Program, Abstracts & Conference Information

Recovery of Biological Products XVII

Bermuda 2016

Smart Bioprocessing

19 – 24 JUNE 2016 | FAIRMONT SOUTHAMPTON | BERMUDA

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Recovery of Biological Products XVII

FAIRMONT SOUTHAMPTON
BERMUDA

19 – 24 JUNE 2016

AN INTERNATIONAL CONFERENCE

Sponsored by:

The American Chemical Society
Division of Biochemical Technology

Conference Management Provided by:

Precision Meetings & Events
301 N. Fairfax St., Suite 104
Alexandria, VA 22314
USA

Table of Contents

Conference Chairs

Steven Cramer, Rensselaer Polytechnic Institute, United States
Günter Jagschies, GE Healthcare Life Sciences, Sweden
Phil Lester, Genentech, United States

Keynote Speaker Moderator

Charles Cooney, Massachusetts Institute of Technology, United States

Oral Session Chairs

Ashraf Amanullah, aTyr Pharma, United States
Dorothee Ambrosius, Boehringer Ingelheim Pharma GmbH, Germany
Ernst Broberg Hansen, NovoNordisk, Denmark
Jon Coffman, Boehringer Ingelheim, United States
John Curling, John Curling Consulting AB, Sweden
Christopher Dowd, Genentech, United States
Suzanne Farid, University College London, Great Britain
Gisela Ferreira, MedImmune, United States
Sanchayita Ghose, Biogen, United States
Caryn Heldt, Michigan Tech, United States
Michael Laska, Moderna Therapeutics, United States
Thorsten Lemm, Roche, Germany
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John Moscariello, CMC Biologics, United States
Jill Myers, Fortress Biotech, United States
Marcel Ottens, Delft University of Technology, Netherlands
Lars Pampel, Novartis, Switzerland
Michael Phillips, EMD Millipore, United States
David Robbins, MedImmune, United States
Jeff Salm, Pfizer, United States
Peter Tessier, Rensselaer Polytechnic Institute, United States
Nigel Titchener-Hooker, University College London, Great Britain
Nihal Tugçu, Merck & Co. Inc., United States
Ganesh Vedantham, Amgen, United States
Suresh Vunnum, Amgen, United States
Andrew Zydney, Penn State University, United States

Debate Session Chairs

Nigel Titchener-Hooker, University College London, Great Britain
John Curling, John Curling Consulting AB, Sweden

Poster Session Chairs

Charles Haynes, University of British Columbia, Canada
Abraham Lenhoff, University of Delaware, United States
David Roush, Merck & Co., Inc., United States

A Warm Welcome from the Conference Chairs

Welcome to the 17th Recovery of Biological Products Conference. The theme for RXVII is "Smart Bioprocessing". For more than 32 years the Recovery Conference Series has been the premier international forum for the presentation and discussion of the status, trends and opportunities in the recovery of high value biological products with therapeutic, diagnostic, or industrial use. Our field has experienced significant recent changes including the implementation of biosimilars for protein therapeutics, continuous bioprocessing, disposable technologies, new classes of biological products and personalized medicine. In order to address these significant challenges and opportunities for downstream bioprocessing, our community must become truly "smart" in all aspects ranging from fundamental recovery science to strategic implementation of new technologies and business decisions.

We believe that this Conference is the premier forum for bringing together the top academics, industrial scientists and engineers to stimulate discussions and debate about how best to move our field to the next level. Interest in the Recovery Conference has remained high as evidenced by the number of applications for attendance, the generosity of our corporate sponsors, and the number of abstracts submitted for presentation. We have designed the program to exceed your expectations of scientific excellence, relevance and diversity of topics, and to provide opportunity for informal discussion. In addition to the outstanding oral and poster sessions, we will have several world class keynote addresses as well as an entirely new format, a series of three debates which promise to raise the level of discourse to a new level.

This Conference has required a huge effort on the part of many individuals. We are extremely grateful to our oral session chairs for their help in creating a state of the art program. Our poster session chairs have done a wonderful job in assembling papers that will assure that the Poster Sessions continue to be a centerpiece of the conference. We are very grateful to our many corporate sponsors for their generosity in underwriting the expense of this program to ensure its success. Finally, we thank the team at Precision Meeting and Events for their dedication throughout our two years of planning and developing all the details related to this conference.

Welcome to Bermuda. We hope you will enjoy the Conference, the beautiful venue, the activities and the stimulating discussions.

All our best,

Steve Cramer | Guenter Jagschies | Phil Lester

General Conference Information

Welcome Hospitality

LOCATION: Frangipani Room, Mezzanine Level

On Saturday and Sunday, all participants and guests arriving early to the hotel that are unable to check in are welcome to relax in the hospitality suite. Enjoy a beverage and a chance to put your feet up.

Guest Hospitality

LOCATION: Rose Room, Mezzanine Level

On Sunday from 3:00 – 6:00 PM, registered guests of participants are welcome to gather to meet one another and enjoy a glass of wine and light fare.

Oral Presentations

LOCATION: Mid Ocean Amphitheater, Lower Lobby Level (same location as general session)

Speakers, you may upload and preview your presentations prior to your scheduled talk time. Please make every attempt to hand in your presentation to the conference desk staff via a flash drive no later than the evening before your scheduled time so that it may be loaded to the presentation computer in advance. Should you need to transfer your presentation another way, please let the staff know and we will provide other options. Note that there will only be one presentation computer. We are unable to accommodate requests for use of your own laptop.

Poster Presentations

LOCATION: Poinciana Ballroom I & II

Posters should be in place no later than 8:00 AM, Monday, 20 June and removed by 10:00 AM, Thursday, 23 June. Posters remaining after 12:00 PM Thursday will be discarded.

Policy on Publication

The Conference does not publish Proceedings. Participants should obtain individual permission from presenters if they wish to have copies of slides, posters, or other materials.

Recording and Photography

The Conference Chairs would like to remind participants that both audio and visual recording of any session during the Conference is not permitted. Photography at oral sessions and photographic documentation of posters is not permitted unless by express permission from the presenting author.

Name Badges

Please wear your name badge during the Conference. Badges will be checked upon entrance to all technical sessions and social events.

Conference Registration

Conference Registration will be open Sunday, 19 June from 12:00 PM – 6:00 PM in Poinciana Foyer on the lobby level of the hotel. The Conference Staff is here to assist you with anything you need. 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.

Hotel Direction Information

The daily general sessions will take place in Mid Ocean Amphitheater located on the lower lobby level. Poster sessions will take place in Poinciana Ballroom I & II on the lobby level. Breakfasts and the Sunday Welcome Dinner will be on the Great Sound Lawn under a tent. Lunch and dinner locations vary so please check your schedule for locations. There will also be staff present to direct you.

Tour and Recreational Information

If you have pre-registered for activities, your tour tickets will be included in your registration materials. Please be sure to bring those tickets 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.

Messages

There will be a message board located in Poinciana Foyer near the Registration Desk. Please check the board during breaks. Messages will not be personally delivered and technical sessions will not be interrupted.

Attire

Dress during the conference is casual. Typical high temperatures in Bermuda in June are high 70's or low 80's (°F)/high 20s (°C). Typical low temperatures are in the mid 70's (°F)/mid 20's (°C.) Please bring sunscreen and hats so that you may safely enjoy the beautiful weather.

Hotel Check-Out and Payment

Hotel accommodations from Sunday, 19 June – Thursday, 23 June are included in your registration fee. If you are staying additional nights prior to and/or after the conference, the hotel has been notified of your arrival and departure dates. 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 telephone, fax, Internet access fees, 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.

Fairmont President's Club

We strongly encourage you to join the Fairmont President's Club if you didn't prior to arriving in Bermuda. Joining the recognition club is the only way you will have complimentary wifi access, and access to the fitness center and spa. Unfortunately, the hotel will not grant access to these amenities unless you sign up for the program. Please visit https://www.fairmont.com/fpc/enroll/ to enroll.

Activity Information

We have scheduled activities for Monday and Wednesday. Please see below for descriptions.

Activity Voucher

The Conference Chairs are providing each participant with a credit worth $75.00 to apply towards the RXVII activities. If you have pre-registered for an activity prior to the conference, your credit card was charged the total amount minus the $75.00 credit. If you haven't pre-registered, please visit the Conference Registration desk in Poinciana Foyer to sign up for an activity of your choice.

The activities in or near the hotel, and the ferry boat to Hamilton (the ferry boat is complimentary to all hotel guests) are also wonderful options. Please visit the conference registration desk for assistance. We will assist with applying your $75 voucher to these activities as well.

Monday | 20 June 2016

Water-based activities depart from the Waterlot Dock at the Fairmont Southampton. The St. George tours and the Railway Bike Tour depart from the main entrance of the resort. You will be returned to the resort with time to prepare for dinner.

ST. GEORGE WALKING TOUR

PRICE: $60.00 per person
TIME: 1:15 pm – 4:45 pm

Guests will be guided on a walking tour of St. George by one of the Towne's most well-known residents. Stops include the World Heritage Center, Ordinance Island, Perfumery, forts and boutique museums. It also includes private personalized tours of the State House. Stories told by well-known residents and stops at quaint local eateries and shops.

ST. GEORGE BIKE TOUR

PRICE: $68.00 per person
TIME: 1:15 pm – 4:45 pm

Covers over 10 historic sites, beaches, eateries, museums, cemeteries and coastline in a comfortable, scenic area loop. Includes guide, bike, helmet, locks and baskets.

RAILWAY BIKE TOUR

PRICE: $85.00 per person
TIME: 1:30 pm – 5:00 pm

Explore the history and beauty of Bermuda by joining a guided tour of the historic Railway Trail by bicycle. Includes transportation from the Fairmont Southampton to and from the trail, guide, mountain bike, helmet, bottled water and a 30 minute beach break.

KAYAKING

PRICE: $85.00 per person
TIME: 1:30 pm – 5:00 pm

Kayaking in Bermuda is a great way to explore the coves, wildlife, marine life, coral and more. Kayakers will depart from the Waterlot Dock at the Fairmont Southampton.

GLASS BOTTOM & SIGHTSEEING TOUR

PRICE: $40.00 per person
TIME: 1:45 pm – 4:00 pm

Boat will depart from the Waterlot Dock at the Fairmont Southampton and cruise across the Great Sound, under Watford Bridge, past King's Point and Somerset Long Bay. Commentary will be provided. Once at Daniels Head, group will go down below to view coral and the Vixen shipwreck through the glass bottom. A relaxing cruise back to the Fairmont. Cash bar available (not included in price.)

BEACH DAY

Enjoy the beautiful pink sandy private beach at the Fairmont Southampton. Relaxing beach amenities include lockers, towels, showers, chaise lounge chairs, Cabana Bar & Grill & Oceanclub Restaurant. The Dive Bermuda shop is also on the beach. Should you wish to rent a snorkel kit, please visit the registration desk and we will assist in the arrangement to cover the cost.

Wednesday | 22 June 2016

Water-based activities depart from the Waterlot Dock at the Fairmont Southampton. The St. George tours and the Railway Bike Tour depart from the main entrance of the resort. You will be returned to the resort with time to prepare for dinner.

ST. GEORGE WALKING TOUR

PRICE: $60.00 per person
TIME: 1:15 pm – 4:45 pm

Guests will be guided on a walking tour of St. George by one of the Towne's most well-known residents. Stops include the World Heritage Center, Ordinance Island, Perfumery, forts and boutique museums. It also includes private personalized tours of the State House. Stories told by well-known residents and stops at quaint local eateries and shops.

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BERMUDA SHIPWRECK SNORKEL

PRICE: $70.00 per person
TIME: 2:30 pm – 5:30 pm

Take an hour cruise to the shipwreck site where you will plunge into crystal blue water and view the most popular shipwrecks in Bermuda. You will see some of the most spectacular coral reef and marine life along the way. Since this snorkel is offshore and in deeper waters, it is recommended for strong swimmers only. It is subject to weather conditions. Departs from the Waterlot Dock at the Fairmont Southampton.

BEACH DAY

Enjoy the beautiful pink sandy private beach at the Fairmont Southampton. Relaxing beach amenities include lockers, towels, showers, chaise lounge chairs, Cabana Bar & Grill & Oceanclub Restaurant. The Dive Bermuda shop is also on the beach. Should you wish to rent a snorkel kit, please visit the registration desk and we will assist in the arrangement to cover the cost.

Notes

Schedule

Program Overview

Time	Sunday | Arrival day 19-Jun-16	Time	Monday 20-Jun-16	Time	Tuesday 21-Jun-16
		7:00	**Breakfast** GREAT SOUND LAWN	7:00	**Breakfast** GREAT SOUND LAWN
9:00	**Welcome Hospitality** FRANGIPANI ROOM	8:00	**Session 2: New Materials for Downstream Processing** **SESSION CHAIRS:** Andrew Zydney, Michael Phillips MID OCEAN AMPHITHEATER	8:00	**Session 4: Impact from Alternative Expression Systems** **SESSION CHAIRS:** Suzanne Farid, Lars Pampel MID OCEAN AMPHITHEATER
		10:35	**Bioprocess Research and Practice in the Coming Age of Personalized Medicine** Charles Haynes, University of British Columbia MID OCEAN AMPHITHEATER	10:05	**A Holistic Approach to Targeting Disease with Polymeric Nanoparticles** W. Mark Saltzman, Yale University MID OCEAN AMPHITHEATER
12:00	**Registration Opens** POINCIANA FOYER **Poster Set-Up** POINCIANA BALLROOM I & II	11:20	**Session 3: The Marriage of HTS & Process Modelling** **SESSION CHAIRS:** Marcel Ottens, Ernst Broberg Hansen MID OCEAN AMPHITHEATER	10:50	**Session 5: Priorities in Cost & Performance Improvements** **SESSION CHAIRS:** David Robbins, Sanchayita Ghose MID OCEAN AMPHITHEATER
		12:55	**Lunch break** (boxed lunch grab-n-go in MID OCEAN FOYER and depart for activities)	12:25	**Lunch break** WINDOWS ON THE SOUND RESTAURANT
15:00	**Guest Hospitality** ROSE ROOM	13:25	**Activity Afternoon**	14:25	**Session 6: Purification of non-Protein Therapeutics** **SESSION CHAIRS:** Michael Laska, Caryn Heldt MID OCEAN AMPHITHEATER
16:00	**Conference Opening** **OPENING REMARKS** Steve Cramer, Günter Jagschies, Phil Lester MID OCEAN AMPHITHEATER				
16:20	**Session 1: The Origin of Impurities** **SESSION CHAIRS:** Ganesh Vedantham, Ashraf Amanullah MID OCEAN AMPHITHEATER				
18:30	**Welcome Reception** GREAT SOUND LAWN	18:45	**Dinner** POINCIANA BALLROOM III	17:30	**Dine-Around** (HOTEL RESTAURANTS) Bacci, Newport Room, Ocean Club, Waterlot
19:15	**What's in Your Blood? The Circulating Antibody Repertoire in Humans** George Georgiou, University of Texas				
20:00	**Opening Dinner** GREAT SOUND LAWN	20:00	**Poster Session 1** **SESSION CHAIRS:** Charles Haynes, Abraham Lenhoff, David Roush POINCIANA BALLROOM I & II	19:45	**Debates Session I / II / III (in series)** **SESSION CHAIRS:** Nigel Titchener Hooker, John Curling MID OCEAN AMPHITHEATER
22:00	**End of Day**	22:00	**End of Day**	22:00	**End of Day**

Time	Wednesday 22-Jun-16	Time	Thursday 23-Jun-16	Time	Friday | Departure Day 24-Jun-16
7:00	**Breakfast** Great Sound Lawn	7:00	**Breakfast** GREAT SOUND LAWN	7:00	**Breakfast** GREAT SOUND LAWN
8:00	**Session 7: Purification from Platform to Diversity** **SESSION CHAIRS:** Nihal Tugçu, Dorothee Ambrosius MID OCEAN AMPHITHEATER	8:00	**Session 9: Next Generation Unit Operations & Integrated Processes** **SESSION CHAIRS:** Jeff Salm, John Moscariello MID OCEAN AMPHITHEATER		
10:35	**The future of Biomanufacturing** Jörg Thömmes, Biogen MID OCEAN AMPHITHEATER	10:35	**Trends in the Global Burden of Disease: Early Results from the Global Burden of Disease 2015 Study and Forecasts to 2040** Christopher Murray, Institute for Health Metrics and Evaluation MID OCEAN AMPHITHEATER		
11:20	**Session 8: Process Challenges with Biosimilars** **SESSION CHAIRS:** Suresh Vunnum, Jill Myers MID OCEAN AMPHITHEATER	11:20	**Session 10: Biomolecular Modelling for Manufacturability** **SESSION CHAIRS:** Christopher Dowd, Peter Tessier MID OCEAN AMPHITHEATER		
12:55	**Lunch break** (boxed lunch grab-n-go in MID OCEAN FOYER and depart for activities)	12:55	**Lunch Break** WINDOWS ON THE SOUND RESTAURANT		
13:25	**Activity Afternoon**	14.15	**Session 11: New Developments in PAT and QbD** **SESSION CHAIRS:** Gisela Ferreira, Thorsten Lemm MID OCEAN AMPHITHEATER		
		16:20	**Session 12: Increasing Patient Access to Biopharmaceuticals** **SESSION CHAIRS:** Jon Coffman, Bruno Marques MID OCEAN AMPHITHEATER		
18:45	**Dinner** POOLSIDE	17:55	**End of Scientific Program**		
		19:00	**Closing Reception** FAIRMONT PRIVATE BEACH *Wear comfortable shoes.* *You will be in the sand!*		
20:00	**Poster Session 2** **SESSION CHAIRS:** Charles Haynes, Abraham Lenhoff, David Roush POINCIANA BALLROOM I & II	20:00	**Closing Dinner** FAIRMONT PRIVATE BEACH *Wear comfortable shoes.* *You will be in the sand!*		
22:00	**End of Day**	22:00	**End of Conference**		

Sunday | 19 June 2016

9:00 – 17:00
Hospitality Suite for early arrivals
FRANGIPANI ROOM

12:00
Registration Opens
POINCIANA FOYER

Poster Set-Up
Poinciana Ballroom I & II

15:00
Guest Hospitality
We invite participant's guests to enjoy a glass of wine and get to know one another
ROSE ROOM

16:00 – 16:20
Opening Remarks
MID OCEAN AMPHITHEATER

16:20 – 18:00
The Origin of Impurities
MID OCEAN AMPHITHEATER

18:30 – 19:15
Cocktails & Entertainment
GREAT SOUND LAWN

19:15 – 20:00
KEYNOTE ADDRESS
George Georgiou, University of Texas
GREAT SOUND LAWN

