Elution Heterogeneity
George Pamenter, University College London, United Kingdom

09:20 - 09:45 Overcoming challenges in the development of chromatographic separation of empty, partial, and full AAV capsids for Gene Therapy applications
Vijesh Kumar, Spark Therapeutics, United States

09:45 - 10:15 Mid-Morning Break | Inside of Sage Springs Pavilion

Oral Session 7: Separating without Sticking – Adventures in Non-AdsorptiveSeparations | Sage Springs Pavilion
Elizabeth Goodrich, MilliporeSigma, United States
Caryn Heldt, Michigan Tech, United States

10:20 - 10:45 Developing a Scale-Down Model for Batch Lysis in Plasmid DNA Purification Processes
Ehsan Espah Borujeni, Bristol Myers Squibb, United States

10:45 - 11:10 Model-based optimization of Single Pass Tangential Flow Filtration (SPTFF) for concentration and purification of viral vectors
Akshay Chaubal, Pennsylvania State University, United States

11:10 - 11:35 A unified view of virus and recombinant protein interactions with block copolymer membranes
Daniele Gerion, Terapore Technologies, United States

11:35 - 12:00 Regulatory Considerations for Design and Implementation of Continuous Viral Inactivation Reactors
Scott Lute, FDA/CDER/OPQ/OBP, United States

12:00 - 13:15 Lunch (Boxed) | Sage Springs Pavilion

13:30 - 17:30 Networking Activities | Offsite Participants will Meet at Besson Commons for Bus Pick Up

18:00 - 19:30 Dinner | The Backyard

19:30 - 20:30 Roundtable Readout | Sage Springs Pavilion

20:30 - 22:30 Poster Session 3 | Sage Springs Pavilion

THURSDAY 6 JUNE 2024

07:00 - 08:00 Breakfast | Homestead

Oral Session 8: Capers and Antics Across the Purification Process | Sage Springs Pavilion
Yinying Tao, Eli Lilly and Company, United States
Thomas von Hirschheydt, Roche, Germany

08:05 - 08:30 When the platform doesn't fit: Simultaneous innovation of process and platform during downstream development of a challenging Fc-fusion protein
Julie Robinson, Merck & Co., Inc., United States

08:30 - 08:55 Process Intensification of Recombinant Adeno-Associated Viral Vector Production
Andrew Tustian, Regeneron Pharmaceuticals, United States

08:55 - 09:20 The well-being approach for higher yields – how to make lentiviral vectors comfortable during downstream processing.
Noor Mujahid, University College London, United Kingdom

09:20 - 09:45 Integrated continuous mRNA precipitation-based purification process
Maria del Carme Pons Royo, MIT, United States

09:45 - 10:15 Mid-Morning Break | Inside of Sage Springs Pavilion

Oral Session 9: Manufacturing Control and PAT: Successful Implementation and Continuing Obstacles | Sage Springs Pavilion
Astrid Dueraue, BOKU & Austrian Centre of Industrial Biotechnology, Austria
Emily Schirmer, Catalent Pharma Solutions, United States

10:20 - 10:45 The minimum requirement on PAT's success for industrial implementation: The simultaneous prediction of multiple relevant Product Quality Attributes in real-time
Gang Wang, Boehringer Ingelheim, Germany

10:45 - 11:10 Right every time?! Assessing polishing chromatography under dynamic loading conditions
Christopher McHardy, Roche, Germany

11:10 - 11:35 "Shaken not stirred", why James Bond was right: the next generation PAT solution for downstream processing
Karol Lacki, Repligen Corp, Sweden

11:35 - 12:00 Case studies on combining spectroscopy and modern machine learning in DSP monitoring
Robin Schiemer, Karlsruhe Institute of Technology, Germany

12:00 - 13:00 Lunch | Homestead

Oral Session 10: Instagram vs. reality: Challenges, surprises, and successes in fitting your perfect process into an imperfect manufacturing facility | Sage Springs Pavilion
Olga Paley, Takeda Pharmaceuticals, United States
Brad Stanley, Biogen, United States

13:20 - 13:45 Achieving High-Titer and High-Concentration Monoclonal Antibody Production: an Innovative Journey Integrating with Next-Generation Bioprocessing Technologies
Yinying Tao, Eli Lilly and Company, United States

13:45 - 14:10 How far can we push the limits of connected biopharmaceutical manufacturing? – Unleashing the full potential of an intensified and connected purification process.
Julian Hitzler, Novartis Pharma, Switzerland

14:10 - 14:35 The Balancing act in Biomanufacturing: Maximizing efficiency and flexibility when faced with an unpredictable product mix
Evan Shave, Thermo Fisher Scientific, Australia

THURSDAY 6 JUNE 2024 (CONTINUED)

14:35 - 15:05 Afternoon Break | Inside of Sage Springs Pavilion

Oral Session 11: Innovative Purification Technologies | Sage Springs Pavilion
David Wood, Ohio State University, United States
Brenda Carrillo-Conde, Pfizer, United States

15:10 - 15:35 Asymmetric bispecific antibody purification platforms using avidity effects of protein A and protein L affinity ligands
Mats Ander, Cytiva, Sweden

15:35 - 16:00 Giving Novel Purpose to Protein A: A New Paradigm for Multispecific Antibody Manufacturing
Arch Creasy, Pfizer, United States

16:00 - 16:25 Rational and combinatorial design of peptides for ss-mRNA/ds-mRNA separation and purification
Pankaj Karande, Rensselaer Polytechnic Institute, United States

16:25 - 16:50 Innovative Protein Scaffolds for Single Step Purification from Proteins to Viral Vector Particles
Romas Skudas, Merck Life Science KGaA, Germany

16:50 - 17:15 Closing Remarks | Sage Springs Pavilion

18:00 - 22:30 Closing Dinner | Great Hall

FRIDAY 7 JUNE 2024

07:00 - 08:00 Breakfast | Homestead

45TH PARALLEL PROVISIONS

Website: www.45thparallelprovisions.com

45th Parallel Provisions proudly presents handcrafted, small-batch specialty pepper products: Candied Jalapeños, 3-Pepper Relish, and Oregon Trail Candy. Elevate dishes with unique flavors, all made with care.

AD GLASS & DESIGN

Website: www.ad-glass.com

AD Glass & Design is the first and only glass studio in Central Oregon open to the public. We offer experiences, lessons, and a variety of unique hand-blown glass art. We are dedicated to preserving the ancient art of glassblowing and sharing our knowledge with our local community and the next generation of our youth.

AD Glass & Design has a wide portfolio of products including glassware and barware such as pint glasses, stemless wine glasses, and decanters. Vases, bowls and plates in a wide variety of styles, shapes and colors are always available. Clear glass cups with clean lines offer timeless appeal, while unique drinking glasses display a touch of personality.

Old World engraving techniques of cutting and polishing glass by hand with no molds are used to elevate your next craft drink with hand cut glassware.

Artwork- Sculptural pieces with hand drawn imagery. Unique hand blown and functional glass art and 1 of a kind blown/sculpted gallery pieces. Gaffer Aaron Duccini's current artistic focus is a classic Swedish style with hand drawn imagery carved deep into multiple layers of color and clear glass. These pieces are created and cooled off before imagery is drawn and carved. They are then picked up hot and shaped into their final form. These pieces are especially suited for storytelling and social issues.

BROADUS BEES

Website: www.broadusbees.com

James Broadus Wilkie V, Owner & Head Beekeeper, has had a fascination with bees and their importance to the environment ever since he studied wildlife biology at Western State College in Colorado. He wants to educate people about the importance of bees to our production of food. Jimmy feels like it is his responsibility to give back to the very thing that gives us life. Jimmy has created a sustainable business through honeybee products, hive installation and maintenance, and educating the public about the critical role bees play in producing the food we eat.

THE CROW'S CROFT

Website: www.thecrowscroft.com

Nestled in Redmond, Oregon, The Crow's Croft is a family-run farm dedicated to sustainable and ethical practices. Specializing in raising healthy, pasture-raised pork, and crafting small-batch lard-based soap and body products. Committed to providing wholesome, earth-conscious offerings, The Crow's Croft invites you to experience the taste and quality of their farm-to-home creations.

THE FLAMING WICK CANDLE CO.

Website: www.theflamingwickcandleco.com

Selling great smelling candles in Redmond and the Central Oregon area since January 2020. Candles are hand poured in small batches. Products range from soy wax candles, dough bowls, wax melts, car diffusers and room sprays using nontoxic fragrance oils and essential oils.

GIFTED + CO. & BEX CUSTOM DESIGNS

Gifted + Co. Website: www.facebook.com/giftedandcompany

Bex Custom Design Website: www.facebook.com/profile.php?id=100068343120594

Gifted + Co. supplies handmade leather goods and jean jackets. Bex Custom Designs incorporates beautiful free hand burned hats with designs inspired by Oregon's inspiring outdoor beauty.

HOWL GOODS

Website: www.howlgoods.com

An apparel and leather goods brand focused on using natural materials to create sustainable, durable items; each piece is intentionally designed to be functional yet beautiful and made to last generations. Founder and designer Alicia Renner carefully sources only sustainably produced textiles including wool and organic hemp, ethically harvested hides and furs including from her grandfather in North Dakota, and responsibly manufactured hardware including solid brass fasteners and mother-of-pearl and buffalo horn buttons.

MANDRAKE ROOTS

Instagram: @spacewayv

Kurt Norpchen creates driftwood sculptures, shelves, & propagation stations for plants from one-of-a-kind driftwood pieces. Many of the elements used within his artwork are sourced from planet Earth.

MOUNTAIN HOME ARTS

MOUNTAIN home arts

Website: www.mountainhomearts.com

Jewelry that is slow crafted, from start to finish. Hand collected stones used in jewelry from Jesse & Joell Fence's mine, that is cut and polished and set using fine and sterling silver. Seed bead jewelry is made with glass or clay seed beads and cotton thread. The beads are woven together using various stitches to create intricate designs. Jesse & Joell's jewelry represents their combined love for nature and fine craftsmanship.

MUDLAKE STUDIOS

Mud Lake Studios

Website: www.mudlakestudios.com

Mudlake Studios is home to over 75 artists, primarily ceramic artists, though over the years have welcomed members who work in: painting, photography, jewelry, leather, textiles, and more. It serves as a community ceramics studio, gallery, and supply shop offering workspace in a historic iron works building providing a safe, positive, accessible, environmentally conscious space open to anyone and their creative process to grow as an artist, hone their craft, learn new skills, and engage in welcoming, like-minded community.

RACHEL ELISE ART

Rachel Elise

Website: www.racheleliseart.com

Rachel Elise Mallon is a watercolor artist with a background and degree in interior design. She specializes in creating semi-abstract pieces with soft brush strokes and a limited color palette inspired by the Pacific Northwest.

WYLD TERRA

wyld TERRA

Website: www.wyldterra.com

Wyld Terra is a jewelry brand that creates hand crafted beaded and gemstone jewelry which is inspired by the places on our earth that are still wild. Each piece of jewelry is slow crafted by hand in Kate's home studio in Bend, using only high quality materials that are ethically sourced. From the first bead, to the last piece of packaging, celebrating and supporting Mother Earth is top of mind at Wyld Terra.

SUNDAY 2 JUNE 2024 - Full poster abstracts can be found on page 80

Theme: Viral and Non-Viral Vectors

Poster #1: Overcoming mRNA Degradation: A Leading-Edge Strategy for Rapid Vaccine Development and Worldwide Distribution
Jack Kramarczyk, Moderna, United States

Poster #4: Leveraging flocculation to improve adeno-associated virus (AAV) purification process
Qingxuan Li, Sanofi, United States

Poster #7: Membrane chromatography for preparation of AAV at the manufacturing scale
Mark Schofield, Cytiva, United States

Poster #10: Atomic basis of AAV affinity chromatography: from design to cryo-EM validation
Nathaniel Clark, Repligen, United States

Poster #13: Biophysical investigations to elucidate the role of resin media and feed constituents on AAV affinity column fouling
Harshal Soni, Rensselaer Polytechnic Institute, United States

Theme: Big Data, Data Management and Modelling

Poster #15: The Downstream Data Browser – Enhancing Data-Driven Decisions via Customized Data Handling and Visualization
Andrew Wagner, Just - Evotec Biologics, United States

Poster #19: Manufacturing Facility and Economic Analysis of Process Intensification Strategies
Xuankuo Xu, Bristol Myers Squibb, United States

Poster #21: A priori process development of multimodal chromatography in antibody purification: a multiscale modeling approach
Rudger Hess, Boehringer-Ingelheim, Germany

Poster #25: A Systematic Approach for Estimating Colloidal Particle Adsorption Model Parameters
Federico Rischawy, Boehringer Ingelheim / Karlsruhe Institute of Technology (KIT), Germany

Poster #28: Short-cut methods for obtaining parameter values of mechanistic chromatography model simulations
Shuchi Yamamoto, Yamaguchi University, Japan

Theme: Membrane Processing

Poster #31: Novel all-membrane process for the purification of monoclonal antibodies without protein A
Ruben G. Carbonell, NIIMBL, United States

Poster #33: Addressing Challenges in High Concentration Drug Substance Manufacturing
Zhi Li, Bristol Myers Squibb, United States

Poster #37 Application of upstream and downstream filtration technologies as solutions for batch and continuous bioprocessing
Julie Kozaili, Asahi Kasei Bioprocess America, United States

Poster #39: High Pressure Increases Likelihood of Parvovirus Breakthrough for a Platform Virus Retentive Filtration by Viresolve® Pro
Valerie Cusick, Regeneron Pharmaceuticals, United States

SUNDAY 2 JUNE 2024 - Full poster abstracts can be found on page 80

Theme: Chromatography and PAT

Poster #43: Real-time process analytical technology (PAT) aggregate monitoring for cation exchange pooling
Moira Lynch, Thermo Fisher Scientific, United States

Poster #46: Solving the Mystery of Multi-Peak Elution Behavior in CEX Chromatography: A Case Study on mAb Process Development
Ronak Gudhka, Amgen, United States

Poster #49: New concept of pH-independent affinity separation of antibodies
Sonja Berensmeier, TU Munich, Germany

Theme: Novel Proteins and New Modalities

Poster #52: Size based sterilization of large therapeutic particle formulations
Volkmar Thom, Sartorius Stedim Biotech GmbH, Germany

Poster #55: Developing a novel non-chromatographic purification for antibody drug conjugates: Moving beyond UF/DF
Brandon Coyle, Gilead Sciences, United States

Poster #58: Performance Evaluation of Continuous Counterflow Centrifugation for Advanced Human Pluripotent Stem Cell (hPSC) Bioprocessing
Alexandra Stuetzer, Sartorius Stedim Biotech GmbH, Germany

Poster #61: How to build a future proof and robust NANOBODY® molecule CMC platform
Willem Van de Velde, Sanofi, Belgium

Theme: mAbs and Bispecifics

Poster #64: Development of a Versatile Downstream Process Toolbox for Control of Specific Polysorbate-Degradation Enzymes
Michael Rauscher, Merck & Co., Inc., United States

Theme: Continuous Processing

Poster #66: Towards Platformization of a Continuous Monoclonal Antibody Purification Process with Capture via Precipitation and Polishing via Subtractive Adsorption
Matthew Mergy, Rensselaer Polytechnic Institute, United States

Theme: Harvest Technologies

Poster #73: Abstract - Scaling Up the Purification of Mammalian Cell Culture Through a Robust PrA EBA Step
Curtis Phippen, UCB, United Kingdom

Poster #75: Development and Implementation of the Single Use Centrifuge for Harvest and Proof of Concept Perfusion Capability
Ryan Hundley, Genentech, United States

Theme: Manufacturing Strategies

Poster #78: Towards Fully Automated Process Development
Brian Murray, Sanofi, United States

MONDAY 3 JUNE 2024 - Full poster abstracts can be found on page 80

Theme: Viral and Non-Viral Vectors

Poster #2: Development of purification process using diverse adsorptive media for novel non-viral DNA gene therapy modality
Khushdeep Mangat, Sanofi, Genomic Medicine Unit, United States

Poster #5: A Novel Harvest-Capture Process for Rapid Clarification and Recovery of rAAV9 from Cell Lysate Using a Macroporous Filtration Device and AVIPure Affinity Resin
Philip Yuen, Repligen Corporation, United States

Poster #8: Improved AAV Release and Lysate Clarification applying an enhanced Cell Lysis Strategy
Martin Sallabus, Sartorius, Germany

Poster #11: A Case Study on the Impact of AAV Triple Transfection Process Changes on Downstream Processing
Matt Teten, BridgeBIo, United States

Theme: Big Data, Data Management and Modelling

Poster #14: A unified data management strategy for downstream development, from early research to market launch
Anton Sellberg, Novo Nordisk, Denmark

Poster #17: Host cell proteins in model based process development of biopharmaceuticals
Marcel Ottens, Delft University of Technology, Netherlands

Poster #20: Driving improvement and optimization of production scale processes via modelling and experimentation
Rune Lorits, Novo Nordisk, Denmark

Poster #23: All-Atom Modelling of Methacrylate-Based Multi-Modal Chromatography Resins for Isotherm Parameter Prediction
Tim Ballweg, Karlsruhe Institute of Technology, Germany

Poster #26: Lord of the rings – Optimization of rAAVv plasmid purification process with a combination of HTS and mechanistic modeling
Sabrina Koch, Roche Diagnostics GmbH, Germany

Theme: Membrane Processing

Poster #29: The ChromaWeb® platform, a modular, linearly-scalable, high-resolution membrane chromatography cassette for purification of viral vectors.
Gastón de los Reyes, SPF Technologies, United States

Poster #32: Optimization and Characterization of a High-concentration UF/DF Process to Overcome the Gibbs-Donnan Effect on Multiple Excipients
David Brown, KBI Biopharma, United States

Poster #35: Microfluidic Filtration Device for High Throughput Vaccine Process Development
Nusrat Jahan, University College London, United Kingdom

Poster #38: Analyzing Sterile Filtration of mRNA-LNP Therapeutics Using a New Modeling Framework
Andrew Zydney, Pennsylvania State University, United States

Theme: Chromatography and PAT

Poster #41: Peptide-based Host Cell Protein (HCP) Removal Technologies for Biomanufacturing
Stefano Menegatti, North Carolina State University, United States

Poster #44: Advanced Multi-Mode Separation Platform using Block Copolymer Approach
Jerald (Jerry) Rasmussen, Solventum, United States

MONDAY 3 JUNE 2024 - Full poster abstracts can be found on page 80

Poster #47: Structure-based multimodal ligand design: an iterative approach towards novel selectivity
Eva Heldin, Cytiva, Sweden

Poster #50: Impact of 3D printed stationary phases in integrated downstream processing of monoclonal antibodies – a process modelling approach
Mariachiara Conti, The University of Edinburgh, United Kingdom

Theme: Novel Proteins and New Modalities

Poster #53: Elevating Precision in Extracellular Vesicle Research: Unveiling a Tailored Affinity Chromatography Approach Monitored by Novel Analytical Tools
Rita Fernandes, IBET, Portugal

Poster #56: Nanoparticulate separations: the challenges of larger dimensional space
Daniel Bracewell, University College London, United Kingdom

Poster #59: A scalable membrane process for the purification of extracellular vesicles
Cristiana Boi, Università di Bologna, Italy

Theme: mAbs and Bispecifics

Poster #62: Development of a High-Capacity Downstream Toolbox for Purification of Bispecific Antibodies
Chris Furcht, Bristol Myers Squibb, United States

Poster #65: Strategies to control Polysorbate 80 degradation in mAb bulk drug substance
Erich Blatter, GSK, United States

Theme: Continuous Processing

Poster #67: A lab-scale model and GMP-scale proof of concept for PCC Protein A using a dynamic column loading strategy
Joseph Costanzo, Eli Lilly & Company, United States

Poster #70: AUTONOMOUS OPERATION AND ADVANCED CONTROL OF INTEGRATED CONTINUOUS DOWNSTREAM PROCESSES
Bernt Nilsson, Lund University, Sweden

Theme: Harvest Technologies

Poster #72: Evaluation of a single-use disk stack centrifuge for improved efficiency and sustainability at 1000 L GMP manufacturing scale
Brad Stanley, Biogen, United States

Theme: Manufacturing Strategies

Poster #76: Integrated Strategy of Raw Material Interchangeability for Resilient Manufacturing and Global Supply
Xiaoxiang Zhu, Amgen, United States

Poster #79: Buffer recycling in biopharmaceutical downstream processing for cost reduction and increased sustainability
Madelene Isaksson, Lund University, Sweden

WEDNESDAY 5 JUNE 2024 - Full poster abstracts can be found on page 80

Theme: Viral and Non-Viral Vectors

Poster #3: Why not both? Harnessing the powers of affinity- and size- based separations to transform the manufacturability of lentiviral vectors
Kelli Luginbuhl, Isolere Bio, United States

Poster #6: Bridging the translation gap: The development of a toolkit to accelerate process development and provide early manufacturability insights for AAV gene therapies
Jayan Senaratne, MeiraGTx, United Kingdom

Poster #9: Use of flowthrough anion exchange chromatography for the enrichment of full capsids in Adeno-Associated Viral Vector purification
Andres Martinez, Roche, Germany

Poster #12: Highthroughput Development and Scale-up of a Novel Anion Exchange Chromatography Method for Removal of Empty Capsids from full rAAV Capsids.
Amith Naik, Asklepios Biopharmaceutical Inc., United States

Theme: Big Data, Data Management and Modelling

Poster #16: Enhancing Biopharmaceutical Process Control and Efficiency through Integrated Data Analytics and Predictive Modeling: Insights from the BioRaptor Platform
Yaron David, BioRaptor.AI, Israel

Poster #18: A novel isotherm for hydrophobic interaction chromatography that improves prediction precision across operation conditions and facilitates sensitivity analyses
Johannes Buyel, University of Natural Resources and Life Sciences, Vienna (BOKU) , Austria

Poster #22: Mechanistic modeling-based characterization of size-exclusion-mixed-mode adsorbents for antibody fragment separations
Steven Cramer, Rensselaer Polytechnic Institute, United States

Poster #24: Integrating QSAR Modeling with High Throughput Screening for Rapid Development of Polishing Chromatography Steps for Protein Therapeutics
Michael Hartmann, Merck, United States

Poster #27: Efficient Simulation of Extra Column Volume in Small-Scale Multi-Column Chromatography
Juliane Diehm, Karlsruhe Institute of Technology, Germany

Theme: Membrane Processing

Poster #30: Scale-Up and Applications of Nanofiber Membrane Chromatography
Steve Burton, Astrea Bioseparations Ltd, United Kingdom

Poster #34: Capabilities and limitations of IR for enhanced process monitoring during UF/DF of proteins
Yi Zhang, AstraZeneca, United States

Poster #36: Post-affinity-capture depth filtration: it's just there to remove solids, right?
Brian Bowes, Eli Lilly and Company, United States

Poster #40: Informing Ultra Scale Down depth filtration device design using high resolution imaging
Thomas Johnson, University College London, United Kingdom

Theme: Chromatography and PAT

Poster #42: Streptococcus pneumoniae surface protein and nanobody affinity resins for Capture and Purification of fully assembled secretory immunoglobulin A
Nico Lingg, BOKU, Vienna, Austria

Poster #45: Novel Approach to Affinity Capture Elution Design
Wei Lu, Takeda Pharmaceuticals, United States

WEDNESDAY 5 JUNE 2024 - Full poster abstracts can be found on page 80

Poster #48: Advancing Efficiency and Mitigating Risk: Raman Spectroscopy-based Analysis of Monoclonal Antibody and Excipient Concentrations throughout Downstream
Kristina Pleitt, Thermo Fisher Scientific, United States

Poster #51: The impact of elution pH on product quality of Fc containing proteins.
Alvaro Cruz-Izquierdo, Purolite, United Kingdom

Theme: Novel Proteins and New Modalities

Poster #54: END-TO-END SCALABLE PURIFICATION PLATFORM FOR EXTRACELLULAR VESICLES
Sanket Jadhav, Sartorius Stedim, Netherlands

Poster #57: Optimization of Post-conjugation Polishing Steps for Peptide-antibody Conjugates
Qin Gu, Amgen, United States

Poster #60: Biosimilars development challenges and opportunities
Mahsa Rohani, Amgen, United States

Theme: mAbs and Bispecifics

Poster #63: Host cell proteins profiling from a group of Monoclonal and Bispecific antibodies
Jean Aucamp, Lonza, United Kingdom

Theme: Continuous Processing

Poster #68: Enhancing Virus Filtration Efficiency in Continuous Processing through Serial Filtration with High Area Ratio
Sal Giglia, MilliporeSigma, United States

Poster #69: Integrated Control and Scheduling of a Multi-Column Chromatography Operation with Numerical Dynamic Optimization
Ian Gough, McMaster University, Canada

Poster #71: Development of Temperature-Controlled Batch and 3-Column Counter-Current Protein A System for Improved Therapeutic Purification
Stephen Goldrick, University College London, United Kingdom

Theme: Harvest Technologies

Poster #74: From Challenges to Opportunities: Evaluation and Implementation of New Single-use Harvest Technologies with a Holistic View on Portfolio and Network Impact
Jerome Bill, Genentech, United States

Theme: Manufacturing Strategies

Poster #77: INNOVATIVE RISK-BASED APPROACHES TO ACCELERATE DOWNSTREAM BIOPROCESS DEVELOPMENT
Angela Lewandowski, Bristol Myers Squibb, United States

Poster #80: Next Generation Small Footprint facilities and Simplified Workflow utilizing Continuous Processing and Integrated Buffer Management
Vinit Saxena, Sepragen Corporation, United States

In keeping with the conference aims of fostering and supporting knowledge sharing, discussion and networking, RXX will host 5 roundtable sessions. The Roundtables will give participants a chance to 'dig a little deeper' into areas they are passionate about, as well as build and strengthen connections with like-minded conferees. Using engaging, open-ended topics, conferees will share their experiences, brainstorm questions, yield new ideas and have conversations in a very different way to oral or poster sessions. There will be five simultaneous topics discussed in separate meeting rooms.

Recovery XX Roundtable 1: What Do We Do Today to Be Ready for the Next Pandemic? | Abbot

We will engage in dynamic discussions aimed at drastically reducing the time to market for vaccines and drugs. This session is an urgent call to address the critical questions of pandemic preparedness in the context of CMC development and manufacturing. We will explore the technological advancements in recovery and purification, and standardization of platform technologies across various modalities such as proteins, mAbs, mRNA, and viral vectors and delve into the accelerated CMC pathways that have emerged from the crucible of COVID-19, analyzing innovative regulatory and quality approaches. Operations will take center stage as we discuss the optimization of supply chains, the urgency of rapid capacity deployment and the potential of decentralized manufacturing. The three breakout sessions will focus on:

- Technology Advancement & Platform Standardization: Discover cutting-edge advancements in recovery and purification, and the push for platform standardization that's revolutionizing vaccine and drug modalities from proteins to mRNA and beyond.
- Accelerated CMC Development: Examine the fast-tracked pathways of CMC development, and discuss the innovative regulatory frameworks (e.g. platform technology designation) and quality approaches enabling this rapid progress.
- Operational Excellence in Supply Chain and Manufacturing: Explore strategies for optimizing supply chains (in both raw material sourcing and product distribution), rapidly deploying manufacturing capacity, decentralizing production, and managing the workforce to meet the demands of a health crisis.

We will discuss the following questions in each break out session:

- What are the new technologies, new ways of working developed in our industry that are significantly impactful overcoming the critical challenges imposed by the COVID-19 pandemic?
- What challenges and gaps still exist? What actions can we take today to close the gaps. What can we do across industry, countries, governments, companies and academia?
- What key processes, systems, and ways of collaboration should we build or improve today to sustain the agility demonstrated in responding to the COVID pandemic?

Recovery XX Roundtable 2: Go Big or Go Home – Evolving Bioprocessing for New Modalities | Homestead 1

New therapeutic modalities, such as viral vectors, lipid nanoparticles, multi-specific assemblies, and others, present new opportunities to treat, cure, and prevent serious health conditions and greatly improve quality of life around the world. With these opportunities come process and manufacturing challenges that will dictate how fast these new treatments can be developed, tested, and commercialized. The new classes of therapeutics are larger, more complex, and have more complicated biochemistry compared to the current major classes of biotherapeutics such as mAbs. Let's discuss the best ways to accelerate process development of these new modalities!

The round table will focus on three general areas to develop a set of recommendations for the wider conference audience.

Discussion area #1: Finding order in chaos – defining new modality spaces

- How will the new modality space evolve?
- How new is new?

Discussion area #2: Is legacy a valuable companion or excess baggage?

- Scale-up vs scale-out?
- To platform or not to platform?
- Regulatory framework evolution vs revolution?

Discussion area #3: Let's get down to business!

- Are near and future bioprocessing priorities the same in these spaces?
- Top 3 priorities and trends in bioprocessing strategy and technology for the future
- Bottom 3 priorities and trends in bioprocessing strategy and technology for the future

Recovery XX Roundtable 3: What is the Future of Modeling for Recovery of Biological Products? | Homestead 2

One may assume that with advances in scientific knowledge and a plethora of data for calibration that first principles will be employed in developing models for recovery of biological products. The goal of this roundtable is to foster a debate about the future state of modeling and the strategy on how to translate the opportunities into tangible and sustainable deliverables.

For example, if first principles are feasible for model development who and where will these be developed (Industrial CMC, Academia, Collaboration, Scale-up) and maintained over the lifecycle? https://onlinelibrary.wiley.com/doi/abs/10.1002/bit.27520. Standardization and reference state/system for model development? How good is good enough? What are the acceptance criteria, and do they differ depending on context of use? What is the role of ML and AI in model development? Mathematically consistent but physically relevant? Initial forays have revealed some limitations:
https://www.npr.org/2023/02/02/1152481564/we-asked-the-new-ai-to-do-some-simple-rocket-science-it-crashed-and-burned

Recovery XX Roundtable 4: How Can We Drive Down Biopharma Costs to $1 Per Dose for Wider Access? | Homestead 3

Join us for an engaging roundtable session focused on the pressing issue of bringing down biopharma manufacturing costs and ensuring wider access to advanced biotherapeutics in low and middle-income populations. In this roundtable we will explore innovative strategies, insights, and potential solutions to make advanced biotherapeutics more affordable and accessible; this will cover modalities ranging from mAbs through to viral vectors, nucleic-acid therapies and beyond. We will delve into cost-effective manufacturing techniques, draw inspiration from lower margin industries, and examine the possibilities of newer modalities in driving costs down. Additionally, we will explore the potential of distributed manufacturing as a means to improve equitable access to advanced medicines for underserved populations. Don't miss this opportunity to be part of the conversation shaping the future of global healthcare access.

We will be tapping into your experiences and insights on these two core themes, addressing questions such as:

Theme 1: Innovative Approaches to Cost-Effective Manufacturing

- While the cost of manufacturing is only a very minor contributor to the cost of drugs in high-income countries, it will be limiting profitability in low and middle-income countries. What are the main levers to reduce the cost of manufacturing that can enable access to underserved populations?
- What valuable insights can we gain from lower margin industries like the food industry or industrial enzyme production about cost-efficient biopharmaceutical manufacturing?
- How can the vaccine industry's experiences with high volume/lower cost manufacturing inform strategies for expanding access to advanced biologics in resource-constrained settings?

Theme 2: Striking the Balance: Cost Efficiency for Wider Access

- With the emergence of mRNA-based therapies, do we anticipate lower production costs compared to traditional protein therapeutics, and if so, how can we leverage this potential for wider accessibility?
- Is distributed manufacturing a viable solution to improve equitable access to advanced medicines in underserved populations, and what are the challenges and opportunities associated with this approach?
- How can we foster collaboration among stakeholders to ensure wider access to advanced biotherapeutics?

Our goal is to facilitate a lively and interactive discussion where we encourage open dialogue and sharing of experiences from all participants. We will start with a quick check-in with participants for additional suggestions and prioritization of topics for the group to tackle. Please feel free to forward comments to us before the meeting.

Recovery XX Roundtable 5: How Do We Implement Sustainable Production that is Compatible with Reduced Costs? | Landmark

The main objectives of bioprocesses in biopharmaceutical manufacturing are to reliably deliver drugs in a relatively short time frame with high quality, and to avoid batch failures within a tight regulatory framework. Bioprocesses are highly complex, the level of automation is highly varying, and there is constant pressure to improve efficiency and reduce costs. In addition, climate change and resource scarcity require a reduction of the environmental footprint of bioprocesses and manufacturing facilities.

The global environmental impact of the entire healthcare industry has been almost completely unaddressed. The American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable has reported that the global warming potential for e.g. a US-based 2000 L process is approximately 22.7 tons CO2eq per 1 kg drug substance. Process intensification and better plant utilization are key to reducing environmental impact. For a 2000 L single-use bioprocess, the estimated single-use systems account for 769 kg per batch compared to 226 kg for the chemicals used. Also, measures must be taken to prevent emissions from increasing further despite the growth of the sector.

In the biopharmaceutical industry, two extreme production scenarios are often employed: a fully disposable factory with the characteristics of small batch sizes, great flexibility, and speed, or a fixed large-scale plant with high capacity. Forward-looking solutions and ideas will be discussed on how to combine environmentally friendly practices and technologies with new bioprocesses for the benefit of the patient, supply security and profitability.

Three topics will be explored in this Roundtable, considering the current best practices used for established biologics production and ways to implement sustainable production that are compatible with high quality and reduced cost:

- Metrics for assessing the environmental footprint of a bioprocess in context of societal impact of biopharmaceuticals
- How to improve the environmental footprint through more sustainable process design and manufacturing practices and understanding the consequences for production costs
- Implementation of a circular bioeconomy in bioprocessing with focus on contributions possible through downstream processing improvements (e.g., reduce, reuse, recycle)

Session 1 - The Adventure Begins at Harvest

Arick Brown, Amgen, United States
Brandon Christensen, Visterra, United States

1. Single-use continuous centrifugation harvest for high density cell culture

*Oliver Kaltenbrunner, Amgen, United States
Xiaoyang Zhao, Amgen, United States
David Ladoski, Amgen, United States

Intensified high density cell culture in a single-use setting is a highly productive means to producing biological therapeutics. However traditional single-use technologies are not suitable for high density cell culture harvest operations. Direct depth filtration is limited by the high solids content in the feed. Microfiltration is difficult to perform robustly with either product sieving or filter fouling limitations. While continuous discharge centrifugation is an established technology in stainless steel manufacturing, an equivalent technology for single-use production was not available until recently. In collaboration with a manufacturer, we guided the development of a robust single-use centrifuge for cell culture harvest. We will discuss harvest performance for a wide range of cell densities and cell culture viabilities, as well as process control and robustness challenges compared to stainless steel centrifugation. Our analysis is based on many centrifugation harvests of several molecular entities at pilot, clinical, and commercial scale with feed solids content across a wide range packed cell volume, utilizing both Single-Use and Stainless Steel centrifuges. Harvest performance and operational controls will be compared to stainless steel centrifuge harvest.

2. Efficient clarification strategies for high solid content cell culture fluids

*Haikuan Liu, WuXi Biologics, China

High titer production of monoclonal antibodies through continuous cell culture processes is typically achieved by maintaining high Viable Cell Density (VCD, typically > 60 × 106 cells/mL) over long culture durations. However, these high titer processes also come with new challenges for the following harvest step with high solid content. Usually, flocculation combined with depth filtration is used to handle the high solid content cell fluids. However, the load capacity of the depth filters and the recovery yields are typically low, and the Cost of Goods (CoGs) is high. Meanwhile, cell lysis and target protein loss caused by flocculation treatment are observed in some cases. To overcome these challenges, three clarification strategies were scouted and compared for clarification of the high solid content (up to 35%) cell culture fluid, including continued discharging centrifugation followed by depth filtration, continued discharging centrifugation followed by flocculation and depth filtration, and perfusion harvest using ATF. The results of the above three methods are remarkable, and the recovery yield of the target protein can reach higher than 90% with lower cell lysis risk and CoGs, with no impact on the product quality. These results illustrate that our efficient strategies can be successfully used in the clarification step for the recovery of high solid content and high cell density cell culture, and they can be easily integrated into current mAb purification platforms, effectively alleviating other related downstream processing challenges.

3. 5-in-1 application of an off-the shelf charged fibrous device allows for hyper-intensified production of recombinant Adeno-Associated Viral vectors

*Daniel Hurwit, Bristol Myers Squibb, United States
Rebecca Hochstein, 3M , United States
Nikki Sitasuwan, 3M , United States

Adeno-associated viral vectors have emerged as the leading gene delivery modality for in vivo gene therapy and ex vivo, gene-edited, cell therapy applications. Consistent and considerable investment have advanced the field, leading to: an enhanced understanding of AAV biology, an explosion of clinical programs in a growing diversity of indications, the commercial approval of six (and counting) groundbreaking therapies, and the development of scalable, robust, and modern manufacturing processes. However, sizeable opportunities to recognize the potential of these therapies remain, especially in the realm of rAAV manufacturing. Despite advances in vector production, manufacturing is regularly on the critical path to the clinic, and often a factor in commercial viability. Furthermore, as the modality matures, quality standards continue to rise. In the quest for 'more, better, faster, and cheaper', process intensification must be considered. Excited by the challenge, we aimed to combine lysis, clarification, DNA removal, full enrichment, and concentration and buffer exchange into one operationally simple process step. We aimed not only to combine but also to improve the cost, speed, quality and robustness in comparison to the standalone unit operations; for example, improving the yield from typical detergent-based lysis, and eliminating the common and highly expensive utilization of nuclease for DNA clearance. Here, we review the design, screening, development, and optimization of a commercially available, charged, non-woven fiber filter to create the combined, 5-in-1 process . We will present a thorough analysis of our efforts and results, including favorable cost and performance comparisons relative to other technologies across and in consideration of a wide range of metrics. We hope to share our work to expand the toolbox for rAAV manufacturers and, perhaps, that of other modalities as well.

4. A new multifaced player in the field of AAV harvest recovery and viral clearance

Alex Berrill, Pfizer, United States
*Yulia Ivanova, Pfizer, United States
Hannah, Bare, United States

Primary recovery out of harvest is first and the least understood step in downstream purification of AAV vectors. Complexity of lysed cell culture coupled to relatively low protein concentration of AAV product makes it very difficult to optimize process performance. A novel detergent was identified to support process development need for much higher AAV recovery in comparison to recoveries observed with standard Triton/DB combination. New detergent, in combination with divalent cation salt was shown to play dual role, act as lysis and recovery reagent as well as a DNA flocculation agent. First identified in small scale screens, best condition was scaled up and demonstrated to perform comparably at 1L, 10L, 250L and 2000L scale. Overall recovery was improved 3-5-fold without any adverse changes to the AAV product quality. Identified detergent was also shown to have comparable to Triton viral clearance ability, thus providing alternative route to viral clearance. Identified detergent/salt combination was tested with different serotypes and showed positive results in all tested cases.

Session 2 - Advances in Structure-Function Understanding and Developability Assessment for Novel Biologics

Chen Wang, AbbVie, United States
Peter Tessier, University of Michigan, United States

1. Developability Evaluation of Discovery Biologic Candidates by

*Rajeeva Singh, AbbVie, Inc., United States

Screening of discovery-stage biologic drug candidates for developability is an important tool for the selection of lead candidates with desired physicochemical characteristics, enabling further drug development. We have optimized the developability screening to support a pipeline of highly varied biologic drug classes, including monoclonal antibodies, bispecific antibodies, antibody-drug conjugates, fusion proteins, and AAV gene therapies. Based on the requirements for diverse therapeutic areas, suitable screens have been implemented to assess physicochemical properties and accelerated stability under stress conditions. Developability evaluation of discovery biologic candidates is challenging due to the constraints of small sample amounts and the limited time available for comprehensive comparison of multiple candidates. We have used both experimental and sequence-based tools to enable efficient developability screening. Case studies will be discussed, such as ultra-high concentration feasibility screening of biologic candidates based on low viscosity, and chemical liability evaluation under appropriate accelerated stability stress conditions to assess storage and physiological stability risks. Benchmarking studies to validate the screening criteria will be discussed using examples of commercial biologics. The early developability screening of biologic candidates in discovery programs ensures the selection of efficacious and developable lead candidates for further pre-clinical and therapeutic evaluation. The developability screening tests for the candidates are customized based on the requirements of the therapeutic areas, such as high concentration liquid formulation to enable delivery by pre-filled syringe, sub-cutaneous versus intravenous delivery, or lyophilized formulation. A collaborative evaluation by research and development teams allows an overall developability assessment of discovery biologic candidates based on physicochemical, pharmacokinetic, and pre-clinical safety properties. Furthermore, implementation of the developability evaluation in the discovery screening funnel enables rapid advancement of the lead candidates to the clinic.

2. Evaluation of descriptors and machine learning strategies for monoclonal antibody chromatography process developability prediction

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Sean Burgess, Genentech, United States
Amy Wang, Genentech, United States

Purification process development for biotherapeutics conventionally relies on wet lab experiments in order to understand the relationships between protein sequence/structure and molecular properties such as chromatographic binding affinity. Empirical workflows have been greatly improved with high- throughput techniques, resulting in increased data volume. This, along with improved molecular descriptors, has enabled the development of robust machine learning pipelines to predict purification behavior from the amino acid (AA) sequences of molecules. In this presentation, we describe a novel approach for generating downstream process predictions from AA sequence. We employ quantitative structure property relationship modeling (QSPR) to predict monoclonal antibodies' (mAb) binding behavior on 5 different chromatography resins in the presence of two different background salt species across a range of pH and salt concentration covering the purification design space. Models were developed with a diverse set of 50 mAbs, and evaluated with a withheld subset of 10 mAbs (randomly selected with stratification in order to ensure a diverse and rigorous test). Model development benchmarked 4 different whole-mAb descriptor sets including structure-based descriptors from two commercially available software packages, and sequence-based descriptors from two open source protein language models. The evaluation demonstrates that, in many cases, QSPR may be used in lieu of performing a batch-binding screen, and provides a valuable tool for ranking molecular binding affinity, identifying molecules with similar binding behavior, flagging potentially challenging to develop outlier molecules, and providing meaningful insights to inform purification process development from sequence alone.

3. Modeling the chromatography behavior of monoclonal antibodies in hydrophobic interaction chromatography

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Thomas Chin, Takeda Pharmaceutical Company, United States
Mick Eamsureya, Takeda Pharmaceutical Company, United States
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Christina Alves, Takeda Pharmaceutical Company, United States
George Parks, Takeda Pharmaceutical Company, United States

Monoclonal antibody (mAb) therapies require a high level of purity for regulatory approval and commercialization. High-molecular weight (HMW) species are a common impurity encountered in the downstream purification of mAbs. Hydrophobic interaction chromatography (HIC) resins are often used to remove these HMW impurities. Selecting a suitable HIC resin to use for HMW clearance while maintaining high percent recovery of mAb monomer can require screening across multiple resins and buffer conditions. It would expedite the bioprocess development of novel mAb therapies to, instead, use predictive models based on a few measurements of the physicochemical properties of mAbs and resins to guide the selection of HIC resins. In this study, we measured a set of physicochemical properties of seven mAbs and thirteen resins and correlated those physicochemical properties with percent flow-through recovery and HMW clearance when using a high throughput screening technique. We explored thermal stability, surface hydrophobicity, and surface charge as descriptors for the mAbs and hydrophobicity, zeta potential, and vendor as descriptors for the resins. The thermal stability of the mAbs was assessed by determination of the melting temperature (Tm) and temperature of the onset of aggregation (Tagg). The surface charge of the mAbs was assessed using both pI and capillary zone electrophoresis and the relative surface hydrophobicity (RSH) was qualitatively assessed using a HPLC HIC assay. The hydrophobicity of each of the resins was evaluated using an analysis of retention factor of our set of mAbs on the HIC resins. Predictive models were generated for percent recovery and HMW clearance for individual mAbs and resins. The physicochemical properties correlated well with the behavior of mAbs on HIC resins. The models were successfully applied in selecting a resin for HMW clearance for a novel mAb. Poor thermal stability (low Tm and Tagg) was correlated with generation of HMW species upon purification with HIC resins. When modeling percent recovery and HMW clearance for the mAbs across a single resin, RSH alone could be used to account for most of the variance in our data. However, the inclusion of a surface charge descriptor into the model was critical for accurate predictions. We did identify unique mAb-resin interactions that could not be modeled using our modeling approach. Notably, attempting to model the HMW clearance for the mAbs which generated large amounts of HMW species resulted in poor fits for the models. We were also able to model percent recovery and HMW clearance of a single mAb across our set of resins using resin descriptors of hydrophobicity, zeta potential, and vendor. To our knowledge, this is the first report of zeta potential measurements on HIC resins and was critical in generating accurate predictions for percent flow-through recovery and HMW clearance. These models were used to predict an optimal resin for a test mAb using just measurements of the mAb's RSH and surface charge and applied in a small-scale chromatography run. This modeling approach can accelerate bioprocess development and reduce time to patient.

4. Assessing the developability of fractionated monoclonal antibody proteoforms: Impact of charge, hydrophobicity, and glycans on aggregation susceptibility

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Monoclonal antibodies play a vital role in patient therapeutics and the industry is experiencing positive annual growth. Virus filtration is a dedicated virus reduction step in mAb production and effectively removes both enveloped and non-enveloped viruses by size exclusion. However, current process development for virus filtration relies on experimental approaches including excipient evaluation or prefiltration to mitigate premature virus filter fouling. Denatured hydrophobic variants, plugging aggregates and reversible aggregates have previously been reported as major foulants of virus filters. This study addresses the gap in understanding related to self-association and aggregation behavior of post-translationally modified mAb variants (proteoforms). This study fractionated a highly hydrophobic mAb pool into sub-pools enriched with different proteoforms. The effectiveness of different chromatography resins (cation exchange, anion exchange, and mixed mode) in fractionating the proteoforms was then validated using capillary electrophoresis characterization. Correlations were established between the relative abundance of specific proteoforms, sub-pool hydrophobicity, and diffusion interaction parameter (KD). The relative hydrophobicity of sub-pools was determined by retention time on a hydrophobic interaction phenyl column. Charge and glycoform parameters were utilized to evaluate the proteoforms of interest. Hydrophobicity and KD were indicative of reversible and irreversible aggregation propensity. This study provides a framework for selecting appropriate pH, conductivity, buffer, and excipients to mitigate monomeric self-association propensity of mAbs. By developing process conditions that minimize these interactions we move towards virus filtration process development based on mAb characteristics, rather than brute-force experimental approaches.

Session 3 - Mission Impurity Characterization: Advances, Challenges, and Regulatory Insights across Modalities

Kristin Valente, Merck and Co., Inc., United States
Andre Dumetz, GSK, United States

1. Quality By Design for Control of Polysorbate Degrading Host Cell Protein in Biologicals

*John Mattila, Regeneron Pharmaceuticals, Inc, United States

Chinese Hamster Ovary (CHO) Host Cell Proteins (HCP) with enzymatic activity present challenges to patient safety and product stability if they are not addressed during process development and scale up. A class of CHO HCP has been reported in literature to degrade polysorbates, which may reduce levels of this stabilizing excipient or result in formation of insoluble fatty acid particulates (Jones et al., 2021). These insoluble fatty acid particles appear as subvisible particles governed under U.S. Pharmacopeia guidance for parenteral products, and these HCP may limit shelf life when present at single digit ppm levels. While these HCP with enzymatic activity could be present in any CHO derived biologic, this presentation will provide retrospective analysis and data visualization suggesting commonalities and differences across protein modalities. They have been shown to persist in drug substance despite multiple orthogonal manufacturing steps intended to reduce impurity levels, suggesting an interaction or co-purification with protein therapeutics. Lack of control of these impurities can be shown to result in highly variable product shelf life. As such, process development is prioritized consistent with Quality by Design to ensure control of these HCP for higher risk protein therapeutics. Trusted analytical methods are foundational to process development and control. Rapid, effect-based analytical methods allow optimization of a manufacturing step for the class of polysorbate degrading HCP with minimal bias towards specific proteins. These methods allow mathematical models to be constructed for performance simulations to estimate process capability based on input variation. This presentation will feature case studies illustrating how multiple downstream steps may contribute to control of CHO HCP that degrade polysorbate. Affinity rProtein A may remove non-specifically bound HCP and offers potential for impurity removing wash solutions. Adsorptive depth filters have demonstrated capability to reduce CHO HCP when used in combination with further purification steps. Multiple chromatography modes may be used to exploit well-understood mechanisms and may also contribute to control. Studies have shown careful regeneration procedures are essential to ensure reproducible performance during repeated use of chromatography resin. This presentation will show control of CHO HCP that degrade polysorbate to illustrate a Quality by Design approach for process characterization. This approach may be broadly applicable to other novel product-related impurities.

2. Tailoring Polishing Steps for Effective Removal of Polysorbate-Degrading Host Cell Proteins in Monoclonal Antibody Purification

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Ensuring the quality and safety of biopharmaceutical products requires the effective separation of monoclonal antibodies (mAbs) from host cell proteins. A significant challenge in this field is the enzymatic hydrolysis of polysorbate in drug products. This study addresses this issue by investigating the removal of polysorbate-degrading host cell proteins (HCPs) during the polishing steps of downstream purification, an area where knowledge of individual HCP behavior is limited. Using a highly sensitive LC-MS/MS method, we identified polysorbate-degrading hydrolases in different formulated mAb model products. Stable overexpression and purification of these hydrolases allowed for detailed characterization and risk assessment. We then examined the separation of five different mAbs from four individual polysorbate hydrolases using cation exchange (CEX) and mixed mode chromatography (MMC) polishing steps. High-throughput binding screenings were conducted across various pH and salt concentrations, using the partition coefficient (Kp) as a key indicator of protein binding or flow-through. The analysis revealed distinct binding behaviors of polysorbate-degrading HCPs and mAbs, emphasizing the importance of understanding the binding characteristics to optimizedownstream processing steps. A three-step strategy was employed to eliminate polysorbate-degrading HCPs in polishing steps. First, high-throughput screenings were performed for mAbs to compare their binding behavior to the screening HCPs. Second, a Design of Experiment was applied to 600 µL miniature columns to transfer the data to a packed bed column format and optimize the separation conditions. Optimal pH and counterion concentrations were identified and validated for each polishing step using packed bed columns. This strategy successfully identified optimal separation conditions, achieving satisfactory HCP reduction levels, while maintaining high mAb recovery rates (> 96 %). Our findings highlight the critical role of the antibody's inherent properties for successful separation. These results underscore the need to tailor the purification process to leverage the slight differences in binding behavior and elution profiles between mAbs and HCPs. This approach can lead to the production of high-quality mAb products, addressing the challenge of enzymatic polysorbate degradation in biopharmaceuticals.

3. Progress and challenges towards characterization and control of impurities in antibody-drug conjugates

*Michaela Wendeler, AstraZeneca, United States

Antibody-drug conjugates (ADCs) represent one of the fastest growing classes of biopharmaceuticals. Combining the targeting specificity of monoclonal antibodies with the cytotoxic effects of highly potent small molecules, they are emerging as powerful tools for the treatment of cancer. For process developers, these modalities present unique challenges with regard to impurity characterization and control: In addition to aggregates, fragments, and complex product variants that need to be cleared, the conjugation process introduces highly toxic impurities and other chemicals that must be reduced to extremely low levels. Furthermore, analytical characterization requires novel approaches in the face of complex molecular properties, and CMC strategies need to adapt to evolving regulatory expectations. Here we describe process and analytical strategies to characterize and control product-and process- related impurities in ADCs. We illustrate development challenges presented by the large variety of impurities, describe characterization strategies, and demonstrate how an integrated control strategy that spans across the small and large molecule process guarantees high product quality. We demonstrate the benefits of including modeling of impurity clearance in process design. And finally, we illustrate how enhanced understanding of impurity characteristics can lead to reduced process mass intensity and significantly improved sustainability, while ensuring patient safety and regulatory success.

4. Advances in the Purification of Antisense Oligonucleotides

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Following on the commercial success of Spinraza® (treatment for spinal muscular atrophy) and most recent Qalsody (treatment for ALS), antisense oligonucleotides (ASO) are now a critical part of Biogen's drug portfolio that enables us to better treat neurology or neuromuscular diseases. Development of the ASO platform process employs aqueous-based orthogonal chromatography (HIC, AEX) steps to achieve industry high purities while maintaining good yield along with required detritylation and UF/DF steps. To date, we've now leveraged this platform process to purify 13+ ASO molecules that contain different sequences, chemistries, and most recently incorporating conjugation reactions. Having completed process characterization for several ASO candidate along with intensification for 1 ASO, we've achieved a greater understanding of the process, it's capabilities to clear process and product related impurities and understand the mechanisms that control the chromatography. On the manufacturing side, Biogen recently validated the process for Qalsody using single-use disposable technologies which included traditional scale-up, modeling and PAT as part of the control strategy. Several cases studies will be presented on learnings from development on controlling the process steps, incorporating both process modeling and intensification, lessons learned regarding use of a liquid API, incorporation of a conjugation reaction into the purification process along with some at scale performance metrics.

Session 4 - Escapades of a Continuous Nature

Andrew Tustian, Regeneron Pharmaceuticals, United States
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1. Integrated and continuous purification: the journey from hybrid to fully continuous, and from vision to reality

*Jason Walther, Sanofi, United States
Chad Varner, Sanofi, United States
Shashi Malladi, Sanofi, United States
Akshat Mullerpatan, Sanofi, United States
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Peter Firmin, Sanofi, United States
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Kevin Brower, Sanofi, United States

Continuous manufacturing has been gathering momentum for the production of biologics since the early 2010s. Following other industries that have made the transition from batch to continuous, examples of integrated and continuous bioprocessing have appeared in both clinical and commercial settings. Often these examples rely on a hybrid approach, where continuous operations comprise a portion of the process, and batch methods are used for the remainder. At Sanofi, we have a vision and a plan to take the next step and design, test, and implement a fully integrated and continuous purification platform to fully unlock the benefits of continuous manufacturing for drug substance processing (increased automation, reduced footprint, lower residence times, etc.). This poster will discuss that journey, with a particular focus on recent steps toward implementation. The following topics will be discussed:

- Full-scale, proof-of-concept runs have been executed, demonstrating the feasibility of the continuous purification platform and identifying potential gaps
- A flexible, modular skid has been designed and prototyped, and has been successfully integrated within the continuous platform at commercial-scale levels of throughput. New data has been generated demonstrating that this skid is capable of operating continuous chromatography or filtration operations, and will enable a transition of this technology to a GMP environment
- Advanced process control strategies have been evaluated to increase operational responsiveness while simultaneously decreasing development time and reducing complexity for the user
- The continuous purification platform has been implemented during the development of a pipeline biologic for first-in-human trials. This opportunity has allowed the team to assess the feasibility of rapid process development for continuous operations. This effort culminated in the pilot-scale continuous production of drug substance, enabling product quality comparisons and a technical assessment of the capabilities and gaps of the platform While significant advances have been made, exciting opportunities still await. Continued progress will be best realized as we work and partner together in this industry to increase capabilities and understanding across various dimensions, such as control, automation, equipment, sensors, and regulation.

2. Turning the crank using a hybrid continuous purification platform

*Michelle Najera, Just-Evotec Biologics, United States
Megan McClure, Just-Evotec Biologics, United States
Beth Larimore, Just-Evotec Biologics, United States

The benefits of process intensification via the coupling of perfusion bioreactors to continuous chromatography include enhanced time and material efficiency, reduced cost of goods, and reduced impurity burdens. Despite the unrivaled performance of continuous processes, this strategy is not commonly incorporated into platform purification processes for the manufacture of clinical material for antibody-like biologics. One reason lies in the perception that they are inflexible and too complex, particularly around automation. Just-Evotec Biologics has developed and scaled up hybrid continuous processes to the 500 L scale and has successfully manufactured over 15 different products in this format. This talk provides an overview of the Just-Evotec hybrid continuous chromatography platform with an emphasis on the Protein A capture and low pH viral inactivation steps. One key requirement of a robust platform process is flexibility, especially for contract manufacturing and development organizations (CDMOs) that are likely to handle a wide range of molecular formats and process inputs. Another key requirement is simplicity of design, such that execution in manufacturing is sufficiently straightforward. One obstacle to adoption of continuous strategies could be the perceived necessary additional automation complexity to allow intensified cycling over the capture resin. However, by using a streamlined workflow to achieve automation optimization, we have found a continuous capture chromatography platform can easily be configured at the lab-scale, tested for robustness, and scaled-up for manufacturing. A key challenge in the CDMO space is ensuring a favorable platform fit over a range of molecule formats and productivities. Platform process adjustments to accommodate a variety of process inputs such as high and low titers, low binding affinities, impurity levels, and residence time variability will be discussed. For Just-Evotec, a platform capture step that is configurable for either 3- or 4-columns has allowed for favorable process performance for all molecules manufactured thus far. From our experience with continuously perfused bioreactors, we have gained considerable experience around time-based process fluctuations that can be tolerated within a single bioreactor run. Specific case studies include temperature control to mitigate Protein A leaching, specialized wash steps, sanitization procedures to enable continuous processes, and considerations for next generation resins. Key benefits of coupling continuous protein A with continuous viral inactivation will also be considered, alongside bottlenecks in the remainder of the downstream purification process. Finally, a discussion of equipment design tradeoffs will be included as well as recommendations for desirable next-generation technologies to add to the current capabilities available today.

3. Truly continuous purification platform – beyond the PoC

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Keith Morgan, AstraZeneca, United States
Alison Hinton, AsttraZeneca, United States
Taylor Bloom, AstraZeneca, United States
Michelle Chen, AstraZeneca, United States
Makenzie Lewis, AstraZeneca, United States
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The truly continuous platform for downstream bioprocessing of monoclonal antibodies (mAbs) is based on nanoparticles, as a vehicle to separate mAbs from process impurities, and filtration membranes (tangential flow filtration and dialysis) that enable separation by size. It has the potential to reduce cost-of-goods for mAbs by half or more over conventional chromatography-based separations. This nanoparticle-based method also promises higher productivity, lower capital and facility costs stemming from reduced-scale equipment, and enhanced process sustainability compared to current generation batch processes. Here we show the proof-of-concept (PoC) using a novel self-assembling affinity nanoparticle designed to bind and elute a mAb, and resist neutral and low pH, while being used as a vehicle to separate the mAb from the process-related impurities through a series of membrane filtration, at bench-scale, in a continuous operation for many hours. We investigated scores of nanoparticles. An analytical toolbox was developed to inform our nanoparticles' design and expression, to support screening and to characterize structure after purification. Once they were combined with the mAb and processed through filtration membranes, we measured binding capacity, stability, elution at low pH and fouling. With multiple stage operation, we showed >95% recovery of mAb. The development of this innovative platform follows a stagewise approach, starting on the PoC to focus on low concentrations to determine nanoparticle capacity and membrane fouling; followed by the product mass index (PMI) targets for water of <300, possible to achieve by concentrating the feed (mAb and nanoparticle); then the integration across affinity capture phases and with the upstream process (bioreactor), including the process control and automation; expansion to the polishing steps, where different nanoparticles are used; considering scalability and ultimately the GMP implementation. We will discuss our strategy to build a nanoparticle library, that includes collaboration, and how we envision to progress this platform into large scales and GMP implementation, in particular because the engineering solutions for the equipment and consumables already exists even thought this is a novel application. Also, as a result of the decreased water use, the purification equipment is significantly smaller, and presumably cheaper. An economic assessment with those key considerations will be presented to show how we can enable this new bioprocess architecture.

Session 5 - Principles, Strategies and Highlights of Modelling and Data Analytics in Biomanufacturing

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1. Process development using an autonomous process optimizer

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Time to market timelines are getting shorter and complexity of molecules for development is growing. To be able to develop scalable and robust processes under these circumstances, efficient setup and execution of screening experiments is of crucial importance. Normally, the experimental design is setup prior the screening experiment, the screening is performed and analyzed, and optimization of the process is done based on the developed models. Using this approach, the model quality may not be sufficient, or it shows higher uncertainties in particular areas of the model. This can be a problem especially if the optimum of the process is in the area of higher uncertainties. To circumvent such problems, we present an autonomous screening system on our liquid handling platform. In this approach, an initial small experimental design is generated using our in-house developed SMART PD platform. The design is automatically translated into files readable by the liquid handling platform. The files are then used in predefined scripts and the first screening round is performed. The analytical results are automatically transferred back to the SMART PD platform and processed. An initial model is built, and a new experimental design is generated based on the model. Using this design, a new iteration on the liquid handler is automatically initiated. In each iteration the developed model is retrained until a sufficient model quality is obtained. This approach can be either used to increase the overall model performance or to increase the model performance around the optimum of the process. Therefore, a screening without manual interaction can be performed with a more efficient usage of experiments obtaining models of higher quality and less uncertainties. Based on the implemented feedback loop the model improves itself by an automated learning process. This enables transfer of more robust processes to large scale and more efficient usage of models during process characterization studies.

2. Automated generation of digital twins and their use in real-time monitoring of process chromatography

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The advent of the Quality-by-Design [1] and Industry 4.0 [2] paradigms signify a need for change in the approach to manufacturing of biopharmaceuticals. More emphasis needs to be placed on process understanding by means of mechanistic modelling of unit operations, on-line analysis for quality assurance and advanced monitoring of process performance. In this regard, downstream processes in biopharmaceutical production pose a major challenge. The main unit operation used in downstream processing, chromatography [3], is a time-consuming step with real-time data limited to UV and conductivity measurements at the outlet of the chromatography column, making real-time process monitoring a difficult task [4]. For these purposes, the implementation of digital twins of downstream process systems is of particular interest [5]. A framework for automated generation of model structures representing the individual unit operations and tubing in a chromatographic system has already been explored [6]. We have built on this framework by implementing an automated procedure for the calibration of adsorption parameters of multiple components in ion-exchange chromatography. This procedure makes use of the digital representation of the unit operations to both perform experiments in the lab and to simulate the corresponding experiments. The adsorption parameters as well as dead-volumes in the process are estimated by comparing the real experimental data with its simulated equivalents. The resulting digital twin can be used for process development, optimization, and open-loop control of the chromatographic system.
The output from the digital twin's mechanistic description of the process can be updated to better match reality by creating a continuous, real-time flow of data from the UV absorbance and conductivity sensors of a chromatography process. Kalman filters were employed to achieve this. A Kalman filter works by predicting the states of a process, e.g., the salt and protein concentrations in the column, based on a mechanistic model of the process, e.g., a digital twin. The Kalman filter then reads the sensor data and updates the prediction to match. This cycle of prediction and updating is repeated for each data sample obtained form the sensors, resulting in an improved model fit to the data than running only the prediction, i.e., in open loop. We were able to obtain improved digital twin fits to data in real time by implementing a linear Kalman filter (LKF) to estimate the salt concentration, and an extended Kalman filter (EKF) to do the same for the protein concentrations. Using separate filters reduced instabilities due to non-linearities in the protein adsorption model and improved the algorithm's computational time. As a result, we were able to improve the digital twin's fit to the column elution profiles, achieving a robust and reliable method of monitoring the elution profiles of co-eluting, individual proteins in real time.

3. Universal Hybrid Chromatography Modeling Framework for Optimization of Multi-column Chromatography Systems

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First principle modeling of chromatography, using the general rate model and traditional isotherms, is scientifically mature (Shekhawat, 2019). However, development of these models requires detailed understanding of the specific binding interactions present in the system as well as a mechanistic understanding of the diffusion pathways in the binding phase material. Hybrid models combining data-driven and physics-based components have emerged as strong performers for creating digital twins of upstream processes (Richelle, 2022). Similar attempts to hybridize AI models and physics have been made in digital twins for chromatography as well (Narayanan, 2021). However, the numerical challenges of simulating high dimensional, stiff partial differential equations have limited the practical efficacy of training hybrid physics/AI chromatography models. We have developed a chromatography modeling framework that hybridizes AI components, describing binding interactions and diffusion, with physics-based components to capture column flow characteristics and maintain mass balances. Our approach uses state-of-the-art back propagation and neural network techniques to overcome numerical issues that have been reported in literature. We have drawn inspiration from convolutional neural networks used in image analysis and dynamic neural networks used in process control to achieve good overall predictive performance. The approach uses a unique, coarse spatial discretization scheme that resolves stiffness problems in spatial dimensions. Stiffness in the temporal dimension is addressed by using a local linearization technique drawn from control literature. We have demonstrated that, used together, these approaches provide an efficient solver. Importantly, we have demonstrated that the solver is implementable in a numerical graph environment enabling automatic differentiation. Consequently, error between observed and simulated fractions can be backpropagated to parameters in the underlying binding and diffusion models enabling use of gradient descent algorithms to train these AI models from outlet fraction data. Additional heuristic methods for model pre-training have been developed in parallel with the numerical solver. These pretraining approaches greatly speed-up the training convergence of the AI model. The proposed modeling framework is being developed for use cases ranging from process development to manufacturing monitoring. Four areas of particular focus are scale-up during process development, transition from batch to multi-column continuous platforms (such as Sartorius' BioSMB and BioSC devices), monitoring for deviations at manufacturing scale, and development of optimal adaptive recipes (control strategies) for connected continuous multi-column processes. In this work we show experimental results and the corresponding model predictions demonstrating efficacy for these applications including examples with both membrane and resin binding modalities.