20:00 – 22:00
Welcome Dinner
GREAT SOUND LAWN

Monday | 20 June 2016

7:00 – 8:00
Breakfast
GREAT SOUND LAWN

8:00 – 10:05
New Materials for Downstream Processing
MID OCEAN AMPHITHEATER

10:05 – 10:35
Refreshments
POINCIANA FOYER

10:35 – 11:20
KEYNOTE ADDRESS
Charles Haynes, University of British Columbia
MID OCEAN AMPHITHEATER

11:20 – 12:55
The Marriage of HTS & Process Modelling
MID OCEAN AMPHITHEATER

12:55
Boxed Lunch (Grab-N-Go)
MID OCEAN FOYER

13:00 – 18:30
Activities (optional)

18:45 – 19:45
Dinner
POINCIANA BALLROOM III

20:00 – 22:00
Poster Session 1 & Dessert
POINCIANA BALLROOM I & II

Tuesday | 21 June 2016

7:00 – 8:00
Breakfast
GREAT SOUND LAWN

8:00 – 9:35
Impact from Alternative Expression Systems
MID OCEAN AMPHITHEATER

9:35 – 10:05
Refreshments
POINCIANA FOYER

10:05 – 10:50
KEYNOTE ADDRESS
W. Mark Saltzman, Yale University
MID OCEAN AMPHITHEATER

10:50 – 12:25
Priorities in Cost & Performance Improvements
MID OCEAN AMPHITHEATER

12:25 – 14:25
Luncheon
WINDOWS BY THE SOUND RESTAURANT

14:25 – 16:30
Purification of non-Protein Therapeutics
MID OCEAN AMPHITHEATER

17:30 – 19:30
Dine-Around
HOTEL RESTAURANTS: BACCI, NEWPORT ROOM, OCEAN CLUB, WATERLOT

19:45 – 22:00
Debate Sessions
MID OCEAN AMPHITHEATER

Wednesday | 22 June 2016

7:00 – 8:00
Breakfast
GREAT SOUND LAWN

8:00 – 10:05
Purification from Platform to Diversity
MID OCEAN AMPHITHEATER

10:05 – 10:35
Refreshments
POINCIANA FOYER

10:35 – 11:20
KEYNOTE ADDRESS
Jörg Thömmes, Biogen
MID OCEAN AMPHITHEATER

11:20 – 12:55
Process Challenges with Biosimilars
MID OCEAN AMPHITHEATER

12:55
Boxed Lunch (Grab-N-Go)
MID OCEAN FOYER

13:00 – 18:30
Activities (optional)

18:45 – 19:45
Dinner
POOLSIDE

20:00 – 22:00
Poster Session 1 & Dessert
POINCIANA BALLROOM I & II

Thursday | 23 June 2016

7:00 – 8:00
Breakfast
GREAT SOUND LAWN

8:00 – 10:05
Next Generation Unit Operations & Integrated Processes
MID OCEAN AMPHITHEATER

10:05 – 10:35
Refreshments
POINCIANA FOYER

10:35 – 11:20
Keynote Address
Christopher Murray, Institute for Health Metrics and Evaluation
MID OCEAN AMPHITHEATER

11:20 – 12:55
Biomolecular Modelling for Manufacturability
MID OCEAN AMPHITHEATER

12:55 – 14:00
Luncheon
WINDOWS BY THE SOUND RESTAURANT

14:15 – 15:50
New Developments in PAT and QbD
MID OCEAN AMPHITHEATER

15:50 – 16:20
Refreshments
POINCIANA FOYER

16:20 – 17:55
Increasing Patient Access to Biopharmaceuticals
MID OCEAN AMPHITHEATER

19:00 – 22:00
Closing Banquet
FAIRMONT PRIVATE BEACH
(bus transportation will be provided for those that prefer not to walk)

Friday | 24 June 2016

7:00 – 10:00
Breakfast
GREAT SOUND LAWN

Notes

Keynote Speakers

George Georgiou
University of Texas

Sunday, 19 June, 19:15 – 20:00

GREAT SOUND LAWN

Charles Haynes, Ph.D., P.Eng., FCIC, FRSC
University of British Columbia

Monday, 20 June, 10:35 – 11:20

MID OCEAN AMPHITHEATER

W. Mark Saltzman
Yale University

Tuesday, June 21, 10:05 – 10:50

MID OCEAN AMPHITHEATER

Jörg Thömmes
Biogen

Wednesday, June 22, 10:35 – 11:20

MID OCEAN AMPHITHEATER

Christopher Murray
Institute for Health Metrics and Evaluation

Thursday, June 23, 10:35 – 11:20

MID OCEAN AMPHITHEATER

George Georgiou

BIOGRAPHY

George Georgiou is a Professor at the University of Texas, Austin. He received his B.Sc. from the University of Manchester, U.K. and Ph.D. from Cornell. His research is focused on understanding the serological antibody repertoire (as well as the BCR and TCR repertoires) in human health and disease and on the discovery and preclinical development of enzyme and antibody therapeutics for cancer and for inborn errors of metabolism.

Professor Georgiou was elected to the National Academy of Engineering (2005), National Academy of Medicine (2011) and the American Academy of Arts and Sciences (2016). He is also a Fellow of the American Institute for Biological and Medical Engineers (AIMBE), the American Academy of Microbiology and the American Association for the Advancement of Science (AAAS). He is the author of >240 research publications and co-inventor of 87 issued or pending US patents, more than 65% of which (comprising 24 distinct technology suites) have been licensed to 27 pharma & biotech companies. He founded GGMJD (1999; acquired by Maxygen in 2000), Aeglea Biotherapeutics (2013-Present; NASDAQ: AGLE) and Kyn Therapeutics Inc. (2015-Present) and currently serves as a Director and Chairman of the SAB for both companies. In 2013 Georgiou was selected as one of the top 20 Translational Researchers by Nature Biotechnology.

ABSTRACT

What's In Your Blood?
The Circulating Antibody Repertoire in Humans

George Georgiou

Antibodies are present in blood at high concentrations (about 10 mg/ml) and are critical for defense against pathogens. Notably, the main mechanism of protection to infection elicited by nearly all approved vaccines is the production of circulating antibodies that bind to, and neutralize the pathogen.

The human immune system can generate well over 10^{12} different antibodies, yet only a relatively small set (which we estimate is of the order of $2x10^4$ antibody proteins) are present in the blood of an individual at any time. Remarkably, more than 100 years since the discovery of antibodies, it is only possible to determine whether an individual has an antibody response to a pathogen (say, HIV or strep tests) but not the number of distinct antibodies, their amino acid sequences, relative amounts or biological functions of the pathogen-specific antibodies produced in that person. Understanding the nature of antibodies elicited by disease or vaccination is very important for therapeutic and prophylactic purposes.

We have developed a suite of proteomic, microfluidic, protein engineering and informatics technologies that has enabled the deconvolution of the identities and relative amounts of antibodies in biological fluids and the delineation of the relationships between antibody production and the relevant B cell immunological mechanisms. This unique research toolset has enabled us to address a plethora of fundamental issues related to human health and the development of therapeutics and vaccines. Topics that will be discussed in this talk will include:

1. How he antibody repertoire informs on strategies to improve the seasonal influenza vaccine.
2. Identification of therapeutic antibodies directly from patients that have overcome disease
3. How is antibody immunity shaped by age and by persistent infection or inflammation

Charles Haynes, Ph.D., P.Eng., FCIC, FRSC

BIOGRAPHY

Dr. Charles Haynes is a Professor and holds the Canada Research Chair in Interfacial Biotechnology within the Michael Smith Laboratories and the Department of Chemical and Biological Engineering of the University of British Columbia. The central objective of Dr. Haynes' research program is to improve our understanding of the interfacial and binding-recognition behavior of biomolecules and cellular systems, and to use this fundamental knowledge to invent new technologies and instruments for isolating and analyzing biological analytes from complex samples.

From that work, he has pioneered and licensed a number of technologies to efficiently localize, purify, or analyze biomolecules that have achieved widespread use in the biotechnology and healthcare industries. Companies created based on those inventions include EIChrom Inc., CBD Technologies Inc., and, more recently, AbCellera Inc., which uses novel microfluidic enabling technologies developed in the Haynes lab to discover and characterize monoclonal antibodies, and 3Ci Diagnostics Inc., a new personalized medicine company that markets digital PCR-based tests for genetic variations prognostic or theranostic of life-threatening diseases, including a range of cancers. Dr. Haynes is a Fellow of the Royal Society, and recipient of several awards, including the Winnaker Technology Transfer Award, the Les Shemilt Award, and the BC Gold Medal in Innovation.

ABSTRACT

Bioprocess Research and Practice in the Coming Age of Personalized Medicine

Charles (Chip) Haynes
Michael Smith Laboratories
University of British Columbia

The potential for predictive or precision medicine to improve patient care and speed development of new biologic treatments has only begun to be realized. Fueled by major initiatives such as the Precision Medicine Initiative, 'omics science is now rapidly discovering genes harboring polymorphic or somatic variations that contribute to human disease, linking genetic variations to patient response to dozens of treatments, and identifying the molecular causes of major diseases, particularly certain forms of cancer. This is allowing development of powerful diagnostic tests of genetic and gene-product variations that are prognostic or theranostic of patient response to targeted therapy. The opportunities provided by personalized medicine are tremendous to improve health and health outcomes, but the challenges to its effective implementation are also tremendous. Adoption of personalized medicine is challenged by the lack of a comprehensive strategy to deliver its benefits to patients. That strategy must include ways to rapidly and cost-effectively develop and manufacture new targeted therapies that actually improve treatment efficacy without complications, and to optimize treatment by steering patients to the right drug at the right dose at the right time. Initiatives in our and other laboratories aimed at addressing needs within this strategy are under way, and I will describe those needs and their importance to overcoming current challenges to realizing the personalized medicine concept, and then present engineering-based technologies in development that might serve to help realize the promise of personalized medicine to improve care while keeping costs in check. These include creation of clinically suitable technologies for multiplexed multi-loci testing for genetic variant signatures for common, complex diseases, assembly of an ensemble of existing and new classes of biologic drugs and new ways to more rapidly and effectively screen them to identify promising leads, and creation of scaled-down platforms that greatly accelerate bioprocess design, including clonal selection and testing, culture protocols, and challenging elements of the downstream process.

W. Mark Saltzman

BIOGRAPHY

W. Mark Saltzman is an engineer and educator. His research has impacted the fields of drug delivery, biomaterials, nanobiotechnology, and tissue engineering: this work is described in more than 300 research papers and patents. He is also the author of three textbooks: *Biomedical Engineering* (Second Edition, 2015), *Tissue Engineering (*2004), and *Drug Delivery* (2001).

Mark Saltzman graduated with distinction from Iowa State University with a B.S. in chemical engineering (1981), He received an S.M. in chemical engineering (1984) and a Ph.D. in medical engineering (1987) from MIT. He has served as a faculty member at Johns Hopkins University, Cornell University, and Yale University. At Yale, Dr. Saltzman was appointed as the Goizueta Foundation Professor of Chemical and Biomedical Engineering in July of 2002, and served as the founding chair of Yale's Department of Biomedical Engineering in 2003-2015.

Dr. Saltzman has been recognized widely for his excellence in research and teaching. He has received the Camille and Henry Dreyfus Foundation Teacher-Scholar Award (1990); the Allan C. Davis Medal as Maryland's Outstanding Young Engineer (1995); the Controlled Release Society Young Investigator Award (1996); the Professional Progress in Engineering (2000) and Professional Achievement Citation in Engineering (2013) Awards from Iowa State University (2000). He has been elected a Fellow of the American Institute for Medical and Biological Engineering (1997); a Fellow of the Biomedical Engineering Society (2010); a Member of the Connecticut Academy of Science & Engineering (2012); a Fellow of the National Academy of Inventors (2013), and a Member of the US National Academy of Medicine (2014).

ABSTRACT

A Holistic Approach to Targeting Disease with Polymeric Nanoparticles

W. Mark Saltzman
Department of Biomedical Engineering
Yale University, New Haven, CT 06511, USA

The primary goal of nanomedicine—using materials such as polymer nanoparticles—is to improve clinical outcomes. For example, targeted nanoparticles can be engineered to reduce non-productive distribution while improving diagnostic and therapeutic efficacy. Paradoxically, as the field has matured, the notion of 'targeting' has been minimized to the concept of increasing affinity of a nanoparticle for its target, and most applications have focused on intravenous delivery, where this kind of targeting is difficult to achieve. Here, I present examples of alternate approaches, in which nanoparticle design is used to achieve a positive outcome in the treatment of brain tumors, targeted correction of a single-gene disorder, long-lasting, safe prevention of UV-mediated DNA damage in skin, and antibody-mediated targeting of inflamed endothelium. These examples all involve targeted nanoparticles, but with unique designs that guide their biological activity.

Jörg Thömmes

BIOGRAPHY

Dr. Jorg Thommes is currently the Senior Vice President of Technical Development at Biogen. This function is responsible for development of Biogen's manufacturing processes at lab and pilot scale for all therapeutic modalities ranging from proteins to oligonucleotides and small molecule pharmaceuticals. Previous positions include Senior Vice President of Operations Technology and Innovation, Vice President of Global Engineering and Facilities, and Vice President of Biopharmaceutical Development. Jorg graduated from University of Bonn, Germany with a degree in Chemistry (Diplom-Chemiker) and holds a doctorate in natural sciences from the same university. Post doctorate, Jorg spent nine years at the Institute of Enzyme Technology at University of Dusseldorf, Germany and received an advanced research and teaching degree (Habilitation) in Biochemical Engineering. Jorg is currently the chairman of the board of the Recovery of Biological Products Conference series.

ABSTRACT

Manufacturing of Biological Products – Lessons from the Past and Visions for the Future

Jorg Thommes

From the point of view of a process development scientist, the development of biopharmaceutical manufacturing over the past four decades is nothing short of amazing. From the time when manufacturing of gram quantities of recombinant proteins required very large volume bioreactors and purification was considered an art to today's reality with more than 10 metric tons of annual therapeutic protein manufacturing, our industry has experienced a more than 1000 fold increase in productivity. However, if one were to analyze this history from an outsider's point of view, then the conclusion could be that nothing has changed over four decades: the fundamental technology is still based on large stirred tank reactors followed by chromatography. From afar it may appear that innovation has been remarkably absent, which an outsider could explain by the absence of any market pressures.

Both these observations are true in the context of the observer's environment; the question is, what can we learn from both interpretations of our industry's history and can we make some predictions of what the future holds?

Significant debate has occurred in literature and at conferences on whether small volume flexible manufacturing will replace traditional fixed manufacturing infrastructure. Many have postulated that disruptive innovation is a must in biomanufacturing, while others have found that existing infrastructure and technologies can be refined to deliver on the promise of biotherapeutics. Continuous protein manufacturing, while around for decades without receiving much attention, has come into focus both in industry and at regulatory agencies; it is discussed oftentimes as the next generation of manufacturing processes. What role can these different approaches play in the future of biotherapeutics?

This presentation will try to analyze how our industry reached the present state of biotherapeutics manufacturing and attempt to draw some conclusions on what the next steps for biomanufacturing might be. We will explore how different therapeutic fields may pose different challenges to manufacturing and which manufacturing solutions could meet them. From localized small volume manufacturing for rare diseases where accessibility is the driver, to very large volume manufacturing for millions of patients with affordability drivers, to biosimilars manufacturing with sales price in mind, solutions will vary from flexible factories to operational excellence of traditional facilities and approaches employed in the manufacturing of industrial enzymes. We will also try to expand the discussion from protein manufacturing to novel (or not so novel) modalities.

Biomanufacturing translates the promise of science into products for patients. The full breadth of therapeutic opportunities in our industry will most likely not be met by a one size fits all future manufacturing approach. Solutions will have to be developed in the context of the therapeutic area, its market size, and economic pressures. This presentation will attempt to span the breath of challenges and potential solutions for our industry.

Christopher J L Murray

BIOGRAPHY

Christopher J.L. Murray, MD, DPhil, is a Professor of Global Health at the University of Washington and Institute Director of the Institute for Health Metrics and Evaluation (IHME) whose career has focused on improving health for everyone worldwide by improving health evidence. A physician and health economist, his work has led to the development of a range of new methods and empirical studies to strengthen health measurement, analyze the performance of public health and medical care systems, and assess the cost effectiveness of health technologies. Dr. Murray is a founder of the Global Burden of Disease (GBD) approach, a systematic effort to quantify the comparative magnitude of health loss due to diseases, injuries, and risk factors by age, sex, and geography over time. He led the collaborative of almost 500 researchers from 50 countries that produced the Global Burden of Diseases, Injuries, and Risk Factors Study 2010 (GBD 2010).

In his earlier work, Dr. Murray focused on tuberculosis control and the development with Dr. Alan Lopez of the GBD methods and applications. From 1998 to 2003, Dr. Murray worked at the World Health Organization (WHO), where he served as the Executive Director of the Evidence and Information for Policy Cluster while Dr. Gro Harlem Brundtland was Director-General. He went on to become Director of the Harvard Initiative for Global Health and the Harvard Center for Population and Development Studies, as well as the Richard Saltonstall Professor of Public Policy at the Harvard School of Public Health, from 2003 until 2007. Dr. Murray has authored or edited 14 books, many book chapters, and more than 250 journal articles in internationally peer-reviewed publications. He holds Bachelor of Arts and Science degrees from Harvard University, a DPhil in International Health Economics from Oxford University, and a medical degree from Harvard Medical School.

ABSTRACT

Trends in the Global Burden of Disease: Early Results from the Global Burden of Disease 2015 Study and Forecasts to 2040

The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) is the largest and most comprehensive effort to date to measure epidemiological levels and trends worldwide. The results of the GBD study show major progress in combating infectious disease, but also significant gaps in tackling causes of death and disability that could be reduced with increased access to drugs, vaccines, and primary health care. Countries around the world continue to undergo an epidemiological transition from communicable to non-communicable diseases as the major contributors to the burden of disease. The GBD is a valuable resource for understanding trends and patterns of disease burden and allows users to understand and visualize the disease profile of a given population. Consequently policy officials, government leaders, and other public health advocates are better able to articulate the need for resources in order to target and reduce the causes of death and disability in that population.

New research from the Institute for Health Metrics and Evaluation (IHME) at the University of Washington will develop future disease burden scenarios that will create a better understanding of population and country disease profiles over the next 25 years. Once baseline scenarios are constructed, users will have the ability to propose what-if scenarios to model future trends. For example, health system managers could analyze the impact of introducing a new drug or technology on the future burden of disease of a population. The possibilities for this work have clear implications for drug, vaccine, and technology development.