4. pH Transients and Elution Profiles in Protein A Affinity Chromatography: Experimental Observations, Modeling, and Approaches to Elution Buffer Engineering

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More than 40 years after its commercial introduction, Protein A affinity chromatography is still the workhorse for the recovery and purification of antibodies and related biomolecules. The molecular mechanism of the interaction between Protein A and IgG is well understood and numerous studies have addressed the adsorption capacity and adsorption kinetics of different Protein A resins. More recently, some investigations have also modelled the entire purification step including elution. However, pH transients and the ensuing peculiar elution profiles that can be encountered in protein A chromatography have only barely been touched upon. In particular, the relationship between the composition of the elution buffer and the range of pH values over which the antibody actually elutes remains elusive. Understanding and being able to predict this relationship can be invaluable to design elution buffers engineered to elute the antibody over desired pH ranges as well as to direct pH adjustments for post-Protein A steps. To this end, we have performed a systematic investigation of elution profiles using different elution buffers with different molarity, pH, and NaCl concentrations for a broad range of commercially available Protein A resins including MabSelect PrismA, MabSelect SuRe, ProSep and Amsphere, and for both polyclonal and monoclonal antibodies. The central observation of these studies is that, depending on the buffer used, the pH wave can travel through the column far slower than the conductivity wave delaying elution and causing the antibody to elute at pH values that are substantially higher than the eluent pH. The spread between the two waves ranges between 0.5 and 3 column volumes and strongly depends on the elution buffer composition, the ligand density, and the amount of bound antibody. The underlying reasons for these behaviors are the interplay of ion exchange and adsorption processes occurring upon addition of the elution buffer. Protein A and bound IgG essentially form a complex poly-buffer system due to the local high concentration of charged amino acid residues. Titration effects of specific amino acids result in further transition points and bending of the pH wave. The selection of a buffer system allows controlling the pH that the antibody is exposed to elution. In extreme cases, e.g., when using weakly buffering glycine, the entire bound antibody can be eluted at nearly neutral pH. However, this comes at the expense of an increased elution volume. We also observed that the addition of even small NaCl concentrations to the elution buffer can have dramatic effects on the peak shape, especially for glycine and acetate buffers, resulting in unexpected peak fronting akin to anti-Langmuirian behavior. This can be explained by shielding repulsion of the histidine residues that are responsible for the elution and increasing hydrophobic effects generally favoring interaction rather than desorption. To better understand these phenomena, we have developed a mechanistic model of the elution process based on a detailed description of the interactions between the buffer components with both the immobilized Protein A ligands and with the adsorbed antibody along with the thermodynamics of interaction between the antibody itself and the Protein A ligands. The model explains the experimental findings and provides a useful tool for engineering the elution buffer. Our results suggest that mass transfer effects play only a minor role in the elution process. Thus, the model is largely based on the assumption of local equilibrium.

5. Implementation of Mechanistic Model-Informed Chromatography Process Development and Validation: Successes and Challenges

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Mechanistic models have gained increased attention for their potential to reduce time-intensive experimentation while maintaining or increasing process understanding. Integrated, realized, process development to validation workflows have not yet been fully detailed. In this talk, a comprehensive strategy for leveraging mechanistic chromatography models to increase development and validation efficiency along with process understanding will be described and illustrated. This strategy consists of utilizing data collected during process development, with a significant portion of data collected on high throughput RoboColumns, to calibrate mechanistic models for prediction of product quality attributes and process indicators of interest. The initial model is calibrated with a focus on speed and qualitative prediction of attributes relevant to step development, and used to support process parameter optimization and provide an early read on step robustness. The mechanistic model is further refined for use in process validation, where additional experimental data may be incorporated, protein subspecies and impurities may be added, and a more thorough optimization, quality assessment and verification of the model is performed. The verified model is used to perform risk ranking and filtering, identifying impacting and non-impacting process parameters, and inform design of a worst-case corner only process validation study. This streamlined validation study (compared to a DOE approach) serves to confirm process parameter effects and acceptable operating ranges, and also provides confirmatory data on the robustness of the step for non-modeled attributes such as host cell protein. Finally, the model is qualified to predict at-scale manufacturing performance. Real, applied, case studies will be presented to illustrate the strategy in action. Model development data consisted of RoboColumn studies supplemented by a small set of AKTA-scale chromatography experiments, and enabled a confirmatory AKTA-scale validation study with a significant reduction in runs compared to the traditional approach. The considerations used to evaluate and apply the model will be discussed, including development of quantitative and qualitative model evaluation criteria, design of the confirmatory AKTA-scale validation study, selection of simulation conditions for the model application, and strategies for non-modeled attributes. A comprehensive framework for application of models in process development and validation is proposed, setting the stage for a future paradigm of model-informed process development and validation integrating molecule-specific high-throughput experimental data, cross-molecule process knowledge, and mechanistic modeling.

Session 6 - Adsorptive Separations Across the Universe of Biologics

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1. Cleavable affinity tags can revolutionize biologics manufacturing – but how do we convince the FDA?

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Affinity tag methods have shown incredible power in simplifying recombinant protein purification, and variations are now used in virtually every protein production laboratory in the world. Yet, with the single exception of blinatumomab (Blincyto®), they are never seen in biologics manufacturing. The main obstacle is the removal of the tag, which is generally considered a requirement for FDA approval due to the potential immunogenicity of the tag. Conventional tag removal methods would require validated protease enzymes, as well as additional steps, which have made this approach impractical. In the case of blinatumomab, the low dose and end-of-life indication was enough to gain approval with a polyhistidine tag in place, but this exception is unlikely to become the rule. These difficulties have been largely eliminated through the use of self-removing tags, which provide an untagged target protein in a single column step using a highly reusable affinity resin. All that is required is a small shift in pH, which triggers release of the cleaved target protein from the immobilized tag. This method can therefore be used to purify virtually any protein for which a convenient affinity partner does not exist. Previously difficult proteins, including scFvs, cytokines, fusion proteins, antibody fragments and fusions, nanobodies, and even engineered bifunctional T-cell engagers (like blinatumomab, but without the His tag), can now be easily purified at scale with minimal process development. Published case studies have shown successes of this approach, using proteins and glycoproteins expressed in microbial and mammalian hosts, with high yields and extraordinary purity(1). As this method is evaluated for clinical material however, an important question arises: How can we provide a validated method to demonstrate complete tag removal as part of an IND application to the FDA? It is obvious that tag removal will be a critical quality attribute, and that any release assay must be robust and reproducible. But there are special challenges when quantifying the removal of a small segment of protein from a much larger target, and this is unexplored territory for the FDA. In this presentation, we will examine this question and provide unpublished preliminary data on a variety of potential approaches. These include immunoaffinity approaches, such as western blots and ELISA assays, as well as MALDI, LC/MS and other chromatographic approaches. Our initial results using western blots and intact protein MALDI suggest that the cleaved target has a nearly undetectable level of remaining tag (due to the high affinity of the tag for the resin), but the development of more sensitive ELISA approaches will provide a clearer insight. Also in this presentation, we will provide case studies on the production and purification of several complex glycoproteins, as well as data on Host Cell Protein and DNA clearance, as well as quantitative data on tag removal. The end result will be a clear road map to enable the use of this powerful technology in the development of new clinical proteins.

2. 3D-printed matrices for the purification of plasmid DNA via steric exclusion chromatography

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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 plasmid pVAX-eGFP (3,685 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 3D-printed monolithic columns with 0.70 mL bed volume 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. In conclusion, the proposed method significantly mitigates the relaxation effects in plasmids observed in current purification procedures required for isolating sc pDNA, which makes it a promising and innovative approach for the development of a fast, high-capacity, and cost-effective pDNA capture step.

3. Lentiviral Vector Determinants of Anion-Exchange Chromatography Elution Heterogeneity

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Lentiviral Vectors (LVs) are membrane-enveloped and are used throughout the Cell and Gene Therapy industry for ex vivo modification of patient cells. Manufacturing difficulties, particularly effective purification of LV drug substance, have contributed to restricted global supply. The industry predominantly employs convective anion-exchange (AIEX) adsorbents for primary product capture, yet inconsistent and generally low recoveries are reported. This stems from a poor understanding of the complex adsorption behaviours related to the large size and intricate structure of LVs. These include product loss from time-dependent irreversible binding and binding heterogeneity, which is frequently characterised by a broad salt gradient elution profile consisting of weak (peak 1) and strong (peak 2) binding peaks. To understand which LV components mediate AIEX sorption behaviour. We began experiments with LVs devoid of pseudotype protein (VSV-G), often assumed to be a key envelope-associated protein involved in AIEX binding. However, the “two peak” elution profile remained consistent indicating that there are other components in the envelope that contribute to the interaction. Upon sequential protease digestion, the strongly interacting “peak 2” population converges to a single weak binding “peak 1”. A literature review of the membrane glycocalyx composition identifies highly charged long-chain glycosaminoglycans (GAGs), covalently attached to base protein units to form membrane proteoglycans, as potential strong binding species. Heparanase I/III and Chondroitinase ABC digestion of these GAGs causes all LV to elute in a single weakly interacting peak (peak 1), challenging the assumption that variations in multipoint attachment of envelope proteins causes LV binding heterogeneity. The data substantiates the hypothesis that distinct components of the LV envelope govern binding behavior, wherein the “two peak” profile emerges from a subset of LVs interacting via highly charged glycosaminoglycans (peak 2) along with a weaker binding population likely interacting through the phospholipid membrane and envelope protein (peak 1). Further experiments isolating material from each binding population and re-injecting them onto AIEX adsorbents yields homogenous single peaks with retention points corresponding with their location in the initial “two peak” gradient profile. This demonstrates that binding heterogeneity originates from distinct subpopulations of LV present in the cell culture supernatant, perhaps due to differences in glycosylation state. Finally, we demonstrate product loss due to increased irreversible binding at prolonged periods of adsorption cannot be mitigated by digestion of GAG species, but reduces with increasing membrane occupancy, alluding to a conformational spreading rather than a “Velcro” type loss mechanism. In conclusion, our data establishes that LV binding heterogeneity stems from heterologous populations with differing envelope compositions. Contrary to current assumptions, the most strongly interacting LV populations on AIEX adsorbents are shown to bind via negatively charged glycosaminoglycans rather than envelope proteins such as the commonly used VSV-G. As removal of these species does not mitigate product loss mechanisms, our findings advocate for a redesign of AIEX adsorbents, specifically targeting lower ligand densities for LV applications, to achieve high-recovery AIEX processes.

4. Overcoming challenges in the development of chromatographic separation of empty, partial, and full AAV capsids for Gene Therapy applications

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Gene therapy offers a promising approach to address many unmet medical needs. Recombinant Adeno-associated viruses (rAAV) have emerged in the past decades as one of the most popular viral gene transfer vehicles for gene therapy, due to its relative high efficiency, low immunogenicity and good safety profile. Recent analysis showed >150 registered clinical trials on going using rAAV. However, the purification of rAAV vectors is particularly challenging, given capsids without therapeutic transgene (empty capsid) or an incomplete copy of the transgene (partial capsid) can make up 70-90% of the total output from the bioreactor. The current framework for purification process development remains inefficient. Product consistency also necessitates proper control of the product profile in the final drug substance, calling for a scalable purification method that can separate these product variants. Anion exchange chromatography (AEX) is widely used to separate empty and full capsids. However, the enrichment and separation of empty and partial capsids with anion exchange chromatography with higher yield and purity are rarely demonstrated in the literature. In this talk, we will present separation strategies for two rAAV constructs and how to overcome the yield and purity challenges using different separation modalities on AEX resin. The separation strategies can be built in three stages. The first is optimizing the selectivity of capsid variants by screening optimum resin chemistry. We found that for rAAVs, the presence of kosmotropic agents and metal ions is not only critical for better selectivity but also enhanced stability of intact capsid during purification. The two different constructs of rAAV in our case showed a different selectivity behavior in the presence of additives. Secondly, the transport limitation due to the large size of rAAVs has to be overcome by the appropriate selection of resin pore size or monolith media. In contrast to using a bigger pore size resin, AAV exclusion from the effective smaller pore resin, established from the mechanistic model, was found to be more suitable for higher resolution. Using resin pore size that excluded capsid or using a monolith column provided lower residence time and better resolution among capsid variants and yielded better product recovery. Lastly, after optimizing the optimal selectivity; flow-through, step, and gradient elution strategies were evaluated using the mechanistic model. Overall, we will provide the best modalities to achieve >95% empty capsid removal with a yield greater than 80%. We will also illustrate the removal of the partial capsid by greater than 40% using the three-column displacement chromatography and single-column two-step elution method aligned with the approach adopted above.

Session 7 - Separating without Sticking – Adventures in Non-Adsorptive Separations

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1. Developing a Scale-Down Model for Batch Lysis in Plasmid DNA Purification Processes

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Plasmid DNA (p-DNA) has been widely used as a critical component in production of therapeutic proteins. Recent advances in genetic therapeutics like gene and cell therapy have created a large global demand for this critical component across biopharmaceutical industry. However, current p-DNA production processes pose limitations on the large-scale manufacturing capacity of p-DNA, which makes meeting this uprising demand challenging. One of those limitations is the scale-up of the lysis unit operation in downstream process wherein the harvested cells are lysed via mixing with an alkaline solution to release the intracellular p-DNA, followed by partial cellular impurities removal via precipitation. Batch mixing has been the most common approach used for this unit operation with mixing quality impacting the quality and quantity of the recovered p-DNA. Mixing quality can also potentially affect the performance of subsequent unit operations via carried-over cellular impurities. Therefore, robust design and scale up of lysis mixing operation becomes crucial. However, due to the complex viscoelastic properties of lysate, and shear sensitivity of both p-DNA and genomic DNA, developing a representative lysis scale down model becomes challenging. Although computational modeling approaches have been previously used for designing mixing-based unit operations in bioprocesses, it would be extremely challenging to apply them in this case due to the dynamic and complex rheological properties of lysate. In this work, we will present a creative approach where a 3D-printed small-scale lysis set up, mimicking the design of the lysis container at scale, was developed using several geometric scaling parameters. The scale-down model was then used for defining the lysis operation parameters at lab scale followed by the lysis scaling up to the pilot plant and GMP facilities, using several operational scaling parameters. The developed scale-down model could successfully predict the lysis performance at scale with comparable process and product quality attributes.

2. Model-based optimization of Single Pass Tangential Flow Filtration (SPTFF) for concentration and purification of viral vectors

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The recent approval of several viral-vector-based therapeutics has heightened the focus on the imperative for more efficient strategies in manufacturing these groundbreaking gene therapy products. Viral vectors are generated at relatively low titers, and the tangential flow filtration (TFF) process is frequently employed to concentrate the viral vector to the desired concentration, both for crude and purified viral vectors. Compared to the conventional TFF process, Single-Pass Tangential Flow Filtration (SPTFF), which concentrates the product along the length of the module in a single pass without any recycling, is superior in maintaining product quality due to lower process shear. Additionally, SPTFF can lead to reductions in manufacturing costs and greater manufacturing flexibility [1]. In this study, SPTFF was evaluated using two viral-vector modalities: adeno-associated viral vectors (AAV) and lentiviral vectors (LV). Both clarified cell lysate (CCL) and purified product streams were evaluated to assess the impact of host-cell impurities on SPTFF performance. Vector yield, HCP removal, and membrane fouling were studied during SPTFF using both cellulosic and polyethersulfone membranes with different nominal molecular-weight cutoff. Single-membrane flux-stepping experiments were then performed to evaluate the impacts of process conditions on vector yield and membrane performance. The study results demonstrated that the maximum operating flux increased when increasing feed flow rate and decreasing vector concentration. The study results aligned with a newly developed modified concentration polarization model that accounts for the variation in transmembrane pressure and vector concentration along the length of the module [2]. These findings were then applied to the design of internally-staged SPTFF modules, incorporating permeate flow control strategies to maintain the vector concentration on the membrane surface below a critical level. After successfully demonstrating a proof-of-concept for SPTFF, process intensification was evaluated by coupling the SPTFF unit operation to upstream and / or downstream unit operations. This seamless integration holds the potential to significantly enhance the downstream manufacturing process for high-value gene therapy products.

3. A unified view of virus and recombinant protein interactions with block copolymer membranes

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Virus filtration (VF) in downstream biomanufacturing is a process that exists on a knife edge. On the one hand, at least 99.99% of viruses larger than 20nm must be rejected by the filter. On the other hand, valuable biotherapeutics with sizes up to 15nm are expected to pass through the filter at rates greater than 99%. For the past twenty years, VF manufacturers have responded to this challenge by providing a variety of products with increasingly better performance. Yet, recent anecdotes paint a story where VF offerings are falling short in situations that are becoming mainstream. These include newer recombinant therapeutics and processing conditions, such as at very high conductivities or at low process flux. These shortcomings manifest themselves in either suboptimal viral retention (LRV<4), markedly reduced protein throughputs, or both. The response to these challenges has been confined largely to empirical feed adjustments that may be difficult to implement on the factory floor. The efforts directed towards membrane engineering are hampered by the complex system of retention and fouling mechanisms at play during the virus filtration process. TeraPore Technologies has developed a proprietary technology that uses self-assembled block copolymers to produce membranes that address the most complex separation challenges in biopharmaceutical production. This technology gives us full control of key membrane attributes such as pore density, uniformity, and surface chemistry. This offers a unique opportunity to study the fundamental interactions between the membrane and biomolecules. A clear picture emerges of a unified description of the interaction mechanisms in virus retention and protein fouling, that include pore-blocking and adsorption for both biomolecules. We have demonstrated that monoclonal antibodies (mAbs) fouling of isoporous membranes is dominated by adsorption caused by hydrophobic forces between the mAbs and the membrane. This fouling mechanism is always present but can be greatly minimized by a proper chemistry treatment of the membrane. A second fouling mechanism is pore-blocking, which is marginal for monomeric feeds but dominates when the feed contains even a fraction of small aggregates. These two protein fouling mechanisms have analogs for virus-membrane interactions. Virus rejection by size exclusion can be viewed as an interaction mechanism similar to pore-blocking by protein aggregates. We then explored the extent to which hydrophobic forces contribute to virus interactions with membranes by using PP7 and □X-174 phages and self-assembled block copolymer membranes that were surface modified to adjust their hydrophobicity and pore size. Our data support the interpretation that there are at least two mechanisms of viral retention: (1) Size exclusion, which plays an overwhelming role in robust virus retention, and (2) virus adsorption, which is a significant driver to push the LRV up to 5-7 for hydrophobic membranes. Our findings allow us to design VF devices that are predominantly driven by size exclusion rather than adsorption and provide clues on the cause of virus breakthroughs at high conductivity and low flow rates that have been reported. They also offer the prospect of a rational design of VF membranes with robust and predictable performances.

4. Regulatory Considerations for Design and Implementation of Continuous Viral Inactivation Reactors

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There is an ongoing paradigm shift away from batch processing and towards continuous manufacturing (CM) to produce therapeutic protein drug products. Fundamentally, conversion of a batch process such as low pH chemical inactivation to a continuous process includes switching from the static batch tank hold of fluid to a dynamic continuously flowing fluid stream. Physically, this means converting a static stirred-tank model to a dynamic pipe-flow model while maintaining critical process parameters (CPP) such as time, temperature, and pH. One such model, the continuous virus inactivation reactors (cVIR) rely on residence time distribution (RTD) models to demonstrate process control and ensure the CPP are met. Understanding of the cVIR design, tracer selection, and RTD modeling has been a regulatory challenge that must be met to prepare for the paradigm shift towards continuous manufacturing of therapeutic proteins. Our work aims to understand the impacts of cVIR development and scale up on the process parameters of the continuous viral inactivation process, as well as identifying potential failure modes that may impact viral safety and efficacy. There is also a need to understand the impact of tracer sensitivity on the RTD model, particularly when determining the minimal and maximal residence times achievable by the cVIR. Preliminary data demonstrate that the minimum residence time (MRT) approach for cVIR characterization differs by the selection of tracer utilized from Acetone, phosphate buffered saline and riboflavin. This is demonstrated via the difference in curve profile observed at similar flowrates of each tracer through the same column configurations. Additionally, tracer detection sensitivity can impact MRT determination, with acetone and riboflavin have a detection range of 99% to 0.1%, or up to 3 log10. To address this, we are using bacteriophage PP7 and PR772 as tracers with a detection limit of 99% to 0.0001% breakthrough, or 7 log10. These data will help to determine if tracer sensitivity and tracer particle size can impact MRT. By understanding how cVIR design and tracer selection impact critical process parameters such as MRT, this study hopes to provide scientific understanding to potential concerns that may arise regarding applying a cVIR for low pH inactivation to support regulatory acceptance of this advanced technology.

Session 8 - Capers and Antics Across the Purification Process

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1. When the platform doesn't fit: Simultaneous innovation of process and platform during downstream development of a challenging Fc-fusion protein

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Early phase programs historically have relied on a rapid purification platform fit to enable molecules to reach the clinic as quickly as possible. However, new constructs such as antibody fragments, fusion proteins, and multi-specifics present unique product quality profiles where the platform sometimes proves inadequate. In such cases, advancement of both the purification process as well as the development strategy/tools used to arrive at the end process is required to deliver on accelerated first in human (FIH) timelines. This talk exemplifies parallel innovation of process and development workflow through the case study of a challenging heterodimeric Fc-fusion protein. A low pI, heavy glycosylation and substantial HMW content (~ 40%) necessitated extensive process development beyond the typical platform. The integration of in silico and high throughput experimental techniques was a key enabler for the on-time delivery of a robust, scalable downstream process despite the additional scope of development. Both empirical and mechanistic models were leveraged to rapidly identify and optimize non-platform conditions for both polishing chromatography steps. PAT was implemented at lab and pilot scale to provide real time monitoring of HMW clearance. Process development efforts started with re-design of the Protein A step for both product capture and purity enrichment. The optimized Protein A step reduced HMW levels by more than 10% prior to polishing chromatography. The second column utilized MMAEX, rather than the platform AEX, in flow through mode at low pH to achieve HMW, residuals, and viral clearance while maintaining the charge variant profile and sialic acid content. A CEX polishing step was rapidly optimized to handle variable feed impurity composition with the aid of mechanistic modeling. The final process showed consistent performance across scales, from 50L to 500L to 2000L. Importantly, the innovations in the development workflow for this Fc-fusion protein case study resulted in advancements to the general FIH strategy to accommodate other challenging molecules. Furthermore, the integrated experimental HTS/computational modeling approach also has the potential to further expedite purification process development for molecules that fit the platform.

2. Process Intensification of Recombinant Adeno-Associated Viral Vector Production

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Recombinant adeno-associated viral vectors (rAAV) are currently the preferred mode of delivery for in-vivo gene therapy treatments. However, large-scale manufacturing of rAAV to support late-stage clinical trials and commercial production remains a significant challenge due to low production yields, limited scalability, and high facility and consumable costs. The average cost-of-goods for a gene therapy treatment is estimated to be in the range of USD 0.5-1 million per patient, and the treatments themselves range from USD 0.8 – 3.0 million per patient. Therefore, process intensification is vital if rAAVs are to become accessible to large patient populations, especially in less developed economies The talk details innovations in production of rAAV in order to reduce cost-of-goods during production for AAV by triple transfection in HEK293 cells, including (i) reducing or eliminating the need for endonuclease treatment during cell lysis and harvest via use of single-use chromatographic clarification (ii) coupling of single-pass tangential flow filtration (SPTFF) to affinity capture to reduce processing time, and (iii) development of anion exchange chromatographic workflows, applicable to a variety of serotypes, to enable enrichment of full capsids to more than 75%, and up to 98%, from input feedstreams containing less than 15% full capsids.

3. The well-being approach for higher yields – how to make lentiviral vectors comfortable during downstream processing.

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Many cell and gene therapies use lentiviral vectors (LV) to deliver therapeutic genetic material to host cells. Although LVs have been approved for clinical use, their manufacturing process faces major bottlenecks, particularly in the purification stage, where recoveries below 30% are typical. This presentation aims to discuss how a better understanding of the surrounding environment and the interaction between LV and its process conditions can enhance LV recovery during clarification, concentration/diafiltration using TFF, and anion exchange chromatography while also considering stability and robustness. So far, we have achieved a 2-3-fold improvement in LV yields; this is a significant achievement considering the challenges in performing LV studies. Our key enablers are scale-down methodologies and manufacturing our own upstream feed conditions ranging from transient transfect third-generation VSV-G LV in both adherent and suspension cultures to stable cell lines (WinPAC). The presentation will show unpublished results, and the majority of these will be first reported here. To improve clarification recoveries, we investigated the role of membrane chemistry in reducing LV loss. One key innovation is the novel application of membrane surface zeta potential techniques to bioprocess characterisation. We aimed to understand the impact of membrane ionic charges on the rate and extent of fouling of different LV feeds. Results obtained from our experiments revealed a relationship between membrane material, in-process conditions, and recovery. For instance, PES had a recovery rate of 93%, while Nylon only had a recovery rate of 67%. This was correlated with their respective membrane zeta potential, showing that the nylon surface had a positive surface charge, which differed from the negative LV crude feed. This could result in a higher adsorption rate and interaction with the membrane surface, leading to a loss of functional vector particles. We also used confocal and scanning electron microscopy to visualise the fouling on the membrane surface and quantified these using foulant-specific dyes and fluorescent antibodies. In our TFF experiments, we studied the interaction between processing sequence (i.e., UF-DF or DF-UF), mode of operation, and membrane properties on LV yield, productivity, and vector quality. We demonstrated, both at ultra scale-down and larger systems (up to 50-fold membrane area), that the sequence of TFF unit operations plays a vital role in obtaining high recovery. Additionally, we found that operating TFF at higher TMP (up to 2 bar) to increase productivity did not lead to a significant loss in functional titre, further confirming our recently published work that LVs can withstand high shear and are more robust than previously thought. To the best of our knowledge, this will be the first time that a whole bioprocess analysis on LV recovery will be presented, showing how a range of upstream conditions impact downstream recovery and the impact of buffer conditions during TFF and subsequent anion exchange chromatography on aggregation, stability, and recovery. Our findings demonstrate that LV recovery and product profile can be improved by selecting the right upstream and downstream conditions (i.e., a suitable processing environment can be determined for the “well-being” of LVs during bioprocessing).

4. Integrated continuous mRNA precipitation-based purification process

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In recent years, mRNA-based therapeutics, including mRNA vaccines, have emerged as cutting-edge technologies for treating various diseases, ranging from cancer and gene therapy to cardiovascular and autoimmune conditions. Current downstream processing relies on chromatography strategies, such as size exclusion chromatography, ion pair reverse-phase chromatography, ion exchange chromatography, or affinity-based separation, coupled with tangential flow filtration. However, purification remains challenging due to working conditions that can lead to denaturation, high costs, limited scalability, or gel formation and fouling during filtration steps. These extensive intermediate steps significantly impact overall yield and RNA integrity, thus influencing final production costs. Therefore, the need for innovation in manufacturing processing is crucial with the advent of such a new therapeutic modality. In recent years, precipitation has become an interesting, flexible, easy-scalable, and cost-effective alternative to chromatography systems for purification of therapeutic products such as monoclonal antibodies. Precipitation aligns with the current trend of continuous manufacturing and sustainability, as it can be performed in a fully continuous mode, reducing energy and material consumption. On the laboratory scale, precipitation serves as a straightforward and cost-effective method for concentrating RNA, involving salts like ammonium acetate or lithium chloride. However, these methods do not allow for the removal of impurities, such as aberrant mRNA, mRNA fragments, or dsRNA, and the scalability of such processes still needs to be addressed. We will present our integrated and continuous manufacturing process for mRNA purification. These methods involve combining various precipitating agents, such as PEG and different cations. Precipitation is carried out in a tubular reactor, followed by continuous flow filtration for both concentration and washing. This system is integrated at the benchtop scale for the continuous purification of mRNA. “This research was supported by the U.S. Food and Drug Administration under the FDA BAA-22-00123 program, Award Number 75F40122C00200.”

Session 9 - Manufacturing Control and PAT: Successful Implementation and Continuing Obstacles

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1. The minimum requirement on PAT's success for industrial implementation: The simultaneous prediction of multiple relevant Product Quality Attributes in real-time

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Efforts to develop and implement Process Analytical Technology (PAT) have emerged in the biopharma industry, building upon pioneering work in academia. New sensors combined with multivariate data analysis hold great promise for future biopharmaceutical downstream manufacturing. However, despite the evident advantages of implementing PAT, convincing successes of tracking multiple and specific critical quality attributes in an industrial implementation have yet to be demonstrated. This may be attributed to the inherent nature of PAT and its application scenarios. PAT aims to substitute analytical methods in a real-time manner, but its impact on resources, capacities, and sample savings would be insignificant if it only addresses a limited number of relevant product quality attributes. Moreover, if PAT fails to replace the current input-output control strategy comprehensively due to insufficient coverage of quality attributes, Process Characterization Studies would still need to be carried out, resulting in no significant wet-lab savings but only a leaner analytical panel. Thus, the impact of PAT appears to be more akin to a Heaviside-function rather than linearly correlated with the degree of implementation observed in other emerging technologies. This presentation proposes the minimum requirement for PAT implementation in both GMP and non-GMP use cases: the ability to address all relevant product quality attributes. This requirement directly translates to the expected return on investment, justifying the upfront investment in PAT development and implementation. To the authors' knowledge, this study is the first to demonstrate the implementation of a Raman spectrometer in preparative Protein A chromatography for real-time measurement of a monoclonal antibody capture step ready to be used in GMP environment. The machine learning model trained on in-line Raman signals, accurately predicted quality attributes such as antibody concentration, size variants, charge variants, N-glyco variants, and HCP. The model's predictions were validated against reference off-line analytics. The experimental setup included Raman sensors connected to a liquid-handling station and an ÄKTA device. The signal pre-processing method and model calibration approach were described, and the quality of the model's predictions was assessed.

2. Right every time?! Assessing polishing chromatography under dynamic loading conditions

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Due to an increasing world population, higher life expectancies, increased occurrence of chronic diseases and limited access to modern medicines in income-poor countries, healthcare systems all over the world are under pressure to deliver state-of-the-art therapies at reasonable costs and in sufficient amounts to societies. Also, global warming entails the urgent requirement to reduce the environmental impact of operations and products. Owing to its social responsibility, the biopharmaceutical industry has to provide and implement solutions for these challenges. In this context, the industry is exploring new ways of manufacturing, among others continuous and semi-continuous manufacturing technologies, which offer the potential for cost- and resource-efficient and robust operations by requiring reduced manufacturing equipment size, footprint and material demand, and relying on fully automated manufacturing processes with real-time quality monitoring. On the upstream side, improved cell lines in conjunction with novel processing technologies enable higher titers and increase bioreactor productivity. The inherent variability of upstream processes in conjunction with the continuous supply of harvest material poses a challenge to downstream processing, which now has to deal with a continuous inflow of material of varying composition, while being traditionally set up exclusively for batch-mode purification of well-mixed load materials. To date, there is a lack of experience within the industry regarding end-to-end continuous manufacturing. Data demonstrating the robustness of downstream operation under time-varying conditions is hardly available, especially within the constraints of existing manufacturing facilities. To enable chromatography operation within this framework and to assess its robustness, we developed a lab-scale experimental setup, consisting of an AEKTA chromatography skid, which is additionally equipped with several technologies. These additional technologies include different PAT sensors (slope spectroscopy, online liquid chromatography), as well as controllers for adaptive column loading and the real-time conditioning of column load material. Using this fully automated setup, we experimentally investigate the impact of unsteady load composition on process robustness for different chromatography modes such as bind/elute or flow-through chromatography. Different dynamic patterns of load quality are created by another chromatography step further upstream, which also operates in bind/elute or flow-through mode. As a surrogate measure for product quality, we developed ultra-fast online-SEC and applied this technique at multiple points in the downstream train. Our results allow us to draw conclusions about the operability, quality and robustness of chromatography operated in different modes and with unsteady load composition. The presented technologies and findings of the small-scale study ultimately support the cause of implementation of advanced downstream processing technologies in manufacturing.