Oral Abstracts

Session 1 | The Origin of Impurities

SESSION CHAIRS:
Ganesh Vedantham, Amgen
Ashraf Amanullah, ATyr Pharma

1. The Sources of mAb Aggregate and Process Control Strategy

**Min Zhu, MedImmune, United States*
Guillermo Miro-Quesada, MedImmune, USA
Sanjeev Ahuja, MedImmune, USA
Tizita Mammo, MedImmune, USA
Adrian Man, MedImmune, USA
Karim Nakuchima, MedImmune, USA
Allen Bosley, MedImmune, USA
Kara Shafer, MedImmune, USA
Erica Hackner, MedImmune, USA
David Robbins, MedImmune, USA

Protein aggregate is a critical quality attribute (CQA) that needs to be adequately controlled during manufacturing of monoclonal antibody (mAb) product. The types of the aggregate can be covalently formed oligomers (e.g. disulfide-linked), or reversible or irreversible non-covalent dimer, trimer and eventually oligomers, typically mediated by hydrophobic interaction or mAb denaturing. Aggregate formation is observed in many manufacturing process steps from cell culture, purification, and formulation unit operations as well as during process holds. This presentation will incorporate understanding of aggregate formation to illustrate and explain how manufacturing conditions turn into the source of the aggregate formation. The work was built on both well-established principles and experiences in the field. These conditions include 1) cell culture medium components and feed strategy, 2) interfacial stresses such as foaming and mixing condition, 3) sensitivity to pH, temperature and duration during the low pH viral inactivation step; 4) elution pH for Protein A and CEX chromatography; 5) sensitivity to photostress under process relevant conditions; and 6) duration and temperature for in-process hold. Several analytical techniques, such as mass spectrometry (MS), differential scanning calorimetry (DSC) and circular dichroism (CD) are utilized to connect process observation with aggregate formation mechanism. Each mAb sub-class shows different level of the sensitivity to specific manufacturing conditions and aggregation mechanisms. In addition, through empirical and molecular modeling analyses, the inputs on the design of the molecule to avoid inherent aggregation will be discussed. The artificial aggregation phenomena due to sample handling, specifically for cell culture fluid samples will be covered. In the end, a control strategy as a holistic and integrated approach is proposed based on the in-depth understanding of the sources of the aggregate formation. The strategy begins with molecular design, balancing an appropriate level of the risk at each unit operation; eventually demonstrates the aggregate formation is in control.

2. Optimising Cell Design Steps for Efficient Removal of E. coli Host Cell DNA Impurities from Fab' Fragment Process Streams

Desmond, Schofield, UK
Ernestas Sirka, UCL, UK
Eli Keshavarz-Moore, UCL, UK
John Ward, UCL, UK
**Darren N. Nesbeth, University College London, United Kingdom*

We previously used cell-engineering approaches to construct an Escherichia coli (E. coli) Fab' fragment production strain in which exogenous Fab' and staphylococcal nuclease are both expressed in the periplasmic space via translocation by the SEC pathway. The strain was capable of post-homogenisation clearance of genomic DNA, with a resultant reduction in feedstock viscosity and improvement in clarification performance, but also increased Fab' leakage to the surrounding growth medium. We sought to improve Fab' retention in the engineered strain by using a different translocation route for periplasmic nuclease expression. We directed translocation of nuclease by the signal recognition particle (SRP) route and tested if route choice influenced periplasmic leakage of the recombinant Fab' fragment. In 5L scale fermentation experiments, SRP-routed nuclease co-expression coincided with reduced Fab' leakage relative to the SEC-routed nuclease strain. Mathematical modelling of periplasm filling and overload predicted the rate of intracellular accumulation of Fab' and predicts the onset of Fab' leakage from cells at 6% periplasm occupancy. We conclude that exploitation of different translocation pathways can improve industrial performance of engineered E. coli production strains that feature recombinant proteins intended to effect impurity removal in addition to recombinant protein products.

3. Isolation and Characterization of Incompletely Assembled Bispecific Antibody Variants

**Thomas von Hirschheydt, Roche Innovation Center Penzberg, Germany*

The formation of unsymmetrical bispecific antibodies requires specific protein engineering to establish the right assembly of the protein chains. However, these novel antibody formats often come along with new types of product related byproducts caused by the protein engineering which may not be addressed by generic purification protocols. Bispecific CrossMAbs represent a new member of the IgG family. Key feature of bispecific CrossMAbs is a domain crossover in the Fab region. While the fully assembled bispecific CrossMAbs behave like standard IgGs, new types of possible side product were found for CH1-CL-crossing: incompletely assembled CrossMAb fragments lacking the crossed light chain, (3/4 antibodies) or lacking both light chains (heavy chain dimers). This observation is surprising because a prerequisite for antibody secretion is their assembly into a defined quaternary structure, composed of two heavy and two light chains for IgG. Unassembled heavy chains are actively retained in the endoplasmatic reticulum (ER). Buchner et al. showed, that the CH1 domain of the heavy chain is intrinsically disordered in vitro, which sets it apart from other antibody domains. It folds only upon interaction with the light chain CL domain. Therefore, in natural IgGs lacking a light chain cannot be expected to be secreted into the medium during the fermentation process. In contrast, bispecific CrossMAbs have been observed to show a different behavior and the incompletely assembled "¾ antibody" fragments can be detected in the fermentation supernatant. In addition the correct folding of the CH1

Oral Abstracts

domain of the natural heavy chain can be supported by the engineered heavy chain to form a stable heavy chain dimer without any light chains. Independent chromatographic methods for depletion of "¾ antibodies" and the targeted expression and isolation of heavy chain dimers will be presented as well as biochemical characterization and structure elucidation by transmission electron microscopy.

Session 2 | New Materials for Downstream Bioprocessing

SESSION CHAIRS:

Andrew Zydney, Penn State University
Michael Phillips, EMD Millipore

1. Evaluation of Next Generation Hybrid Filters for Simultaneous Clarification and Purification of Biologics

**NRIPEN SINGH, Bristol Myers Squibb, United States*
Michael Peck, Bristol Myers Squibb, United States
Abhiram Arunkumar, Bristol Myers Squibb, United States
Michael Borys, Bristol Myers Squibb, United States
Zhengjian Li, Bristol Myers Squibb, United States
Alexei Voloshin, 3M, United States
Jonathan Hester, 3M, United States

Recent progress in mammalian cell culture process has resulted in significantly increased product titers, but also resulted in substantial increases in process and product related impurities. Due to the diverse physicochemical properties of these impurities, there is an ever increasing need for new technologies that offer increased productivity and improved economics without sacrificing process robustness required to meet final drug substance specifications. This work examined the use of new generation hybrid filters modified with a high binding capacity of quaternary amine (Emphaze™ AEX) and novel salt tolerant biomimetic (Emphaze™ ST-AEX) ligands for host-cell protein (HCP), residual DNA, soluble aggregate removal and viral clearance. It combines three technologies: anion-exchange (AEX) functional polymers, fine-fiber nonwoven materials, and multizone membranes to deliver an all-synthetic clarifying product containing both an AEX ligand and a bioburden-reduction membrane. Binding isotherms using bovine serum albumin (BSA) were obtained to develop adsorption isotherm models as a function of ionic strength for hybrid filters and compared against traditional positively charged depth filters. The ionic capacity of these depth filters was measured and correlated with their ability to remove impurities for multiple molecules. The AEX capacity of hybrid filters significantly exceeded that of conventional depth filters, providing substantially higher reduction of soluble anionic impurities including DNA and anionic HCPs. Implementing Emphaze™ AEX hybrid filter with quaternary amine functionality at the clarification stage significantly enhanced the performance of Protein A chromatography capture, easing the challenges faced by downstream polishing steps. Furthermore, Emphaze™ ST-AEX filter with guanidinium functionality demonstrated improved process related impurity removal and viral clearance at both low and high conductivity post Protein A chromatography. The consequences of this radically enhanced process performance are far reaching as it permits restructuring and simplification of the downstream polishing train.

2. 3D-Printed Agarose Cation-Exchange Monoliths for Protein Capture from Solids-Laden Feeds

Anne Gordon, University of Canterbury, New Zealand
**Conan Fee, University of Canterbury, New Zealand*
Simone Dimartino, University of Canterbury, New Zealand
Tim Huber, University of Canterbury, New Zealand
Suhas Nawada, University of Canterbury, New Zealand
Don Clucas, University of Canterbury, New Zealand

Conventional preparative-scale chromatography media are typically comprised of randomly packed, spherical particles that vary in size and present mobile-phase flow channels that are complex and ill-defined. Furthermore, packed beds are unsuitable for processing solids-laden feed streams such as fermentation broths. In contrast, additive manufacturing (3D printing) offers the ability to exert control over the size, shape, orientation and spatial placement of solid-phase geometric elements within porous media. Clearly, this implies corresponding control over the mobile-phase channel geometry and thus both solid and mobile phase geometries can be designed a priori. In this presentation, we demonstrate the performance of 3D-printed, agarose, cation exchange monoliths with uniform mobile-phase flowpath and solid-phase geometries, designed to capture proteins while allowing passage of suspended solids. The printed monoliths were 1.0 cm in diameter, with a series of designed lengths of the order 10.0 cm, and the geometric features of the solid-phase and the mobile-phase flowpaths were identical, with characeristic diameters of approximately 200 microns. Moreover, the geometric features were identical in the x, y and z directions. The agarose solid-phase was modified with 6-aminohexanoic acid to impart cation exchange functionality and the monolith was then used to separate cytochrome C and bovine serum albumin as model basic and acidic proteins, respectively, in the presence of yeast cells. Unlike other hybrid systems such as expanded bed adsorption, operation was stable and simple, requiring no special procedures or equipment compared with conventional packed-bed chromatography, despite the presence of cells. We present optical and electron microscope images to show the fidelity between the final prints and the original computer-aided designs and the solid-phase pore structures, respectively. The static protein adsorption capacities are given as Langmuir isotherms and the effects of operating conditions on dynamic binding capacity and loading/elution peak shapes are described. Residence time distribution studies, including cell passage/retention, are also presented. The results show that 3D printing has significant potential as a method for producing preparative-scale monolithic chromatography columns with fine control of solid- and mobile-phase geometries at a resolution suitable for protein capture in the presence of a cell suspension.

3. Application of Smart Polymers in the Downstream Processing of Biological Products

**Milton Hearn, Monash University, Australia*
Pankaj Maharjan, Monash University, Australia
Eva M Campi, Monash University, Australia
Roshanak Sepehrifar, Monash University, Australia
Reinhard I Boysen, Monash University, Australia
William R Jackson, Monash University, Australia

Over the last decade, advances in in the field of biochromatography as it impacts on the analytical and preparative capabilities of downstream processing of

Oral Abstracts

biological products have largely been driven by development of new separation materials and better understanding of the underlying separation mechanisms. The aim here has been to achieve greater selectivity, higher resolution, faster speed, enhanced handling capabilities and overall improved productivity. Of these factors, control over selectivity has remained the single most challenging objective. A highly desirable approach to manipulate selectivity and scalability at the same time as achieving multi-dimensionality in separation-based adsorption phenomena is to use 'smart' stationary phases that have tunable surface properties. In this manner, separation selectivity can be better adjusted to accommodate variation in feedstock composition or operational conditions, thus providing improved access to separation systems that serve as more efficient platform technologies with lower levels of waste generation. This presentation examines recent progress addressing these objectives from the perspective of design, synthesis and use of several new classes of stationary phases that act as stimuli-responsive polymeric (SRPs) adsorbents. Also, the basis of the prevailing separation mechanisms will be contrasted for several new SRP adsorbents[1], formed from the immobilisation onto suitable porous support materials of pre-formed block co-polymers or alternatively in situ grafted polymeric systems, which exhibit changes in their properties in response to an external stimulus. Results will be presented based on batch binding studies, packed bed investigations in different formats and larger scale process applications as well as insights into the controlling capture and release mechanisms where the potential of these novel stimuli-responsive chromatographic materials has been documented for energy efficient purification of biological products generated by recombinant DNA/cell culture methods or during process stream recovery. [1] Hearn, M.T.W., Woonton, B.W., Maharjan, P., De Silva, K., & Jackson, W.R. US Patent 8,877,477 B2 and patents in other national jurisdictions.

4. Novel Ultrafiltration Membranes Produced by Electrospinning

**Mikhail Kozlov, MilliporeSigma, United States*
Alex Xenopoulos, MilliporeSigma, USA
William Cataldo, MilliporeSigma, USA
Clif Ngan, MilliporeSigma, USA

We report the first ultrafiltration membranes based on electrospun nanofibers of extremely small diameter. Highly uniform, composite nanofiber mats were produced by electrospinning a polyamide solution, with the fiber diameter of retentive layer on the order of 10 nm. The nanofiber mat samples were characterized by SEM imaging, water permeability, liquid-air porometry, macromolecule rejection, and performance in model tangential flow filtration processes. The results show that these electrospun nanofiber membranes have the properties of open ultrafiltration membranes. The performance of the nanofiber membranes in ultrafiltration and diafiltration applications was evaluated using a series of dextran feeds and compared to conventional solution-cast polyethersulfone (PES) UF membranes of similar nominal molecular weight cut-off (NMWCO). The nanofiber UF membranes had about two-fold advantage in water permeability and ultrafiltration process flux compared to the benchmarks. These encouraging first results may pave the way for the next generation UF membranes with enhanced performance in purification of biological products such as vaccines and recombinant proteins.

Session 3 | The Marriage of High Throughput Screening and Modeling

SESSION CHAIRS:

Marcel Ottens, Delft University of Technology
Ernst Broberg Hansen, NovoNordisk

1. The Application of High Throughput Process Development Datasets for Smarter Process and Molecular Design

**Gregory Barker, Bristol-Myers Squibb, United States*
Joseph Calzada, Bristol-Myers Squibb, United States
Zheng Ouyang, Bristol-Myers Squibb, United States
Joseph Lomino, Bristol-Myers Squibb, United States
Nate Domagalski, Bristol-Myers Squibb, United States
Stanley Krystek, Bristol-Myers Squibb, United States
Michelle Wang, Bristol-Myers Squibb, United States
LeLand Paul, Bristol-Myers Squibb, United States

The development of biological therapeutics continues to pose significant technical challenges. These include traditional challenges such as scale-up but also the early assessment of manufacturability, building quality by design (QbD) into the process, and ensuring the rapid delivery of biological candidates to the clinic and market (speed to patient). Ensuring each of these aspects are addressed with equal rigor is paramount. High throughput techniques have evolved for most, if not all, of the unit operations used in the cell culture, recovery, and purification of biological molecules. These high throughput techniques can be effective methods to assist with each of the development challenges above and to ensure the rapid progression of candidates but also the development of robust processes. Several key workflows have been introduced in the development of biological molecules at Bristol-Myers Squibb. Specifically, multiple methods have evolved to directly measure the solubility of a candidate molecule as a function of solution conditions, while other techniques focus on understanding chromatographic separations and filtration. These workflows are now mature enough to support a rapid progression of molecules through development. As such, for each of these workflows, we have compiled large datasets for multiple biological molecules. In a similar way, molecular modeling of biologicals has improved with computational power and the ability to mine molecular level descriptors. Advanced computational tools such as molecular dynamics simulations have enabled a more detailed understanding then previous approaches. All of this has led to an additional store of molecular level data that can be readily mined. In this work, we demonstrate novel approaches of data mining and exploration for the comprehensive HTPD and molecular datasets. HTPD datasets are mined independent of molecular information to inform future high throughput activity and understand possible mechanisms for process performance. At a first level, protein solubility mining shows that certain buffer species, pH, and counterion concentrations drive solubility almost independent of structure. Next, the biological molecular descriptors are added to enable further understanding of biochemical and molecular level drivers for process outcomes. These approaches will further demonstrate the utility of HTPD datasets to build smarter processes and biological molecular design.

2. Mechanistic Chromatography Modeling for Industrial Applications – A Lean Approach to a Complicated Tool

Chris Williams, Genentech, United States

Mechanistic chromatography modeling, with the right approach, can potentially provide significant savings in the time and resources required for optimization and characterization of a chromatography operation. However through an industrial perspective, time is invaluable and the opportunity costs of informative but inefficient chromatography modeling activities can be high. A balance between the value that a mechanistic model brings to a controlled biopharmaceutical manufacturing process and the cost to build in additional complexity is critical. The preferred approach would provide the most essential phase appropriate information for industrial applications from the fewest resources, even if not achieving academic expectations. One lean approach enables the very rapid development of models that provide accurate predictions of key process indicators and quality attributes within controlled manufacturing ranges. The approach begins by using a high throughput batch binding screen to assess the binding behavior of a mAb or other biomolecule on the chromatography resin of interest. Binding behavior is used to design small-scale or RoboColumn-scale experiments that can be used for model calibrations. The calibration itself focuses on matching experimentally observed key process indicators (KPIs) and quality attributes of interest, as opposed to chromatogram curve fitting. In this approach, tools have been developed to couple parameters with shared interactions in order to allow the efficient navigation of a multivariate parameter space with direction built on scientific understanding rather than mathematical algorithms. This lean approach enables the adaptation of a generic chromatography model for a given biomolecule in less than 1 week in most cases. The ease with which this approach can be applied by novice modelers is a further advantage. Expanding pH ranges, adding quality attributes, and other phase appropriate modifications to model complexity are only applied when needed.

3. Experimental and Computational Lysate Characterization for Fast Bioprocess Optimization in Tailored Manufacturing

**Pascal Baumann, Karlsruhe Institute of Technology (KIT), Germany*
Tobias Hahn, Karlsruhe Institute of Technology (KIT), Germany
Kai Baumgartner, Karlsruhe Institute of Technology (KIT), Germany
Juergen Hubbuch, Karlsruhe Institute of Technology (KIT), Germany

With biopharmaceutical industry moving away from large quantity blockbuster production of therapeutic agents (‘one drug fits all approach’) to a more personalized medicine (‘tailored manufacturing’), finding a generic approach for purification process development is a key challenge. Straightforward routines, like antibody platform processes, might not be applicable anymore and thus all stages of process development for biopharmaceuticals might require individual and tailored productions. Conventional approaches based on heuristics, high-throughput experimentation (HTE) and statistical modeling alone hardly keep pace with this trend. Expert knowledge, HTE and mechanistic modeling constitute a good foundation for fast and effective process development for new products, but their full potential can only be realized in a combination of these procedures. Upstream processing and product formation is hard to predict with a physico-chemical model which is why this step still requires experimental screening using high-throughput cultivation experiments. Subsequently, the large number of candidate lysates need to be scored in terms of downstream performance. To that end, we first developed deterministic process designs for chromatography based on experimental lysate characterization. In this context, characteristic key parameters serve as a basis to generate new optimized processes for any complex feed stock without any prior knowledge of the feed composition and the physical and chemical nature of the product or impurities. The pH was found to be the central parameter for ion-exchange chromatography optimization shown in a case study of a complex Pichia Pastoris supernatant. Analogously, hydrophobic interaction chromatography processes were determined to be mostly driven by protein solubility, shown for different proteins of acidic, neutral and alkaline isoelectric point. Besides experimental lysate characterization, we developed a hybrid approach combining high-throughput experimentation and in silico lysate scoring. By merging these two technologies, up- and downstream processes could be optimized in an entirely integrated fashion. The evaluation and characterization of a large set of different feed stocks generated by high-throughput cultivations was performed by in silico lysate characterization. This methodology was used in an integrated process optimization study for a phase II liver enzyme produced in Escherichia coli. In summary, we propose two advanced strategies for integrated up- and downstream process optimization. The first is based on deterministic experimental lysate scoring, the other on in silico lysate characterization. Both approaches are qualified for fast and effective process development and identification of critical impurities while requiring minimal sample consumption and experimental effort.