3. “Shaken not stirred”, why James Bond was right: the next generation PAT solution for downstream processing

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Quality and process control of downstream processes rely on extensive analytical efforts. Combination of several off-line and in-line measurements need to be used in concert before a drug is released. This approach does provide the desired level of product safety, but at the same, it is resource and time intensive. According to ICH guidelines, the PAT is to be seen as “a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality”. While the modern PAT approaches rely on several detectors each monitoring a specific attribute, there is still a need to replace off-line analysis with the real-time detectors that deliver relevant information about the process and product quality attributes to minimize the risk to produce out of specification products. This talk will present several examples of how a single spectrophotometric technique can be used for simultaneous monitoring, in real time, of various product and process attributes including; concentrations of product and/or process related impurities, key and critical process parameters and process performance. As such, the power of this technique has the potential to deliver a leap in the field of downstream process analytics. A single device that quantifies product and impurities, both process and product related, and at the same time monitors key process parameters all in real-time, makes the concept of real-time batch release realistic. The examples will cover both chromatography and ultrafiltration/diafiltration unit operations. In addition, the talk will discuss the technical challenges associated with introducing the new PAT device into a GMP operation, including aspects such as robustness, scalability, and limits of detection and quantification. Examples of how the spectroscopic technique together with mechanistic modelling can be used for process control will be presented.

4. Case studies on combining spectroscopy and modern machine learning in DSP monitoring

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Optical spectroscopy stands as a valuable Process Analytical Technology (PAT) in biopharmaceutical manufacturing, capable of monitoring processes from upstream to downstream. Paired with machine learning (ML), it can be used to e.g. resolve chromatographic elution profiles with multiple components or track the progress of chemical reactions in real-time. However, spectroscopy-based models often require extensive manual tuning or may lack the selectivity to resolve all desired quality attributes. In addressing these challenges, we present case studies involving spectral data augmentation and automated model optimization. These methods aim to improve and streamline spectroscopy-based model development. Furthermore, we demonstrate the integration of a spectroscopy-based model with a mechanistic process model, creating an adaptive soft-sensor capable of compensating for process or model deviations. The first case study evaluates convolutional neural network (CNN) models for process monitoring of two chromatographic separation processes of proteins and introduces a novel method for generating in silico spectral data from experimental data sets. The proposed data augmentation method is shown to produce highly realistic spectral data by estimation and recombination of pure component profiles. The resulting CNNs are shown to reduce the accuracy for the quantification of different protein species in multi-component mixtures by 20-45% in two different case studies. In the second case study, Raman spectroscopy-based models are used for the quantification of Hepatitis B virus core antigen virus-like particles (VLPs) during precipitation from crude feedstock. The collected Raman spectra are prone to variance due to the biological variance of the clarified cell lysates and interferences induced by the addition of ammonium sulfate (AMS) and hence require extensive preprocessing. In addition to the solution environment, the Raman spectra are shown to be strongly affected by the precipitates forming in solution. We demonstrate the systematic and automated identification of a suitable preprocessing pipeline in combination with a ML model, reducing the interferences caused by the complex mixture and improving model accuracy in small-scale batch and fed-batch experiments. In the last case study, we showcase the combination of a UV/Vis spectroscopy-based ML model with a kinetic reaction model to monitor a site-directed antibody-drug conjugate (ADC) conjugation reaction [1]. Using an extended Kalman filter (EKF) to merge predictions from the ML model and the kinetic reaction model, the monitored reaction species are dynamically updated, leading to a substantial reduction in prediction errors by up to 23% compared to the kinetic model. Despite the ADC species not being distinguishable by UV/Vis spectroscopy, the developed soft-sensor is able to dynamically update all relevant reaction species. The developed framework is shown to enhance robustness against noisy sensor measurements or incorrect model initialization and could successfully be transferred from batch to fed-batch mode.

Session 10 - Instagram vs. reality: Challenges, surprises, and successes in fitting your perfect process into an imperfect manufacturing facility

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1. Achieving High-Titer and High-Concentration Monoclonal Antibody Production: an Innovative Journey Integrating with Next-Generation Bioprocessing Technologies

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The monoclonal antibody manufacturing landscape, shaped by decades of process development, continues to evolve with an unwavering commitment to optimizing specific facets of the drug substance production process. This ongoing effort seeks to concurrently reduce manufacturing costs and elevate the overall process efficiency. Recent breakthroughs in cell line engineering, coupled with innovations in cell culture and purification techniques, have ushered in an era where manufacturing productivity has soared to unprecedented heights, transcending the limitations of traditional methods. Moreover, a deepened comprehension of molecular properties within solution formulations has pushed the boundaries of drug substance and drug product concentrations, enabling more convenient subcutaneous delivery for patients. In this presentation, we will delve into a compelling case study illustrating the successful integration of next-generation bioprocessing technologies into a high-volume drug substance manufacturing process, all accomplished within a challenging development timeline. This presentation will shed light on the transformative journey of harmonizing these state-of-the-art technologies across both upstream and downstream processes, culminating in the first-time achievement of a high-titer (10g/L) and high-concentration, high-quality drug substance process for GMP manufacturing. Key focus points will encompass the enabling technologies in realizing this high-titer manufacturing process, including advanced cell lines, optimized cell culture media, the N-1 continuous cell culture processes, high-titer capture and polishing chromatography, and high-throughput membrane filtration. Equally compelling is our account of the successful tech transfer of these innovative technologies into clinical and prospective commercial manufacturing facilities. We will candidly dissect the challenges that beset us on the path to enabling high concentration drug substance production, while also revealing the ingenious solutions that fostered robust process control, ensuring consistent drug substance concentration across multiple batches. Furthermore, we will cast a spotlight on the pivotal role played by real-time learning and adaptability in the fine-tuning of our processes, facilitating incremental adjustments that not only amplified efficiency but also ensured seamless facility compatibility. Our goal in sharing these experiences is to underscore the transformative potential inherent in these emerging bioprocessing technologies and their remarkable adaptability to large-scale manufacturing. Their role in advancing the monoclonal antibody manufacturing landscape is pivotal, serving the ultimate objective of enhancing the well-being of patients on a global scale.

2. How far can we push the limits of connected biopharmaceutical manufacturing? – Unleashing the full potential of an intensified and connected purification process.

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Our mission is to improve people's wellbeing by providing access to high-quality medicines. To allow more patients to benefit, reducing manufacturing costs of biologics is essential. Consequently, Novartis has implemented a connected biomanufacturing concept that optimizes capacity utilization, enables multi-product flexibility, and is highly automated for cost efficiency. This concept is based on a High-Density Perfused Batch (HDPB) cell culture process, continuous capture chromatography, and connected-downstream unit operations. As such, this concept is already operational in a GMP facility. However, despite the first-generation process resulting in comparable productivities to traditional fed-batch processes the ever-rising costs of single use items have posed a challenge in further reducing manufacturing costs. While single-use technology is here to stay, alternative cost reduction strategies need to be explored. One such approach is increasing integration and throughput in both upstream and downstream processing. Our second-generation process exploits synergies of recent cell line development and process intensifications, resulting in an unprecedent productivity. However, processing high product masses whilst maintaining the facility drumbeat puts challenges on the existing connected downstream train. Furthermore, time constraints during transfers and limited facility space restrict significant changes in plant setup . In this presentation, we will showcase the development of an intensified and connected downstream process using available ready-for-GMP technologies and smart scale down models. Their use enabled us to significantly accelerate development, increase facility throughput, while in parallel reducing disposable costs , and leading to significantly lower cost of goods compared to earlier connected DSP processes.

3. The Balancing act in Biomanufacturing: Maximizing efficiency and flexibility when faced with an unpredictable product mix

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Despite all the efforts of designing flexibility into manufacturing facilities and platform standardization, biomanufacturers can still face challenges due to not always having clear sight to exactly what product their facilities will be making. Changes in the pipeline priority and introduction of new molecules/modalities from internal/external development can lead to significant product volume or mix changes in a given facility, sometimes rapidly. The unpredictability of product volume, mix, and technology requirements is more pronounced in CDMO facilities, which are expected to offer flexible volumes and technology capabilities to a wide variety of molecules in the market while maintaining on time delivery of products to customers. This presentation will discuss how to tackle this challenge with a multi-prong approach that includes: a). focusing certain facilities or production lines in the network towards high mix/low volume, or high volume/low mix production to balance flexibility and efficiency. b). enhancing the technology transfer workflow to improve right first time (RFT), c). modular standardization to gain efficiency and reliability, d), leverage end to end capabilities in the value chain to offer opportunities for reduced development and commercialization timelines. Several case studies will be presented to elucidate these strategies. 1). Using an improved tech transfer workflow to deliver a bispecific antibody under a compressed timeline, 2). Using a scale-out approach in cell culture to meet a high-volume demand, high titer fusion product while overcoming various downstream challenges. Specifically, coping with the increased upstream throughput, inactivating an unexpected host cell protease and a high drug substance concentration of 250 mg/mL. 3). Driving modular standardization of single use components to gain efficiency (design and set up time reduced by >80%). As a CDMO operating a network of multi-product manufacturing facilities, it is critical to develop strategies to effectively balance efficiency and flexibility. Our approaches may offer useful insights and tactics for other biomanufacturers faced with similar unpredictable product mix scenarios.

Session 11 - Innovative Purification Technologies

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1. Asymmetric bispecific antibody purification platforms using avidity effects of protein A and protein L affinity ligands

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The number of asymmetric bispecific antibodies have exploded in recent years. They have a great potential for becoming the next big leap for antibodies. However, purification of asymmetric bispecific molecules is more complicated compared to standard monoclonal antibodies due to the need of correct pairing of the heavy and light chains. One way to purify these molecules is by using avidity effects on affinity protein A and protein L resins. In this work, we show newly developed tools and a systematic approach that can be used to achieve high purity of the correctly paired antibody already in the capture step. Both protein A and protein L bind to the variable part of the antibody. Protein A binds to the fragment crystallizable (Fc) region and the variable heavy (VH) chain of the VH3 sequence family while protein L binds to the variable light chain of kappa class (VL-k). We have created a novel protein A resin, without the classical Fc interaction, that binds only to the VH3 domain of the antibody. We show that it is possible to allow binding to one heavy or light chain and avoid binding to the other heavy or light chain with the affinity ligands for VH3 and VL-k and by using the sequence variability in the variable part of the antibody. Utilizing an avidity effect, correctly paired heterodimers can be separated from incorrectly paired homodimers for asymmetric bispecific antibodies. We will also show the molecular structure of the VH3 and VL-k interactions with the affinity ligands and how to design-in or design-out binding by varying the antibody sequence.

2. Giving Novel Purpose to Protein A: A New Paradigm for Multispecific Antibody Manufacturing

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Interest in multispecific antibodies as the next generation of therapeutic biologics has grown dramatically over the past decade [1]. This interest is driven by their unique capability of binding to two or more antigens or epitopes simultaneously, thereby delivering enhanced or new therapeutic effects. While multispecifics hold great promise in delivering breakthrough medicines for patients, there is a degree of complexity in the manufacture of these molecules since they typically require two separate cell cultures and extended in-process hold times which can increase clinical manufacturing timelines. Efforts to reduce this complexity with multispecifics have resulted in the development of One-Pot Capture Redox technology. This technology involves the co-culture of two cell lines, with each cell line producing a single homodimeric antibody, and a redox reaction to rearrange the antibody chains into the heterodimer during the protein A affinity chromatography step. The focus of this talk is on the novel capture redox method which consists of reducing the interchain disulfide bonds in both antibodies, while they are bound to the protein A resin, and the successful heterodimerization and reformation of the disulfide bonds during the elution step. The capture redox combined with the co-culture process enables electro-steering based multispecifics to be delivered with timelines comparable to a well-behaved mAb, with high yield and robust product quality. The robustness has been demonstrated across bispecifics and trispecifics consisting of both IgG1 and IgG2 backbones. Moreover, this technology has been successfully demonstrated in a continuous small-scale system for two different multispecifics. Implementation of the one-pot capture redox technology has the potential to enable platform processes within both fed-batch and continuous manufacturing strategies for a diverse pipeline of multispecifics.

3. Rational and combinatorial design of peptides for ss-mRNA/ds-mRNA separation and purification

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Objective: The development of a generalizable, fast, effective, scalable, translatable and economic purification platform is critical for the technical and economic success of mRNA-based therapeutics. Our goal in this work is to develop downstream bioseparation strategies to separate dsRNA impurities from ss-mRNA, the preferred therapeutic form. To achieve this goal, we aim to identify peptide ligands that are selective towards dsRNA and couple these peptides to polymer filtration membranes to demonstrate the feasibility of purifying labile ss-mRNA from dsRNA at high yield and purity in a scalable fashion. Methods: Two strategies were employed for the design and selection of peptide ligands. The first, a rational and focused approach, was based on mimicking the active binding pocket of naturally occurring dsRNA specific binding proteins. Protein design templates were selected based on the following criteria: (i) binding affinity to dsRNA; (ii) binding selectivity/specificity to dsRNA versus ss-mRNA; (iii) structural information available; and (iv) length/size of the protein. Four proteins/domains were identified as templates including a double stranded RNA binding domain and three RNA silencing suppressors. A library of 16-mer peptide candidates was generated using linear epitope mapping of the target proteins with particular focus on the peptides that cover the key interacting regions of the protein. A second approach based on combinatorial phage display was employed to screen a vast pool of randomized peptide sequences. Pure ss-mRNA and dsRNA were challenged against a commercial phage library for negative and positive selections. Lead peptide candidates identified from the two design strategies were synthesized and screened for dsRNA binding affinity and selectivity in an immobilized format on microarrays. Results: Peptides that exhibited strong binding to dsRNA while displaying weak or no binding to ssRNA were identified through high-throughput screening and further validated in an ELISA or microarray format. Lead peptide structures were mapped on to their parent protein templates and revealed a strong consensus with the dsRNA binding site. Interestingly, a few ssRNA-selective peptides were also identified, originating from the dsRNA-selective binding proteins but from different regions of the protein when compared to the dsRNA binding site. Lead sequences shared several common motifs and/or amino acid propensity. The role of secondary structures, especially alpha helices, in the differential binding of the lead peptides to dsRNA was also observed. Lead peptides identified in this study will be further optimized through site-specific mutagenesis to further enhance their affinity and selectivity to dsRNA. Dissociation constants of the lead peptides to dsRNA were determined through fluorescence polarization assay. The selectivity of the peptides was further validated using competitive screening methods including competitive fluorescence polarization assay. Conclusion: dsRNA-selective peptides with high affinity and selectivity were successfully identified using two distinct strategies involving rational design and combinatorial library screening. These peptides demonstrated strong binding to dsRNA and, in some cases, showed selectivity for dsRNA over ssRNA.

4. Innovative Protein Scaffolds for Single Step Purification from Proteins to Viral Vector Particles

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Affinity chromatography is a well-known approach to streamline downstream processing with a clear advantage for purifying biomolecules resulting in high purity, yield and effectiveness. While it has long been the go-to method for purifying monoclonal antibodies, there is a lack of appropriate affinity purification solutions for novel drug modalities. A wave of new gene-therapy-based biopharmaceutical entities such as adeno-associated viruses for viral vectors-based gene therapies or extracellular vesicles as next-generation drug delivery systems (exosomes), require innovative purification solutions. This study introduces an innovative and universally applicable technology based on the affitin protein scaffold that broadens the scope of affinity capture beyond antibodies. Case studies presented showcase the development of a streamlined, single step purification process for a mutant form of streptolysin O (SLO), adeno-associated viruses and exosomes, highlighting its potential for universal applicability. The novel protein scaffold library provides specificity for the target of interest and was developed from a small robust protein, facilitating to meet current needs for specifications like affinity strength, dissociation rates, and caustic stability. An adaptable conjugation to different chromatographic materials was developed at different process scales. The single-step affinity purification process consistently resulted in high-purity (>90%) while also offering notable advantages over the conventional three-step process with smaller footprint and reduced process times (high productivity). The described benefits of such a novel affinity material are highlighted with a case study of SLO with a yield increase from 0.04 g to 0.31 g of SLO per kg of harvest broth. Another exemplary case study is based on a highly productive purification of adeno-associated virus 2 (AAV2) from cell culture supernatant. Custom demands in outperforming binding capacity of >2 E+13 vp/ml throughput as well as host cell impurity depletion through high specific affitin ligand materials could be identified. The custom affinity system described in this study holds the potential to be applied to any biologic for which a specific affinity ligand can be identified. This establishes a versatile platform with significant implications for the production of novel biopharmaceuticals.

Session - Poster Session

Ana Azevedo, Instituto Superior Tecnico, Portugal
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1. Overcoming mRNA Degradation: A Leading-Edge Strategy for Rapid Vaccine Development and Worldwide Distribution

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The global distribution of mRNA vaccines poses unique challenges due to the necessity for ultra-cold storage to prevent changes in product quality attributes, most notably mRNA purity and associated impurities. This presentation introduces an innovative strategy, implemented by Moderna, that addresses these issues, enabling worldwide vaccine distribution at -20C and 5C without necessitating time-intensive end-of-shelf-life clinical studies. Key to this approach is the close collaboration between Chemistry, Manufacturing and Controls (CMC) and Clinical Development teams. This partnership allows for the integration of shelf-life considerations into dose selection and clinical study design. Using first-order kinetics, changes in mRNA purity at varying storage temperatures are predictable, allowing for accurate estimations of mRNA purity at each clinical administration. By administering a commercially-representative range of mRNA purity, we can validate patient-centric specifications. Shelf-life is further extended through process improvements enhancing product quality at release. Importantly, we ensure that degradation products do not negatively impact the vaccine's biological responses. Incorporating data from both mouse and human studies, we provide a comprehensive understanding of the vaccine's performance. This strategy allows Moderna to distribute vaccines globally with a changing product quality profile without delaying market delivery.

2. Development of purification process using diverse adsorptive media for novel non-viral DNA gene therapy modality

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Viral vectors, particularly recombinant adeno-associated viral (rAAV) and lentiviral vectors, play a crucial role in gene therapy for rare diseases, dominating regulatory approvals. Despite their wide use, challenges like AAV packaging issues, hepatotoxicity, and lentiviral oncogenic or immunogenic responses persist. Recent advances in vector technology have made the non-viral gene therapy approaches particularly appealing for chronic diseases such as Hemophilia. The non-viral vectors could potentially circumvent the limitations associated with viral vectors and provide the flexibility of inserting a large transgene such as human FVIII together with the regulatory elements for the long-term persistent expression. A novel non-viral DNA vector was generated in the baculovirus-insect cell system in the form of closed-end DNA (ceDNA). Unlike rAAV vector, the ceDNA vector has no packaging constraints imposed by the limiting space within the viral capsid and also drives long-term persistent transgene expression in vivo. However, complexities arise for large-scale purification due to DNA size, shear sensitivity, and unique expression system. Manufacturing intricacies, including purification challenges due to cell lysis, are addressed through a robust and scalable process, aiming to yield high-quality DNA drug substance. Given the size of the DNA and viscous feed streams, anion exchange convective adsorptive media were investigated for the initial capture step. Membranes, monoliths, and perfusive resins were evaluated, with a membrane-based adsorptive media with a wide pore size rating identified as the top choice due to its high binding capacity and high-resolution separation potential. A two-step polishing process was employed to further purify full-length DNA by removing smaller DNA fragments and residual host cell nucleic acid impurities. Perfusive resins and monoliths based on hydrophobic interaction chromatography (HIC) were assessed for the intermediate polishing step. HIC monolith candidate was discounted due to scalability issues, and the process was refined using HIC perfusive resins with various ligand chemistries. The intermediate HIC polishing step demonstrated versatility in both bind-and-elute and flow-through modes without compromising product recovery or impurity clearance. However, flow-through mode was preferred for its increased loading capacity and reduced concerns about product stability and precipitation concerns due to high ammonium sulfate concentrations. For the final polishing step, core-shell resins with size exclusion and binding properties were evaluated. Loading conditions were established allowing the core shell resin's utilization as a final polishing column. Process parameters were further optimized globally to enable an integrated purification process, including the capture, intermediate polishing, and final polishing steps. This comprehensive approach ensures product recovery and impurity clearance, contributing to a robust and integrated purification strategy for DNA drug substance.

3. Why not both? Harnessing the powers of affinity- and size- based separations to transform the manufacturability of lentiviral vectors

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The rapidly growing cell and gene therapy field has the potential to target a wide range of previously incurable diseases and cancers, due in no small part to the use of lentiviral vectors (LVs). LVs can efficiently transduce a variety of dividing and non-dividing cell types and can carry large genetic payloads. While they are predominantly used for ex vivo transgene delivery, in vivo applications are growing, putting even more stringent requirements on the concentration factor and quality. Furthermore, the fragility of LV envelopes, a critical component to their high potency, makes manufacturing difficult and current processes yield only 10-20% of function recovery. These low LV recoveries put greater demand on upstream production volumes, a bottleneck that is limiting the availability and affordability of curative cell and gene therapies. We have developed a chromatography-free purification platform using our novel affinity reagent, IsoTagTM LV, which streamlines downstream purification into a two-step TFF process. The process leverages both size and affinity- based separation through a single recombinant protein with unique phase behavior properties and specificity for the VSV glycoprotein. The first step, a concentrate and wash of the crude harvest material, has a step recovery of over 80% and provides a three-log reduction in host cell proteins (HCP) and a four-log reduction in dsDNA. The second step removes the IsoTagTM LV reagent and simultaneously provides for further concentration and formulation, yielding highly pure LV with a step recovery of 90%. The entire process concentrates and purifies LV by 200X with a total functional recovery of over 70%. This process has been validated using several different feed streams from collaborators, showing strong performance with both traditional lentivirus vectors as well as virus-like particles. Improved recoveries are attributed to the use of gentle buffers as well as the inherent virus-stabilizing properties of the IsoTagTM LV reagent. When mixed with lentivirus, IsoTagTM LV enhances viral stability, improving infectivity compared to controls by over 2X in high salt conditions and for over a month at 4-8 degrees Celsius. Furthermore, IsoTagTM LV improved vector stability during freeze-thaw cycling and improved transduction efficiencies in T cells by 5x compared to formulations with no IsoTagTM LV present. This technology offers a paradigm shift in lentivirus manufacturing, with a multi-faceted approach to addressing LV manufacturability limitations. Successful implementation of this platform could significantly reduce the bottleneck currently limiting the development and growth of the cell and gene therapy industry.

4. Leveraging flocculation to improve adeno-associated virus (AAV) purification process

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The recombinant adeno-associated viruses (rAAVs) with precise genome editing and unique cell-virus interaction have gained importance as the effective delivery tool for gene therapy. However, rAAV manufacturing process development has its unique challenges in contrast to traditional biologics, e.g., monoclonal antibodies (mAbs). First, rAAV is produced intracellularly and requires cell lysis to release product. The heavy amount of host cell impurities after breaking cellular membrane challenges the subsequent clarification and purification processing. Significant amount of endonuclease is required for host cell DNA removal, which increases the cost of goods for rAAV production. The intrinsic interaction of rAAVs with impurities causes severe product aggregation and further impacts product recovery. In addition, cell culture not only produces rAAVs with full length genome (full capsid), but also rAAVs packed with no genome (empty capsid), or partially packaged genome (partial capsid). Leveraging subtle charge difference to enrich full capsids from empty or partial capsids on ionic exchange chromatography is essential for sufficient therapeutic efficacy. The interference from impurities makes the capsid separation extremely challenging. Controlling the host cell impurities at the early stage of purification is mandatory for successful and robust rAAV manufacturing. Flocculation has been employed for mAbs purification processes to remove host cell impurities and debris but has not been fully explored in gene therapy purification. In this work, flocculation by acidification in AAV purification process has been successfully implemented from bench top to 500 L scales. The benefits have been demonstrated, including more than 10-fold host cell impurity reduction without use of endonuclease, efficient filtration without flux decay, 5-times higher affinity resin lifecycles due to the low impurity in the load material. Furthermore, with less interference from impurities, the subtle charge difference between three kinds of capsids enables a higher resolution and better enrichment of full capsids. By controlling impurities level at upstream feed stream, rAAV viral vectors demonstrate improved stability with minimal aggregation at low conductivity, which further improves process recovery. With cleaner feed stream, the downstream intermediates achieve more than 10-times lower turbidity values with maintained rAAV titers, enabling easy filtration and manufacturing robustness. Flocculation has been successfully demonstrated as an innovative rAAV manufacturing technology for multiple AAV serotypes. The implementation in rAAV process platform gains not only superior product quality, significant benefits to downstream recovery, but also a major cost of saving in rAAV manufacturing.

5. A Novel Harvest-Capture Process for Rapid Clarification and Recovery of rAAV9 from Cell Lysate Using a Macroporous Filtration Device and AVIPure Affinity Resin

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Recombinant adeno-associated virus (rAAV) vectors are a leading platform for gene delivery for a variety of human diseases and have been the focus of hundreds of clinical trials over the past several decades. The manufacturing of rAAV therapies at the industrial scale involves a number of labor intensive and expensive steps, such as clarification by depth filters or centrifugation, UF/DF by tangential flow filtration, and chromatography. To optimize the manufacturing of rAAV, Repligen has developed a novel process combining the clarification of rAAV from cell lysate and affinity chromatography using a macroporous filtration device and AVIPure affinity resin in a single step. In roughly 3 hours, >90% of rAAV9 capsids were recovered directly from 1-L of cell lysate. Scale down experiments have also been shown to display an average volume reduction of ~10x from initial cell lysate to final product recovery. The benefits of this process include elimination of depth filters or centrifugation for clarification and high clearance of host cell proteins and host cell DNA. The process has been designed to be fully scalable so that a single filter can be used for manufacturing runs of up to 2000-L, requiring only 10-L of AVIPure affinity resin. The novel harvest-capture methodology will provide value to the rAAV vector space by reducing the number of unit operations, time, and cost of the manufacturing process without negatively impacting product yield and purity.

6. Bridging the translation gap: The development of a toolkit to accelerate process development and provide early manufacturability insights for AAV gene therapies

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Many AAV based gene therapies stumble during the translation from the pre-clinical/discovery phase to Phase I leading to program delays or cancellation. A significant number of these are due to manufacturability concerns such as poor process productivity or product quality issues (e.g., full capsid ratio, truncated transgenes, variant capsids). This presentation will discuss how a toolkit consisting of high throughput process development (HTPD) methods, mechanistic modelling and reliable high throughput analytics can be used to enable manufacturability assessments during the pre-clinical phase and accelerate the development of the early clinical production process. Generating enough material to assess new product candidates during the pre-clinical phase is complicated by the propensity of AAVs to have low capture recoveries. This means that the candidate assessment may limited to evaluating efficacy without considering manufacturability and product quality. The low process recoveries are typically due to non-specific interactions which can be negated by using additives such as salts and surfactants. As the optimum additive scheme (types, concentrations) is hard to predict, extensive screening is required. We have established a microwell plate based HTPD method to enable this screening during early development. Material generation at this phase is typically performed at the ~ 200 mL scale. Using this method and < 10 mL of the initial material, the optimum additive scheme can be identified. This additive scheme can then be used to maximise the product capture from the remaining material, which in turn enables further assessment of the candidate for manufacturing and quality related aspects. A key quality attribute for the AAV gene therapies is the proportion of capsids that carry the therapeutic transgene (full capsids). While we have established a platform process step based on anion exchange chromatography to enrich the packaged capsids, considerable experimental effort is still required to identify the exact processconditions that maximise resolution between the closely related empty and full capsids. This delays the transition of the product into the clinic. To minimise the experimental effort required, the microwell based method mentioned above is employed to determine the optimum additive scheme but is also adapted to determine the optimum elution salt for resolving the full and empty capsids. We have established a mechanistic modelling workflow which is then used to determine the remaining process parameters (load, wash, and elution conditions) with a minimal number of training experiments. The adoption of the above approaches creates a large analytical burden which typical methods such as qPCR, ELISA and AUC are not well suited to handling. We have overcome this hurdle by developing calibration free HPLC based assays that can handle large sample numbers while generating data of a high enough quality for use in training mechanistic models. The use of this toolkit helps to identify candidates with a higher likelihood of successful manufacture while accelerating their transition into clinical production.

7. Membrane chromatography for preparation of AAV at the manufacturing scale

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The recent FDA approval of several adeno-associated virus (AAV)-based gene therapies is driving demand for AAV production. One of the biggest manufacturing challenges is removing “empty” capsids, which do not contain the gene of interest. Anion exchange chromatography has emerged as a solution for scalable empty and full capsid separation. Here we develop a process for baseline separation of empty and full AAV capsids using Mustang Q anion exchange membrane chromatography. This process development approach utilizes AAV serotypes 5, 8, and 9 and traverses initial screening using Mustang Q XT 0.86 Acrodisc unit up to manufacturing-scale processes with Mustang Q XT 140. Process development of a two-step elution was performed via response surface DoE, exploring conductivity and the length of the first elution step. Empty and full capsid percentages were quantitated with ELISA and ddPCR assays as well as mass photometry. The results from response surfaces were used to construct statistical models of the process operating space. These models provide optimal conditions for recovery and purity, both of which can exceed 80%. Model predictions were then validated at small scale prior to scale-up. We present the results from our scale-up purification and show that purity and yield are consistent with the results obtained from the response surface model. To demonstrate that Mustang Q membrane chromatography can be a robust manufacturing technique, we tested three different feedstreams of AAV5 on three unique lots of Mustang Q. The feedstreams varied by cell line (HEK and SF9) and cell culture technique (adherent and suspension). Drastic differences in the behavior of feedstream sources are identified, and we performed mass spectrometry to deepen the understanding of these phenomena. Here we show that despite the differences in feedstreams, reproducibility and robustness are high across membrane lots. We demonstrate the ability to cycle Mustang Q membrane nine times to achieve reproducible, back-to-back separations. The presence of empty capsids is a challenge for both manufacturing and drug product understanding. Two prevalent methods for quantifying full to empty capsids ratios are the use of capsid ELISA and ddPCR ratios, and UV 260 nm to 280 nm ratios. To further understand these techniques and their relationship, we built regression models using both metrics. Ultimately, we compare those measurements to mass photometry and analytical ultracentrifugation (AUC) to highlight why orthogonal analytical methods are needed to accurately measure full capsid percentage.

8. Improved AAV Release and Lysate Clarification applying an enhanced Cell Lysis Strategy

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The lysis of mammalian host cells to release intracellular products such as adeno-associated viruses (AAV) is accompanied by the release of numerous cell debris and dissolved impurities. Depending on the type of lysis method, cell lysis can have a strong impact on the subsequent lysate clarification and product purification steps. Established lysis strategies are limited by various factors, including the elimination of the widely used lysis surfactant TritonX due to safety concerns [1], long lysis durations of greater than two hours or longer [2], and relatively low filter capacities of lysates with increasing cell concentration for improved upstream processes [3]. Therefore, improvements of cell lysis are needed to intensify the industrial production of viral vectors. An additional challenge in the development of lysis strategies is the usually unknown initial quantity of viruses in the non-lysed cells. Therefore, a specific virus recovery for corresponding lysis approaches cannot be determined, which limits the comparability of different lysis strategies between different batches. In order to intensify chemical cell lysis, a holistic lysis strategy was developed using a human embryonic kidney (HEK293) suspension cell line with more than 50% viability at harvest for production of AAV2. The lysis approach consists of two subsequent short lysis steps: In the first step, concentrated lysis buffer with a non-toxic detergent and a small amount of nuclease were added to initiate cell lysis. The cell suspension was then homogenized and tempered at 37 °C for one hour. In the second step, the partially lysed cell suspension was acidified. The low pH approach generated near immediate cell lysis. Therefore, the subsequent clarification process using a depth filter (8.0 + 0.8 µm) followed by a sterile filter was started after approximately five minutes of acidification. Overall, the two-stage lysis approach achieved complete lysis of the cells within less than 70 minutes, which is significantly shorter than established methods. The functional virus titers were up to 50% higher compared to two different single step lysis methods tested in parallel. In addition, the acidification caused precipitation of impurities, which facilitated their removal and resulted in a more than 50% lower concentration of DNA and host cell protein impurities after the clarification step. Furthermore, it is highly likely that other impurities such as cell debris were also aggregated, which could be an explanation for the more than twice higher filter capacities compared to lysis without acidification. It should be noted that, the application of two-stage lysis on a process scale could be further simplified by using the bioreactor for mixing, temperature control, and low pH titration. In summary, we show the high potential of an intensified viral vector production by the development of the two-step lysis approach combined high functional virus titer with reduced impurity levels as well as rapid streamlined processing.