Session 4 | Alternative Expression Systems: Strategic Impact for Biopharmaceuticals?

SESSION CHAIRS:

Suzanne Farid, University College London
Lars Pampel, Novartis

1. Alternate Reality: Alternate Expression Systems and the Future of Biomanufacturing

Chapman Wright, Biogen, USA
Catie Bartlett, MIT, USA
Venkatesh Natarajan, Biogen, United States
Heather Saforrian, Biogen, USA
J. Christopher Love, MIT, USA
**Matthew Westoby, Biogen, United States*

CHO-based manufacturing has been the workhorse for biopharmaceutical production. Despite its success, production with CHO has several challenges, including limits to productivity, high media costs and extensive purification requirements. Recent work has focused on improving productivity and cost by adapting CHO to perfusion-based cell culture and running in single-use, continuous manufacturing formats. While these efforts improve flexibility, they result in only incremental improvements in productivity and cost. In addition, these efforts have not been readily transferable to high volume production. To achieve the next level in biopharmaceutical production, alternate high productivity expression systems should be considered. Biomanufacturing 2020 is an initiative at Biogen to create a disruptive, high productivity biomanufacturing platform. Within this initiative, we are identifying and integrating new technologies and processing paradigms that result in at least a 10-fold increase in productivity and significant cost reductions compared to current processes. A critical component of this project is the selection and development of an expression system. In this talk, we describe our process of identifying expression systems of interest, developing selection criteria, and defining a development path for these non-mammalian systems. In addition, we illustrate how an alternate host could enable a smarter, streamlined downstream process and lead to novel manufacturing platforms.

2. Lessons Learned from Developing a Platform Purification Approach for Domain Antibodies

**Andre C. Dumetz, GSK, United States*
Jeff T. Kurdyla, GSK, USA
Gerald J. Terfloth, GSK, USA

The emergence of novel classes of biotherapeutics creates new challenges for the development of purification processes meeting aggressive development timelines. For domain antibodies (dAb) derived from VH or VL domains and expressed in E. coli, a toolbox approach was developed during the last five years to accelerate early phase process development. After an initial risk assessment, a pre-established decision tree was used to define a first intent purification sequence based on the molecular construct. The results from the small scale evaluation were used to select pre-established high throughput (HTP) and small scale column experiments to establish a final process. The initial capture was performed using either Protein A, Protein L or mixed mode chromatography followed by either one or two chromatographic steps. The rapid optimization of the capture step was enabled by a fundamental understanding of the dAb's binding capacity on protein A and protein L resins. The differences in the polishing steps' resin binding properties were characterized using a HTP plate approach. Gradient elution was adopted by first intent. Lastly, the observed variability in HCP levels was addressed by adopting a comprehensive HTP approach to characterize HCP elution profiles to guide polishing step optimization. In all cases, pre-defined work packages leveraged the same raw materials. As a result, raw materials for GMP campaigns could be ordered shortly after the initial small scale evaluation removing process development from the critical path. This approach was implemented successfully for several projects under very aggressive development timelines, demonstrating its ability to address the development challenges due to the diversity in the dAbs.

3. Purification of Secretory IgA from Lemna Minor (duckweed)

*Michel Eppink, Synthon Biopharmaceuticals BV, Netherlands
Meng Liu, Synthon Biopharmaceuticals BV, Netherlands
Guy de Roo, Synthon Biopharmaceuticals BV, Netherlands
Kim Burgers, Synthon Biopharmaceuticals BV, Netherlands
Bram Kamps, Synthon Biopharmaceuticals BV, Netherlands
Danielle van Wijk, Synthon Biopharmaceuticals BV, Netherlands
Gerard Rouwendal, Synthon Biopharmaceuticals BV, Netherlands
Gerry Ariaans, Synthon Biopharmaceuticals BV, Netherlands

In recent years, secretory IgA (SIgA) antibodies have attracted increased attention as potential therapeutics for infectious and malignant diseases. Despite this, SIgA antibodies have not been commercially advanced. One of the main problems hampering work with SIgA antibodies is the lack of established methods for production and purification. Here we present Lemna duckweed as a promising platform for production of SIgA antibodies. The production process comprises (1) expression of SIgA in stably-transformed duckweed, (2) extraction of SIgA by disruption of the plant material (3) removal naturally abundant impurities by acidic precipitation (4) clarification by depth filtration and TFF (5) purification by affinity chromatography followed by polishing steps and (6) formulation in a stable buffer.

Session 5 | Priorities in Cost and Performance Improvements

SESSION CHAIRS:
David Robbins, MedImmune
Sanchayita Ghose, Biogen

1. A Downstream Approach for Maximizing Recoverable Titer and Facility Throughput

*Amit Mehta, Genentech, United States
Asha Radhamohan, Genentech, United States
Benjamin Sackett, Genentech, United States
Chris Williams, Genentech, USA
Mary Mallaney, Juno Therapeutics, United States
Rachel Specht, Genentech, USA
Yinges Yigzaw, Genentech, USA

Over the last two decades, antibody titers have increased by almost a 100-fold which has significantly increased the throughput of manufacturing plants in the biotechnology industry. Key options to further increase throughput include continued increase in titers and/or pursuit of continuous manufacturing. Downstream process bottlenecks have been highlighted as a key challenge with further increase in titers and the industry conversation may have thus pivoted in the direction of continuous manufacturing. Benefits such as robust product quality, better process controls and ease of scaling have also been highlighted with continuous manufacturing. While continuous bioprocessing in principle offers several advantages, its broader adoption will likely be hindered by several technical, operational and near-term economic challenges. Some of these challenges include retooling of an established manufacturing base, fitting complex processes for novel drug formats into continuous manufacturing platform, development of continuous processes in an environment that is shooting for higher productivity and ever shortening clinical development timelines. This talk will focus on a downstream process concept that meets the industry desire for higher throughput by maximizing recoverable titer and leverages recent innovations in protein purification and separation media to overcome downstream bottlenecks. This process concept in combination with recent facility design innovations and single-use technologies can also reduce the capital expenditure by providing high throughput at a reduced scale. Data will be presented with several molecules including molecular format diversity. Overall this transformative but not operationally disruptive process concept can lead to higher return on manufacturing assets, is broadly implementable, accommodates a diverse and novel molecule portfolio, syncs with the ever shrinking development timelines and furthermore has the potential to be semi-continuous for additional facility throughput increases.

2. GMP: Get the Most Out of Your Plant (by integrating unit operations)

Ron Kowle, Eli Lilly, USA
Dayue Chen, Eli Lilly, USA
Samantha Streicher, Eli Lilly, USA
Purbasa Patnaik, Eli Lilly, USA
**Jace Fogle, Eli Lilly, United States*

This talk will highlight two novel approaches for reducing downstream process cycle time and cost – the use of depth filters for clarification as well as retrovirus clearance and a tandem anion exchange/hydrophobic interaction flow through column step. First, the ionic capacity of five commercially available depth filters was measured using a unique counterion displacement method. Small scale virus spiking experiments showed that parvovirus clearance by depth filters may be influenced by process conditions (pH, conductivity) as well as the filter media charge characteristics. However, complete retrovirus clearance was observed on all five filters. A PCR-based assay was developed to directly measure the reduction in Type C retrovirus-like particles across the depth filters in actual process streams. Finally, a tandem anion exchange-hydrophobic interaction chromatography column was designed for an antibody fragment molecule with unique challenges related to HCP clearance and product-related variants. By combining the anion exchange and hydrophobic interaction chromatography unit operations, a highly non-platform process was fit more easily into the manufacturing schedule without compromising product quality. Both the tandem flow through chromatography step as well retrovirus clearance on depth filters have been demonstrated at scale in GMP clinical manufacturing.

3. Meeting Challenges in Productivity and Product Quality Improvements for the Manufacture of a Recombinant Enzyme

**Michaela Wendeler, MedImmune, United States*
Xiangyang Wang, MedImmune, USA

Economical process design can be challenging for non-monoclonal antibody products, especially when low titers conflict with high product demands, and complex separations of product-related impurities are required. This case study illustrates process development for a recombinant enzyme that had to address significant challenges to meet cost and quality targets. The initial process resulted in vastly insufficient productivity due to low expression level, poor enzyme stability, and the presence of closely related protein variants that necessitated low efficiency separations. Systematic advancements in product understanding paved the way for integrated upstream and downstream optimization. We discuss the challenges and opportunities presented by the change to a perfusion upstream process, the evaluation of continuous downstream options, and the implementation of a robust manufacturing process with significantly improved productivity and product quality.

Session 6 | Purification of Non-Protein Therapeutics

SESSION CHAIRS:
Michael Laska, Moderna Therapeutics
Caryn Heldt, Michigan Tech

1. Improving Washing Strategies of Human Mesenchymal Stem Cells Using Negative Mode Expanded Bed Chromatography

Bárbara Cunha, iBET, Portugal
Ricardo J.S. Silva, iBET, Portugal
Margarida Serra, iBET, Portugal
John Daicic, GE Healthcare Bio-Sciences AB, Uppsala, Sweden
Jean-Luc Maloisel, GE Healthcare Bio-Sciences AB, Uppsala, Sweden
John Clachan, GE Healthcare Bio-Sciences AB, Uppsala, Sweden
Anna Åkerblom, GE Healthcare Bio-Sciences AB, Uppsala,, Sweden
Manuel T. Carrondo, iBET, Portugal
Paula M. Alves, iBET, Portugal
**Cristina Peixoto, IBET, Portugal*

The use of human mesenchymal stem cells (hMSC) in clinical applications has been increasing over the last decade. Their immunomodulatory characteristics, as well as capacity in secreting bioactive molecules with anti-inflammatory and regenerative features, have been making them attractive candidates for autologous and allogeneic therapies. However, to be applied in a clinical setting hMSC need to comply with specific requirements in terms of identity, potency and purity. The main aim of this work is to improve established tangential flow filtration (TFF)-based washing strategies, further increasing hMSC purity, using negative mode expanded bed adsorption (EBA) chromatography with a new multimodal prototype matrix based on core-shell bead technology. The matrix was characterized and a stable, expanded bed could be obtained using standard equipment adapted from what is used for conventional packed bed chromatography processes. The effect of different expansion rates on cell recovery yield and protein removal capacity was assessed. The best trade-off between cell recovery (89%) and protein clearance (67%) was achieved using an intermediate expansion bed rate (1.4). Furthermore, we also showed that EBA chromatography can be efficiently integrated on the already established process for the downstream processing (DSP) of hMSC, where it improved the washing efficiency more than 10-fold, recovering approximately 70% of cells after total processing. This strategy showed not to impact cell viability (> 95%), neither hMSC's characteristics in terms of morphology, immunophenotype, proliferation and adhesion capacity and multipotent differentiation potential. Overall, negative mode chromatography represents the beginning of a promising platform for cell therapy applications, where new adsorbents can be designed to have affinity with target impurities (e.g. BSA) and not to the final product itself, the cells. This means that the methodologies herein developed can be adopted to other type of cell products relevant for cell therapy applications.

2. Challenges in the Development and Scale-up of an Attenuated Live Virus Vaccine Candidate

*Marc Wenger, Merck & Co., Inc., United States
Adam Kristopeit, Merck & Co., Inc., United States
Janelle Konietsko, Merck & Co., Inc., United States
Justin Ma, Merck & Co., Inc., United States
Tung Nguyen, Merck & Co., Inc., United States
Katherine Phillips, Merck & Co., Inc., United States
Matthew Woodling, Merck & Co., Inc., United States
Joseph Joyce, Merck & Co., Inc., United States

Prophylactic live attenuated vaccines (LAV) have been successfully developed for multiple viral disease targets, offering an advantage over subunit vaccine approaches by simultaneously stimulating innate, humoral and cellular immune responses. However, the development of robust manufacturing processes for LAVs at commercially viable scales can be challenging, particularly because of the need to use novel and/or adherent cell lines, the inefficient performance of conventional chromatography for processing large viral particles, the presence of similarly sized host-cell microvesicle contaminants, and the complexity of product characterization. Further adding to these challenges, closed-system aseptic processes are required for those viruses too large for terminal sterile filtration, thereby limiting processing options. Highlighting these challenges, we present here on the development of a scalable, fully sterile purification process for a candidate LAV vaccine. In the process development of this vaccine, a variety of purification approaches were evaluated including membrane and monolith supports, newly designed bead-based media for viruses and large biomolecules (e.g. Capto Core 700), and non-chromatographic methods such as selective precipitation and large-pore tangential flow filtration. The final purification unit operations were selected based not only on usual performance criteria such as purification potential and yield but also on their ease to be performed aseptically, with a premium placed on ready-to-use, single-use technologies. However, in one instance, a ready-to-use, single-use format was not available for a promising resin-based chromatography, therefore requiring us to adapt this step to the sterile processing environment. The final purification process enables a scalable path for commercialization, while exceeding targets for purity and yield.

3. Use of Ultrafiltration for the Purification of Conjugated Polysaccharide Vaccines

*Andrew Zydney, Penn State University, United States
Mahsa Hadidi, Penn State University, United States
John Buckley, Pfizer, United States

Capsular polysaccharides from pathogenic bacteria have been used to produce vaccines against important diseases such as pneumonia and meningitis. Traditional polysaccharide vaccines are largely ineffective for children < 2 years of age and elderly people. Effective vaccination can be provided using a conjugate vaccine generated by chemically coupling the polysaccharide to a highly immunogenic protein (e.g., CRM197, a non-toxic mutant of diphtheria toxin). The development of multivalent conjugated vaccines requires the coupling of each purified polysaccharide with the immunogenic protein, with any unreacted (free) polysaccharide removed in the purification process. This purification can be a major challenge in the commercialization of conjugated vaccines. The objectives of our work were to evaluate the potential of using membrane ultrafiltration for the purification of these conjugated vaccines and to develop a fundamental understanding of the factors controlling the transmission of both free polysaccharides and their corresponding conjugates through commercially available ultrafiltration membranes. Experiments were performed using several pneumococcus polysaccharide serotypes (with different size and electrical charge) over a range of solution ionic strength. Polysaccharides were characterized using both dynamic light scattering and size exclusion chromatography. Ultrafiltration data were obtained in a stirred cell using composite regenerated cellulose and polyethersulfone membranes. Polysaccharide transmission in dilute solutions was a strong function of filtrate flux due to concentration polarization effects, with the data in good agreement with the stagnant film model. Polysaccharide fouling became significant at high filtrate flux and when using more concentrated solutions, consistent with the presence of a critical wall concentration for fouling for each serotype. The flux and polysaccharide transmission were also strong functions of solution ionic strength due to a combination of inter- and intra-molecular electrostatic interactions between the charged polysaccharides and the charged membrane. Additional insights into the effects of solution ionic strength were obtained from the changes in effective hydrodynamic volume of the polysaccharides and conjugates as determined by size exclusion chromatography using the different buffer solutions. Ultrafiltration results showed significant opportunities for enhanced separation by exploiting both solution conditions and concentration polarization to maximize the selectivity of the membrane process. These results provide important insights into the factors controlling the ultrafiltration behavior of bacterial polysaccharides and a framework for the design of effective membrane processes for the purification of polysaccharide-based vaccines.

4. Re-designing Purification Processes for Oligonucleotides; What's Achievable?

Robert Gronke, Biogen, United States
Ratnesh Joshi, Biogen, United States
Kris Ruanjaikaen, Biogen, United States
Yannick Fillon, Biogen, United States
Carson Tran, Amgen, United States
Firoz Antia, Biogen, United States

Purification of antisense oligonucleotides has traditionally been based on the philosophy that a single step chromatography process, along with precipitation and detritylation steps would be sufficient to deliver an active pharmaceutical ingredient (API) of sufficient purity. Regulators, however, are paying more attention to product-related impurities that in the past were once considered an acceptable part of the API. Recently, we have developed a much improved downstream processing of antisense oligonucleotides by employing an aqueous based capture step that gives surprisingly high resolution of product-related impurities. Then, in an effort to further enhance purity, a second, orthogonal step is employed. Overall, this process design achieves control of several key product-related impurities and provides robustness over earlier platforms without sacrificing yield or productivity. Details of the optimized process will be presented.

Session 7 | Purification – from Platform to Diversity

SESSION CHAIRS:
Nihal Tugçu, Merck & Co., Inc.
Dorothee Ambrosius, Boehringer Ingelheim Pharma GmbH

1. Modular Approaches for Diverse Molecules: Reinventing Smart Bioprocessing

**Stefan Schmidt, Rentschler Biotechnology, Germany*

The biopharmaceutical industry is in the middle of a revolution as novel complex protein based therapeutics are threatening the dominance of monoclonal antibodies. These designer molecules are difficult to purify as in many cases they lack a common motif that would enable the installation of a platform process based on affinity. In order to accommodate this molecular diversity we have chosen an optimization strategy focusing on individual characteristics of the respective proteins. Our concept does not necessarily rely on sequential brute force high throughput process development only, but on the intelligent deconvolution of general purification issues in manageable chunks that are systematically rearranged to form a logical sequence of minimally required steps to achieve the intended quality and purity profile. Multiple modules are then assembled according to the specifics of each new molecule or class of molecules to redesign a quasi-platform downstream process. Irrespective of the molecule type, the challenges of enabling a fast and cost effective capture, HCP reduction, virus inactivation and aggregate removal remain the same. For instance we can chose from several individual virus inactivation steps, taking into account the different sensitivities of protein molecules. Another example are multiple modules to eliminate aggregates based on their interaction type. Although the HCP load is highly dependent on upstream conditions and the choice of a cell line, we created a toolbox to quickly reduce HCP content. Furthermore we achieve cost savings by implementing generic buffer systems that are applicable in all purification steps. I will present the current status of our modular approach to transfer well proven elements from platforms into downstream processes of non-antibody molecules. I will show case studies from our highly diverse portfolio of complex molecules ranging from enzymes and fusion proteins to vaccines, addressing the issues mentioned above and exemplifying our strategy.

2. Leveraging HTPD to Streamline Implementation of Non-platform Therapeutics to Minimize Impact to Development Timelines

**Jennifer Pollard, Merck & Co, United States*
John Welsh, Merck & Co, United States
Michael Rauscher, Merck & Co, United States
Sandra Rios, Merck & Co, United States
David Roush, Merck & Co, United States
Nihal Tugçu, Merck & Co, United States

External pressures on the industry have challenged research and development to accelerate molecules to the clinic. Historically, these accelerated timelines were achieved through the use of purification platforms, most readily for full length monoclonal antibodies (mAbs). As a wider range of novel biologic targets are pursued, a more diverse set of therapeutic modalities such as antibody fragments, fusion proteins, peptides and nanobodies provide challenges to downstream development. These challenges can be related to unique impurities such as product fragments or more typical impurities such as residuals or aggregates. As more non-platform molecules are desired, a different approach is needed to address the time and resource challenge.