9. Use of flowthrough anion exchange chromatography for the enrichment of full capsids in Adeno-Associated Viral Vector purification

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Adeno-associated viral vectors have seen a surge in interest as promising gene delivery agents in the last decade due to their non-pathogenicity and low immunogenicity. Commonly manufactured using mammalian cell cultures, the resulting gene vector material coming from upstream includes the target capsids containing the therapeutic DNA payload, as well as empty and partially full capsids. Empty or partial capsids are associated with a higher immune response risk and adverse clinical outcomes. Resin based ion exchange chromatography, traditionally used for antibody purification, has previously been successfully used for the enrichment of full capsids under operation in a bind-elute mode. To reduce costs and time and improve efficiency, it is desirable to intensify the use of chromatographic resin and minimize material hold times, which might affect vector stability. We demonstrate the use of an anion exchange resin in frontal chromatography mode for the enrichment of full capsids, operating in non-binding conditions for the empty capsids, also called “overload chromatography”. A flowthrough of >99% empty capsids was measured, indicating that the chosen conditions favor binding of full capsids, also resulting in a displacement effect of empty and partial capsids. An enrichment factor of over 4x and a vector genome recovery of >65% were achieved. Furthermore, we show the potential for this chromatographic mode for the clearing of partially filled capsids. We study the effect different divalent cations at different concentrations have on the overload capacity and specificity, and the use of different commonly used anion exchange chromatography resins. Finally, we estimate the impact an intensified polishing step can have on the overall purification process. Overall, we show that overload chromatography can be applied as an effective, fast and high yielding purification method for gene therapy, using chromatographic resins and buffers well known in the biopharmaceutical industry and well suited for large-scale manufacturing. Applying this chromatography mode to continuously increasing bioreactor titers and sizes will support the long-term industry goal of considerably cutting the manufacturing costs of AAV-based gene therapies.

10. Atomic basis of AAV affinity chromatography: from design to cryo-EM validation

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To improve downstream processing of AAV feed streams we developed a suite of alkali-tolerant affinity resins for AAV serotypes 2,5,6,8, and 9. The ligands were identified through screening diverse libraries of low-molecular-weight protein scaffolds. The initial set of ligands was filtered through secondary assays to identify the best ligands in terms of recovery, elution, clearance of host cell proteins and DNA, and high stability in sodium hydroxide. The resulting affinity resins offer best-in-class process economics because they maintain performance through 20+ clean-in-place cycles. This reusability can increase patient access to AAV therapies by lowering manufacturing costs. To validate that the ligands bind specific AAV epitopes, we determined a high-resolution cryo-EM structure of the AAV2 ligand bound to AAV2 capsids. The resulting structure validates that specific protein: protein interactions are the basis for affinity capture. The AAV2 ligand binds to the side of the cleft formed by protrusions around the 3-fold symmetry axis of the AAV2 capsids. The shape of the helical ligand is complementary to the shape of this cleft. The ligand contacts AAV2 residues 265-268, 503, 512-515, 527, 575, 581, and 592 through hydrogen bonds, van der Waals interactions, and a salt-bridge. Structure determination for AAV5,6, 8, and 9 ligand: capsid complexes is ongoing, and we will present available low-resolution cryo-EM structures for these ligands. At the lower resolutions, the maps show the footprint of the ligands on the capsids, but not the specific amino acid interactions. These cryo-EM structures enable rational pairing of affinity resins and engineered capsids by identifying the capsid residues which interact with the affinity ligands. This presentation will offer an ‘under the hood’ look at how novel AAV affinity resins are created, and how these resins engage capsids at the atomic level.

11. A Case Study on the Impact of AAV Triple Transfection Process Changes on Downstream Processing

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As a promising and increasingly utilized modality, adeno-associated virus (AAV) has seen rapid advancements addressing productivity and scalability in both upstream and downstream unit operations. The implementation of these new technologies into the upstream process places a burden on downstream process development teams to ensure comparable critical quality attributes and impurity profiles in the drug substance. A case study will be presented here showcasing the implementation of process changes to critical upstream process materials and critical process parameters in a HEK293 triple transfection process. The transfection conditions used in initial upstream process development encountered scaling challenges, resulting in a transfection change late in development that both decreased initial percent full and increased residual packaged DNA species. This change challenged the developed downstream process and resulted in required rapid further development to accommodate the changes in feed material. This work outlines our approach to quickly identify impacts to the downstream process and implement mitigating steps to address these challenges with minimal impact to timelines.

12. Highthroughput Development and Scale-up of a Novel Anion Exchange Chromatography Method for Removal of Empty Capsids from full rAAV Capsids.

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Separation of empty capsids from full recombinant Adeno-associated Virus (rAAV) capsids is one of the challenging areas in the downstream processing of rAAV gene therapy production. The work presented here describes the development, process understanding and scale-up of a chromatographic based method for removal of empty AAV capsids. The separation mechanism on anion exchange (AEX) is different from the traditional mode of AEX operation as it involves use of weak acids to partition the empty/full capsids resulting in selective retention of full rAAV capsids. This method offers advantages over currently reported methods in terms of increased robustness towards variability in load composition and mobile phase composition. DOE (Design of Experiment) and OFAT (One Factor at Time) based methods were used to understand the impact of process parameters (viz. weak acid concentration, pH, concentration, buffer components), make process improvements and establish robust operating ranges. The parameters identified from HTS (high-throughput screening) on 0.1mL robocolumns to achieve ≥ 80% enrichment were scaled-up through small scale, pilot scale and production scale columns. A full rAAV capsid enrichment of 85% (by AUC (Analytical Ultracentrifugation)) and > 60% recovery (ddPCR) was obtained at 250L scale demonstrating successful scaleup of a robust and an AEX chromatographic method for empty/full AAV capsid separation.

13. Biophysical investigations to elucidate the role of resin media and feed constituents on AAV affinity column fouling

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The increasing demand for Adeno-Associated Virus (AAV) therapies necessitates a need for robust, cost-effective purification processes. Most commercially available AAV affinity columns are expensive and offer suboptimal product recovery with limited column reusability. In our recently published work, we demonstrated that the reuse of POROS CaptureSelect AAV affinity columns can affect the product eluate quality due to the co-elution of aggregates and host cell impurities (Soni et al., 2023). Here we carry out a detailed biophysical investigation to elucidate the role of resin media, AAV-ligand interactions, and feed constituents on AAV affinity column performance and fouling. We achieved this through applying a multi-faceted biophysical approach. Confocal microscopy was first employed to shed light on the differences in AAV transport within the chromatographic beads. Interestingly, while AAVs were found to fully penetrate the polystyrene-based resin materials during loading, the AAVs remained primarily on the surface of the agarose-based resin material. These studies were complemented with structural imaging and analysis of resin architecture and associated transport mechanics, visualized in 3D using nanoscale X-ray computed tomography (CT). This was supported by measuring the 3D pore size distribution of each resin from X-ray CT imaging. Confocal microscopy was also employed to study column fouling and to identify the location of the AAV products, host cell proteins (HCPs), aggregates and DNA in the AAV affinity resin materials. Upon examining the post-CIP resin samples, it was found that the polystyrene resin samples exhibited a greater degree of fouling compared to the agarose resin samples. While foulants constituting AAVs, HCPs and DNA residuals were seen to increase in the polystyrene resin samples obtained from cycle one to cycle four, the AAVs located primarily on the surface of the agarose beads were found to be efficiently removed during the elution step. These results indicated that fouling was not just a function of the ligand interactions with the feed, but that resin pore size and channels can also contribute to column fouling. We also employed surface plasmon resonance (SPR) to study the binding behavior between a range of AAV ligands and AAV model vectors. This data clearly demonstrated key differences between the pan-AAV and serotype-specific AAV affinity ligands. Further, new AAV affinity ligands were shown to exhibit improved desorption kinetics under less harsh elution conditions. The data obtained from these various techniques demonstrates an interesting interplay between the effects of mass transport, affinity, and kinetics on the fouling of these systems as well as their chromatographic behavior. In addition, these results demonstrate that AAV column performance degradation is likely due to fouling caused by the high impurity burden, strong AAV-ligand interactions, resin base matrix structure and ineffective column cleaning. Finally, this data informs useful approaches for improving AAV affinity column performance as well as future directions for improved resin/ligand design.

14. A unified data management strategy for downstream development, from early research to market launch

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During the development of a new active pharmaceutical ingredient (API) data is generated in different phases of the API development process and in different parts of our organization. In our traditional workflow, the data is summarized in reports, while the actual raw data is archived on local or shared drives with little documentation and metadata attached. In practice, such raw data is difficult to interpret for scientists in later phases, and consequently experiments are often re-run. This causes extra works and challenges traceability. This contribution presents a new data management infrastructure and workflow for downstream processing at Novo Nordisk. The infrastructure allows us to leverage all relevant data throughout the value chain to build the best knowledge base for developing our downstream processes. The infrastructure is instrument vendor agnostic and works across instruments of different sizes. Schematically, the infrastructure consists of two parts namely a data lake and a harmonization layer. The data lake contains copies of all our experimental data from early research to GMP production. Automatic data pipelines connect both laboratory and pilot equipment with this data lake. Since there is no human involved in the upload the data lake is completely representative. At arrival in the data lake, the raw data is indexed such that data is findable and accessible for both humans and machines. The harmonization layer utilizes equipment metadata and instrument knowledge to expose the data in a uniform fashion. The harmonization layer e.g., translates vendor specific naming conventions and converts units thus enabling interoperability between different equipment and process scales. Moreover, the harmonization layer is used to enrich the raw data with a context e.g., associating a preparative chromatogram with one or several analytical chromatograms. Seamless integration with mechanistic or statistical models is achieved after the data harmonization. A case study consisting of the complete downstream process of a single-domain antibody is presented. The case shows how high throughput process development data is seamlessly combined with data from laboratory column experiments, and pilot scale purification to create a mechanistic model for each of the purification scales. At each process scale, the model was refined as new data entered the data lake. Finally, the model was used to optimize and validate against a GMP production.

15. The Downstream Data Browser – Enhancing Data-Driven Decisions via Customized Data Handling and Visualization

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High-throughput screening methodologies have accelerated downstream development for monoclonal antibodies (mAbs) by enabling parallelized evaluation of chromatographic resins across a range of conditions. However, scientists must now interrogate large datasets that may require complex statistical analysis to interpret results in a meaningful and consistent way. Moreover, accessing, comparing, and combining historical datasets can be cumbersome without standardized approaches to data capture. Selection of the best resin and operating conditions is a multivariate optimization problem that ideally leverages model fitting procedures to predict resin capacity, selectivity, and mode of operation for a given mAb. Here we present a flexible web-based visualization and analysis tool, the downstream data browser. This tool automates visualization of high-throughput datasets, fits response surface statistical models, standardizes report results from a high-throughput screening method and facilitates comparison across molecules. The Just-Evotec platform-in-plate (PiP) concept is an automated high throughput batch-binding workflow which evaluates several chromatography resins in a single 96-well plate (Gillespie, 2017). For flow-through polishing resins, 24 buffer conditions are explored to evaluate protein-resin interactions and raw output data for each well (absorbance and size-exclusion signals) can be transformed to partition coefficients to draw conclusions about resin performance. The downstream data browser automatically fits pH and sodium chloride factors to a response surface model and reports outcome measures including partition coefficients, separation factor of contaminant species, and solution stability, which are visualized by a pre-defined set of contour plots. The tool is equipped with detailed documentation to assist in consistent decision making and data communication. The tool also provides fit parameters to assess model quality, and a residual plot to interrogate the model – these features have helped identify cases where additional modeling with more factors has been necessary. Models are built in real time and allow historical datasets to be viewed at once, enabling comparison of results between molecules and even detection of opportunities for platform improvement. The downstream data browser is an ideal case study in a database strategy to extract, visualize, and standardize results reporting from datasets. Coupling high-throughput screenings with sophisticated, customized data capture, visualization, and analysis has ensured the benefits of parallelized workflows are fully realized across a network of scientists.

16. Enhancing Biopharmaceutical Process Control and Efficiency through Integrated Data Analytics and Predictive Modeling: Insights from the BioRaptor Platform

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Background: The complexity of biopharmaceutical processes necessitates a robust, integrated approach to data management and analysis. BioRaptor introduces an innovative platform designed to revolutionize process design and control by harnessing the power of integrated data analytics and predictive modeling. Methods: Integration of Digital Process Signatures: BioRaptor utilizes soft sensors for real-time data collection, which, in conjunction with advanced analytics, generates Digital Process Signatures. These signatures are complex digital representations that encapsulate the intricate dynamics and interactions of bioprocesses, offering a holistic view of upstream (USP) and downstream (DSP) procedures. Optimization through Design of Experiments (DOE): The platform employs DOE across all unit procedures to systematically identify optimal process conditions. This methodological approach enhances process understanding and enables parameters optimization to improve yield, quality, and efficiency. Predictive Modeling with Digital Twins: Employing dynamic digital twins, BioRaptor provides predictive insights into process behaviors. This feature allows for the anticipation of potential issues and the proactive implementation of solutions, significantly reducing the risk of deviations and enhancing overall process control. Results: In-process Advancements: The implementation of BioRaptor's process health sensor marks a significant in-process advancement. This sensor leverages a combination of in-line, at-line, and offline data to continuously monitor Critical Quality Attributes (CQAs). The resulting real-time insights into process health enable immediate adjustments, ensuring consistent quality and reducing the likelihood of deviations. Cross-Unit Procedure Optimization: Outside the realm of immediate process control, BioRaptor has successfully integrated digital twins with sequential Design of Experiments (DoE) to optimize across various unit procedures. This strategic combination allows for a holistic optimization approach, considering the interdependencies and cumulative impacts of each process stage. The result is a more efficient, cohesive production process with improved overall yield and quality. Conclusion: BioRaptor represents a paradigm shift in biopharmaceutical process management. By integrating comprehensive data analytics with advanced predictive modeling techniques, the platform sets a new standard for process optimization. The use of Digital Process Signatures and soft sensors facilitates a deeper understanding of complex bioprocesses, enabling a more proactive and precise approach to biomanufacturing.

17. Host cell proteins in model based process development of biopharmaceuticals

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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 using automation and miniaturization via for example liquid handling robots in so called High Throughput Process Development – HTPD. Combining this experimental HTPD with mathematical models based on sound physical-chemical phenomena captured in so called mechanistic chromatographic models, 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 running 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 such a hybrid model based HTPD approach, method 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.

18. A novel isotherm for hydrophobic interaction chromatography that improves prediction precision across operation conditions and facilitates sensitivity analyses

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Hydrophobic interaction chromatography (HIC) is a highly relevant separation technique that provides orthogonal selectivity to widely used ion exchange chromatography (IEC). However, in contrast to the latter, the mechanisms underlying hydrophobic interaction are difficult to capture in the form of an isotherm, probably due to the complex nature of the mechanisms involved. This complexity is, for example, reflected by the plethora of hydrophobicity scales that have been developed for amino acids [1]. In 2016, Wang et al. have published a HIC isotherm [2] that expands on previous work by Mollerup [3] and Deitcher [4]. This modified isotherm improved the prediction accuracy by incorporating the activity of the bulk like water molecules that are released during the adsorption process. However, we found that it resulted in implausible predictions in other situations. For example, when altering the protein concentration during salt gradient elution the location of the elution peak shifted drastically. We investigated the underlying assumptions made during isotherm development and found that using the bound protein concentration as a substitution for the activity of the bulk like water molecules was most likely causing the unrealistic model results. We then formulated an alternative isotherm based on the assumption that the released water molecules are indistinguishable from the water molecules making up the mobile phase. Accordingly, our isotherm utilizes the activity of the surrounding water molecules to describe the activity of the released bulk like water molecules. We evaluated this new isotherm on in silico and experimental datasets. Using the new isotherm eliminated the unrealistic predictions and outperformed the 2016 HIC isotherm on two out of three experimental datasets based on metrics of the elution peak height and peak position. Additionally, our new isotherm can be a drop-in replacement for the Mollerup and Deitcher isotherms. Specifically, individual model assumptions can be excluded by setting the respective parameters to zero. This includes, but is not limited to, the parameters KP and KS from the Mollerup isotherm, which modify the activity of the solved protein based on the solved concentration of protein and salt respectively. Also, parameters $\beta 0$ and $\beta 1$, which modify the influence of the released bulk like water molecules on the binding dynamics, can be assessed individually. This enables a comparison of isotherm performances and facilitates parameter sensitivity analyses that can be conducted in a single combined parameter estimation step. Lastly, we implemented the isotherm in the latest version of CADET, so it can be easily used from within the software suite, once the manuscript about the isotherm will be published.

19. Manufacturing Facility and Economic Analysis of Process Intensification Strategies

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Downstream process intensification technologies provide options for faster, more productive, and more sustainable biomanufacturing than conventional batch operation. A quantitative analysis of the possible benefits obtained from the implementation of these technologies is fundamental to making critical decisions related to process and facility designs. Nevertheless, such an analysis can be highly dependent on manufacturing scale, facility capabilities, and particular production scenarios. In this work, key performance indicators (KPIs) are defined and used for developing a framework for techno-economic feasibility analysis to assess the detailed impact of various process intensification options for protein A capture (e.g., multi-column chromatography, MCC), polishing chromatography, and viral filtration. These KPIs include Costs of Goods (COG), productivity, manufacturing cadence, Full-Time Equivalent (FTE) utilization, and Process Mass Intensity (PMI) that measures resource usage efficiency. The simulation comprising three blocks that exchange information with each other is based on a combination of process knowledge, facility operation and scheduling parameters, and cost modeling. KPIs are compared for scenarios featuring different facility schedules, upstream titers, and downstream process intensification strategies, where results are presented for decision-makers to easily identify the best process alternatives for a given production scenario. For the conditions evaluated in this work, we found that scheduling practices and technological intensification strategies have the greatest impact on process productivity and operating costs, respectively. While intensification of individual unit operations can yield benefits from a sustainability and cost perspective, it appears necessary to implement intensification strategies for both downstream process and scheduling practices to achieve an optimum plant throughput. We will also present a productivity and cost analysis for upstream scenarios comparing fed-batch and steady-state perfusion production and discuss its implications to biomanufacturing process intensification.

20. Driving improvement and optimization of production scale processes via modelling and experimentation

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Mechanistic models are used to provide advanced process understanding for improvement and optimization by representing a physical system through its physical phenomena. In the pharmaceutical industry, the mechanistic models together with process knowledge and data are used sequentially at different scales and phases for design, scale-up and optimization studies. Ideally, for continuous improvement as a function of time, mechanistic models can transfer knowledge and capture system dynamics in moving from lab-scale experiments to full-scale manufacturing. Improvement and optimization at the manufacturing scale is usually driven by business needs and executed within a framework of cross functional-collaborations to ensure high quality products and robust solutions. Within the process of improvement/optimization, models are only used reactively when the time and availability of data allows. Most of the times modelling activities are limited to statistical methods which are fast to develop and they require high data quality to ensure that they describe the nonlinearities of complex processes. The objective of this abstract is to discuss and demonstrate the importance of the development and the application of mechanistic models at full-scale manufacturing and the opportunity which arise when they are used proactively. For their development, we leverage multiscale analysis of the production lines and analysis of business goals to identify areas of interest [1]. They are derived using literature, experts knowledge and data. The models are either fully physics-informed or hybrid models. The models are then used iteratively until they reach their validation point to generate valuable insights to drive the improvement and optimization projects. The discussion will focus on how models can be used to assist decision making for projects, to facilitate cross-functional collaboration and to design targeted experiments. In decision making, the models are used to generate insights and ideas for process improvement/optimization by quantifying the operation space and testing different scenarios. Those insights are used to enhance cross-functional collaboration and communication by evaluating and visualizing the effects of various inputs. To design experiments, the models are applied to map areas with high uncertainty due to limited knowledge/data and generate knowledge by proposing tests at lab or industrial scale with minimum risk. Finally, we will discuss the challenges and the opportunities in applying models at industrial scale. Throughout the presentation, industrial application of modelling different unit operations such as chromatography, reaction, filtration, precipitation and drying will be shown as examples.

21. A priori process development of multimodal chromatography in antibody purification: a multiscale modeling approach

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Multimodal chromatography has emerged as a powerful method for the purification of therapeutic antibodies. However, process development of this separation technique remains challenging because of an intricate and molecule-specific interaction towards the multimodal ligands, leading to time-consuming and costly experimental optimization. This study presents a multiscale modeling approach to predict the multimodal chromatographic behavior of monoclonal antibodies based on their sequence information. Linear gradient elution experiments were performed on the Capto adhere resin for 59 full-length antibodies, including five different antibody formats at pH 5.0, 6.0, and 7.0 that were used for isotherm parameter determination in a linear adsorption regime. Quantitative structure-property relationship (QSPR) modeling was utilized to correlate the isotherm parameters with up to 1374 global and local physicochemical descriptors calculated from antibody homology models. The final QSPR models employed less than eight descriptors per model and demonstrated high training accuracy ($R2 > 0.93$) and reasonable test set prediction accuracy ($Q2 > 0.83$) for the isotherm parameters. Model evaluation revealed the significance of electrostatic interaction and hydrophobicity in determining the chromatographic behavior of antibodies, as well as the importance of the HFR3 region in antibody binding to the multimodal resin. Chromatographic simulations using the predicted isotherm parameters showed good agreement with the experimental data for the vast majority of antibodies not employed during the model training. The results of this study demonstrate the potential of sequence-based prediction for determining chromatographic behavior in therapeutic antibody purification. This approach leads to more efficient and cost-effective process development, providing a valuable tool for the biopharmaceutical industry.

22. Mechanistic modeling-based characterization of size-exclusion-mixed-mode adsorbents for antibody fragment separations

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Modernization of biopharmaceutical production has driven the development of effective platform processes. Despite this, there are still obstacles that are yet to be overcome. One of these obstacles is the development of platformable methods that robustly remove fragmented antibodies from therapeutic antibody process streams. Antibody fragments have been considered a remarkably difficult product-related impurity to remove [1]. This difficulty is attributed to structural similarity with the monomeric antibody [2], having similar physicochemical properties relevant for purification. Because fragments can negatively impact product critical quality attributes [2,3], they must be removed during antibody purification. Consequently, this requires the use of robust, efficacious downstream processes. Chromatographic purification is the workhorse of these operations, with a heavy reliance on affinity chromatography steps. While affinity media provides high selectivity, they can have significant disadvantages such as low binding capacity, low elution pH, and ligand leeching [4-6]. Clearly, improved adsorptive materials are needed to address this gap in the purification toolbox. In this work, a novel size-exclusion-mixed-mode (SEMM) resin [7] was characterized (experimentally and in silico) with respect to its effectiveness in removing both antibody fragments and high molecular weight (HMW) species from a mAb protein A eluate feed. Inverse size-exclusion chromatography showed that the silica-based resin had a narrow pore size distribution and a median pore radius of roughly 6.2 nm—slightly greater than the mAb's hydrodynamic radius of 4.5 nm. Simulations were carried out with the Chromatography Analysis and Design Toolkit (CADET) software [8], using the general rate model of chromatography and the multicomponent Langmuir isotherm. Isotherm and transport parameters were obtained from fitting column breakthrough curves, at multiple residence times, for a mixture of mAb, HMW, F(ab')2, Fab/Fc, and Fc fragments. Model validation was carried out with a scaled-up column using a different set of operating conditions compared to the calibration runs. Results indicated that the mechanistic model performed well in both interpolative and extrapolative scenarios. The utility of the adsorbent was also demonstrated—achieving 90% mAb yield, 37% HMW removal, 29% F(ab')2 removal, 54% Fab/Fc removal, 100% Fc fragments removal, and a productivity of 72.3 g mAb/L/h. Simulated batch uptake experiments then showed that resin penetration depth was directly related to fragment size and that separations were driven by differential pore diffusion rates. An array of simulations was then carried out to characterize the dependence of impurity removal on protein load density, feed composition, and column dimension. Removal was shown to be highly dependent on load density, where optimal purification was achieved below 100 mg protein/mL column. Additionally, removal was dependent on column volume, but agnostic to whether column length or diameter was altered. Lastly, the relationship of feed composition with impurity removal was shown to be complex. While removal was inversely correlated with fragment and HMW feed mass fraction, the extent of this relationship depended on protein size. In summary, this investigation both illustrated the utility of the SEMM resin in removing mAb fragments and HMW species and elucidated relationships with key operational parameters using model-based characterization.

23. All-Atom Modelling of Methacrylate-Based Multi-Modal Chromatography Resins for Isotherm Parameter Prediction

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Chromatographic separations rely on highly specific interactions between the target molecules and the chromatography resins. Multi-modal chromatography (MMC) further increases the complexity of these interactions by combining various interaction types, making the prediction of optimal binding and elution conditions during initial process development challenging. Molecular-scale simulations have become crucial in this context, providing detailed insights into biomolecular adsorption mechanisms on resin surfaces and aiding in predicting molecular affinities towards chromatographic matrices. While previous studies have shown promising predictive capabilities for peptides and proteins in ion exchange and MMC, the extensive computational effort required for time-resolved molecular simulations limits their practicality for high-throughput screenings. Furthermore, accurately modeling chromatography resins at the molecular level is challenging due to limited knowledge of their complex three-dimensional all-atom surface structures. Therefore, resins are typically depicted in simplified models, where the ligands are directly tethered to a planar surface. However, it is important to consider that, in reality, resins neither possess a planar surface nor a perfectly uniform ligand density, attributed to the nanoscale structure of the backbone. Moreover, for polymer-based chromatography resins, like methacrylate-based resins, the backbone itself can exhibit hydrophobic characteristics, which can influence the overall molecular affinity towards target molecules. Addressing these challenges, we developed two automated, sequential molecular modeling workflows for predicting Langmuir constants. The first workflow creates all-atom models of methacrylate-based chromatography resins through a multistage simulation approach. The process starts with coarse grain simulations, encompassing the polymerization reaction of the backbone and the subsequent ligand attachment. It then progresses with a conversion from coarse grain into all- atom resolution and concludes with an energy minimization. As an example, we created an all-atom model of the resin surface for the commercially available multimodal resin TOYOPEARL MX-Trp-650M from Tosoh Bioscience, measuring 15 nm in diameter. The second workflow facilitates high-throughput screenings of binding poses and energies between the resin model and target molecules. It employs molecular docking simulations for an initial binding pose identification, followed by a force-field-based minimization of the resin-target-molecule complex with continuum solvation modeling. The workflow concludes with the calculation of the binding energy through single-point semiempirical quantum mechanics simulations. For an initial validation, we selected 12 linear peptides with varying molar masses as target molecules. Our findings indicate that including the polymeric backbone in the model enhances van der Waals interactions and reduces electrostatic repulsion with zwitterionic peptides. The binding energy calculations demonstrated a high predictive accuracy for experimental determined Langmuir constants, with a coefficient of determination of 0.96. The all-atom resin model notably decreased the root mean square error in Langmuir constant predictions compared to previous models lacking a backbone. Furthermore, the workflow offered insights into the conformational changes of both the resin and target molecules during adsorption. Future efforts will aim to adapt the workflow for proteins of different sizes, thus expanding its utility in chromatographic research.

24. Integrating QSAR Modeling with High Throughput Screening for Rapid Development of Polishing Chromatography Steps for Protein Therapeutics

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In recent years, there has been significant growth in the field of protein therapeutics, encompassing various complex modalities such as multi-specifics, fusion proteins, and protein conjugates. However, the expansion into these diverse modalities poses several challenges for purification process development, which stems from the unique biophysical properties of these novel modalities relative to monoclonal antibodies (mAbs) as well as distinct impurities profiles. Given these challenges, it is imperative to rapidly develop non-platform polishing chromatography processes that can effectively isolate the desired product with adequate yield and purity. Experimental high throughput screening (HTS) capabilities such as slurry plate screens and robocolumn chromatography have become important tools to rapidly assess operating conditions for polishing chromatography. Predictive modeling enhances HTS capabilities for identifying operating conditions by enabling exploration and prioritization of large design spaces in silico and therefore picking promising conditions to screen experimentally. This talk presents the development and implementation of a quantitative structure activity relationship (QSAR) model that leverages over 10 years of internal Kp screening data and can predict the partition coefficient as a function of protein, resin, and mobile phase conditions. The model contains ~10,000 screened conditions on more than 40 resins. A diverse set of >40 proteins are represented in the data, including mAbs, Fc-fusion proteins, host cell proteins, viruses, and other modalities. Overall, the model has a test set R2=0.91 and can predict elution and strong binding conditions across the protein property and resin space with 95% classification accuracy, enabling extensive design space reduction for HTS experiments. Intentional diversification of the training data has expanded the applicability window of the model and enabled the prediction of both product and impurity partitioning, including aggregates, low molecular weight fragments, and HCPs. The prediction of both product and impurity partitioning facilitates computational assessment of other metrics describing the purification process, including separability and orthogonality between multiple steps. Overall, this work outlines how predictive modeling can be used in conjunction with HTS experimentation to guide the development of polishing chromatography steps. These models can be applied to a diverse set of proteins and across the development lifecycle, either by proposing novel approaches for a given separation or supporting process changes later in development.

25. A Systematic Approach for Estimating Colloidal Particle Adsorption Model Parameters

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The estimation of cation-exchange model parameters can be crucial to enable efficient model assisted biopharmaceutical downstream development. While steric mass action (SMA) isotherm estimation methods exist, there is a pressing need for a systematic approach to estimate model parameters for the emerging Colloidal Particle Adsorption (CPA) isotherm. Current methods are hindered by model limitations combined with parameter correlations, leading to time-consuming repeated estimations. This study presents a novel strategy that addresses this challenge, offering significant improvements. Through a comprehensive parameter sensitivity analysis, we identified key levers for determining appropriate CPA parameters, enabling the coverage of elution behavior across a wide range of process-relevant conditions. This analysis also revealed the correlation structure of parameters, allowing us to establish a required minimum experimental data set for parameter estimation. Our workflow leverages a surrogate-assisted-global-optimization tool, minimizing computationally expensive function evaluations during parameter fitting. Furthermore, we employed a customized error function, specifically adapted to the model structure and sensitivity results, to enhance the solver's performance. The novel strategy was tested on three proteins of varying size using a standard cation exchange resin, demonstrating its effectiveness and robustness. This pioneering approach for estimating CPA model parameters has the potential to accelerate model calibration for downstream development in the biopharmaceutical industry.

26. Lord of the rings – Optimization of rAAVv plasmid purification process with a combination of HTS and mechanistic modeling

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Smart protein purification process development is nowadays a combination of different technologies such as high throughput chromatography systems, specific and fast analytical methods as well as advanced data analytics. In this industrial case study, we present a highly sophisticated workflow for the development of a platform GMP process for rAAVv plasmids. Plasmids are the key starting material for viral vector manufacturing. However, due to the rapid growth of the gene therapy field and thus increased demand for pure, high quality pDNA the supply of plasmids has become a significant bottleneck. Therefore the development of a scalable and robust purification process is critical. Process characterization was done using a seamless combination of HTS and mechanistic modeling. Initial process parameter spaces were identified with Kp batch binding screens on a liquid handling station including offline analytics. The obtained results were used for designing the calibration runs for mechanistic modeling. The dataset included linear gradient runs at different conditions such as gradient slope and load density. Mechanistic modeling was done in the commercially available GoSilico chromatography simulationTM software package. The optimized process consisted of a robust step elution depleting RNA as well as host cell protein. The suggested process was transferred into GMP and marks one of the key elements for Roche's fast growing gene therapy while facilitating to decrease the costs of gene therapy products.

27. Efficient Simulation of Extra Column Volume in Small-Scale Multi-Column Chromatography

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Recently, the focus of chromatography modeling has expanded to include the extra column volume (ECV) alongside the actual chromatography column volume. This is particularly important for small-scale systems, where the ECV often represents a significant portion of the total volume and can have a negative impact on overall system performance due to additional dispersion effects. Traditionally, ECV is accounted for using 1D models such as dispersed plug flow reactor (DPFR) or continuously stirred tank reactor (CSTR) models, or a combination of both [1-2]. However, recent studies have shown that sometimes it is advisable to apply more complex models. For instance, under certain conditions, laminar flow in capillaries cannot be described by a DPFR model and a 2D model, that considers the radial flow rate profile should be applied instead [3]. Additionally, 3D computational fluid dynamics (CFD) models have been applied to accurately model dispersion effects due to bends and diameter changes, as they occur in the ECV of most chromatography systems [4]. While CFD models undoubtedly provide the most accurate results and are particular useful for small-scale single-column chromatography, their application in multi-column chromatography systems encounters computational hurdles, especially for continuous operations or optimization tasks requiring multiple simulations of the ECV under varying conditions. Striking for a balance between accuracy and computational efficiency, we investigated whether the 2D laminar flow modeling could be extended to account for dispersion effects caused by the ECV's 3D geometry. For this purpose, we adapted the radial flow rate profile to the observed residence time distribution of the respective ECV. We tested various geometries, such as bends, abrupt diameter changes, merges, splitters, and their combinations, which have previously been shown to affect elution profiles [4-6]. As a gold standard, we first conducted one CFD simulation for each geometry to calculate the radial flow rate profiles of the corresponding 2D model. Subsequently, the established 2D models were then tested with various flow rates and inlet profiles and the results compared to CFD simulations, DPFR models, and laminar 2D models. The results demonstrate the versatility of our approach across a broad spectrum of conditions and geometries, showcasing its applicability. Especially when the geometries contain bends, the prediction of concentration profiles is significantly improved. For example, within a section of the ECV in a small-scale simulated moving bed chromatography system with complex geometry, containing two diameter changes, a bend, and a merge, we were able to reduce the deviation in the outlet concentration profile in regard to the CFD simulation from 5.6% with the laminar 2D model to 1.3% with the adjusted 2D model. However, the computational times of the 2D models were significantly lower, taking only minutes compared to several hours for CFD simulations. Thus, we see this approach as a valuable addition to the existing repertoire of ECV models, offering an effective balance between accuracy and computational efficiency.

28. Short-cut methods for obtaining parameter values of mechanistic chromatography model simulations

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Mechanistic model based simulations for chromatography processes of biological products such as proteins and bio-nanoparticles are very important and promising for process understanding, accelerated process design, and process trouble shooting. Although these advantages/merits as well as mechanistic chromatography models have been known for many years, the model simulations were not frequently used as the computers were not strong enough. Currently, because of substantial improvements in performance of personal computers, and commercial or open-source soft wares, it is possible to carry out model simulations. However, it is still not easy to obtain the parameter values needed for the model simulations properly or quickly. For example, experiments for the data acquisition are usually carried out based on prior knowledge. Basically, we need two types of parameter values, the isotherm parameters and the mass transfer-related parameters. As for the isotherm parameter, our short-cut method for determining the two isotherm parameters from linear gradient elution (LGE) experimental data is widely used for ion-exchange chromatography (IEC) and multimodal chromatography. Extended methods were also developed. On the other hand, short-cut methods for determining mass transfer parameters have not been fully examined. One method is to convert the peak width in LGE to the width entirely due to mass transfer by considering the zone sharpening effect. This method was already verified, and applied to several model experiments. We investigated how we can obtain both the isotherm parameters and the mass transfer parameters from LGE curves by using short-cut methods simultanesouly. In addition to the stoichiometric displacement model (SDM) or Steric Mass Action (SMA) model, where the distribution coefficient K is given as $K=AI^{-B}$, the formula $K = a\exp(bI)$ was used as the model equation (A, B, a and b are constants, and I is the salt concentration). The zone sharpening factor for $K = a\exp(bI)$ was also correlated with several parameters. The methods were applied to LGE of monoclonal antibodies (mAbs) by IEC, and the results were compared with the values obtained by the conventional methods to confirm the validity of the short-cut methods. We also examined how to reduce the number of LGE experiments in order to obtain the parameter values needed for the model simulations quickly with a small amount of samples.

29. The ChromaWeb® platform, a modular, linearly-scalable, high-resolution membrane chromatography cassette for purification of viral vectors.

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Gene therapy treatments are expected to grow rapidly with at least 4 treatments already approved and many more in the pipeline. Currently available affinity and ion exchange chromatography media are not optimal for purification of viral vectors due to the small pore size (20~40 nm range) that preclude access to the larger viral vectors (25~350 nm). Therefore, there is a need for large-scale viral particle purification methods that leverage adsorptive media having pores in the micron scale. This is the domain of adsorptive membranes and certain monoliths. Membrane chromatography devices have an inherently shallow bed of 2~10 mm due to the high hydraulic resistance (HR) of membrane media compared to conventional beads. As a result, commercially available membrane chromatography devices have poor chromatographic resolution leading to large elution pools of 6~12 MVs. Furthermore, none are linearly scalable. Therefore, there is a need for membrane chromatography devices that overcome these two limitations. The ChromaWeb modular chromatography platform is a stackable, planar, linearly-scalable cassette having a novel flow distribution network that delivers a very narrow residence time distribution in spite of having a shallow bed and an array of multiple cassettes in parallel. As a result, ChromaWeb cassettes have HETPs on the order of 40 µm, 10 times smaller than the best commercially available devices, peak asymmetry very close to 1 (vs. > 3) and elution pools of about 1 MV. The modular and linearly-scalable structure of the ChromaWeb cassette enables results obtained on a 1⁄2-mL lab-scale cassettes to be directly translatable to process development-scale (10~35 mL), to clinical-scale (100~500 mL) and to process-scale cassettes (1~10 L). In this presentation we describe the structure of the ChromaWeb cassette, including its novel distribution network, and how it is capable of delivering linear-scalability and high chromatographic resolution. Experimental results will be presented with a model protein in a bind-and-elute process at a 10-mL scale demonstrating high productivity, high eluate concentration, and low buffer consumption. The ChromaWeb platform is under development at SPF Technologies, and in search of commercial partners to bring it to market.

30. Scale-Up and Applications of Nanofiber Membrane Chromatography

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Chromatography supports used in biomanufacturing processes traditionally comprise porous, usually spherical, particles of various types. However, the porous nature of these supports means contact with the binding surface is dominated by diffusive flow which creates limitations in terms of residence time, dynamic binding capacity and productivity. In more recent times, convective flow materials such as membranes and monoliths have been developed. However, whilst such materials address some of the shortcomings of porous particulate supports, they are often compromised by available surface area, resistance to flow, robustness and scalability. In view of these challenges, we have pursued the development of high-performance, robust, scalable and sustainable nanofiber membranes. Polymers such as cellulose acetate (CA) can readily be electrospun to provide fibers with sub-µm diameters that can be formed into non-woven fiber mats and subsequently regenerated to cellulose by alkaline hydrolysis. However, such materials lack sufficient strength for use in bioprocessing and are difficult to process and package into housings. By inclusion of a small amount of a re-enforcement polymer such as polyacrylonitrile (PAN), either as a second fiber or in the form of a single composite fiber, the mechanical properties of the membrane can be significantly improved, allowing the fiber-mat density to be optimised to suit the passage of larger biotherapeutic modalities. By judicious choice of polymers and electrospinning solvents, composite nanofiber membranes can be sustainably produced at scale by established electrospinning methods. These materials can be rolled or folded without damaging the membrane which enables continuous membrane derivatisation by roll-to-roll processing and folding into intricate shapes to provide compact membrane cartridges containing relatively large membrane volumes and minimal void volumes. The high surface area of nanofibers in combination with their ability to support high flow rates (1 mv/sec), very short residence times (1 second), relatively wide inter-fiber flow channels (approx. 1 µm) and compatibility with NaOH sanitisation makes these materials ideal substrates for the processing of therapeutic proteins and larger therapeutic modalities such as viral vectors, pDNA and exosomes. Currently there is much interest in the purification of relatively large viruses such as lentivirus (80 –100 nm) but yields with traditional methods are currently relatively low (20-30% functional recovery). Clarified and concentrated Lentivirus containing feedstock (expressed in suspension HEK293 cells) was applied at a flow rate of 5 mv/min (12 second residence time) to a LentiHero® 1 AEX composite nanofiber membrane device. A dynamic binding capacity of 1.6 E+11 vp/ml mv was obtained @ 15% breakthrough with a vp recovery of 70%, functional recovery of 53%, HCP reduction of 98% and 82% removal of DNA impurities, with a total processing time of approximately 80 min; a significant improvement compared to established LV processing methods. Other examples of the use and advantages of composite nanofiber membranes for the highly efficient capture and purification of biotherapeutic products will be presented.

31. Novel all-membrane process for the purification of monoclonalcantibodies without protein A

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There is a strong interest in innovative approaches for the purification of recombinant proteins that can increase productivity and at the same time reduce buffer use, production costs, and process mass intensity of downstream process. This effort is driving the development of novel membrane-based operations that are single-use and can be operated in flowthrough mode for the capture of impurities, as well as in bind-and-elute mode to capture or purify the product. Membranes with high binding capacity can be operated at much shorter residence times than columns and provide more than 10X increases in productivity. At the same time, it is advantageous to minimize diafiltration steps for buffer exchange, and the use of affinity ligands that require harsh elution conditions that can cause aggregation or denaturation of the product and increase the need for additional polishing steps. This presentation will summarize advances in commercial membranes based on nonwoven fabrics for the effective removal of host cells and cell debris, HCPs, DNA, and other impurities. It will also describe novel nonwoven membranes with ion exchange and multimodal ligands with dynamic binding capacities in the same range as those of resins, but capable of operation at residence times of less than 1 minute. To illustrate the point, we compare two processes for the purification of a mAb from clarified cell culture fluid. One process uses the traditional resin-based chromatography platform process for mAb purification involving a protein A capture step, virus inactivation, a bind-and-elute cation exchange polishing step, and a flowthrough anion exchange polishing step. The second process only uses nonwoven membranes and features a capture step with a novel multimodal ligand with mild elution conditions, virus inactivation, a flowthrough anion exchange purification step, and a final bind-and-elute cation exchange polishing and concentration step. The multimodal ligand product capture membrane exhibited a DBC10% of 59.2 mg/mL for the mAb in the supernatant with no pH or conductivity adjustment, at a residence time of 0.5 min. Under the optimized RT, arginine concentration for membrane washing and elution pH, a satisfactory recovery of 94.3%, a high HCP removal of 1.0 LRV and DNA removal of 1.8 LRV as well as a great reduction of aggregate% from 5.4% to 0.9% were achieved. This capture step was followed by a flowthrough anion exchange nonwoven membrane (AEX-TEA) step and a multimodal bind-and-elute cation exchange nonwoven (MMC-MPCA) step to concentrate and further purify the product. The three-step membrane chromatography process in total reduced HCP from 327000 ppm in the supernatant to 93.5 ppm (3.5 LRV), DNA from 994.7 ppm to 6.8 ppb (5.3 LRV), %aggregates from 5.4% to 0.4% with no detected fragments, thereby achieving the common purity requirements (HCP<100 ppm, DNA<10 ppb, % aggregate<1%) for mAb products. A side-by-side comparison with a standard mAb purification by using protein A resin and two ion exchange resins, showed that the three-step membrane processes outperformed the standard process with much less process time (3.8 h vs. 13.1 h), 14% higher overall recovery (88.3% vs. 77.5%), fewer steps (no diafiltration for buffer exchange) and fewer aggregates in the final product despite the similar HCP and DNA removal.

32. Optimization and Characterization of a High-concentration UF/DF Process to Overcome the Gibbs-Donnan Effect on Multiple Excipients

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Drug development for biologics is increasingly moving toward high concentration formulations (>100mg/mL) to support subcutaneous delivery of therapeutics. Compared to typical low protein concentration formulations, higher concentrations often present development and manufacturing challenges due to higher viscosity, increased risk of product aggregation, and increased prevalence of Gibbs-Donnan effect, which results in an undesirable shift in excipient concentrations and/or pH during UF/DF. To address these challenges a combination of formulations tailored to reduce viscosity / aggregation and a well-controlled UF/DF step are needed. Here we present a collaboration between Visterra (a biologics research and early-stage clinical development company) and KBI Biopharma (a contract development and manufacturing organization) to develop a formulation buffer and UFDF step for one of Visterra's IgG2 drugs to achieve a 200 mg/mL drug substance concentration for a product that initially presented challenges with a standard histidine, NaCl, sorbitol, PS80 containing formulation. Visterra contracted KBI to provide process development, formulation, and manufacturing services for an IgG2 biologic, which was initially formulated at 25 g/L. After the first manufacturing run, Visterra and KBI's formulation and process development groups began work to increase the concentration. Initial efforts to bring the product up to 200 mg/mL in its original formulation buffer demonstrated feasibility, however the viscosity became unacceptably high at concentrations >170 mg/ml. KBI and Visterra worked together to design a plan for improving the formulation to achieve the desired 200 mg/mL target; a four-step approach was taken to screen a variety of excipients for their ability to stabilize the drug at high concentrations. First, mixed excipient systems that included various amino acids including arginine, glycine, proline, and glutamic acid were tested. Second, the ability of surfactant to protect against agitation and freeze-thaw stress was evaluated. Third, the top performing candidates were stressed with heat and the data input into a statistical model to determine the statistically optimal formulation. Fourth, the top four optimal formulations were stressed using slow freeze-thaw to determine the best candidate. The chosen formulation included multiple amino acid excipients: histidine, arginine and glutamic acid to achieve the desired reduction in viscosity. Once the best candidate was chosen a UF/DF process was developed by the process development group. Initial development focused on optimizing key step parameters including feed flow rate, operating TMP and diafiltration concentration. However, a pH shift was observed in the final retentate, and amino acid analysis revealed an amino acid excipient content shift. To understand and maintain control over individual amino acid excipient concentrations in the final product more extensive optimization and characterization of the step was performed, and ultimately an altered diafiltration buffer was used to match the target amino acid concentration in the BDS. This presentation highlights how Visterra and KBI communicated and collaborated effectively throughout to design a formulation and UFDF process to ultimately meet the product (and excipient) concentration targets.

33. Addressing Challenges in High Concentration Drug Substance Manufacturing

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Compared to intravenous administration, subcutaneous administration of biologics therapeutics offers advantages in patient experience and medicine accessibility. For protein therapeutics that require high dosages, the drug substance needs to be formulated at a high concentration to allow a low drug product volume and enable subcutaneous administration. The manufacturing of high concentration drug substance faces challenges in the last steps of the manufacturing process, including ultrafiltration/ diafiltration (UF/DF) and final fill. For the UF/DF step, the excipient concentration in the diafiltration buffer needs to be empirically determined to address the excipient losses due to the Donnan effect and volume exclusion. In addition, the high concentration targeted for the product pool, combined with the excipients required to stabilize the product protein under high concentration, result in high viscosity of the product stream, which leads to longer process time and higher system pressure. For final fill, the highly viscous formulated drug substance poses challenges to the homogeneity of the filtered drug substance. Both the UF/DF and final fill steps share the same difficulty in recovering the highly viscous protein solution to achieve a high yield. In this presentation, case studies on multiple high concentration monoclonal antibody drug substance manufacturing processes are shared. The measures explored to overcome the above challenges will be discussed. For UF/DF, the operational parameters within the current manufacturing process design, for example, membrane loading, crossflow flux, optimal final concentration target, and recovery flush volume, were evaluated on their impact on the system pressure, product quality, and final concentration. More disruptive solutions have also been evaluated as future improvement opportunities, including different types of membrane cassettes and single-pass TFF. Moreover, product loss in the UFDF system was examined at manufacturing scale and improvements were made to minimize the loss. For final fill, opportunities to reduce product loss were evaluated. Buffer and product flush volumes were optimized to ensure homogeneity of the filtered drug substance pool while minimizing product loss. The filter train was also re-designed to reduce hold-up volume. These process changes resulted in significant yield improvement in the final fill step. The findings and best practices presented here will provide insight to designing high concentration drug substance manufacturing processes.

34. Capabilities and limitations of IR for enhanced process monitoring during UF/DF of proteins

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Ultrafiltration/diafiltration (UF/DF) is a common unit operation in biologics manufacturing for protein concentration and/or buffer exchange operations, such as for drug substance formulation. One of the challenges often associated with UF/DF at manufacturing scale is a lack of real-time process monitoring. This is especially so for high protein concentration UF/DF. The process is typically monitored via the weight of the retentate (feed) and permeate vessels to estimate protein concentration and buffer exchange progress. Monitoring of other attributes (e.g. pH, conductivity, protein and excipient concentrations) requires removal of grab samples for off-line measurements. Some of the off-line assays can be time and labor intensive, and may fall short at high protein concentrations. In addition, due to mixing issues, there is a higher chance of sampling inconsistencies at high protein concentrations which can further complicate process control. As a result, it is difficult to have an early detection of non-standard behaviors and response to troubleshooting can be delayed. Process analytical technologies (PAT) can help mitigate these challenges by allowing more comprehensive real-time measurement of multiple attributes of the product and process. Infrared (IR) spectroscopy is one type of PAT that allows for monitoring of multiple protein, buffer and/or media component concentrations with a single instrument, even in the presence of high protein or component concentrations. In this work, we explored the capabilities and limitations of using IR to monitor protein and excipient concentrations during UF/DF process of a monoclonal antibody. Our approach involved acquiring reference spectra of the various buffers (single- and multi-component) and protein solutions, then monitoring UF/DF processes of the antibody in these solutions in real-time. We will share the spectra from these experiments and our approaches to IR data analysis for real-time UF/DF process trending. This work will highlight the benefits of enhanced process monitoring using IR during UF/DF step.

35. Microfluidic Filtration Device for High Throughput Vaccine Process Development

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Sustainable vaccine manufacturing requires cost-effective, scalable, robust, and efficient processes. While process development efforts have largely focused on improving bioreactor yields, downstream yield is equally important since it can account for a significant contribution to the overall manufacturing costs. Key challenges in downstream processes are the optimisation of filtration and chromatography purification steps. For chromatography development, there are well-established microscale experimentation tools such as resin slurry plates and microscale columns to enable efficient screening studies. However, for tangential flow filtration (TFF), there is a lack of well-understood and cost-effective small-scale screening devices that can identify optimal process parameters for achieving efficient filtration yields for industrial production. Microfluidic tangential flow filtration (µTFF) devices can be engineered to optimise filtration unit operations and reduce the cost of process development for large molecules downstream processing due to some of their key characteristics, such as low feed volume, laminar flow across the system and the opportunity to integrate sensor technology. These characteristics will enable the study a variety of filtration products with different biochemical and physical properties, using µTFF devices as screening tools. However, there has not been enough efforts to bring these µTFF devices forward. We have developed and characterised a novel parallel µTFF device for high throughput filtration process development. This device can be used to screen optimal conditions for the purification of viral vectors and vaccines. The prototype has been tested in concentration mode (5-10x with BSA feed concentration up to 10 g.L-1) at fluxes ranging between 29.6 LMH to 101.1 LMH (70 to 240 µl.min-1) at constant 15 psi transmembrane pressure (TMP). Results have shown no aggregation of protein during filtration with minimum retentate yields of 40%. Furthermore, robust operation was achieved (e.g. no internal leaks observed) which suggest that higher yields are achievable at low flow rates with constant TMP. Integration with sensing technology allows the implementation of automated control strategies, removing operator-induced variability, therefore improving reproducibility and data quality. Our approach has the potential to deliver scale-relevant data, significantly reducing development times and process costs.

36. Post-affinity-capture depth filtration: it’s just there to remove solids, right?

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With the advent of LC-MS techniques to detect and quantify host-cell proteins (HCPs) and the growing recognition that certain HCPs may pose immunogenicity or product stability risks, purification development scientists have been tasked with achieving ever-lower targets for HCP content in mAb drug substances. Significant efforts have explored various chromatographic techniques to reduce HCPs including improved affinity column washes and better characterization of existing chromatographic modalities, in particular, yielding improved understanding of the power of HIC techniques. However, this presentation explores whether untapped opportunities exist elsewhere in the typical mAb process. Purification processes for mAbs and their variants (bispecifics, Fc fusion, etc.) overwhelming start with affinity capture chromatography (protein A, protein L, etc.). Typically, products are eluted via acidic solutions, further acidified for viral inactivation, and then neutralized to the pH 5-8 range, frequently resulting in a turbid solution. In the Lilly mAb process, post-affinity-capture depth filtration was introduced to clarify such turbid solutions, and impurity removal was typically not much better than that of membrane filtration. However, for one mAb, evaluation of a stronger titrant for acidification led to a significant increase in HCP after depth filtration; while this was clearly not a desired result, fortuitously, it suggested that the solution conditions could have a significant effect on HCP reduction. It was hypothesized that the stronger titrant may have unfavorably increased the ionic strength (IS) leading to the question of whether reducing IS could improve HCP reduction. Reducing the IS of the feed solution to the depth filter can be achieved via buffer exchange or significant dilution, but a more manufacturing-friendly solution was sought. To achieve this goal, a careful and holistic examination of the solutions used for capture column elution, acidification, and neutralization was required, balancing performance of each solution for its individual purpose against the overall goal of achieving low ionic strength and reasonable processing volumes at the point of depth filtration. The approach proved remarkably effective on a test molecule, begging the question of how broadly applicable the approach might be. Interestingly, IgG subclass effects were revealed, with the approach proving quite effective, achieving single-digit ppm levels in some cases, for IgG1s (including bispecifics), but not for IgG4s. While the molecular basis for the difference is not fully understood, some insights have been gained through molecular modeling and heavy chain mutations seeking to make IgG4s more IgG1-like. Additionally, some insights into the role of filter properties are revealed based on their differences in performance. The beauty of the approach is that it simply involves replacement of eluents and titrants, minimally disrupting process operations. There is flexibility to leverage the approach to improve HCP reduction, but also to reduce plastic waste from filters. Ultimately, the power of the approach to reduce HCP was so compelling that it was implemented on an ultra-rapid timeline as a key enabler for greater production of Lilly’s Covid mAbs by facilitating removal of one of the polishing columns.

37. Application of upstream and downstream filtration technologies as solutions for batch and continuous bioprocessing

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Filtration technologies have long been used in biomanufacturing as a tool for both upstream and downstream unit operations. With the increased popularity of intensified processes, the need for membranes that can accommodate larger throughputs, while maintaining good recovery and reduced fouling has become more prominent. Filtration technologies are a great fit for both batch and continuous operations with applications spanning cell retention steps, sterile and virus filtration, as well as ultra filtration/diafiltration. This work highlights two filtration solutions: one for upstream perfusion cell culture and another for downstream virus filtration. Successful perfusion cell culture harvest requires several considerations, including but not limited to: continuous filtration for extended durations, consistent product sieving, minimizing or delaying membrane clogging and scalability. Data presented will show how using Bio-OptimalTM MF-SL membranes under tangential flow filtration (TFF) mode in perfusion cell culture harvest can result in low product sieving decay and reduced plugging, confirming the superior performance of the membranes. Virus filtration is a critical downstream purification step to assure the safety of biotherapeutics. Successful nanofiltration removes smaller, non-enveloped viruses based on a size-exclusion mechanism and achieves a 4 log or greater reduction in virus while still allowing excellent filterability and product recovery. A variety of parvovirus removal filters with unique membrane structures and chemistries are commercially available and have various performance characteristics. Some filters show rapid fouling, reduced flux and virus breakthrough under variable feed stream conditions, while other filters achieve high flux but suffer from inconsistent fouling behavior, which shows that their superior performance is dependent on feed characteristics. Other membranes have broad applicability but are limited to moderate flux and require cumbersome post-use integrity testing. The recently launched PlanovaTM S20N filter was specifically designed to improve operational efficiency and flexibility by providing higher throughput, robust virus removal capability and stable protein filterability. Here, various case studies using the Planova S20N filter alongside other membranes are presented. Test conditions included various protein types and concentrations with varying throughputs (up to 600 L/m2) and process pause durations (30 min to 3 h), as well as virus removal using minute virus of mouse (MVM) or porcine parvovirus (PPV) spiking. Data presented show virus removal under both constant pressure and constant flux mode. Under these conditions, Planova S20N consistently provided robust virus removal, excellent filterability and stable flux profiles at various operating pressures. From the data presented, Bio-Optimal MF-SL and Planova S20N can offer two great solutions for applications in intensified bioprocesses.

38. Analyzing Sterile Filtration of mRNA-LNP Therapeutics Using a New Modeling Framework

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Sterile filtration is one of the critical steps in the production of lipid nanoparticle (LNP)-based biotherapeutics. However, LNP fouling can severely limit the overall capacity of the sterile filter. The objective of this study was to examine the sterile filtration of mRNA-LNP during both constant flux and constant pressure filtration experiments. The complete pore blockage model effectively described the fouling behavior of the dual layer Sartopore 2 XLG membrane at constant pressure, with the rate of pore blockage decreasing with increasing pressure. However, a novel modification of the complete pore blockage model was needed to describe the pressure increase during constant flux operation, with the rate of pore blockage a function of both the instantaneous pressure and the pressure gradient. This new model not only successfully described the transmembrane pressure (TMP) profiles during constant flux operation but also predicted the observed maximum in capacity at intermediate filtrate flux. This newly established framework was then extended to describe a novel “bimodal” fouling behavior that was seen with some sterile filters and with some batches of LNP drug product by incorporating the growth of a compressible LNP deposit in the model. This mathematical description not only provides new insights into the underlying physics describing LNP filtration, it also provides a framework for facilitating the design and optimization of the sterile filtration process for LNP therapeutics.

39. High Pressure Increases Likelihood of Parvovirus Breakthrough for a Platform Virus Retentive Filtration by Viresolve® Pro

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Virus retentive filtration (VRF) is a critical unit operation that contributes to viral safety of purification processes for biotechnology products. It is considered a robust and effective virus removal step that is largely based on a size-exclusion mechanism, which makes it readily applicable for platform processes. There is a wealth of information with studies of commercially available virus filters that show robustness of viral clearance across a wide range of operating conditions. Despite the collective experience in our industry on VRF, there remains a lack of consensus on whether pause, low pressure, and/or high pressure constitute a worst-case condition. Furthermore, it is a regulatory expectation that Sponsors who are building a prior knowledge platform claim thoroughly understand the critical parameters that may affect risk of virus passage through the membrane. The Millipore Sigma Viresolve® Pro filter is an asymmetric, dual layer, polyethersulfone-based flat sheet membrane designed to retain small viruses less than or equal to 20 nm. In Regeneron processes, the virus filter is typically operated at constant pressure from 20 to 35 psi, which is within manufacturer recommended limits of 10 to 60 psi. Regeneron has accumulated more than fifteen years of internal prior knowledge concerning the performance of Viresolve Pro by evaluating constant pressure VRF at small-scale virus spiking studies for more than 200 independent experiments characterizing at least 30 validated monoclonal antibody processes. Within this internal dataset, VRF process parameters that have been reviewed retrospectively for impact on minute virus of mice (MVM) removal include: virus challenge (log10 PFU), filter volumetric loading (L/m2), low or high transmembrane pressure (TMP, psi), and pause (min), among others. Regression analysis shows no process parameters have significant impact to viral clearance log10 reduction factor (LRF) over the wide ranges studied. Although not statistically significant for LRF, high pressure, defined as transmembrane pressure greater than 40 psi, increases the likelihood of MVM breakthrough for Viresolve Pro. With extensive experience, Regeneron has confirmed robustness of the Viresolve Pro filter and identified only one condition that led to increased probability of MVM breakthrough: high transmembrane pressure. This work contributes to understanding of worst-case conditions by the Viresolve Pro filter and aids in the development of prior knowledge claims for viral clearance.

40. Informing Ultra Scale Down depth filtration device design using high resolution imaging

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X-ray computed tomography was applied to visualize and characterize the impact of knife edge seals on flow behavior within depth filtration capsules and their importance in providing structural rigidity whilst reducing process fluid bypass. Four different capsule sizes, ranging from process development tools to full scale industrial manufacture, were imaged in 3D. For each device in this study, knife edge seals were observed to compress the edges of the depth media. Small scale filtration capsules were imaged following barium sulfate flow through devices, providing further understanding to potential flow paths process fluids may take. 3D printed reconstructions of knife edge seal zones were fabricated for high resolution imaging of the compressed media in these zones. Using the same approach, imaging and digital flow simulation on Ultra Scale Down devices for depth filtration was performed, with an internal diameter of 6 mm. Potential fluid bypass was highlighted as a potential issue due, supported by model particle filtration experiements at diameters of 2 µm and 0.2 µm that were imaged using confocal microscopy following constant pressure filtration. An updated version was produced to incorporate a knife edge seal zone and drainage support disc, whilst retaining an effective filtration diameter of 6 mm. Both flow simulations and particle retention from imaging datsets suggested greatly improved usage of the intended filtration chamber when compared to the original device. A further design iteration was created to incorporate a dual-layer depth filtration system whilst retainign idealized flow properties.

41. Peptide-based Host Cell Protein (HCP) Removal Technologies for Biomanufacturing

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High-titer expression of engineered biotherapeutics is accompanied by a complex profile of host cell protein contaminants (HCPs), that can persist throughout manufacturing processes in various ways. Their presence at drug product stages can not only trigger unwanted immunogenicity upon dosage, but also contribute to enzymatic product truncation - thus inactive product variants or even destabilize formulations. The need for understanding and removing such HCP effects is simultaneously fueled by the increasing diversity in expression systems such as yeasts, mammalian cells (P. pastoris, CHO, HEK293 etc.) - and a growing repertoire of therapeutic modalities (multi-specific antibodies, gene therapies, mRNA etc.). To alleviate HCP-associated effects and aid product-agnostic biomanufacturing, we introduced flow-through affinity chromatography – enabled by GuardTM adsorbents functionalized with peptide ligands - designed to capture a broad spectrum of HCPs. In this presentation, we demonstrate the mode of operation and performance of LigaGuardTM (CHO, HEK293), PichiaGuard, and discuss our findings across cell culture harvests containing mAbs, bispecific Abs, viral vectors and other recombinant proteins. With a binding capacity of 20-30 mg HCP/mL resin, GuardTM resins afford product recoveries > 85% in mAb formulations, up to 3 log reduction of HCPs across ~200 CVs loading, and a residual HCP titer as low as 8 ppm when used in combination with affinity capture steps. Furthermore, we detail how proteomic approaches allow us to map and understand species-level clearance of high-risk HCPs such as cathepsins, histones or various proteases and conclude with our views on process and economic considerations for developing a technology like GuardTM for biomanufacturing applications.

42. Streptococcus pneumoniae surface protein and nanobody affinity resins for Capture and Purification of fully assembled secretory immunoglobulin A

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Secretory immunoglobulin A (sIgA) is a potential candidate for passive immunization, because it is suited for inhaled administration to generate the first line of defense in protection the mucosal epithelium from pathogens. In the mucosa, the SIgA is a predominant immunoglobulin consisting of two IgA monomers linked together by a joining chain, and a secretory component, resulting in a molecular mass of approximately 450 kDa for . The proteins has been overexpressed fully assembled in CHO cells with a titer of 0.5 to 1 g/L and subsequently purified. Following purification, the hydrodynamic radius was determined as 10.2 ± 0.9 nm by dynamic light scattering with an isoelectric point of 5.5 after desalting using electrophoretic light scattering. However, the industry standard of capturing antibodies from culture supernatant, staphylococcal protein A affinity chromatography, exerts weak interaction with IgA and therefore not well suited. Specific affinity chromatography materials such as Capture Select IgA, and Capture Select IgA-CH1 chromatography media with camelid-derived single-domain antibody fragments comprising the 3 CDRs domain antibody fragment with a 13 kDa or 14 kDa as ligand have been developed but the technology is still in its infancy regarding the selection of the best-operating conditions with both ligands. The hydrodynamic radius of SIgA is twice that of IgG and therefore a high pore diffusion-limited process can be expected. Due to the small pores of about 30 nm of the agarose-based Capture Select resins, effective diffusivity of sIgA is limited. This results in very low dynamic binding capacities and resin utilization. To alleviate this, a novel resin was developed by immobilizing a Streptococcus pneumoniae surface protein as affinity ligand on a macroporous resin with 100 nm pore size. Influence of ligand density on equilibrium and dynamic binding capacity will be shown. The larger pores of the polymethacrylate resin led to greatly improved resin utilization and allowed us to develop a capture process directly from clarified culture supernatant and a highly purified full length correctly assembled SIgA was obtained. The new affinity resin could form the basis of a platform process for sIgA production, greatly improving the developability and manufacturability of these monoclonal antibodies.

43. Real-time process analytical technology (PAT) aggregate monitoring for cation exchange pooling

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BioPhorum members, through an industry-wide collaboration, have devised a proof-of-concept for controlling product collection during cation-exchange (CIEX) bind-and-elute chromatography. Our approach to this task, using real-time multi-angle light scattering (RT-MALS) technology, was presented at Recovery XIX. It involves directly monitoring aggregate content - a critical quality attribute (CQA) - and applying a defined breakthrough or cumulative threshold to stop pooling. The intent of this partnership is to show that direct monitoring of a suitable CQA enables recovery of good material, currently lost due to conservative pooling practices. In addition to yield improvements for this polishing unit operation, the resulting pools should be more consistent batch-to-batch with respect to aggregate content. In this presentation, we share results from Phase I of the project. MALS technology measures the intensity of scattered light produced by a sample at different angles. The scattered intensity relates to the molar mass and size. Averaged over a sample, the reported value is the weight-average solution molar mass (Mw) which will increase with increasing aggregate content. In our work, the in-line MALS detector reports the change in Mw in real-time allowing us to “see” when the aggregate population starts to break through. This is already an improvement over traditional UV which does not differentiate between protein populations. We could have set a reasonable threshold limit to the Mw signal and used it to trigger stop pooling and moved on with our work. But the team took a more ambitious approach to use the Mw measurement in a more accurate way to calculate the cumulative aggregate content in the pool and use a cumulative aggregate threshold to trigger stop pooling. The more ambitious approach ensures tighter consistency of aggregate content in pools batch-to-batch, irrespective of peak shape. This approach also reduces the need for conservative pooling since stop pooling is triggered by the actual CQA (pool % HMW). The accuracy the team is aiming for requires model training over an expected range of pH, conductivity, and protein concentrations. A Design-of-Experiments (DoE) scheme was developed and executed along with associated off-line analytics to provide enough data coverage across a defined operating space to start building models. Initial attempts at modeling were met with challenges. Additional experiments were needed to fill in sections of the data where values were rapidly changing, and finer fractionation was needed. Multivariate and Machine Learning (ML) modeling strategies were evaluated, each providing only partial success when it comes to robustness and predictability. A hybrid strategy that combines Mechanistic and Machine Learning modeling has shown promise as a path forward. Beyond the technical implications of this work, this cross-industry collaboration has developed approaches and learnings applicable to other PAT or technology proof-of-concepts. Here we present our Phase I results as well as challenges faced, lessons learned, and benefits realized through execution of an industry-wide project.