The utilization of high throughput process development (HTPD) for non-platform molecules provides a strategy to mitigate the impact to the timelines. This high throughput workflow can reduce the gap in development time between a platform and a non-platform molecule and allow for more experiments with a fixed amount of resources. HTPD allows for earlier program engagement due to its ability to utilize small amounts of material, enabling the use of transiently produced protein. This workflow also facilitates a more empirical approach to development and optimization, which may be needed, as early in development, impurity characterization is lacking. In this paper, case studies will be employed to demonstrate the impact on time and resources. The case studies presented will show the impact of HTPD on both the development of a non-platform mAb and a fusion protein. For the non-platform mAb, this workflow was applied to develop an additional polishing chromatography step for aggregate removal as well as optimize the final ultrafiltration conditions. For the fusion protein, HTPD was used to successfully identify a chromatography step to clear product related fragments.

3. Evolution of a Standard Downstream Purification Process for Knob-in-Hole Bispecific Antibodies

*Asif Ladiwala, Genentech, United States
Kimberly Kaleas, Genentech, United States
Glen Giese, Genentech, United States
Amit Mehta, Genentech, United States
Josefine Persson, Genentech, United States
Chris Dowd, Genentech, United States
Michelle Butler, Genentech, Inc., United States
Philip Lester, Genentech, United States

Bispecific antibodies have gained a lot of interest in recent years due to their ability to bind to two different targets, thus opening the door to unique targeting strategies with greater potential for therapeutic success that are otherwise unavailable with traditional monospecific antibodies. Knob-in-hole bispecific antibody technology employs engineering of the CH3 domains of the half antibodies to preferentially drive heavy chain heterodimerization to form the bispecific while minimizing homodimer formation. To minimize the possibility of light-chain mispairing, the two intact half antibodies are produced separately in different production cultures and assembled to form the bispecific antibody. The assembly approach results in unique product-related impurities such as unassembled half antibodies and homodimers that must be removed by the downstream purification process. The conventional mAb purification process was found to be unsuitable for this purpose, necessitating further process development for early knob-in-hole bispecific antibodies. Employing high throughput process development tools for the optimization of the assembly process as well as for the development of downstream purification steps for multiple bispecific antibodies enabled the identification of a generic bispecific production process for CHO and E.coli host derived molecules. This presentation will describe the evolution of a standard downstream purification process for assembled knob-in-hole bispecific antibodies. Select case studies will be presented including some “deviations” from the standard process due to molecule and/or format-specific challenges related to undesirable assembly byproducts as well as high levels of host cell proteins related to expression host differences.

4. A Miniaturized Process on µl-scale Representing the Entire Process Chain from Upstream to Downstream Processing

**Cornelia Walther, BOKU & Boehringer-Ingelheim-RCV, Austria*
Martin Kellner, Boehringer-Ingelheim RCV, Austria
Matthias Berkemeyer, Boehringer-Ingelheim RCV, Austria
Cecile Brocard, Boehringer-Ingelheim RCV, Austria
Astrid Duerauer, BOKU & Austrian Centre of Industrial Biotechnology, Muthgasse 18, Austria

Process optimization should be carried out in a holistic way taking the whole chain of unit operations into consideration. Integration of DoE will lead to numerous experiments and thus such optimization can be only managed in µl-scale. The so-called "new formats" are extremely diverse molecules compared to antibodies and require flexibility for process development. These formats are often expressed in E. coli and the low cost production with high yield can be realized especially when the product is expressed as inclusion body. Harvesting of inclusion bodies is simple and the product is already present in high purity. IB formation and their characteristics depend on multiple parameters. During process development the interpretation of upstream optimization DoEs is often based solely on fermentation titers and not on the entire process chain. High titers do not warrant success of the subsequent downstream processes. A laboratory platform was developed which connects extensive DoE setups in upstream development to recovery of active protein from inclusion bodies at a very early stage of process development. Here, we present a miniaturized and parallelized inclusion body recovery process consisting of mechanical cell disruption in a bead mill and subsequent inclusion body wash procedure. Optimizing the cell disruption via parameters such as shaking frequency and biomass concentration led to high comparability of this small scale method to the bench scale high pressure homogenization in respect to DNA, HCP and product release. Using this high throughput platform the correlation of upstream conditions for up to 24 fermentations with recovery properties of IBs can be determined in parallel per 96 well plate. In the end, the inclusion bodies can be further processed in high-throughput methods to set up optimized solubilization, refolding and capture conditions. This fast and material saving platform method provides a high-quality evaluation of fermentation screenings and reduces the initial barrier for IB processes opening potential for new products. The principle of operation of the platform will be demonstrated with two examples – an affinity scaffold and a single-chain antibody.

5. A Molecular Properties Perspective to Aid in the Development of Chromatographic Strategies for the Purification for Bispecific Antibodies

**Tiago Matos, Downstream Technology, Global Research, Novo Nordisk A/S, Denmark*
Hanne Sophie Karkov, Downstream Technology, Global Research, Novo Nordisk A/S, Denmark
Gorm Andersen, Downstream Technology, Global Research, Novo Nordisk A/S, Denmark
Lars Sejergaard, Mathematical Modelling Department, CMC, Novo Nordisk A/S, Denmark
Haleh Ahmadian, Downstream Technology, Global Research, Novo Nordisk A/S, Denmark
Steven Cramer, RPI, United States

Bispecific antibodies (bsAbs) have emerged as a promising alternative for multiple targeting of complex diseases. In contrast to the downstream bioprocessing of monoclonal antibodies (mAbs), the separation of the two parental mAbs from the bsAbs requires the development of new purification strategies due to the similarity of the molecules. In this work, a wide variety of chromatographic resins and conditions were examined to determine the selectivity of these systems for several bsAbs separation challenges. Further, in order to understand the selectivity trends obtained with these various mixtures and resin systems, a detailed protein surface property analysis was carried out using a range of novel molecular descriptors recently developed in our lab. By examining the protein surface property maps associated with each key molecular descriptor for a wide range of parental mAbs and the associated bsAbs, we were able to identify some of the underlying reasons for these selectivity trends. The results of the experimental screening and in-silico analysis indicated that while traditional hydrophobic interaction (HIC) and ion-exchange chromatography (IEX) exhibited some selectivity for these mixtures, the results were in general not sufficient for developing efficient downstream bioprocesses. In contrast, multimodal (MM) resins, particularly Capto MMC ImpRess were shown to exhibit good selectivity, capacity and recovery for the desired bsAb molecules. Finally, a generic process for the purification of bsAbs using this MM chromatographic resin is proposed.

Session 8 | Process Challenges with Biosimilars

SESSION CHAIRS:
Suresh Vunnum, Amgen
Jill Myers, Fortress Biotech

1. Transforming Cancer Care: Affordable and Accessible Biosimilars

**Steven Lehrer, Cipla BioTec, United States*

Worldwide less than 8% of diagnosed cancer patients have access to Standard of Care treatment which frequently includes biopharmaceuticals. In the US and EU, only about 25% of the population is treated, with cost of therapy as the major barrier to treatment. Cipla BioTec continuing Cipla's tradition of ensuring affordable and accessible medicine for all, best illustrated by making HIV/ARV medicine available worldwide for $1/day in 2002, is developing a portfolio of $1/day equivalent world class biosimillars which will be approved and commercialized worldwide. Cipla BioTec is implementing a "World Class Quality", "World Class Economics", "Be Local" approach to biosimilars.
The presentation will outline Cipla Biotec's overall efforts and approach including some of the innovations and partnerships used by Cipla to deliver $1/day treatment for HIV, how this learning is being applied to Cipla BioTec and Cipla BioTec's focus on Engineering Optimization to improve production of Biopharmaceuticals.

2. Challenges and Approaches in the Development of a Biosimilar Purification Process

*Russell Shpritzer, Pfizer, United States
Kim Sterl, Pfizer, United States
Priscilla Jennings, Pfizer, United States
Brian Korniski, Pfizer, United States
Daniel LaCasse, Pfizer, United States
Timothy Iskra, Pfizer, United States
Richard Wright, Pfizer, United States

While many of the important product quality attributes of a potential biosimilar are driven by the upstream process, the downstream process plays a critical role in the removal of product- and process-related impurities and the control of product-related isoforms. Pfizer's platform purification process served as the starting point for the purification of all five of our Phase 3 monoclonal antibody biosimilars. In addition to streamlining the development process and enabling facility fit at the commercial scale, the platform process routinely reduces process-related impurities to acceptable levels with minimal development. However, additional strategies may be required when considering the levels of product-related impurities and isoforms that may be present in the innovator's drug product. This presentation will discuss some of the approaches that were taken, beyond the platform process, to meet the requirements of each unique biosimilar.

3. Advances and Challenges in the Analytical Characterization of Biosimilar Products

*Jeff Allen, Pfenex Inc, United States

Biosimilar development is substantially based on demonstrating analytical similarity to the reference product with abbreviated clinical studies utilized to remove residual uncertainty. Pfenex Inc, a leader in biosimilar development, routinely deploys its extensive in house analytical infrastructure in support of product development. Significant challenges arise in the process of demonstrating the comprehensive analytical similarity of a given biosimilar to its reference product. In some cases, reference products contain excipients such as Human Serum Albumin (HSA) that interfere with analytical similarity assessment due to the large amount of this protein excipient relative to the active drug protein. In order to enable analytical similarity analysis between Pfenex's interferon beta-1b biosimilar candidate and the marketed HSA-formulated reference product, an HSA-depletion method was developed by Pfenex to remove interferences from this excipient and allow additional characterization at all levels of protein structure, including hydrogen/deuterium exchange (H/DX) to elucidate higher order structural (HOS) forms. Exploration and characterization of possible protein complexes, protein form distribution, and activity for both formulations was performed by 2-dimensional analysis across the product profile. The data from these experiments demonstrate that Pfenex's biosimilar candidate shows high similarity to the reference product based on the analytical methods developed and employed.

Session 9 | Next Generation Unit Operations & Integrated Processes

SESSION CHAIRS:
Jeff Salm, Pfizer
John Moscariello, CMC Biologics

1. Perturbations of Steady States in Continuous Bioprocessing

**Mark Brower, Merck & Co., Inc., United States*
Finn Hung, Merck & Co., Inc., United States
Adrian Gospodarek, Merck & Co., Inc., United States
Nuno Pinto, Merck & Co., Inc., United States
David Pollard, Merck & Co., Inc., United States

Implementation of continuous bioprocessing integrated with sophisticated automation strategies is within reach. Advances in technology for traditional unit operations such as cell-retention devices in perfusion cell culture, continuous multi-column chromatography and single-pass tangential flow filtration have led to pilot-scale demonstrations of both semi-continuous and fully-continuous protein production processes operating at periodic steady states. One obstacle for implementation of continuous bioprocessing is the lack of characterization tools for unit operations that are truly dynamically linked to one another. To address this challenge, we present here on the development of a methodology for characterizing the holistic downstream process performance through perturbation analysis where response to an upstream stimulus is measured at several unit operations simultaneously. By connecting the permeate stream from a perfusion bioreactor directly to a continuous downstream purification train and applying a temporal steady state assumption, the entire process can be characterized in terms of purity and quality over time. Case studies for the purification of a monoclonal antibody will be employed to demonstrate the establishment of unit operation residence times and deviation propagation times throughout the continuous bioprocess. The case studies will not only highlight the ability to characterize connected process performance, but also, the ability to utilize the extracted information to design control strategies that will tolerate major process deviations and material segregation. This methodology, coupled with multivariate data analysis techniques will ultimately lead to dynamic process control strategies that will reliably deliver protein with highly consistent quality and purity profiles from continuous bioprocesses.

2. A Multi-Stage Hybrid Integrated System for Downstream Processing of Monoclonal Antibodies from a Continuous Perfusion Bioreactor

**Rob Fahrner, Pfizer, United States*
Jeffrey Salm, Pfizer, United States
Bob Kottmeier, Pfizer, United States
Marcus Fiadeiro, Pfizer, United States
Jill Kublbeck, Pfizer, United States
Raquel Orozco, Boehringer Ingelheim, United States
Scott Godfrey, Boehringer Ingelheim, United States
Jon Coffman, Boehringer Ingelheim, United States

This talk will describe a multi-stage hybrid integrated downstream system for manufacturing ~1 kg batch of monoclonal antibody (mAb) from a 100 L continuous perfusion bioreactor over a 5 to 15 day period. The key to this design is integrated operational effectiveness, where each stage and sequence of the system maximizes its individual advantages while minimizing any disadvantages. We will present how the system has been engineered for efficient use of space, time, equipment, and reduced operational complexity. Highlights of the system include the ability to operate under a wide range of bioreactor parameters, including a bioreactor productivity of 1–4 g/L/day, which accounts for variation of Qp, sieving decay, and reactor VVD. The system has been developed as a hybrid of three operational stages: continuous, periodic, and batch. During the continuous stage: The bioreactor permeate is continuously harvested by TFF and is alternately loaded onto two 1 Liter protein A columns, where the loading phase switches between the two columns and does not pause. It has the ability to self-adjust for variable product concentration and flow rate at the system inlet. During the periodic stage (which occurs every 4 to 12 hours): After loading, the first protein A column is eluted (while the second protein A column is being loaded in parallel) and runs continuously through 1) a tankless hold, 2) a low pH virus inactivation chamber, 3) a 0.5 Liter weak partitioning anion exchange column, 4) an SPTFF unit, 5) in line pH and conductivity conditioning, and 6) is collected into a single use mixing bag. While the product stream moves continuously through this section, each individual unit operation is taken offline when not containing product, and is regenerated and sanitized in parallel with the other unit operations, which permits significant flexibility for variable product streams and maximizes the periodic stage productivity. The periodic stage then pauses until the second protein A column is ready for elution. During the batch stage: After the complete 1 kg batch is processed through the periodic stage, it is taken through VRF, UF/DF, and filtration operations for final formulation. This allows a clear definition of a batch for release testing and disposition. The overall process operation is simplified so that dynamic control is minimized, and the automation is integrated across all stages. The complete integrated system occupies a footprint of 8 m x 5 m, including a buffer concentrate system built in a cube format. Data presented from prototype pilot runs will demonstrate the ability to successfully operate such a system with product quality, including HCP, leached ProA, DNA, dimer, and aggregate that is comparable to product produced in batch mode. The pilot runs were able to produce purified mAb with a product concentration of ≥ 60 g/L at ≥ 70% downstream yield through the SPTFF in the periodic stage, turbidity <6 NTU at all unit operations, and consistent achievement of pH targets. The principles of the design and operation of this integrated, hybrid system provide an effective method for downstream processing of continuous perfusion bioreactors.

3. Alternative Downstream Processing Based on Continuous Coupled Precipitation–Filtration Capture Operations

Qin Gu, Carnegie Mellon University, United States
Zhao Li, Penn State University, United States
**Todd Przybycien, Carnegie Mellon University, United States*
Andrew Zydney, Penn State University, United States

Capture via continuous coupled precipitation-filtration offers a new, alternative paradigm for the downstream processing of recombinant proteins. In this process, target protein is captured from pre-concentrated clarified cell culture fluid, with concentration >10 to 100 g target/L, in a series of paired tubular mixer-hollow fiber filter stages in which the target is preferentially precipitated, the precipitate is de-watered and washed to remove soluble entrained contaminants, and the purified precipitate is re-dissolved. Re-dissolution conditions can be set to achieve target concentrations up to 100 to 250 g/L, depending on target solubility and viscosity limitations. Keys to efficient process operation include: (1) pre-concentration of the target stream to enhance the yield and purity of the target precipitate phase; (2) use of synergistic pairings of precipitants, here reversible cross-linkers and volume excluders work well, to reduce total precipitant needs; (3) use of a plug flow format in precipitation to ensure that each fluid element experiences the same steady state Camp number, or product of residence time and average shear rate, to produce consistent precipitate morphologies; (4) tailoring of the precipitate morphology, including size distribution and "stickiness", to optimize filtration performance during de-watering and washing stages; and (5) augmentation of the washing and re-dissolution stages with additional mixer-filter pairs to permit use of multiple washing and/or re-dissolution solutions and to allow counter-current operation to reduce buffer requirements. This capture operation can be integrated with an initial single-pass tangential flow filtration pre-concentration operation and with subsequent polishing chromatography operations, e.g., using membrane adsorbers or continuous slurry-based chromatography, to result in a fully continuous purification process. We have conducted preliminary work to realize this process. We have modeled the synergistic solubility effects of proteins in solutions of $Zn2+$, a reversible cross-linker, and poly(ethylene glycol) or PEG, a reversible volume excluder; we have formed $Zn2+$/PEG precipitates of mock targets with similar yields and purities in the presence of clarified CHO culture fluids, but with different morphologies, to demonstrate the importance of contacting conditions during precipitation; and we have connected precipitate morphologies with filtration performance and fouling mechanisms. We have further used process simulation software to compare the CAPEX, OPEX and water/reagent usage for a fully continuous precipitation-filtration-based monoclonal antibody purification process with a fully continuous protein A chromatography-based process and with the current protein A-based platform process. We expect the envisioned process to be generalizable to a wide range of protein- and proteinaceous particle-based products and to offer significant advantages in performance, raw materials usage, simplicity, and cost relative to packed bed chromatography-based capture. This process has particular implications for production of biotherapeutics in low-resource environments or with stringent limits on total cost-of-goods.

4. Implementation of an End-to-End Continuous BioProcessing Platform Using Novel Technologies

**Engin Ayturk, Pall Corporation, United States*

The next frontier for the biopharmaceutical industry is the widespread adoption of integrated continuous bioprocessing for biologics manufacturing. The key to its success, however, is the availability of novel upstream and downstream technologies that will not only reduce facility footprint, capital expenses and product cost of goods (CoGs), but also will increase process productivity, flexibility and further facilitate the utilization of single-use and/or disposable technologies. In this context, the suite of cutting-edge technologies we have evaluated to enable cost effective and reliable implementation of continuous bioprocessing of biological drugs, included the Cadence™ Inline Concentrators within the single-pass TFF (SPTFF) platform, the BioSMB® multicolumn continuous chromatography platform, acoustic wave separation (AWS), a disruptive cell culture clarification technology, and novel continuous diafiltration strategies, to address the innovation gap to provide a simplified solution for the continuous final formulation step. By utilizing a 20L CHO fed-batch cell culture bioreactor with cell density range of $25x10^6$ – $30x10^6$ cells/mL and 65 to 90% cell viability, multiple in-house feasibility runs were conducted through a novel integrated continuous bioprocessing train of unit operations. For instance, while achieving ≥90% continuous

Oral Abstracts

clarification yield for the processing of a batch with 1.25 g/L titer, 25x10^6 cells/mL & ~70% viability, aforementioned, process platform was able to deliver ≥ 2 g/h mAb for the continuous purification train utilizing a stable 4-fold continuous concentration step for the integration of continuous clarification and continuous capture trains. With the coupling of the novel continuous polishing, continuous viral clearance and continuous final formulation steps, such platform, with the current PD-scale bioreactor capacity, will generate ≥ 1 g/h mAb. Coupled with the process economics modeling, this presentation will provide a risk-based and data-driven overview of an integrated continuous bioprocessing platform and highlight its subsequent requirements, challenges and opportunities for product development, process monitoring, validation, control and automation.