44. Advanced Multi-Mode Separation Platform using Block Copolymer Approach

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High precision chromatographic separations form the backbone of the biopharmaceutical purification processes. As the need for simplified and intensified manufacturing systems becomes critical to rapidly moving candidate pipelines through the development and commercialization journey, so does the need for evolved chromatographic separation systems based on new supports and new separation principles. Additionally, the emergence of biopharmaceutical treatments based on modalities beyond classic mAbs requires development of novel chromatographic technologies that are inherently designed around physical and chemical properties of these systems. Recently, we have introduced commercial technologies that enable chromatographic separations of large particles at clarification stage of the process using fiber functional media. However, a number of challenges remain, such as effective flow-through polishing platforms that can work across modalities and candidate pipelines. Here we present the development of novel chromatographic separation platform that combines functional ligands grafted on convective synthetic substrates, such as membranes and fibers, with diffusive control of a PEG near-surface diffusion layer. We graft block copolymers to membrane and nonwoven substrates in order to enable selective separations due to the two polymer blocks with precision that is capable of screening out mAb and larger particles by size, while allowing host cell protein to pass through the PEG layer and to access the ligand chemistry near the convective support surface. This way the separation system acts as a convective system at long distances but has a diffusive control near the surface. We demonstrate this on several chemistries, including AEX and CEX, with respect to separations of mAbs and viral particles for host cell proteins and other impurities clearance. This technology enables creation of intensified flow-through purification and polishing platforms as any chemistry can used for process related contaminant reduction without regard to binding of the product molecule instead. Additionally, we can tune the diffusion layer and process conditions to control the molecule size range for exclusion. This makes this approach especially useful for separations of large modalities of mAbs and beyond.

45. Novel Approach to Affinity Capture Elution Design

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Affinity Capture is the preferred method for primary capture in biotherapeutic downstream processing, including for antibodies, complex fusion proteins, therapeutic enzymes, viral vectors, etc. A common affinity elution procedure involves lowering the pH, altering the ionic state, or adding organic solvents to disrupt the binding interaction between ligand and product of interest. Both low pH and organic solvent may be incompatible with product stability and negatively impact product quality. Additionally, elution buffer with high ionic strength is inconvenient for subsequent polishing chromatography which may necessitate significant dilution to reach appropriate conductivity. Novel and complex biologics are often more sensitive to process conditions and may not fit neatly into a platform process. Optimal affinity elution buffer for such products may need to be product specific, but limited guidelines exist for elution buffer design of this type. Process optimization for such products can leverage high throughput screening (HTS), however small chromatography columns for HTS may not have representative performance and the screening outcomes still need to be verified at bench scale. The overall screening process can consume significant time and resources. We propose a novel approach to the design of affinity elution buffers for these types of challenging products by leveraging our discovery that affinity resins display a significant ion exchange effect at the start of elution. When the low pH elution buffer contacts the resin and immobilized product, both become positively charged and begin to function as an anion exchanger. Consequently, negatively charged ions associate with the resin and are depleted from the mobile phase which drives major shifts in local conductivity or pH during elution. These can directly affect performance through yield loss, product quality degradation, peak splitting, or column fouling. The magnitude and impact of this effect will vary depending on the ion composition of the chosen elution buffer. With our proposed strategy, the elution buffer formulation is optimized specifically for each challenging product to maintain product quality, achieve high yield, and assure maximum compatibility with the subsequent purification step. To the best of our knowledge, this is the first study to elucidate this mechanism enabling us to accelerate elution buffer design and consequently process lock on the path to IND or pivotal manufacturing. This novel strategy is a highly advanced guideline for affinity elution buffer design supporting rapid and cost-effective purification process development.

46. Solving the Mystery of Multi-Peak Elution Behavior in CEX Chromatography: A Case Study on mAb Process Development

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Drug Substance purification process often undergoes significant process improvements over the course of lifecycle of a monoclonal antibody (mAb) from first in human (FIH) phase to commercial manufacturing. These process changes are typically driven by requirements from manufacturing facility fit, raw material constraints, product quality enhancement etc. This poster highlights an interesting case study regarding a CEX polishing step for a mAb. The old process used Fractogel® SE Hicap resin in isocratic elution mode and encountered high pressure issues in pilot plant facilities. Use of the CEX resin Fractogel® SO3- resolved a pressure issue observed when an alternative CEX resin, Fractogel® SE Hicap, was operated in isocratic elution mode, but, surprisingly, a multi peak elution behavior was observed. This elution behavior was hypothesized to be due to on-column aggregation based on previous literature (Guo et. al., J Chrom A, 1356 (2014) 117-128). Contrary to this theory, no impact was observed on pool product quality characteristics. Further evaluations hinted at the possibility of viscous fingering phenomenon associated with high protein concentration zones in the column during the elution phase. Relevant process conditions (e.g., flow rate, wash conditions, pH, elution salt molarity, resin lot) were evaluated to understand and mitigate this unusual multi-peak elution behavior. Amongst these process modifications, only the elution pH and the elution salt molarity could improve the chromatographic elution profiles. However, both cases still exhibited a two peak elution behavior with significant tailing. Finally, viscosity of the mobile phase was modulated using fluid phase modifiers in the wash and elution buffer, which resulted in a manufacturing-friendly single elution peak for Fractogel SO3-. The step-by-step process modification approach employed in this study allowed us to gain further insights into mAb chromatography purification and provided a deeper understanding of elution behavior on the column, which could potentially enable similar modifications necessary for other molecules.

47. Structure-based multimodal ligand design: an iterative approach towards novel selectivity

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Biopharma is facing increased purification challenges due to increased molecular diversity such as multispecific antibodies. Stronger requirements to remove product-related impurities will make polishing more important in the future. Mixed-mode chromatography using multimodal ligands can provide unique selectivities and may thus play a key purification role improving the yield vs purity balance. The goal of the present study is to develop an iterative framework for the design and selection of new multimodal resins for future purification challenges. The approach taken is based on empirical modeling of a hybrid framework that combines structure-based modeling, high-throughput process development (HTPD) characterization, and chromatographic purification performance evaluation. A chemically diverse virtual library of more than one hundred novel multimodal cation exchange ligands is created, designed toward varied properties such as hydrophobicity, hydrogen-bonding, etc. The multidimensional molecular diversity is mapped based on 28 ligand descriptors, and this chemical diversity map is used to select a first subset of chemically diverse ligands to be investigated. A high-throughput plate study of low loading protein binding under a wide range of pH and salt conditions generates data that are condensed to a multivariate chromatographic diversity map. To facilitate an efficient, iterative ligand design framework, we have developed multivariate predictive models based on principal component analysis (PCA) and partial least squares (PLS) that allow direct estimation of the most important characteristics of chromatographic diversity from ligand structural descriptors. The chromatographic diversity map allows resin selection for real-world purification challenge testing and can generate leads for further iterations of the ligand design. The purification performance of the selected ligands is compared by investigating the ability to remove high molecular weight (HMW) aggregates while maintaining an acceptable monomer yield. The first-generation ligand library provided preliminary structure-activity understanding and highlighted the importance of tuning the ligand hydrophobicity and the role of other ligand-specific modes of interaction (e.g., π-π interactions). The second-generation library, based on information from the first library combined with new chemistry modifications, generated ligands with significantly improved selectivity between monomer and aggregates, increasing the aggregate removal by 30% at 90% monomer yield. Further purification challenges tested indicate a general purification performance that is significantly better than the starting point ligand. This study provides insights into the future of iterative design and development of new multimodal ligands. The hybrid modeling framework combining HTPD and multivariate predictive models is a machine-learning ready approach for new ligand development, paving the way to quick discovery of novel chromatography solutions.

48. Advancing Efficiency and Mitigating Risk: Raman Spectroscopy-based Analysis of Monoclonal Antibody and Excipient Concentrations throughout Downstream

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Current biopharmaceutical production heavily relies on limited automation along with at-line and offline analytical measurements to control operational parameters and retrospectively inform on process performance. Online and inline process analytical technologies (PAT), such as Raman spectroscopy, can increase process knowledge and understanding, enable automated process control, and reduce process risk [1]. The final ultrafiltration/ diafiltration (UF/DF) unit operation can benefit from PAT implementation particularly at high protein concentrations when non-traditional buffer exchange may be observed (e.g., Gibbs-Donnan effect). While a variety of inline methods have been assessed to improve performance and operation of UF/DF (e.g., UV-vis, NIR, Raman), PAT is not widely adopted for this unit operation due to difficulties with process development, integration with automation systems, and manufacturing usability. While UV-vis measurements are standard in downstream processing, quantification is limited to proteins rich with aromatic residues; more complex streams (e.g., clarified harvest) prevent accurate quantification due to scattering effect or overlapping absorbance. The specificity of Raman spectroscopy offers solutions for identification, quantification, change monitoring, and additional information about product quality for a broader range of therapeutic proteins [1-2]. Here we show how a generic monoclonal antibody concentration Raman spectroscopy model along with buffer component and excipient models can be applied throughout the downstream process to reduce risk, decrease time, and improve process knowledge and understanding through a single, inline measurement. Specifically, real-time (every 18 seconds) prediction of monoclonal antibody (mAb) concentration using inline Raman spectroscopy was demonstrated at bench and pilot-scale for three mAbs in traditional and UV-vis interfering matrices during UF/DF. Error was < 5% compared to benchmarking analysis for concentrations up to 150 g/L in both matrices. Additionally, L-histidine, L-arginine, and sucrose (each < 5% error) were monitored and operation proceeded based on inline measurement to ensure transfer into the correct matrix. Furthermore, application of the models was extended to other points in the downstream process (e.g., quantification of mAb in clarified harvest). The results demonstrate how platform Raman models can accelerate process development and improve operations by providing real-time process information about multiple attributes for monitoring and potentially equipment control with a single inline measurement. Process risk is reduced through removing operator interaction with the process and by enabling data-driven process decision making. Furthermore, the Raman models would also enable continuous processing by providing real-time, inline concentration values throughout the downstream process which could be used for feedback control (from loading clarified harvest onto protein A chromatography columns to the final concentration of purified product).

49. New concept of pH-independent affinity separation of antibodies

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This study introduces a novel proof of concept for antibody purification through potential controlled chromatography on membrane supports, addressing cost and efficiency in large-scale production. A 2.5 V cell potential was able to disrupt Protein A - antibody interactions and achieved ~90% elution rates. Membrane chromatography itself offers faster cycle times, and eliminates mass transfer limitations. Additionally, it reduces reliance on buffer exchange, potentially enhancing sustainability. Its efficiency and sustainability render it a promising alternative for alternative antibody production. Furthermore, the lack of a low pH elution buffers offers promising new adaptations for difficult and pH sensitive antibody species

50. Impact of 3D printed stationary phases in integrated downstream processing of monoclonal antibodies – a process modelling approach

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Biopharma companies are shifting towards more agile and flexible bioprocessing solutions to embrace the diversity of biological products currently under development. While upstream has seen significant improvements in productivity, downstream processing still relies on time consuming chromatography operations with highly priced resins, requiring costly packing and validation procedures [1]. Additive manufacturing, with its unlimited design freedom, offers a feasible way to fabricate reproducible columns with bespoke flow channels to optimise virtually any downstream requirement. A key advantage of 3D printed columns is their ability to process solid-laden feedstocks without the need for separate clarification steps, enabling accelerated processing and increased efficiency[2–5]. However, industrial implementation of 3D printed chromatography stationary phases as real alternative chromatography adsorbents involves a spectrum of considerations such as cost-effectiveness, scalability, and the adaptability of such technology to existing as well as new downstream processes. This study provides a technoeconomic assessment of 3D printed columns formAb manufacturing in process conditions. We first used our platform ink to fabricate ordered stationary phases with 300 µm flow channels functionalised with affinity (protein A) and cation exchange (SO3–) ligands. The 3D printed columns enabled flow through of clarified mAb harvest expressed in the ApolloTM X expression system and eluted fractions were characterised in terms of capacity, yield, HCP clearance, protein A leakage and mAb aggregates formation up to 20 consecutive cycles. The experimental results were inputted in BioSolve process modelling tool to simulate a mAb purification process using 3D printed columns under industrially relevant conditions representative of clinical trials (200 L) and commercial scale (2000 L) productions. In particular, we i) investigated the economic viability of integrating clarification and capture in a single unit operation in terms of time, cost savings and scalability, and ii) projected the potential sale price for 3D printed Protein A capture columns along with expected future binding capacity improvements to assess its profitability and total investment costs compared to existing mAb biomanufacturing process. Our analysis indicates that integration of harvest and capture can alleviate the cost of mAb manufacturing by up to 15% through i) significant reduction in materials costs, ii) increase in productivity, iii) enhanced flexibility employing bespoke 3D printed columns as a fully disposable technology. Also, future 3D printed columns with capacity of 35 g/L can ensure bioprocessing costs on par with current technology even if marketed at a 30% higher price tag than traditional protein A resins (15000 $/L).

51. The impact of elution pH on product quality of Fc containing proteins.

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The antibody capture step is a critical and heavily burdened process within downstream purification. Protein A affinity chromatography is a widely employed technique to achieve this capture due to its high specificity to Fc containing proteins and antibodies. Elution from protein A traditionally requires low pH for efficiency, however, these low pH conditions can have a negative impact on overall product purity, in terms of low and high molecular weight impurities, host cell proteins, and residual DNA. Eluting at a milder pH has been shown to reduce aggregation and associated impurities carry over, increasing both product purity and product recovery. In this paper, application data will be presented on the capture of several Fc containing proteins using Praesto Jetted A50 HipH, a unique protein A resin with mild pH elution capability. Discussion will focus on the observed improvements in terms of both purity and recovery by implementing the use of this resin under milder elution conditions when compared to traditional resins and low pH conditions.

52. Size based sterilization of large therapeutic particle formulations

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Sterile filtration is the sterilization method of choice in the production of most biotherapeutics. Novel therapeutic modalities, e.g. exosomes, liposomes, viral vectors, etc. are often too large to be sterilized by passing the formulation through a sterilizing grade filter leading to considerable yield loss and filter blockage. Alternative sterilization methods based on the exposure to heat, radiation, solvent and/or detergents are also often not suitable for these novel modalieites due to inherent structural lability. Consequently, sterility of the drug substance at the end of the production process for these modalities is currently ensured by running the entire downstream in pre-sterilized and closed single-use systems. We present the development of a novel size exclusion based method that enables the sterilization of formulations containing large biotherapeutic modalities (up to 300 nm diameter) at the end of the production process while maintaining high yields. This method streamlines the purification process and contributes to the overall efficiency of the bioprocess chain and offers additional risk mitigation in the currently used closed single-use processes and offers cost-efficient alternatives in choosing the process setup. Comment: as discussed via email, due to confidentiality reasons we can´t reveal more information at the moment. We expect to be able to share more information by March 2024. We would than expand the abstract describing the principle of the technology accompanied by data that will validate the concept.

53. Elevating Precision in Extracellular Vesicle Research: Unveiling a Tailored Affinity Chromatography Approach Monitored by Novel Analytical Tools

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Extracellular vesicles (EV) are phospholipid involucrum of cytosol, secreted by cells, implicated in inter-cellular communication, and enacting important roles in physiological and pathological processes. EVs can be classified according to their size and biogenesis mechanism in three different categories: apoptotic bodies (50–1000 nm), microvesicles (100–350 nm) and exosomes (30–150 nm). It is suggested they should give rise to a new class of gene therapy products, with clinical trials already under way. Yet, the manufacturing process of such biopharmaceuticals is challenged by their complexity and heterogeneity. For EV the establishment of scalable downstream processes (DSP) is still ongoing. Currently, most of the purification strategies described in literature are based on density gradient ultracentrifugation. However, this method is time-consuming and compromises the EV quality after purification. Thus, there is a need for novel purification approaches, which could cope with the complexity of these particles. Affinity chromatography (AC) can be used to simplify purification process and generate clinical-grade products with high titer and purity. In this work we report for the first time the implementation of a specific AC resin to purify EV from HEK293T and human induced pluripotent stem cells (hiPSC) cell cultures. Firstly, phenotype evaluation was conducted by Single particle interferometric reflectance image sensor (SP-IRIS) to detect potential EV markers. For the AC development, a set of lead candidates were evaluated by small-scale static experiments, and then the most promising was evaluated in a chromatographic set up. At a laboratory scale, different process parameters were assessed and optimized, including the residence time, dynamic binding capacity and impurity clearance. From this first stage, intermediate scale experiments demonstrated the enrichment in EV, for which approximately 30% recoveries were obtained using vesicles from both cell culture systems evaluated. This recovery yield shows the enrichment of vesicles more potentially related to exosomes. Given the complexity of EVs, available analytical tools that could cope with that complexity are urged to develop. Envisioning to solve this challenge, analytical tools based on biolayer interferometry (BLI) and Single particle interferometric reflectance image sensor (SP-IRIS) combined with immunolabelling were developed. BLI showed potential for phenotype specific EV quantification, with less of 30% deviation from SP-IRIS results. Altogether, these analytical tools enabled to monitor the AC development and final product characterization. Overall, this work aims to contributed to the development of specific and efficient purification technologies which could potentiate the advent of EV-based gene therapies.

54. END-TO-END SCALABLE PURIFICATION PLATFORM FOR EXTRACELLULAR VESICLES

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Extracellular vesicles (EVs) are under investigation as novel therapeutics for a variety of diseases. EVs derived from human mesenchymal stromal cells (hMSC-EVs) constitute most clinical investigations. Despite the growing interest, new methodologies for their purification and characterization have only recently started to be investigated. The goal of this study was to develop a scalable purification platform for hMSC-EVs.15L of EV conditioned media (CM) was generated from adherent hMSC culture in the stirred tank bioreactor and purified over six-unit operations as a proof of concept for generating concentrated and potent EVs for a potential therapeutic application. Following EV production cycle, cell culture supernatant was processed through clarification filters to separate EVs from cells and reduce turbidity. Clarified harvest was processed through crossflow filtration (CF) steps for concentration, enzyme treatment for reducing DNA interference, and buffer exchange for chromatography. Chromatographic method purified EVs were further concentrated, diafiltered in the formulation buffer, and sterile filtered prior to freezing at minus 800C. Throughout purification process, particle and protein measurements were used to assess unit operation recovery and purity. Particles were confirmed to be lipid-bilayer vesicles by staining positive for fluorescent membrane dye and recorded by orthogonal techniques. Identity of EVs was confirmed through western blot analysis and potency was measured by % wound closure via scratch wound analysis. CM containing > 1x10^10 particles/mL was generated. Clarification of CM using filters > 3µm achieved > 65% particle recovery with significant turbidity reduction. CF reduced DNA by 94% mainly due to enzymatic reaction while improving protein removal to > 1 x10^11 particles/mg protein. Chromatography module recovered > 95% of particles with enrichment in purity > 3 x10^12 particles/mg protein. Finally, diafiltered EVs solution in the formulation buffer was sterile filtered and frozen at > 6 x 10^11 particles/mg protein. Purified EV identity was confirmed by presence of surface markers (CD9, CD81, and CD63) as well as cytosolic proteins (TSG101 and Alix), and biofunctionality was verified by scratch test wound healing assay. These promising results serve as a baseline for a robust EV purification platform with consistent critical quality attributes.

55. Developing a novel non-chromatographic purification for antibody drug conjugates: Moving beyond UF/DF

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Current antibody drug conjugate (ADC) purification relies heavily on ultrafiltration/diafiltration (UF/DF) for removal the unconjugated potent compounds or free drug related impurities (FDRI)1. As the ADC landscape becomes more complex, novel payloads with variable levels of hydrophobicity, charge, solubility, stability, and toxicity have become increasingly difficult to remove to safe limits through UF/DF alone.2 ADC manufacturers have turned to traditional biologics purification methods such as chromatography to gain increased control on FDRI removal. Chromatography can add significant cost and time to ADC production and potentially impact the quality target product profile (QTPP). By utilizing knowledge from both small molecule and biologics, our team surveyed a range of purification strategies to identify a solution to an ADC with a labile potent compound. The process was required to have high mass throughput and fast processing times to not impact product quality but also allow scale to very large batch sizes >10 kg. A novel non-chromatographic purification technology was identified that reduced FDRI levels while maintaining product quality and having minimal impact to yield. Additionally, the purification helped to maintain the quality target product profile (QTPP) of the molecule through the removal of unwanted product variants. The team has characterized this technology and is beginning to successfully implement it at scale with minimal additional cost or batch production times.

56. Nanoparticulate separations: the challenges of larger dimensional space

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Separating viral vectors and other nanoparticle-based biological products poses unique separation challenges distinct from those encountered with recombinant proteins upon which the industry's foundations are laid. These challenges are particularly evident in adsorption-based separations, where the effects of avidity and multi-point attachment strongly influence separation behaviour. Our focus is on understanding the behaviour of adenovirus and lentivirus systems during ion exchange chromatography. Cryo-electron microscopy applied to adenovirus systems reveals insights into product quality and heterogeneity, indicating damage to the fiber protein projecting from the virus capsid. This damage intensifies over time in the adsorbed state, resembling a "Velcro" type behaviour during bind/elute processes. This emphasizes the risk of damage to non-covalently bound structures during sorption processes, and we propose exploring the role of molecular modelling to better understand these risks. In the case of the membrane-enveloped lentivirus system, a different set of damage phenomena emerges. The data suggests multi-point attachment and spreading behaviour, leading to irreversible binding and virus loss. Experiments to identify the components in the membrane interacting with the ion exchange surface reveal that the VSV-G envelope protein, commonly believed to drive binding, is not the primary driver. Instead, enzymatic removal of charged glycans within the membrane's glycocalyx strongly influences elution behaviour, causing the frequently observed two-peak pattern to converge into a single peak. These interactions, governed by charged sugar polymers, appear to be time-dependent, indicating that increasing points of attachment over time contribute to losses. In conclusion, the data suggests that the morphological properties of adsorbents, coupled with the control of ligand density, will play a more critical role in the separation of nanoparticulate-based biological products compared to their predecessor products.

57. Optimization of Post-conjugation Polishing Steps for Peptide-antibody Conjugates

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Peptide-antibody conjugates are gaining greater attention as a new class of therapeutics. These molecules are highly potent due to their bi-specific nature: peptides are covalently conjugated to bivalent mAb architecture at either naturally or genetically engineered conjugation sites. However, this complex structure poses unique challenges for their manufacturing and purification processes. The traditional mAb downstream process needs to be altered to allow conjugation steps to fit in. Moreover, critical quality attributes including aggregates, variants of different peptide-to-antibody ratio (PAR), and host cell proteins in final DS must be closely monitored and controlled. Here we present the development of polishing chromatography steps post peptide-antibody conjugation reaction to purify PAR variants and high molecular weight aggregates with the goal to enable facility fit and improve overall run rate.

58. Performance Evaluation of Continuous Counterflow Centrifugation for Advanced Human Pluripotent Stem Cell (hPSC) Bioprocessing

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The advancement of cell therapy manufacturing necessitates robust and scalable technologies capable of aseptic cell concentration, washing, and harvest. The Ksep® system is a simple, robust, automated, and scalable continuous counterflow, single-use centrifuge. Its latest iteration, the Ksep®50, addresses challenges in advanced cell therapy processes within a working range of 0.1 to 20 liters. In addition to cell harvest applications, the system is capable of cell clearance and product recovery from media. This study investigates the effectiveness of hPSC cell recovery using the cell harvest mode of the centrifuge system. Human PSCs represent a potent cell source for regenerative medicine due to their unlimited expandability and differentiation potential in vitro. Here, we demonstrate how Ksep®50 can support hPSC expansion and cardiomyocyte differentiation as matrix-free aggregates in stirred suspension cultures. Cell aggregates are superior for the up-scalable, controlled mass production in suspension, but as their size increases, they interfere with the uniform distribution of gases, nutrients, and growth factors. In this proof-of-concept study, we show that cell aggregates can be concentrated, washed, and separated from non-viable cells by continuous counterflow centrifugation. Within the same centrifugation cycle, the washed cell aggregates are dissolved into single cells by addition of a dissociation agent. The separated cells are further concentrated, washed, and harvested. Due to the aseptic, single-use approach the harvested cells can be reintroduced into a bioreactor for further cell expansion and seed train-like process upscaling. This concept of controlled dissociation of cell aggregates in a counterflow centrifuge could alleviate a bottleneck in the manufacturing of hPSCs at GMP-compliant conditions. In conclusion, our study underscores that continuous counterflow centrifugation systems like the Ksep®50 effectively address the critical challenges of cell manufacturing for the development of advanced cell therapy processes.

59. A scalable membrane process for the purification of extracellular vesicles

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Downstream processes play a critical role in the production of biologics, and this is even more evident with the new classes of large therapeutic molecules in the development pipeline. Viral vectors, mRNA, plasmid DNA and extracellular vesicles are not well suited to packed column chromatography and non-adsorptive membrane processes may be suitable alternatives for their purification. This work aims to develop a scalable and cost-effective integrated membrane process based on microfiltration (MF) and ultrafiltration (UF) hollow fiber modules for the purification of large molecules, using nanosized extracellular vesicles (EVs) from different sources as case studies. Extracellular vesicles (EVs) are lipid nanoparticles secreted by all types of cells. EVs can transport a pool of different biomolecules between nearby and distant cells in a site-specific manner. They are gaining increasing attention for their potential use in nanomedicine, nutraceuticals and cosmetics, particularly as drug delivery vectors, diagnostic tools and in tissue regeneration. The discovery that plants also release EVs has opened the door to the use of plant vesicles as carriers of bioactive compounds and genes [1]. The major challenge in this field is the separation of EVs from complex biological fluids. The proposed process considers a cascade of MF and UF modules that are also operated in diafiltration (DF) mode. Considering the difference in size of EVs with respect to proteins and other contaminants, this technique achieves a higher purification level compared to ultracentrifugation, which is considered as the golden standard for EVs isolation. The process was developed using freshly squeezed lemon juice, which was selected as the starting material because of its beneficial properties for human health, in addition to being a cheap and abundant source. In fact, Citrus Limon juice extracts represent an innovative and still poorly studied source of EVs, which have a very promising therapeutic use as they inhibit cancer cell proliferation on different cell lines [2]. Clarified lemon juice was processed by continuous diafiltration with a 0.15 µm PES hollow fiber module to isolate lemon-derived EVs, with 5 diafiltration volumes and a subsequent 5-fold concentration obtaining a purity of 99.97%. The process has been validated for the purification of bovine milk EVs and plant cell cultures. For example, in an analogous continuous diafiltration process, but using a 750 kDa PES hollow fiber module, whey-derived EVs were isolated from clarified milk whey with a removal of 96.5% of protein impurities. After 5-fold concentration, an EV purity of 98.2% was obtained, demonstrating that the membrane process is superior to the conventional ultracentrifugation process. Moreover, the UF/DF process is scalable and amenable to the purification of other class of molecules such as viral vectors.

60. Biosimilars development challenges and opportunities

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At the core of biosimilar development is the production of a biopharmaceutical product that is similar to its marketed reference product. Reducing cost of goods, matching reference product quality, and being first to market are critical for successful commercialization of a biosimilar product and for bringing a lower cost medication to patients. Matching desired product quality attributes (PQAs) for biosimilars is critical for efficacy and function and a successful marketing application. But given these products are generated by another company, it can be extremely challenging to match their PQAs due to differences in host cell lines, cell culture, and purification processes. These challenges provide unique innovation opportunities during process development to meet the reference product PQA ranges. As a pioneer in the development of biosimilars, Amgen has had the opportunity to learn from its broad portfolio of biosimilars and to identify successful strategies to address many of the challenges that come in matching a reference product under accelerated timelines. Very recently, three biosimilar programs in Amgen portfolio successfully completed process validation and marketing application submissions per target timeline despite challenges came up during covid-19 pandemic. Few off-platform resins and filters need to be deployed for development and clinical large scale runs due to material supply constraints during pandemic. This also provided an opportunity for dual sourcing strategy and supply resiliency for future. Off-platform purification processes may also be used for biosimilars to match the reference product ranges for attributes such as high molecular weight species. Case studies will be discussed from challenges and approaches used to ensure successful technology transfer to clinical and commercial manufacturing and having a comprehensive data package for marketing application per target timeline.

61. How to build a future proof and robust NANOBODY® molecule CMC platform

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NANOBODY® containing modalities represent a unique class of therapeutics, which are versatile, flexible and which can be used in different therapeutic settings such as (immune)oncology, immunology and inflammation and rare & neurological diseases. Sanofi Ghent, formerly Ablynx, has a pioneering role and more than 20 years' experience in developing NANOBODY® molecules into therapeutics with one commercial product on the market and several others in different clinical phases. In this period, a robust and mature CMC platform underpinning this clinical development was built through constant feed-back loop improvement. Today we are entering the next chapter: how to adapt our robust CMC platform to cope with a changing (bio pharmaceutical), a more sustainable environment and a diversifying modality landscape, always keeping our eye on Cost-of-Goods, flexibility, and shorter time-to-clinics in a similar manner as classical NANOBODY® molecules. Focus of this presentation will be on DSP and will touch upon new modalities, process robustness in a changing world, in silico methodologies, eco-design, Cost-of-Goods and the power to build further upon our mature and robust CMC platform.

62. Development of a High-Capacity Downstream Toolbox for Purification of Bispecific Antibodies

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Bispecific antibodies represent a rapidly growing segment of the clinical landscape for protein-based therapeutics. Compared to monoclonal antibody drug substance processes, bispecific antibodies can present unique challenges to the development of robust downstream process due to the presence of closely related impurities such as mispaired homodimers. These challenges are further compounded by the desire to accelerate process development timelines to enable earlier access of these biotherapeutics to patients. Here, we sought to develop a downstream process which is comprised of polishing chromatography resins capable of removing various impurities associated with multiple bispecific antibody formats. In addition to defining streamlined process development approaches to support speed to clinic, we also standardized the operation of these polishing chromatography steps to be performed in flowthrough mode to achieve higher column loading capacities. This criterion was deemed essential to ensure facility fit to our intended manufacturing facility, while also supporting sustainability efforts by driving down buffer and consumable utilization. This work culminated in the definition of a downstream toolbox for bispecific antibodies, which is comprised of plug-and-play unit operations capable of meeting process performance and product quality metrics to support an accelerated timeline for first-in-human clinical trials.

63. Host cell proteins profiling from a group of Monoclonal and Bispecific antibodies

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Host cell proteins (HCP) constitute a major group of impurities and, in order to minimise the risk to patient safety and drug efficacy, their removal to an acceptable level is critical in bioprocess development. The clearance of host cell proteins in biomanufacturing have some challenges: (i) HCPs which co-purify with the product are difficult to remove with a platform based chromatography process (ii) high risk HCPs can impact product stability and immunogenicity even at very low levels (iii) intensified cell culture processes such as the high inoculation density (HID) process and full perfusion processes can generate elevated level of impurities. A fundamental understanding of HCP-antibody interactions can lead to improved control strategies which better mitigate against HCP associated risks. In the first case study, a panel of monoclonal antibodies and Fc-based bispecific antibody scaffolds were captured by Protein A affinity chromatography and analysed for host cell proteins by LC-MS/MS. This method is widely used in HCP analysis due to its ability to provide the identity and relative abundance of individual HCPs. The HCP profiling method allowed us to gain a deeper insight in the type of host cell proteins frequently identified in both monoclonal antibodies and bispecific antibodies of different types. The study also revealed the typical level of different host cell proteins and their composition. A clustering analysis of HCPs showed correlations between the presence of host cell proteins and IgG properties. In the second case study, the impact of Protein A resin and various wash buffers on HCP clearance were assessed. Eleven wash buffers were screened in the Protein A capture step using two representative monoclonal antibodies. The results were benchmarked with the platform Protein A affinity capture step. Two wash buffer conditions displayed a significant reduction of host cell proteins without impacting the product quality and step recovery. Based on the LC-MS/MS data, it is possible to identify wash buffer conditions which remove specific host cell proteins. Combining LC-MS analysis with large sample sets allows a better understanding of HCP associated challenges which can be used to design tailored mitigation strategies.