Session 10 | Biomolecular Modeling for Manufacturability

SESSION CHAIRS:

Christopher Dowd, Genentech
Peter Tessier, Rensselaer Polytechnic Institute

1. Synthesis of Computational and Experimental Developability to Support Protein Engineering

**David Roush, Merck, Sharp and Dohme, Inc., United States*
Francis Insaidoo, Merck, Sharp and Dohme, Inc., United States
Suvrajit Banerjee, Rensselaer Polytechnic Institute, United States
Steven Cramer, RPI, United States

A wealth of possibilities exists for computational modeling of proteins including atomistic, coarse-grain and macroscopic Quantitative Structure Retention Relationships (QSRR). The key to successfully developing a therapeutic protein from Discovery to Commercialization is the synthesis of the appropriate computational and experimental strategies to rapidly achieve this goal. Protein ligand interactions can significantly influence the biophysical properties of the protein. For biologics development, this perturbation can influence the purification profile, solubility properties, and stability profile of the protein. The focus in early development is on affinity and selectivity of the therapeutic protein to its cognate ligand, followed by developability measured through experimental techniques. Currently, in pharmaceutical development strategies, the goal is to rapidly assess the impact on platform fit or manufacturability. Under these conditions, the goal is to discern subtle changes in the protein properties (ex. charge

Oral Abstracts

distribution) on stability, ligand binding (type of ligand) and formulation. Hence, the combination of atomistic scale molecular simulations coupled with High Throughput Screening (HTS) encompassing purification, formulation and analytics is essential. Ultimately these biophysical tools can be employed to define the impact of primary liabilities and to select the best molecule, which is the endpoint for Protein Engineering in the Discovery phase. Once the field of potential modalities for analytics and process has been defined, the focus shifts to optimization of productivity. This second stage of development requires a translation from the atomistic level of information into macroscopic parameters (ex. linkage of ΔG to k' or differences in ΔG to selectivity). Successful development of the second stage requires a different toolset including coarse-grain analysis, QSRR and statistical assessments of the multiple interactions that define purification and formulation. The presentation will provide an overview of the strategic approach defined to span Developability from Discovery through Commercialization.

2. Molecular Basis of Strong Association in Monoclonal Antibodies: Atomistic Computations and Small-Angle Scattering

David Rosenman, University of Delaware, United States
Amit Vaish, University of Delaware, United States
P. Douglas Godfrin, University of Delaware, United States
Daniel Greene, University of Delaware, United States
Sandeep Yadav, Genentech Inc., United States
Isidro Zarraga, Genentech Inc., United States
Norman Wagner, University of Delaware, United States
**Abraham Lenhoff, University of Delaware, United States*

The large number of monoclonal antibody (mAb) therapeutics that have been developed has revealed the great diversity of biophysical properties that may be encountered even for mAbs differing in only a small number of residues; such diversity has also been studied explicitly by site-directed mutagenesis. Of special interest are strongly-associating mAbs, which may aggregate readily and may have anomalously high viscosities in concentrated formulations. A more complete understanding of the molecular origins of such behavior would allow molecular designs that could eliminate the problematic behavior, potentially by mutation of as little as a single residue remote from the complementarity-determining region (CDR), so that biological affinity would not be impaired. In this presentation we will discuss the synergistic use of atomistic simulations and small-angle neutron scattering (SANS) to determine the configuration of mAb-mAb association. We use atomistic molecular mechanics calculations to study a set of three mAbs differing by only a small number of point mutations that appear to control the high-viscosity behavior observed in one of them. A small number of high-affinity mAb-mAb binding configurations were identified and refined, with both non-electrostatic and electrostatic contributions accounted for. The computed binding strength in isolated configurations under experimental conditions corresponding to high-viscosity behavior exceeds 30 kT. A statistical mechanical model is used to show that such binding is sufficient to produce an extensive mAb network in solution for solution concentrations of order 100 mg/mL. In order to investigate the validity of the predicted high-affinity binding configuration, the model predictions are used to generate in silico mAb dimers for which scattering spectra are calculated a priori. SANS data taken on solutions of the strongly-associating mAb show clear evidence of mAb clusters, and the high resolution capability of SANS allows direct comparison of the predicted and measured scattering spectra. Taken together, these methods provide considerable insight into mAb solution behavior that can be utilized along with standard mutagenesis approaches to develop mAbs with both biological efficacy and desirable processing and formulation properties.

3. Mechanistic Model Of Excipient Exchange During Protein Formulation by UF/DF

*Roger A. Hart, Amgen, Inc., United States

The basis for the compositional design of a parenteral drug formulation is focused on the concentration of the active protein ingredient, dosage volume per injection, product stability, and injection tolerability; the later being largely controlled by the viscosity, osmolality, and excipient species and concentrations. Ultrafiltration and diafiltration is typically employed for the manufacture of protein drug products to control the formulation pH, osmolality, and excipient properties. During UF/DF, complex interactions between and among the high-concentration protein molecules and formulation excipients, coupled with volume exclusion effects, cause divergence of the drug substance pH, excipient concentration, and osmolality from that of the diafiltration buffer. This paper describes a UF/DF unit operational model which accounts for the effect of these interactions on excipient retention or rejection throughout the initial concentration, diafiltration, final concentration, and flush recovery. Electrostatic interactions are described using the Poisson-Boltzmann theory with full accounting for electro-neutrality, protein contribution to buffer capacity, protein charge titration, zwitterion excipient speciation, excipient activity coefficients, and protein residue activity. Protein molecular weight and net charge are calculated from the amino acid sequence and excipient properties utilize NIST traceable constants; a single "charge bias" constant is used to calibrate the otherwise entirely mechanistic model. Performance was assessed and qualified for four monoclonal antibodies through statistical comparisons between model predictions and experimentally measured data from DOE multi-factorial characterization studies. Following model assessment, additional process scenarios were modeled to demonstrate the model's potential as a tool for process design and optimization.

Session 11 | New Developments in PAT and QbD

SESSION CHAIRS:
Gisela Ferreira, MedImmune
Thorsten Lemm, Roche

1. From First Principles Chromatography Modeling to Process Control Strategy

*Cenk Undey, Amgen, United States
Oliver Kaltenbrunner, Amgen, United States

Traditional process characterization to support a biological marketing application typically starts with risk-based parameter prioritization followed by the development of an empirical model based on designed experiments that evaluate the effects of the selected parameters. This empirical model is used to understand process responses within the characterized ranges for unit operations in the process. While very effective, these techniques still require considerable amount of resources and time and do not take advantage of the available fundamental understanding of unit operations. It is desirable to utilize the available first principles understanding of the process for improved robustness, predictability and optimization and to integrate this knowledge in process control strategies and regulatory applications. We have been successfully advancing first principles modeling in the area of cation exchange (CEX) chromatography to mimic the results of wet experiments. The focus of this presentation will be a review of our methodology of model development, the development of a regulatory filing strategy, and an overview of feedback received from regulatory interactions. We will articulate how first principles understanding of a unit operation can be integrated in the development and regulatory filing of a bio/pharmaceutical process to improve process robustness and control while reducing wet experiments and development cycle time. This opportunity of leveraging advanced process understanding for efficient process development is demonstrated on industrial case studies of CEX chromatography.

2. Smart2: A Synergistic Life Cycle Approach to Understand and Control Raw Material Variability through Collaborative Process Analytics

**Gunnar Malmquist, GE Healthcare, Sweden*
**Canping Jiang, Biogen, United States*
Mattias Ahnfelt, GE Healthcare, Sweden
Per Lidén, GE Healthcare, Sweden
Roger Lundqvist, GE Healthcare, Sweden
Dave Kolwyck, Biogen, United States
Sarah Yuan, Biogen, United States
Robert Guernard, Biogen, United States

A process is generally considered well understood when (1) all critical sources of variability are identified and explained; (2) variability is managed by the process; and, (3) product quality attributes can be accurately and reliably predicted over the design space established. So far, biologics process development and characterization efforts even under the QbD paradigm have mostly focused on variability associated with the production process itself, leaving significant room for improvement to better understand and control raw material (RM) variability. This leads to a situation where commercial manufacturing is usually the first place to experience the impact of RM variability, resulting in consequences of lengthy manufacturing investigations and batch rejection. In an attempt to solve this conundrum, this case study will illustrate a synergistic life cycle approach to understand and control RM variability through data sharing and collaborative process analytics between a biologics manufacturer (Biogen) and a chromatography resin raw material supplier (GE Healthcare). The essence of this approach is to continuously assess RM variability through the process lifecycle by analyzing combined data from RM and drug substance processes, and to retire RM risk by continuous process understanding and control improvement. To enable this approach, data aggregation systems, multivariate data analysis capability and open collaboration between both sides are critical elements. A pilot case study, encompassing end-to-end multivariate analytics applied across multiple unit operations on a commercial manufacturing downstream process, will demonstrate proof-of-concepts of i) resin variability assessment through multivariate analysis of expanded resin RM data; ii) predictive models to predict drug substance process performance based on resin RM data and drug substance process data; iii) chromatogram feature analysis and its correlation with process performance and consistency; iv) resin cycle performance prediction. We believe this type of process analytics collaboration between drug substance manufacturers and raw material suppliers will more than double the power of smart bioprocessing compared to each party acting alone.

3. Raman Spectroscopy-based Multivariate Analysis as a PAT Tool for Impurity Detection and Adaptive Process Control in Downstream Bioprocessing

**Siddharth Parimal, Biogen, United States*
Sanchayita Ghose, Biogen, United States
John Pieracci, Biogen, United States

The call for increased understanding and better control of biopharmaceutical processes by the regulatory authorities under the QbD framework has paved the way for various PAT tools to be used for monitoring and analysis of critical quality attributes at various steps in the biomanufacturing process. The work presented herein proposes a paradigm shift in the way product quality is typically measured in a GMP facility. Currently, the analysis of downstream intermediates is performed at-line or on-line. In contrast, we use a combination of Raman spectroscopy and multivariate data analysis as a potential enabling technology for fast, non-invasive, in-line analysis during downstream bioprocessing. We develop correlations of Raman spectra with different critical quality attributes (e.g., high molecular weight species, acidic isoforms) for multiple monoclonal antibodies, demonstrating a PAT tool which is easy to use and has minimal manual intervention. We provide examples where in-process measurements of the intermediates can drive feedforward control for the subsequent purification step, resulting in flexible processes where input parameters are modulated to deliver consistent product quality. The approach presented in this work can provide opportunities for real-time, adaptive control strategies to be implemented for robust, continuous control of downstream operations.

Session 12 | Increasing Patient Access to Biopharmaceuticals

SESSION CHAIRS:
Jon Coffman, Boehringer Ingelheim
Bruno Marques, GlaxoSmithKline

1. Broad World Access Requires a Broad Approach to Process Application: Process, Equipment, Automation and New Facility Designs

**Joseph Shultz, Novartis Pharma AG, Switzerland*
Lars Pampel, Novartis Pharma, Switzerland
Mesbah Crietz, Novartis Pharma AG, Switzerland
Urs Wernli, Novartis Pharma AG, Switzerland
Spencer Fisk, Novartis Pharma AG, Switzerland

To sustainably deliver biopharmaceuticals to both industrialized and emerging markets, requires that the entire development cycle must be economically feasible on a global scale. Thus, we must look far beyond yield maximization and raw materials cost reduction. The entire Development and Manufacturing spectrum must be addressed, from molecule design to process development strategies, to the clinical program and manufacturing facility design. The measure is not as simple as targets like COGM of 1% of the selling price, but rather total costs low enough to serve emerging markets, while also serving industrialized markets and making biosimilar entry financially

Oral Abstracts

unappealing. Thus, the scope of process development must expand to also enable cost containment across the organization. Novartis is already capable of drug substance production at less than 1% of sales price, but access to broader markets and efficient distribution chains may be more important. We are driving to a new development and manufacturing paradigm, that can be applied anywhere in the world and address historical challenges, like:

- Underutilized capital and labor that adds cost to every gram produced, regardless of how we design our processes
- Ability to rapidly respond to unexpected demand changes
- Management of flexible drug product presentation costs
- Exposure to program attrition and sunk development costs that must be recouped by a few commercial products
- Limited commercial time under patent coverage As we are not already burdened with a large underutilized infrastructure, we have the opportunity to redefine how we develop and manufacture biopharmaceuticals. Development will utilize a balance of traditional and newer technologies to enable the production of large-facility masses in small, easily built and replicated facilities that can produce a broad range of molecule-types. This will be facilitated with flexibly integrated processes that span from cellular production through drug product packaging and distribution. In fact, the future requires that all components – process, equipment, automation and facility – be integrated as one, to unlock the optimal flexibility and cost profile. We will discuss what we feel are the major economic impactors that will enable broader global access and key aspects of how continuous/integrated processes and advanced automation, allows us to implement commercial manufacturing facilities that will serve a broad range of molecule formats for the future.

2. A Simple GPS for Lowering Capital Cost, Which Is Too High, Even With High Margins

**John Erickson, GSK, United States*
Günter Jagschies, GE Healthcare Life Sciences, Sweden
John Joseph, GE Healthcare Life Sciences, Sweden

The biopharmaceutical industry has debated Cost of Goods Sold (COGS) for a long time, but that measure alone can be misleading. The COGS calculation assumes that capital cost is fully depreciated over a long time. Usually, this makes the depreciation expense comparable to labor and raw materials. That is fine, except when you have to ask for hundreds of millions of dollars to build the factory in the first place. As price pressures continue to mount in the industry, this problem should get worse. Compounding the problem is the fact that plants have traditionally taken about 3 or more years to design, build and validate. This means that major investment decisions need to be made well in advance of actual market demand and sometimes even before products are approved. Because market forecasts are inexact, this leads to building factories that are either too big or too small. If the factory turns out to be too big, there is tremendous pressure to fill up the excess capacity with contract manufacturing. Then, if market demand increases significantly, or if the factory was too small to begin with, the cycle repeats and we are forced to build or outsource manufacturing. Even if capacity is just right, capital cost is a barrier to change. New technologies that require different equipment are difficult to industrialize when significant writeoff of equipment is required, or if buildings have to be modified. This doesn't seem particularly smart. Therefore, no matter whether COGS in a particular market is a large or small fraction of sales, everyone should be concerned about reducing capital cost. Standardization of equipment design across the industry should eliminate redundant engineering costs and decrease leadtime and can be done without any new technological innovations, but requires us all to work together. To guide the journey to even lower and lower capital cost, we propose using the total volume of process equipment as a surrogate for capital cost. If the size of the equipment gets smaller, it should become less expensive and easier to install. The building should be smaller as well. Process volume can be calculated easily from process parameters for individual unit operations or by an overall mass balance for an entire factory. To map out the endpoints of the journey, we calculated the process volume of an existing factory by adding up the process volume of each major piece of equipment. We then used a simple mass balance to calculate a theoretical, smallest possible plant, where all the process equipment had 100 mg/mL of product in it at all times, which is a practical solubility limit. Assuming a cycle time of 8 hours for the entire downstream process in this theoretical plant, compared with one week for the existing facility, the theoretical minimum plant volume, normalized by output, is 5 orders of magnitude less than that of the existing facility. Since buffer makeup and hold tanks constitute the vast majority of current downstream process volume, we start with ways to decrease buffer tankage and will show results of process modeling for various scenarios where buffers are made up continuously. We will also provide simple models showing the effects of resin capacity, residence time, buffer usage and product concentration on process volume. These models can make process development scientists smart enough to develop processes that reduce capital cost, which in turn will make it easier to adapt to whatever the future may bring.

3. Manufacturing gp120 Based Novel HIV Vaccine Candidates: Simultaneously Meeting the Demands of Cost and Rapid Progression From Bench To Bedside

*Abhinav Shukla, KBI Biopharma Inc., United States
Leslie Wolfe, KBI Biopharma Inc., USA
Carnley Norman, KBI Biopharma Inc., USA
Niket Bubna, KBI Biopharma Inc., USA
Sigma Mostafa, KBI Biopharma Inc., USA
Jimmy Smedley, KBI Biopharma Inc., USA
Munir Alam, Duke Human Vaccine Institute, USA
Thomas Denny, Duke Human Vaccine Institute, USA
Barton Haynes, Duke Human Vaccine Institute, USA

Development of a vaccine for HIV has long been a holy grail for immunologists. The complications of dealing with a rapidly mutable virus with multiple mechanisms evolved to evade the human immune system have led to the failure of multiple vaccine candidates in clinical trials. However, recent progress made at the NIAID funded Duke Human Vaccine Institute is paving the way for possible solutions for this devastating disease. Scientists at DHVI have created a series of protein sequences [1,2] based upon the HIV-1 trimeric vaccine envelope glycoprotein (ENV) that are capable of inducing a broad neutralizing antibody (bNAb) response that might finally form a viable pathway to protect against this virus that has had devastating consequences around the world. Introduction of a novel HIV vaccine will have significant consequences for global health and serve patients across economic and geographic spectra. The development and manufacturing of these novel candidates is a key bottleneck in introducing and testing these vaccine candidates in human clinical trials. These trials are an essential step in perfecting a preventative vaccine against HIV which will eventually be an iterative process requiring the production of multiple, evolved sequences that can replicate the pathways by which some resistant patients render the virus ineffective. One-off process development efforts using conventional approaches are clearly inadequate to serve the needs in this space. Costs for development and manufacturing are another simultaneous consideration due to the number of candidates that need to progress into clinical trials. A platform approach was found to be a key necessity to meet with this dual challenge of speed and low cost. However, multiple challenges including the susceptibility to proteolytic clipping, low expression levels, the presence of some difficult to remove host cell proteins, heavy glycosylation and the requirement to maintain binding activity to key bNAb antibodies needed to be overcome. The solution to these diverse challenges lay in the form of a well evolved platform downstream process based purely upon non-affinity chromatographic steps. Multimodal chromatography with selective wash steps [3] provided a unique capture step that simultaneously cleared host cell protein species and improved product stability. The development of dedicated viral clearance steps and polishing steps based on ceramic hydroxyapatite and weak cation exchange chromatography were guided by the use of a surface plasmon resonance (SPR) based binding assay to monitor effective binding to selected bNAbs for each vaccine glycoprotein. Rapid cell culture process development to produce an optimally active glycan profile was achieved by leveraging high throughput miniaturized cell culture bioreactors [4]. Finally, seamless transition into cost optimal clinical manufacturing was achieved by leveraging a single-use manufacturing production train [5]. This presentation will highlight some of the immunological pathways leveraged to create this family of vaccine antigens and describe the rapid development of process and analytical platforms that have taken these candidates from bench to bedside. The economics of rapidly developing and producing clinical trial candidates will be shown to be a key driver throughout the development and manufacturing process.