64. Development of a Versatile Downstream Process Toolbox for Control of Specific Polysorbate-Degradation Enzymes

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Host cell proteins (HCPs) from CHO expression systems include a subset of species that pose a significant process risk for biotherapeutics as they can lead to enzymatic degradation of formulation excipients including Polysorbate-80 or Polysorbate-20. The impact of these polysorbate-degradation enzymes (PSDEs) can also result in a decline in product quality and decrease in shelf life of biotherapeutics. Some PSDEs are highly active even below quantifiable concentrations, which presents a significant challenge in identifying them. Recent advancements in analytical techniques have enabled a more rapid process evaluation. An activity-based protein profiling (ABPP) approach can identify species with a specific activity, though it is low-throughput and not particularly quantitative. A fluorescence-based lipolytic activity assay is higher-throughput and can be more easily integrated into process development workflows, but cannot distinguish between different species. ABPP was used to identify recurrent problematic PSDEs across several programs. High-throughput screening was then used to evaluate partitioning of these species across a range of chromatography resins and mobile phase conditions. This partitioning data has been fed into an existing quantitative structure-and-relationship (QSAR) model built with partitioning data for monoclonal antibodies and other therapeutic proteins. The QSAR model can also be leveraged for more targeted process development when a new problematic HCP is identified. In some cases, separation of these problematic PSDEs may be less effective due to interactions with the product or similarities in properties. In these cases, inhibition or inactivation of active HCPs can be a viable process option. High temperature inactivation for a short time has been demonstrated to reduce lipolytic activity without drastically affecting the quality of the therapeutic product. Plate-based screening identified optimal operating conditions for an inactivation step for process intermediates for two different mAbs. Screening also provided information for optimal placement in a downstream process, showing the impact buffer matrices may have on HCP inactivation. Control of other product quality attributes was also factored into optimization, ensuring there are additional steps for aggregate clearance downstream of the inactivation. The work culminated by comparing the stability of the final formulated product from a process with inactivation to a standard process. In conclusion, various methods have been demonstrated to effectively reduce activity of specific identified PSDEs in formulated biotherapeutics. Combinations of these approaches could be deployed in downstream processes.

65. Strategies to control Polysorbate 80 degradation in mAb bulk drug substance

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Degradation of Polysorbate 80 (PS80) in mAb bulk drug substance (DS) and drug product (DP) has the potential to impact product quality attributes during long-term storage resulting in reduced shelf-life of the DS and/or DP, and remains an ongoing challenge for the industry. This degradation can occur via chemical or enzymatic pathways. While chemical degradation has largely been managed successfully through formulation development, enzymatic degradation occurs at varying rates due to the presence of residual lipases/esterases (HCPs), especially for high-concentration liquid formulations. Further, there are conflicting reports in the literature on the specific HCPs which can cause PS80 degradation and, given the variability in drug substance production platforms across the industry, it is expected that the implicated enzymes belong to a family of lipases/esterases which are expressed and/or cleared to different extents in different drug substance processes. The work presented here details a strategy to manage PS80 degradation through focused development of two chromatography steps in a typical mAb purification process: the affinity chromatography step and a polishing chromatography step. In the affinity chromatography step, a series of wash buffers were evaluated to selectively clear the degrading enzymes prior to product elution. For polishing chromatography, a hydrophobic interaction chromatography step performed in flow through mode was developed, and salt conditions have been identified that may separate the degrading enzymes from the product. The product pools from both steps were analyzed for PS80 degradation, enabling selection of process conditions which showed improved PS80 degradation rates. The results presented here demonstrate a toolbox approach where several process options are targeted to successfully manage PS80 degradation risk across multiple assets. Analytical approaches to support process development will also be discussed.

66. Towards Platformization of a Continuous Monoclonal Antibody Purification Process with Capture via Precipitation and Polishing via Subtractive Adsorption

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New purification processes are required to meet the global need for high-purity, high-volume, high-dose protein therapeutics, such as monoclonal antibodies (mAbs) for the treatment of Alzheimer's disease, high cholesterol, and infectious disease. We developed a novel intensified, continuous purification process that can meet this need by eliminating Protein A affinity chromatography, the bottleneck of the current "platform" mAb manufacturing process. We pre-process harvest cell culture fluid (HCCF) to deplete host cell DNA and remove media components that interfere with mAb precipitation, capture the mAb via precipitation using synergistic bulk precipitants (ZnCl2 and PEG), dewater and wash the precipitate slurry using hollow fiber microfiltration modules to enhance impurity removal, redissolve the washed precipitates at pH 3.5 to enable low pH viral inactivation, and employ two orthogonal flowthrough subtractive adsorbers with minimal intermediate conditioning for polishing. The process can be operated in an integrated, fully continuous mode. It addresses the volumetric throughput, process mass intensity, and cost-of-goods bottlenecks as well as the equipment and supply chain complexities associated with the platform Protein A-based capture step that currently limit global mAb manufacturing capacity. This eminently scalable process also readily accommodates increasing upstream product titers, as the precipitation-based capture step becomes more efficient as mAb concentration increases. We have demonstrated precipitation-based capture and polishing with mAb HCCF feed materials from multiple industrial partners, gaining key insights which support further process development and suggest that the process may be platformable. During HCCF pre-processing, we deplete host cell DNA via CaCl2 precipitation to significantly reduce DNA persistence in the process, which facilitates complete redissolution at acidic pH and allows the redissolved precipitate stream to be directly applied to the first polishing step without further stream conditioning. We also pre-concentrate and diafilter the DNA-depleted HCCF in a single-pass tangential flow filtration step to remove culture media components that interfere with mAb precipitation and to standardize the precipitation feed concentration and buffer matrix, which permits the use of similar, low precipitant concentrations for quantitative precipitation (>95%) for all mAbs studied. For the capture step, we found that the addition of CaCl2 during precipitation leads to the formation of denser precipitate particles, resulting in higher sustainable flux values and better impurity removal in the dewatering and washing operations. We attained conversions of 70-80% in the hollow fiber microfiltration modules, which led to host cell protein (HCP) levels <10,000 ppm for redissolved precipitates. We achieved yield >98%, buffer consumption <400 mL/g mAb, and volumetric throughput >36 g/L/hr (based on wetted system volume) for the precipitation capture step. For polishing, we found that the combination of a hydrophobic adsorbent (activated carbon) and a mixed-mode anion exchanger (e.g., CaptoAdhere ImpRes) resulted in excellent clearance of residual impurities including HCPs, DNA, and aggregate species at high mAb yields. We achieved purified mAb HCP levels <20 ppm and aggregate levels <0.5% with yields >86% for the two-step polishing process. We have performed economic and environmental sustainability assessments and have shown that the precipitation-based process compares favorably to current Protein A-based platform purification processes.

67. A lab-scale model and GMP-scale proof of concept for PCC Protein A using a dynamic column loading strategy

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A greenfield mAb manufacturing facility is being constructed at Eli Lilly & Co. (Lilly) that will have the ability to incorporate batch, as well as intensified and continuous technologies throughout the upstream and downstream portion of the drug substance process to increase manufacturing productivity and throughput (kg/year). These technologies will help boost upstream titers and ensure downstream unit operations can match the increased upstream throughput while using a smaller downstream footprint compared to traditional Lilly mAb manufacturing sites. This presentation will focus on discussing development activities to enable implementation of an intensified downstream chromatography technology in the Protein A (ProA) capture unit operation for a representative mAb. The technology, developed by Cytiva, is a multi-column approach that uses periodic countercurrent chromatography (PCC) and a dynamic product loading control functionality to enable overloading of columns and parallel processing which can decrease resin and buffer amounts required, shrink equipment footprint, and increase throughput compared to traditional batch Protein A unit operations [1]. Previous work has indicated existing lab-scale chromatography models (~ 1 cm inner diameter (ID)) used for batch ProA operations using static (volumetric or time-based) product load control are not representative to PCC ProA applications using a dynamic product load control functionality, specifically when using pre-packed ProA chromatography columns. Work will be presented that involves the development of a refined lab-scale chromatography model specifically for PCC ProA applications when utilizing dynamic load control and pre-packed chromatography columns. For example, product binding capacities observed on larger ID prepacked columns (~ 18 cm ID) were more comparable to binding capacities observed on lab-scale columns packed to higher-than-normal compression factors (e.g., ~1.2 – 1.3). Finally, results from a longer duration PCC ProA run, conducted on a GMP capable PCC system and using larger ID prepacked columns, will be shared to demonstrate how this intensified downstream technology could be operated at commercial scale in the greenfield facility. Results showed an average ProA product load increase of ~ 40% over 36 cycles compared to traditional batch ProA with an ~ 99% yield and similar product and process quality attributes.

68. Enhancing Virus Filtration Efficiency in Continuous Processing through Serial Filtration with High Area Ratio

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This presentation discusses the optimization of virus filtration in continuous bioprocessing by employing a novel approach of serial filtration with a high area ratio between the first and second stage filters. While continuous processes offer numerous advantages over batch operations, integrating traditional batch operations seamlessly remains challenging. Virus filtration in continuous processes often operates at low flux, which can compromise filter virus retention performance and hinder the adoption of truly continuous virus filtration. To address this limitation, we propose a solution that utilizes serial virus filtration with a high area ratio, resulting in improved virus retention assurance. In an experimental study, virus filters were operated continuously (except for planned process interruptions) for 200 hours in a serial configuration at a first-to-second stage filter area ratio of 13:1 and a first stage flux of 5 L/m2. The results demonstrated a log reduction value (LRV) of minute virus of mice (MVM) of approximately 4 across the first stage, with no virus detected in the second stage filtrate, leading to an MVM LRV exceeding 6.7 across the filtration train. The second stage filter was the dominant flow resistance at the start of the run but, as it was protected from foulants by the first stage filter, suffered minimal fouling and the life of the filter train was controlled by the first stage flow resistance. A theoretical case study projected that continuous virus filtration using an optimized serial configuration with a high area ratio would yield about 30% longer filter changeout time, 14% higher productivity (in terms of volume filtered per total effective filtration area), and nearly 6 logs higher LRV compared to single stage operation. The findings of this research are expected to provide valuable insights into optimizing virus filtration in continuous bioprocessing.

69. Integrated Control and Scheduling of a Multi-Column Chromatography Operation with Numerical Dynamic Optimization

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The existing periodic counter current (PCC) and simulated moving bed (SMB) sequencing recipes can facilitate multi-column chromatography operation in principle. For instance, with a constraint that the loading duration be longer than the duration of the other cycle steps combined, the PCC recipe ensures a column is always available to receive feedstock [1]. However, pre-determining the process configuration and operating with a static recipe, or schedule, can limit the overall performance during a dynamic long-term continuous manufacturing run [2]. For example, the optimum process configuration and recipe are sensitive to the feedstock concentration [3]. This fact translates to possibly suboptimal operating performance when a fixed process configuration and recipe are employed as the feedstock concentration inherently fluctuates with time [4]–[6]. Moreover, the column capacity that can degrade over time [7] and thus static recipes must be designed with safety margins to avoid product loss. Clearly, online feedback-based control systems are needed that maintain optimal performance with regards to the real feedstock concentration and column capacity. Recently, Gomis-Fons et al. designed a multi-column configuration around nominal values for the concentration and flow rate, and then adjusted the column loading volume during operation with real-time estimates of the feedstock concentration and flow rate [6]. In a related yet distinct approach, Thakur et al. tackled the same challenge by adjusting the process configuration and schedule among a set of nine pre-determined static recipes, that were tailored to different ranges of feedstock concentrations, based on a real-time estimate of the feedstock concentration [8]. While these feedback-based control systems overcame disturbances in the feedstock concentration, neither were truly optimal with respect to the real-time column capacities. In contrast, implementing a feedback-based integrated control and scheduling strategy has the potential to not only reject process disturbances, but do so in a fashion that is able to account for the real-time process state and economic factors, such as idle time, material consumption and waste generation [9]. To this end, we apply a hybrid column model in a numerical dynamic optimization problem that integrates the control and scheduling design of a multi-column chromatography process. We initially fit the column model to data from separate breakthrough curves where different proteins (e.g., IgG from human serum) were applied to a 1 mL cation exchange resin column (CM Ceramic HyperD F) on the Sartorius BioSMB PD continuous chromatography system. In addition, we apply a state observer to incorporate real-time measurements and state estimates into the rolling numerical dynamic optimization problem. The integrated, dynamic optimization approach is demonstrated in a multi-column chromatography case study with varying feedstock concentration and inherent column capacity degradation. We compare the performance achieved using the integrated control and scheduling design to that from the existing PCC recipe and dynamic loading strategies. We show the integrated, dynamic optimization approach improves process economics while being scalable and flexible. Overall, this work presents a numerical dynamic optimization framework that improves multi-column chromatography operation and economics based on real-time process measurements.

70. AUTONOMOUS OPERATION AND ADVANCED CONTROL OF INTEGRATED CONTINUOUS DOWNSTREAM PROCESSES

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There has been a lot of attention on integrated continuous downstream processing during the last decade, since they promise more efficient processing due to being based on a sequence of integrated purification steps. This enables process intensification, minimization of storage tanks and hold-up times, smaller footprints, decreased buffer consumption and increased sustainability. The result is a complex, integrated sequence of multiple unit operations that performs straight-through processing of the target protein, with minimal time from expression to formulation, often based on an upstream perfusion system. At the same time there is a pressure to decrease the use of resources in process and product development on these new efficient and complex downstream processes. This work addresses this question and presents a platform and methodology for efficient development of complex downstream processes including automation, real-time control, heterogenous data handling and on-line quality monitoring. Complex downstream processes for lab-scale development studies can be configured and implemented using commonly available hardware, like ÄKTA and Agilent systems. The automation can be developed in the research software Orbit based on Python, which creates an open, flexible, extendable, and scalable control system. Orbit gives support for integration of multiple chromatography columns, operation of downstream processes on multiple parallel setups, integrated online analytics, use of advanced feedback control and batch-to-batch control. A case study for continuous production of a monoclonal antibody is used to illustrate the platform and the methodology. The downstream process is based on continuous solvent/detergent based viral inactivation, continuous Protein A capture, and polishing based on mixed-mode, operated in flow- through mode. The automated, continuous downstream process is configured with support systems for automated buffer management, HPLC-based quality analysis and real-time database. The development and implementation of model-based monitoring and control used in the case study will be discussed. The result is an illustration of a downstream process that can be operated (almost) autonomously.

71. Development of Temperature-Controlled Batch and 3-Column Counter-Current Protein A System for Improved Therapeutic Purification

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Optimizing bioprocess chromatography for enhanced product quality is a critical endeavor that involves meticulous control of process parameters and performance attributes. While parameters like bed height, eluate cut points, and elution pH have received extensive attention, the influence of temperature in Protein A chromatography remains underexplored. This study delves into the intricate effects of temperature on monoclonal antibody purification using a commercial chromatography resin, employing both batch and counter-current systems. To achieve precise temperature control, a custom 3D-printed heating jacket was employed, ensuring stable temperatures during loading, wash, elution, and cleaning-in-place (CIP) steps. Experimental observations at 10, 20, and 30°C revealed that higher temperatures correlated with increased dynamic binding capacity (DBC). Mechanistic and correlation-based models were applied to the experimental data, enabling the prediction of optimal operating conditions across a temperature range. These model-based predictions guided the development of a temperature-controlled periodic counter-current chromatography (TCPCC) using three columns, which was subsequently validated experimentally. Operating at 30°C in the 3-column TCPCC system yielded a remarkable 66% increase in DBC compared to 20°C batch chromatography, resulting in a twofold improvement in productivity. Further scaling by increasing the number of columns for higher feed concentrations led to significant productivity enhancements, reaching up to fourfold improvements at 15 g/L feed concentration. This study's temperature-dependent mechanistic models offer valuable insights and optimization tools applicable to both batch and continuous chromatography systems across diverse operating conditions.

72. Evaluation of a single-use disk stack centrifuge for improved efficiency and sustainability at 1000 L GMP manufacturing scale

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Direct depth filtration is an established technology for single-use harvest operation. Advantages of direct depth filtration include familiarity with depth filtration in downstream processes and simplicity of the operation. Drawbacks include low capacity, large footprint, labor-intensive set-up, high water use, and high waste in the form of discarded filters. Single-use centrifugation is emerging as an alternative to depth filtration for the single-use harvest step. Within the single-use centrifugation space, disc stack centrifugation represents the newest entrant. In this study, we evaluated the performance of the GEA kytero single-use disc stack centrifuge to clarify two monoclonal antibody-producing cell culture fluids. The separation performance of the GEA kytero centrifuge varied between the two cell culture fluids, with differences in centrate turbidity and centrate filterability measured. A comparison was then performed to determine resource savings, compared to direct two-stage depth filtration, when using a GEA kytero centrifuge to harvest a 1000 L bioreactor. The analysis concluded that replacement of the first stage of depth filters with a GEA kytero centrifuge has the potential to decrease the required second stage depth filtration area by up to 80%. The decrease in depth filter area resulting from the use of the GEA kytero would result in a decrease in the harvest step footprint, a decrease in buffer volume required to prime and rinse depth filters, and a decrease in the volume of plastic waste. An economic comparison of the GEA kytero single-use centrifuge against a direct depth filtration step found that for a 1000 L harvest step, the GEA kytero centrifuge may reduce costs by up to 20% compared with two-stage direct depth filtration.

73. Abstract - Scaling Up the Purification of Mammalian Cell Culture Through a Robust PrA EBA Step

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The primary recovery of mammalian antibodies includes multiple steps for clarification, from centrifugation to multiple depth filters, before storage of the filtrate pending purification by packed bed chromatography. By reducing the number of steps, it is possible to prevent loss of product, save time during manufacturing, improve water usage, and reduce costs. In addition, preventing the risk of product loss due to storage of the clarified cell culture filtrate. Expanded Bed Absorption (EBA). EBA has been used previously in manufacturing for this very purpose, though fell out of favour due to high water consumption, typically low capacities, and high eluate volumes. Through a collaboration with Biotoolomics we have made advances in updating this technology for more modern mammalian-based projects, via a specialised Protein A (PrA) resin and improvements to the column and running method. Utilising our PrA EBA setup we have consistently shown that we can capture product, up to 40g/L resin, directly from bioreactor feedstreams. Unlike some current versions of PrA EBA, which rely on running the entire setup in upstream, we can perform the elution step in a downflow mode, further reducing buffer usage and producing eluate volumes comparable to those of the fixed bed chromatography. Furthermore, while initial work was performed at lab scale, we have since successfully started scaling up to the pilot scale range. Additionally, as the future of upstream technology moves to more continuous modes of production EBA provides a more ideal fit than the current harvest followed by fixed bed chromatography capture.

74. From Challenges to Opportunities: Evaluation and Implementation of New Single-use Harvest Technologies with a Holistic View on Portfolio and Network Impact

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Applying years of experience and the expectation of robustness, the transition from harvest in traditional stainless steel to new single-use facilities presents both challenges and new opportunities for monoclonal antibody (mAbs) and mAb-like processes. Several new technologies were investigated with an ultimate goal to return to a state that minimizes product-specific development and where prior knowledge can be leveraged to simplify process validation. Using Choosing by Advantages methodology, technologies were compared and prioritized considering multiple factors; emphasizing sustainability, alternate sourcing, and closed processing that accompany a ballroom style facility design for the present and the future. Whole Cell Depth Filtration, Tangential Flow Depth Filtration, and Single-use Centrifugation were selected and implemented for primary clarification and continued to be optimized while cell culture processes were simultaneously increasing cell densities and titers. The need for secondary clarification has also been explored and the potential benefits new defined media bring have been compared against both existing non-defined depth filters and the requirements of future processes. Identified improvements could be transitioned to improve harvest of new AAV or even existing mAb processes in existing stainless steel facilities. The single-use centrifuge was ultimately selected as the primary clarification technology for new 2kL clinical and commercial facilities. The decision comes with a prospective plan towards process validation, flexibility to more seamlessly move new products between single-use and stainless steel facilities, and brings proof-of-concept perfusion capability for additional future applications.

75. Development and Implementation of the Single Use Centrifuge for Harvest and Proof of Concept Perfusion Capability

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Working together with a vendor through collaborative discussion and development over the past 5 years, we were able to convert the expertise in disc stack separation for harvest into a new single-use design. This development has also provided an opportunity to address specific separation challenges that have come about with the increasing cell mass of the upstream processes such as the use of a more efficient disc stack design leading to lower lysis with lower bowl speeds. The new single use centrifuge also addresses the challenge with solids limitations through the continuous solids removal enabled by the shear-thinning properties of the cell solids in place of the intermittent discharge. As part of the development, an implementation effort including both pilot and production scaled equipment was executed and has demonstrated scalability based on standard flow-to-area comparisons. This change has also brought about improvements in the production equipment inlet and outlet flow control leveraging the hermetic design that have further enhanced the robustness of the separation and control. The introduction of the single use centrifuge has presented new capabilities not possible in the stainless steel discharging centrifuges and with modifications has led to successful proof of concept perfusion development work. The modifications to the solids handling and solids to liquid split ratio along with minimizing the temperature deviation being the most significant modifications that were required to enable this use case.

76. Integrated Strategy of Raw Material Interchangeability for Resilient Manufacturing and Global Supply

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The interchangeability of important raw materials in biopharmaceutical manufacturing has long been an area of interest for supply and cost benefits. Through the pandemic it has become clear that the interest has shaped into concrete needs to ensure resilient manufacturing and global supply. To accomplish the interchangeability for raw materials, it requires concerted efforts in multiple fronts, including cross-functional collaborations within the company, industrial cooperations (peer companies and RM suppliers), and negotiation and support from agencies. In this presentation, we provide some case studies at Amgen to demonstrate the integrated strategy to evaluate and implement interchangeability for a few key raw materials in biomanufacturing (resins and filters), where joint efforts were channeled from process development, material science, external supply (vendor relations), manufacturing network, regulatory, and many other organizations and bodies. The successful implementation of the RM interchangeability brings direct benefits for flexibility in manufacturing, savings on Cost of Good Manufactured (CoGM), and most importantly, the resiliency in supplying medicines for the global market.

77. INNOVATIVE RISK-BASED APPROACHES TO ACCELERATE DOWNSTREAM BIOPROCESS DEVELOPMENT

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The efforts across the biopharmaceutical industry to produce effective vaccines and treatments for the Covid-19 pandemic demonstrated that the highly accelerated CMC timelines could be met without sacrificing safety or quality. While such highly accelerated CMC timelines may not be necessary for other biologics treating other diseases; nevertheless the pandemic informed the industry that CMC timeline acceleration can be achievable on an ongoing basis to meet business and patient needs. This presentation will demonstrate the risk-based approaches we have employed strategically across assets to accelerate downstream bioprocess development timelines, reduce book of work, and/or reduce resources. Such approaches include de-risking studies entirely, delaying studies to later in the product life cycle, streamlining study design using a Quality by Design approach, use of prior knowledge based on a wealth of historical data, and leveraging knowledge management tools such as templates, databases, and data visualization platforms. The presentation will focus on key areas where this approach has been applied successfully including viral clearance, impurity control, and process characterization and other process validation readiness activities. Furthermore, the application of such strategies will be shown not just for traditional monoclonal antibodies, but other biologics modalities as well such as multi-specific antibodies, fusion proteins, and microbial-derived proteins.

78. Towards Fully Automated Process Development

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Diya Bhat, Sanofi, United States
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Jason Walther, Sanofi, United States
Kevin Brower, Sanofi, United States

High throughput purification development was the 2010s...Fully automated process development is the 2020s! To address modern biopharma process development challenges, our mission is to develop broadly adoptable, maximally efficient, and highly predictive process development technologies, ultimately working towards fully automated process development. To this end, at Sanofi, we: • Developed a fully automated platform for minicolumn experiments, from buffer and protein solution preparation through post-run pooling and sampling • Demonstrated an in-depth understanding of minicolumns to apply them for process characterization of simple and complex modalities • Combined minicolumns with, to our knowledge, a first-in-industry demonstration of inline UV sensors to enable real-time peak cutting and more predictive minicolumn models • Physically and digitally integrated minicolumns with a bioprocess analytics suite, to enable complete automation from chromatography experimental design to analytical results • Are developing approaches for automated experimental design, starting from a scientist-defined objective function through a complete mapping of the potential operating space with minimal user intervention • Established a vision to integrate our combined purification and analytical suite with high throughput cell culture technologies, to enable fully automated process development, experimentally determining the dynamic process interactions from bioreactor through polishing chromatography. Across our global organization, we have collectively developed and are executing this technology roadmap to bring together diverse ideas from scientists and leadership across sites (and continents!), and to ultimately have all project teams across our global organization move into the future of process development together.

79. Buffer recycling in biopharmaceutical downstream processing for cost reduction and increased sustainability

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The production of biopharmaceuticals is a highly water-intensive process. The water consumption is partly due to the need for many different buffers in large volumes during the downstream process. The shift from batch to continuous operation in biomanufacturing has led to increased productivity and higher equipment utilization, and as a result decreased buffer consumption. Still, very large buffer volumes need to be handled, making buffer management a common bottleneck in biomanufacturing. We have made initial efforts to address buffer management by developing a Buffer Management System, which can automatically prepare and deliver buffers to a downstream process. To address this further, we propose the introduction of buffer recycling in chromatography to reduce buffer consumption. Previous studies have applied buffer recycling in e.g. closed-loop recycling chromatography. However, in closed-loop recycling chromatography, buffer recycling is performed to achieve the required purity, not primarily for reducing buffer consumption. Several considerations must be made before introducing buffer recycling during chromatography. For example, the risk of accumulating impurities should be minimized, making the wash or strip buffers unsuitable for recycling. Furthermore, recycling of buffer during a salt gradient is unpractical due to the varying composition. Another consideration is whether buffers should be recycled in the same unit, or to a different downstream process unit. The chosen implementation must ensure that no impurities from upstream are introduced further downstream in the process. In light of this, the recycling of equilibration buffer to the same unit in a batch-to-batch or multi-column setup is a promising option In this study, we demonstrate buffer recycling during the equilibration step in a 3-column protein A periodic counter-current chromatography process for antibody purification. Commonly, five column volumes of equilibration buffer are passed through the column after cleaning in place, to prepare it for product loading. In our setup, we recover the last column volumes of the equilibration buffer in a separate bottle. This buffer volume has the initial conductivity of the buffer, but a pH above the set-point. After automatic pH adjustment, the recovered buffer is reused in the beginning of the equilibration step of the next protein A column. By introducing buffer recycling, we could reduce the equilibration buffer consumption by almost 50% and have seen no changes in product yield or purity. Hence, through buffer recycling, we can improve process economics, and promote sustainability in biomanufacturing by using less water and chemicals.

80. Next Generation Small Footprint facilities and Simplified Workflow utilizing Continuous Processing and Integrated Buffer Management

Brian Horowski, IPS, United States
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Historically Biological manufacturing processes have been Batch or Fedbatch in the case of mammalian derived products. This batch design has resulted in complex processes, labor intensive workflows with numerous hold steps leading to high operating cost and lowered output and capital intensive relatively inflexible Facilities. This talk is a provocative look at how Process Intensification strategies like 1) Continuous Processing, 2) Integration of buffer making into each unit operation, and 3) Connecting multiple unit operations into a single skid can impact a greenfield or brownfield facility design. We will share what such a novel process design does for Improved Facility layout and utilization, simplified workflow, increased plant output and reduced FTEs, Cycle time and Cost of Goods. Data will be presented from case studies showing the impact of such process design on critical process parameters and quality attributes. The take-away will be that there are simple tools and process choices available today in bioprocess design to positively impact and simplify workflow, increase productivity, improve facility utilization and reduce capital and operating cost.

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Xiaoxiang	Zhu	Amgen	United States
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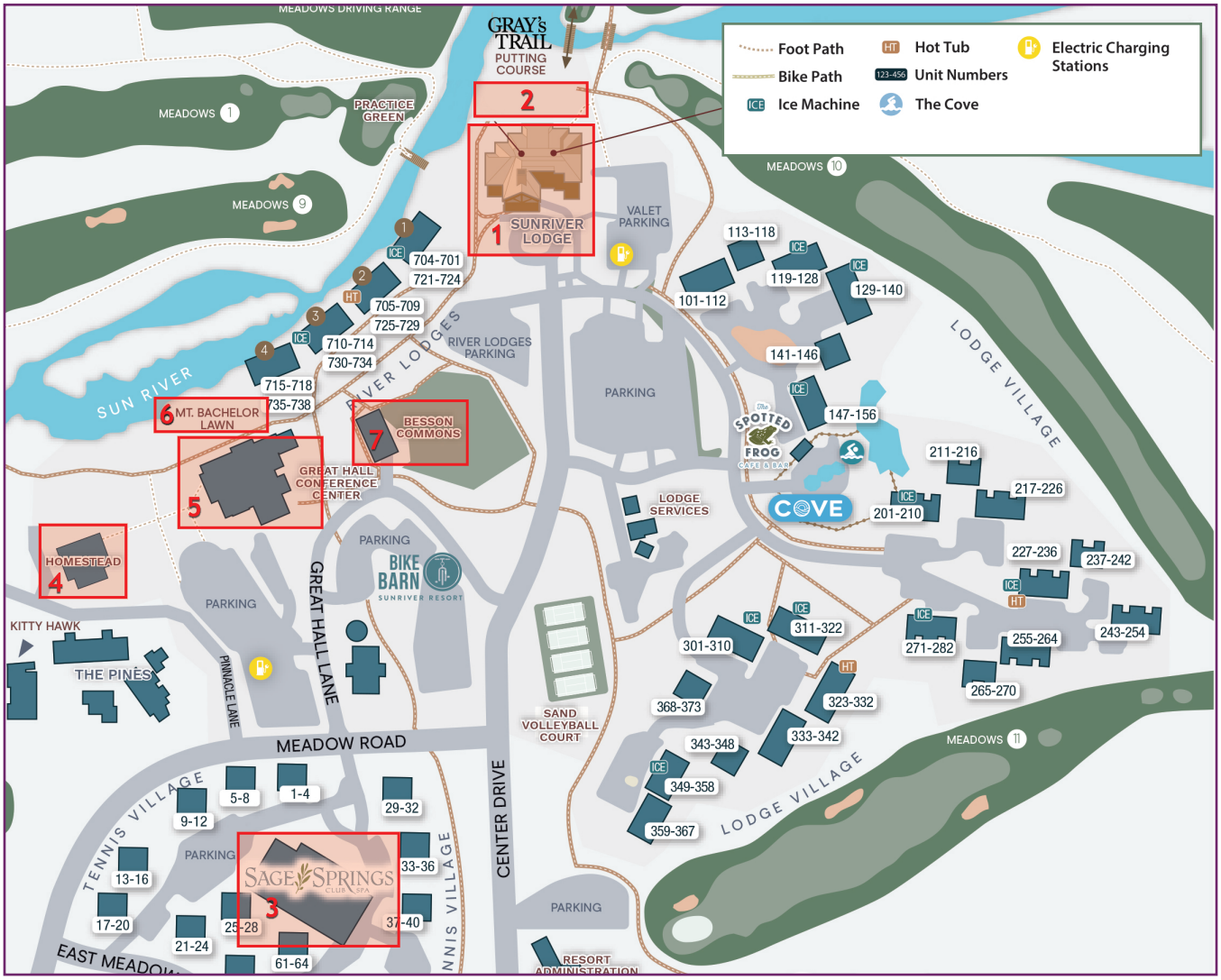
HOTEL MAP:



1. Sunriver Lodge
Hotel Check-In
Saturday/Sunday Conference Registration
Roundtable 1: Pandemic
Friday Airport Shuttle Pick Up

2. The Backyard of Sunriver Lodge
Sunday Dinner
Wednesday Dinner

3. Sage Springs Pavilions
Oral Sessions
Poster Sessions
Monday/Wednesday Boxed Lunch

4. Homestead
Monday-Friday Conference Help Desk
Breakfast Buffet
Tuesday/Thursday Lunch Buffet
Roundtable 2: New Modalities
Roundtable 3: Modeling
Roundtable 4: $1 per Dose

5. Great Hall Conference Center
Roundtable 5: Sustainability
Thursday Dinner

6. Mt. Bachelor Lawn
Monday Dinner

7. Besson Commons
Activities Meeting Point
Tuesday Lunch