1) B. Haynes, G. Kelsoe, S. Harrison, T. Kepler. B-cell lineage immunogen design in vaccine development with HIV-1 as a case study, Nature Biotechnology, 2012, 30(5), 423-433. 2) D. Fera, A. Schmidt, B. Haynes, F. Gao, H. Liao, T.Kepler, S. Harrison. Affinity maturation in an HIV broadly neutralizing B-cell lineage through re-orientation of variable domains, PNAS, 2014, 111(28), 10275-10280. 3) L. Wolfe, C. Barringer, S. Mostafa, A. Shukla. Multimodal chromatography: characterization of protein binding and selectivity enhancement through mobile phase modulators, Journal of Chromatography A, 1340, 151-156, 2014. 4) S. Rameez, S. Mostafa, C. Miller, A. Shukla. High-throughput miniaturized bioreactors for cell culture process development – reproducibility, scalability and control, Biotechnology Progress, 30(3), 718-727, 2014. 5) U. Gottschalk, A. Shukla. Single-use disposable technologies for biopharmaceutical manufacturing, Trends in Biotechnology, 31(3), 147-154, 2013.

The Recovery Conference Debates

Continuous Processing

Mathematical Modelling vs. DoE

Disruptive Technologies

Debate Session

Recovery of Biological Products XVII

BERMUDA, JUNE 19-24, 2016

Debate Session: Tuesday June 21 at 19.45

Session Chairs: Nigel Titchener-Hooker, John Curling

I: Continuous Processing

MODERATOR: John Curling
DEBATERS: Jonathan Coffman and Brian Kelley

Continuous bioprocessing is all the rage! A fully continuous, scalable commercial cGMP process would be unprecedented, a technological tour-de-force. But what problems or benefits does it offer vs. established batch processing? Do we really need a process to be fully continuous to be valuable?

Let's discuss what's behind the hype, and disentangle related but distinctly different topics – disposable unit operations, connected vs. continuous, PAT, etc.

Is the increased investment and additional risk worth the reward?

FOR:

- Continuous processing can make more Kgs and products in the same facility
- Henry Ford's assembly line was also criticized for its complexity
- Continuous processing enables flexible and fast facility construction
- It allows development and clinical manufacturing to get off the critical path with a launch capable process
- Investment in continuous processing technologies mitigates perceived risk

Are we afraid of new things?

AGAINST:

- What problem(s) are we solving with continuous processing?
- It's very complicated … continuous USP + DSP = a flying car!
- Perfusion history is checkered, continuous purification is yet unproven…
- If you don't perfuse, then why use continuous DSP?
- Production flexibility is limited (CMOs, >1 site in an internal network)
- Continuous ≠ Connected ≠ SUT! Choose…

Is there a strong business case or are we looking for things to do!?

II: Mathematical Modelling vs. DoE

MODERATOR: Jürgen Hubbuch

DEBATERS: Karol Łaçki and Arne Staby

Process understanding is crucial to modern bioprocessing. Design-of-Experiments (DoE) and other statistical methods are the industrial workhorses behind process development and process validation efforts for they are intuitive, well described and easy to implement. However do they provide a real process understanding or just give a false perception of being in control? In contrast, mechanistic models based on first principles are seen by many as the ultimate process understanding tool. Advances in basic research and in computational power of standard computers seem to support this expectation, but is it really realistic or does it make sense? While a first principle model of a scalable commercial cGMP process would be unprecedented, it is feasible but what problems or benefits does it offer vs. established DoE?

An industry wide discussion, including both academia and industry, on pros and cons for both approaches and their applicability to process control, trouble shooting, and other related areas is required.

To Do Experiments or not to Do (many) Experiments? That is the question!

FOR: Mechanistic Modelling

- It's complicated… or is it?
- Regulatory agencies understand it (not)
- Why settle for less?
- Methods may be extrapolated outside experimental areas

As long as you know why it really works then.......??

FOR: DoE

- It's simple – everyone can do it!
- Regulatory agencies understand it
- Accuracy good enough
- Who needs to extrapolate anyway!

As long as it works, you really don't need to know why!

III: Disruptive Technologies

MODERATOR: Todd Przybycien

DEBATERS: Hanne Bak and Jörg Thömmes

In therapeutic protein manufacturing the CHO based platform and column chromatography rules. Will it be ever thus? Or will traditional therapeutic protein manufacturing platforms be by-passed by disruptive innovation the way large integrated steel manufacturing or mainframe computers were made obsolete? Will the current platforms be replaced by something more productive and mobile like the PC by mobile computing? But we are saving people's lives in Healthcare, there is no way to compare steel mills and cell phones with innovative medical research in pharmaceutical manufacturing. Or is there? If so, will we be purifying therapeutic proteins by column chromatography forever? Or will our industry learn from industrial enzymes and purification trains will become protein refineries?

Let's pick this apart. Old School versus New (Old) School. Mano a mano.

FOR:

- Healthcare costs are a real issue, not only campaign rhetoric. Manufacturing costs must come down.
- Proteins are moving into therapeutic areas with very large patient populations. We can't manufacture proteins for tens of millions of patients the way we manufacture for hundreds of thousands
- Titers are going up and up, making it hard for column chromatography to keep up and making alternative processes more attractive
- Alternative processing equipment can be really simple and robust
- Have you purchased 500L of protein A resin lately?

Are we afraid of new things?

AGAINST:

- COGs not an issue, don't waste my time
- Therapeutic protein manufacturing is a high margin business and time to market is my key driver. Why risk delaying launch on all these unknowns?
- The CHO platform combined with packed bed chromatography works great and has a lot of upside potential, let's optimize this first
- We are working in a highly regulated environment, why would I upset the apple cart?
- It is about patient safety: purity rules and nothing can touch the resolving power of column chromatography.

What is the driver here?

Poster Abstracts

Session 13 | Poster Session – Oceans of Innovation

SESSION CHAIRS:
Charles Haynes, University of British Columbia
Abraham Lenhoff, University of Delaware
David Roush, Merck & Co., Inc.

1. Development and Lab to Pilot Scale Testing of a New High pI Presequence Useful for Purifying Low pI proteins from E.coli Fermentation

**Christine Bruun Schjødt, Novo Nordisk A/S, Denmark*
Gong Wei, Novo Nordisk A/S, China
Yu Mingrui, Novo Nordisk A/S, China
Chen Jianhe, Novo Nordisk A/S, China
Xia Wenjuan, Novo Nordisk A/S, Denmark
He Xugang, Novo Nordisk A/S, China
Christian Tihic Rasmussen, Novo Nordisk A/S, Denmark
Anja K Pedersen, Novo Nordisk A/S, Denmark

The large majority of E.coli proteins have slightly acidic pI values, presenting a purification challenge when producing recombinant proteins using E.coli as host. In the present work we have developed a new pre-sequence with alkaline pI that will allow the use of cation exchange chromatography as capture step when producing a low pI protein in E.coli. As model protein we have used human growth hormone, a protein of 22 kDa with a pI of approximately 5 [3], similar to a large number of the proteins originating from E.coli. In order to be able to remove the pre-sequence, hence allowing production of a mature protein, a prerequisite for development of the pre-sequence was that it should be possible to remove it enzymatically using diamino peptidase during the downstream purification process. Another requirement was soluble protein expression and preferably high expression level. In order to obtain an increase in pI of approximately one pH unit, it was calculated that the pre-sequence should contain at least three lysine or arginine residues. Previous experiments had shown that diamino peptidase does not work well with peptides containing arginines [2, 4] and hence the tests focused on lysine rich peptides. Subsequently, a series of pre-sequences were tested containing 2, 3, 6 or 7 lysine residues named SEQ1, SEQ2, SEQ3, SEQ4, SEQ5 and SEQ6. As expected from the theoretical calculations, it was found that three lysines indeed provided an increase of pI by one unit and also rendered soluble protein at high expression level. Unfortunately, approximately 10% of the expressed protein was N-terminally formylated, leaving the N-terminus blocked for DAP activity. To mitigate an inherent productivity loss, the pre-sequence was improved by inserting a serine residue as the second residue, thereby enabling removal of the N-terminal methione by E.coli enzymes during protein expression [1]. The resulting pre-sequence SEQ7 was subsequently used for experiments in 1 L, 20 L and 100 L pilot scale to investigate scalability of fermentation as well as recovery process and subsequent removal of the pre-sequence. Comparison of expression, recovery, capture and finally removal of the pre-sequence from lab to 100 L scale showed that the process was indeed very robust and reproducible. Data from the pre-sequence development and the tests in various scales will be presented in the poster. References: 1. Hirel et al (1989) PNAS 86, 8247-8251 2. Yang et al (2011) Protein expression and purification 76, 59-64 3. De Vos et al (1993) 3HHR.PDB 4. US07858747 B2

2. A Self-cleaving Tag System for Complex Glycoproteins

**David W. Wood, Ohio State University, USA*
Changhua (Steven) Shi, University of California at Irvine, USA
Tzu-Chiang Han, Ohio State University, USA
Joseph Taris, Ohio State University, USA
Merideth Cooper, Ohio State University, USA
Yamin Fan, Ohio State University, USA
Atefeh Alizadehbirjandi, Ohio State University, USA

The ability to purify arbitrary target proteins using a simple universal platform has long been a goal in biological research and the biopharmaceutical industry. This platform is provided at laboratory scale through the use of affinity tags, although even at laboratory scale occasional concerns remain regarding impacts of the tag on the target protein activity. More importantly, however, tags generally cannot be used for therapeutic protein manufacture due to the potential immunogenicity of the tag and the expense and process complexity associated with tag removal. For these reasons, a cheap and effective means for tag removal is highly desirable. In our previous work, we have developed self-cleaving tags by combining conventional affinity tags with modified inteins. Although this approach has been highly effective for proteins produced in bacterial expression hosts, these methods have been ineffective for complex glycoproteins expressed in mammalian and other eukaryotic hosts. The primary reason for this is premature cleaving, where pH-sensitive tags self-cleave during protein expression due to the permissive pH and temperature conditions required for mammalian cell culture. Although thiol-induced self-cleaving tags have been developed, the required thiol-containing compounds break disulfide bonds in the target proteins and are economically prohibitive at large scale. Thus, the promise of a truly convenient and effective self-cleaving system has yet to materialize for complex glycoproteins in mammalian systems – until now. In our recent work, we have extensively re-engineered the naturally split intein from the Npu dnaE protein, which has been shown to express well as separate segments, and assemble and splice with extraordinary speed. An important aspect of split inteins is that the separated split intein segments are inactive alone, and are therefore unable to cleave prematurely during protein expression. Once recombined in vitro, however, the assembled segments form an active complex, and can be used to generate self-cleaving tags. Thus, one segment of a split intein can be expressed, purified and immobilized onto a solid support, while the other segment can be used to tag a target protein. Strong and specific association of the tags effectively mimics a conventional affinity resin, where the target protein is captured as the intein segments assemble into an active complex. Although the Npu intein has been developed as a self-cleaving tag by other researchers, it was shown that the intein still required thiol compounds in order to cleave effectively. In our work, however, we have made several point mutations to both segments of this intein. These mutations provide a highly effective means for controlling the assembled intein with only pH in the absence of thiol. Further, we have modified the intein segments so that one intein segment can be covalently immobilized in a specific orientation onto a chromatographic backbone. The other intein segment, which is only 35 amino acids in length, then becomes the target protein tag. Once assembled and washed, our modified Npu intein is induced to rapidly cleave in response to a small pH shift, releasing the target for collection. Importantly, the affinity resin can then be regenerated, where the small tag intein segment is removed to prepare the column for the next target protein. We have successfully used this system to purify several proteins from several expression hosts, including fully glycosylated and active secreted alkaline phosphatase (SEAP) and tissue plasminogen activator (tPA) expressed in HEK and CHO, without any trace of premature cleaving. Further, the availability of a reusable affinity resin, akin to Protein A for non-antibodies, suggests a high potential impact for these methods at both laboratory and manufacturing scales. Finally, we have also incorporated this technology as the first capture step of our DARPA BioMOD device, which will also be discussed.

3. QSAR Analysis of Additive Effects on the Aggregation of Monoclonal Antibodies

Olubukayo Oyetayo, Biberach University of Applied Sciences, Germany
Fabian Bickel, Biberach University of Applied Sciences, Germany
Martina Merg, Biberach University of Applied Sciences, Germany
Oscar Mendez Lucio, Cambridge University, United Kingdom
Andreas Bender, Cambridge University, United Kingdom
**Hans Kiefer, Biberach University of Applied Sciences, Germany*

Aggregates of biopharmaceutical proteins that form upon exposure to various stress conditions can adopt widely different molecular structures. Depending on the mechanism of formation, they also behave differently with respect to aggregation reversibility. We have established small-scale model experiments to produce aggregates of monoclonal antibodies by controlled shifts in pH or ionic strength as well as by exposure to various interfaces. Aggregate secondary and tertiary structure was analyzed by spectroscopic methods. Aggregation kinetics was shown to be highly reproducible and rate constants of nucleation and growth were extracted from kinetic traces. In an additive screen including compounds from three different substance classes, molecular descriptors were correlated with both aggregation rate constants and thermal stability changes using a quantitative structure activity relationship (QSAR) approach. The analysis reveals a correlation between certain molecular properties such as relative and absolute surface polarity and the protective effect of additives tested. Negative correlation with some descriptors, i.e. destabilizing properties, were also identified. This approach will be extended to a larger compound set with the aim to obtain more detailed information, eventually enabling the design of improved additives.

4. Using Quantitative Structure-Property Relationship Analysis and High-Throughput Methods as Tools for Molecular Assessment & Process Development

Lydia Beasley, Genentech, Inc., United States
Brian Connolly, Genentech, Inc., United States
Tom Patapoff, Genentech, Inc., United States
Paul McDonald, Genentech, United States
**Benjamin Tran, Genentech, Inc., United States*

High-throughput partition coefficient (Kp) determination enables the rapid mapping of antibody binding behavior on different chromatography resins. This information can be used to inform chromatographic process development

Poster Abstracts

for antibodies. The historical Kp database generated from these screens can be leveraged using Quantitative Structure-Property Relationship (QSPR) analysis to create an in silico model to predict the binding behavior of new antibodies on commonly used resins from their amino acid sequences alone. This is advantageous during early molecule selection, where material for testing may be limited. In this study, we determined chemical structure information for a library of antibodies using homology and molecular dynamics modeling using antibody primary amino acid sequences. Molecular descriptors were then calculated using specific generated outputs including, but not limited to, residue position, charge, solvent-accessible surface area, and trajectories. These descriptors were then used to train a QSPR-based model to predict antibody Kp values on commonly used cation- and anion-exchange resin. We show how this data can be used in concert with high-throughput batch binding and robocolumn methods to accelerate purification process development.

5. Predicting and Controlling Aggregation for mAbs during Bioprocessing

**Sarah Hedberg, Imperial College London, United Kingdom*
Jerry Heng, Imperial College London, United Kingdom
John Liddell, Fujifilm Diosynth Biotechnologies, United Kingdom
Daryl Williams, Imperial College London, United Kingdom

Protein-protein molecular interactions are known to be involved in protein solution aggregation behaviour and are a common issue for the manufacturing of therapeutic proteins such as mAbs. Much effort has been employed to gain a better understanding of aggregation, however the mechanisms leading to protein aggregation are still not fully understood. The osmotic second virial coefficient (B22) is a fundamental physiochemical property that describes protein-protein interactions solution, which can be a useful tool for predicting the aggregation propensity of proteins. One way of predicting aggregation propensity is self-interaction chromatography (SIC), which recently have shown to be a promising tool for better understanding of phase behaviour of proteins. Another technique, cross-interaction chromatography (CIC), has shown to be an even more high-throughput technique than its predecessor with the same capabilities. The work reported here was performed on two mAbs as well as a polyclonal IgG.
In order to find the best SIC immobilisation strategy a number of different chromatographic resins and solution conditions were screened to established the most effective immobilisation method. For the best employment of these techniques a scale-down study was performed from laboratory scale macro-columns to micro-scale columns, which will enable more efficient screening process by only needing micrograms or milligrams of a mAbs for a full stability study. The major part of this work presents an extensive formulation study of mAbs, varying pH and salt, as well as the presence of different stabilisers as well as different external factors known to induce aggregation. The B22 and B23 values determined from the formulation study are then correlated with aggregation data obtained from size-exclusion chromatography. It was shown that over all test conditions, good correlations could especially be found between B22 and aggregation rate. Finally a few selected solution conditions were chosen that indicated both good and poor stability to further investigate the validity of B22 as an indicator for protein stability. All these studies are performed enabled a better insight into the mechanisms of aggregation via an improved understanding of SIC- and CIC- protein-protein chromatograms obtained. REFERENCES
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Poster Abstracts

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6. High-throughput Methods for Selecting and Optimizing Highly Developable Monoclonal Antibodies

Peter Tessier, Rensselaer Polytechnic Institute, United States

A key challenge in developing monoclonal antibodies (mAbs) as therapeutics is their variable and difficult-to-predict biophysical properties (solubility, viscosity, aggregation, polyspecificity) that collectively determine their developability. Thus, methods for assessing developability at the earliest stages of antibody discovery are critical for reducing problems that may occur later during antibody development. We are establishing several high-throughput methods for assessing the biophysical properties of antibody libraries to guide the selection of highly stable and well-behaved mAb candidates. First, we are developing methods for assessing the biophysical properties of large antibody libraries (millions to hundreds of millions) that are displayed on the surface of yeast. Yeast surface display is increasingly being used to identify high-affinity antibodies, and its compatibility with flow cytometry provides exciting opportunities for unusually high-throughput analysis. We find that selection of high-affinity antibody fragments using yeast surface display often leads to variants with defects in antibody stability and specificity. To overcome this challenge, we have identified conformational probes specific for well-folded antibodies that enable co-selection of antibodies with high affinity and stability, and which greatly increase the reliability of identifying antibody fragments with excellent biophysical properties. We are also developing a second method for screening the biophysical properties of hundreds to thousands of mAb candidates obtained via immunization or related methods. Our approach is to use gold nanoparticles coated with anti-human antibodies to capture mAbs of interest and then assess the colloidal stability of the resulting immunoconjugates to evaluate mAb self-association. Surprisingly, we find that our high-throughput mAb self-interaction measurements are not only correlated with conventional biophysical properties such as antibody solubility but also with non-conventional ones such as non-specific interactions with non-adsorptive chromatography columns. We expect that these and related high-throughput methods will improve the selection and optimization of antibodies that are well suited for the extreme environments (high concentration, extremes in pH and temperature, exposure to various types of surfaces) encountered during antibody purification, formulation and delivery.

7. Molecular Modelling to Predict Chemical Stability

**Lydia Beasley, Genentech, United States*
Amy Hilderbrand, Genentech, United States
Nisana Andersen, Genentech, United States
Bill Galush, Genentech, United States
Vikas Sharma, Genentech, United States
Tom Patapoff, Genentech, United States

Early assessment of the stability and manufacturability of multiple potential molecule candidates can be crucial to the selection of lead molecules that (1) have a high probability of technical success later in development, and (2) enable the use of platform processes that accelerate development. Molecular dynamics (MD) simulations

Poster Abstracts

provide the opportunity to predict the chemical stability of molecule candidates even before the processes to generate a sample of the molecule have begun. MD simulations offer the ability to perform in-depth analyses of physiochemical properties by generating estimates of thousands of conformations of each molecule and tracking the position of each atom over time. This atomic-resolution simulation data can be used to make detailed chemical and structural calculations of each molecule. We demonstrate that careful analysis of MD trajectories can predict the risk of methionine (MET) and tryptophan (TRP) oxidation events that have the potential to impact efficacy and shelf life of antibody therapeutics. Experimental oxidation data was used as a training set to establish the relationship between MD analyses and MET/TRP oxidation. For each molecule, fully atomistic molecular dynamics simulations of the antibody Fv-region were executed. Strong predictive relationships were found between certain MD outputs and MET/TRP oxidation risk. Prediction of additional physiochemical properties using MD outputs will be discussed.

8. Competitive Ion-Exchange Adsorption of Proteins: From Columns to Single Molecules

Ujwal Patil, University of Houston, United States
Lydia Kisley, Rice University, United States
Sagar Dhamane, University of Houston, United States
Katerina Kourentzi, University of Houston, United States
Christy Landes, Rice University, United States
**Richard Willson, U of Houston, United States*

Competitive adsorption of proteins from multicomponent mixtures is the basis of chromatographic separations, but has not been extensively studied in mechanistic detail. We recently introduced the use of single-molecule imaging to the study of protein adsorption, and now have extended single-molecule methods to the investigation of competitive protein adsorption phenomena. We also are developing novel methods of studying macroscopic multi-protein adsorption under conditions of constant competitor concentration, including on agarose ion-exchange chromatographic columns of controlled ligand density. We will present results from each of these approaches, as applied to the same protein/adsorbent systems.

9. QSAR Models For Fab Libraries: Powerful Predictive Tools to Facilitate Process Development and Identify Important Surface Properties

**Julie R. Robinson , Rensselaer Polytechnic Institute, United States*
Hanne Sophie Karkov, Downstream Technology, Novo Nordisk, Denmark
James A. Woo, Rensselaer Polytechnic Institute , United States
Steven M. Cramer, Rensselaer Polytechnic Institute , United States

Our lab recently has reported on the rational design of homologous series of antibody Fab variants and demonstrated that these libraries can serve as powerful tools to investigate selectivity in multimodal chromatographic systems. In this poster, experimental data obtained from linear salt gradient experiments with these libraries are employed to develop robust quantitative structure activity relationship (QSAR) models for the prediction of Fab retention in several chromatographic systems. These models are the first reported to date for the a-priori prediction of retention behavior of large, complex proteins such as Fabs. Model development is enabled by a new class of descriptors that quantified properties in localized patches on the Fab surface. In addition to being well suited for predicting Fab variants not included in model generation, these QSAR models were also successful in predicting deamidated species and different isotypes. QSAR models also were developed to predict the subtle selectivity changes resulting from the use of arginine and guanidine as mobile phase modifiers with these Fab libraries in the Capto MMC and Nuvia cPrime multimodal systems. Significantly, the descriptors selected in these models provided insight into important properties of the proteins that determine the nature of the change in retention in the presence of these modifiers and corroborate existing theories about the mechanisms of interaction. This work demonstrates how computational and experimental tools can be combined into a multi-step framework that can facilitate process development of recombinant biopharmaceuticals. These results also suggest that the selected descriptors can provide important insights into key interaction regions on the protein surface which can further inform future fundamental studies. Finally, this work sets the stage for the development of in-house QSAR models for a range of complex new classes of biological products with important implications for rapid bioprocessing development.

10. Refined Simplex as Superior Process Development Method for Bioprocess Steps And Sequences

**Ajoy Velayudhan, UCL, London*
Spyridon Konstantinidis, UCL, London
David Roush, Merck, Sharp and Dohme, Inc., United States
John P Welsh, Merck, Sharp and Dohme, Inc., USA

Work done in our group has demonstrated that the classical simplex method, which has widely been used for numerical optimisation, can in fact be effective as an experimental optimisation tool. We have refined the simplex method to cope with the discrete or gridded nature of input variables in biomanufacturing. For instance, pH can rarely be controlled to better than +/- 0.1, which is its least count; similarly, salt levels can rarely be controlled to better than +/- 5 mM or even +/- 10 mM. In this presentation, we extend the use of the refined simplex to make the following claims: i) That the refined simplex method is better suited to, and more efficient than, typical DoE approaches to early-phase process development (up to Phase IIa) of individual unit operations in the downstream processing train for macromolecular therapeutics; and ii) That the refined simplex method can also be used effectively to design process sequences within a bioprocess. Experimental results from the Merck bioprocess development group are used to support the first claim. It is shown that, for a variety of polishing chromatography and protein refolding steps, the refined simplex method outperforms quadratic and higher-order regressions to experimental data. The refined simplex method is model-independent, and therefore captures any realistic experimental trend, no matter how complex, in the same way (without altering the method). When more than one optimum exists (as can occur for multimodal resins), the refined simplex finds the global optimum very often; the DoE methods routinely failed to identify the existence of multiple optima and often failed to find effective conditions. When combined with its robustness and ability to cope with missing or even incorrect data, the refined simplex method is found to be superior to experimental designs in finding effective (and usually near-optimal) operating conditions simply and rapidly. Experimental work in our laboratory shows that the development of a two-column polishing train can be achieved in a single set of experiments driven by the refined simplex method. Results for a ternary protein mixture were generated in high-throughput format using a Tecan liquid handler and RoboColumns. With a natural choice of pooling from the first column, the operating variables for both columns were optimised simultaneously. Again, it was found that the refined simplex was able to find effective operating conditions rapidly. Typical experimental designs usually failed to find the global optimum when applied to this simultaneous design of a two-column train, and often failed to fit the experimental trends, thereby suggesting inefficient operating conditions. These results suggest that the refined simplex method is well-suited to finding effective operating conditions rapidly for DSP steps as well as sequences of such steps.

11. Improving Product Uniformity by Integrated Continuous Biomanufacturing

Fabian Steinebach, ETH Zurich, Switzerland
Daniel Karst, ETH Zurich, Switzerland
Thomas Villiger, ETH Zurich, Switzerland
**Massimo Morbidelli, ETH Zurich, Switzerland*

Continuous manufacturing is currently being considered by the Biopharmaceutical Industry not only for the classical reasons which make continuous operation preferred over the batch one, but also for recent initiatives of the regulatory agencies. We discuss here a series of experiments where a perfusion reactor with CHO cells for the production of a monoclonal antibody has been operated in the continuous mode and connected to a two column continuous protein A chromatographic unit for product capture. Different steady states are examined and the use of simulation models for process design and control is illustrated. In such an integrated continuous process, the protein quality of the capture product is different compared to classical batch processing. The lower residence time in the bioreactor and the higher loading in the multi-column capture step lead to post-Protein A pools with more uniform product quality. Hence the performance of subsequent polishing steps is improved. Additionally, it is shown how in a continuous process polishing with the MCSGP process can overcome the purity-yield tradeoff of classical batch chromatography. This cascade of beneficial correlations in integrated continuous manufacturing results in higher productivities and yields of improved product quality.

12. Serendipity in Chromatographic Design: How Development of a Custom Resin for Purification of Fully Human Bispecific Antibodies Led to an Advance in Continuous Processing

**Andrew Tustian, Regeneron Pharmaceuticals, United States*

In the biopharmaceutical industry, there is strong interest in the production of bispecific monoclonal antibodies that can simultaneously bind two distinct targets or epitopes to achieve novel mechanisms of action and efficacy. Regeneron's bispecific technology, based upon a standard IgG, consists of a heterodimer of two different heavy chains, and a common light chain. Co-expression of two heavy chains leads to the formation of two homodimeric IgG contaminants, the removal of which is facilitated by a dipeptide substitution in the Fc portion of one of the heavy chains that ablates Fc Protein A binding. Thereby the Protein A step of the purification process must perform both bulk capture and high resolution of these mAb impurities, a task current commercially available resins are not designed for. This talk details development of a novel Protein A resin, which combines the a base stable, non VH-region binding ligand with a base bead exhibiting excellent mass transfer properties to allow high capacity single step capture and resolution of bispecific antibodies with high yields. During

Poster Abstracts

late-stage testing, it was realized that the high capacity at lower residence times (> 60 g/L), steep mAb breakthrough curve, and base stability of this new resin made it ideal for a much broader application: continuous capture by periodic counter-current chromatography.

13. Continuous Countercurrent Tangential Chromatography for Continuous Purification of Monoclonal Antibody Products

*Andrew Zydney, Penn State University, United States
Oleg Shinkazh, Chromatan, Inc., United States
Boris Napadensky, Chromatan, Inc., United States
Amit Dutta, Chromatan, Inc., United States

One of the main challenges in the development of integrated continuous processes for the production of high value therapeutic proteins is the replacement of traditional batch column chromatography, which tends to dominate current downstream processing, with an effective continuous capture technology. Continuous Countercurrent Tangential Chromatography (CCTC) has been designed to provide truly continuous product capture and purification using a column-free system that overcomes many limitations of traditional column chromatography. All operations in CCTC are conducted on a moving slurry that is continuously pumped through a cascade of static mixers (for mixing and residence time) and hollow fiber membrane modules (for separation of the fluid phase from the resin particles). For example, host cell proteins and other impurities are removed in the permeate collected through the hollow fiber membrane in the washing step, while the bound product is retained by the membrane. Operation is at low pressure (<20 psi), enabling the use of a fully disposable flow path. This eliminates the need for column packing / validation, greatly facilitating operation in facilities designed for single use manufacturing. Contacting in the individual steps is performed in a countercurrent fashion, with the flowing slurry moving from stage (module) 1 to stage 2 while the permeate moves in a countercurrent direction. This significantly increases the overall throughput while improving product yield and purification. All of the chromatographic operations are performed simultaneously, with a fraction of the resin being used for binding while the rest of the resin is in the washing, elution, or regeneration steps. Recent experimental studies have demonstrated that CCTC can be successfully used for initial capture of a monoclonal antibody product from clarified CHO cell cell culture fluid produced in a fed batch bioreactor. Host cell protein removal and antibody yields and purities were similar to that obtained with conventional batch columns, but CCTC provided a several-fold greater productivity (g purified mAb per liter resin per hr). In contrast to multicolumn systems, CCTC provides true steady-state operation, with the product concentration remaining constant over a multi-hour run. Current efforts have been focused on the optimization of the CCTC system to increase productivity while reducing buffer requirements. This includes novel strategies for buffer recycling, the use of small particle size resins to reduce mass transfer limitations, and the optimization of the countercurrent staging. Mathematical models for CCTC performance have been developed and provide a framework for system design and process development efforts. In addition, experimental studies are being extended to couple the CCTC system directly to a perfusion bioreactor, providing truly continuous production of highly purified monoclonal antibody products.

14. Truly Continuous Downstream Processing of Biologics

*Ruben Carbonell, North Carolina State University, United States
Stefano Menegatti, North Carolina State University, United States
Ashton Lavoie, North Carolina State University, United States
Amith Naik, BTEC, North Carolina State University, United States
Tuhidul Islam, North Carolina State University, United States

Truly continuous purification of biologics can be achieved by developing novel steps for impurity removal that result in the complete elimination of unit operations based on the classical "bind-and-elute" mode. Flow-through mode capture of impurities is widely accepted as a polishing step, but product capture via bind-and-elute mode using high capacity affinity or ion exchange media is still dominant, even in so-called "continuous processes" that link together three or more columns in different stages of operation. Bind-and-elute steps capture and concentrate product while significantly reducing host cell protein (HCP), DNA, and other impurities, These processes, however, require binding, wash, elution, and regeneration steps, which are time consuming, and exhibit large footprints for buffer storage, buffer preparation and waste disposal management. Our work explores the development of novel, robust, inexpensive, ligands for the continuous removal of HCPs and other impurities from process streams. Combinatorial libraries are screened for small ligands that capture the whole spectrum of HCPs in a specific cell line without retaining the target product at a given set of solvent conditions (buffer pH, composition, and conductivity). Because of the wide range of molecular weights and concentrations of HCPs, a single ligand is not likely to be effective enough to achieve complete HCP removal, thus a "polyclonal mixture" of HCP binding ligands needs to be developed. These ligands can be used on any type of support, but disposable, single-use membranes would be ideal for this application. HCP-capture membranes or columns can be implemented immediately after the cell removal and clarification steps and can also be used in final polishing steps. In truly continuous operation the process stream would flow through these devices with the implementation of continuous concentration technologies such as single-pass tangential flow filtration. In cell culture, the HCP concentration is approximately ten times lower than that of the typical biological product, and as a result HCP capture devices will be considerably smaller than product capture columns and will be able to process much larger volume of process fluid in less time. Additionally, implementation in a disposable format would save considerable time, space and manpower costs. We have found that commercially available cell removal and HCP removal resins can result in approximately 0.4 logs of HCP removal from supernatant when used in a flow-through mode with little loss of IgG yield. We have also examined the molecular weight and characteristics of the HCPs that can be removed with existing resins to guide ligand identification. Several peptide ligands have been identified that can bind to HCPs from mammalian cell culture without binding to human IgG. Preliminary results will be presented on the removal that can be achieved with these ligands immobilized on chromatographic supports.

15. Optimisation and Integration of a Continuous Post Capture Purification Process

*David Gruber, MedImmune, United Kingdom
Muazzam Khan, MedImmune, United Kingdom
Lucy Evans, MedImmune, United Kingdom
Richard Turner, Medimmune, United Kingdom

As the Biotechnology industry matures and begins focusing on strategies for increasing productivity and reducing cost there has been a move towards continuous processing. The implementation of perfusion cell culture has increased productivity once again shifting the bottleneck onto downstream. The use of periodic counter current chromatography as a capture step is gaining popularity, however this is largely due to the on/off nature of a Protein A affinity step for processing antibody products. Polishing the post capture product is a more complex operation as product must be separated from more closely related impurities such as aggregates. Bind and elute chromatography steps are typically utilised in order to separate aggregates, thus allowing for the development of robust separations. However these operations are limited by the binding capacities achieved (<80g/L). Additionally the use of salts as counter ions for elution limits the conditions available for downstream operations without the need for dilution or UF/DF. Here we describe the development of flow through polish steps as well as a strategy for their integration designed to increase productivity of the overall purification process. A high throughput screening methodology is used to determine the optimal conditions for the polishing unit operations. Using these conditions enables the implementation of integrated high productivity polishing steps that can be used in continuous manufacturing processes.

16. Model-based Conversion of a Single-column Batch Process to 3- and 4-column Periodic Counter-Current Chromatography

*Tobias Hahn, Karlsruhe Institute of Technology, Germany
Gang Wang, Karlsruhe Institute of Technology, Germany
Fabian Görlich, Karlsruhe Institute of Technology, Germany
Juergen Hubbuch, Karlsruhe Institute of Technology, Germany

As fully integrated continuous processing is being adopted by the biopharmaceutical industry, the individual batch-wise processes have to be replaced by continuous equivalents. Typically, chromatography steps are employed to purify the target component from both weaker and stronger binding impurities. While conventional simulated moving bed (SMB) systems can only purify binary mixtures, periodic counter-current chromatography (PCCC) systems are able to cope with ternary mixtures. PCCC has been successfully employed as first capture step and design charts have been developed for such cases with favourable isotherm by Carta and Perez-Almodovar. However, intermediate purification steps based on ion exchange or hydrophobic interaction chromatography do not possess a favourable isotherm and are much more sensitive to small changes in the process parameters. Because of the many degrees of freedoms of PCCC set-ups, such as flow rates, buffer concentrations, cycle duration, etc. model-based process development is the method of choice to identify the design space and optimal conditions. In the presented ion exchange case study using SP Sepharose FF, the target component was an intermediate binding IgG. The model was calibrated using three gradient elutions in linear mode and one in non-linear mode. The optimal process conditions for single-column batch, 3-column (3C) and 4-column (4C) PCCC processing were determined with a multi-objective optimization regarding yield, purity and production rate. The results showed comparable values for yield and purity. However, the production rates of the simulated 3C-PCCC and 4C-PCCC were increased by 100 % and 280 % compared to the batch process. Multi-variate data analysis (MVDA) was employed to identify the main factors of influence on the three sub-objectives. MVDA was unable to explain the whole variation in the PCCC design space, underlining that the optimization problem is highly non-linear. However, applying MVDA to the Pareto fronts allowed to identify different clusters of process variables, the most important ones being related to the conditions while overloading the column.

17. Sterilized Column Chromatography for Continuous Downstream Processing

*James Peyser, Repligen Corp, United States
Dana Pentia, Repligen Corporation, United States
James Rusche, Repligen Corporation, United States*

For continuous production of recombinant proteins at manufacturing scale, sterility at every step of the process is vital. Upstream processing can integrate many available alternatives for sterile systems: bioreactors, filters, etc. For downstream purification part, sterility can be obtained only by using membrane chromatography. To date there is no suitable solution for a sterilized capture step of monoclonal antibodies from the bioreactor. Here we introduce a novel sterilized, pre-packed chromatography solution for downstream capturing step that can be used in a continuous processing at manufacturing scale. This study describes the feasibility of sterilizing a pre-packed disposable column packed with a capture resin by gamma radiation. This involves comparative evaluation of performance of a column sterilized by gamma irradiation with that of a non-irradiated column. We demonstrate that physical properties of the irradiated column, and pressure specifications are not changed. We also demonstrate the long-lasting maintenance of column, and capture media performance, and sterility upon irradiation. Long-lasting stability of column, and resin properties is particularly important in a continuous chromatography, as the columns will be used for extended period of time, sometimes for up to two months during one purification campaign. Operating the sterilized, irradiated column at normal pressure and flow over an extended period of time showed no change in column integrity. Sterility is also demonstrated to be maintained inside the column during extended use at operating conditions. A method of preserving performance of affinity resins allows for maintenance of purification performance upon gamma irradiation with minimal loss of capacity. Feasibility of implementing such sterilized affinity resins in prolonged continuous purification is demonstrated. The study presented here establishes that a capture chromatography solution for closed system continuous process can be achieved, and implemented in a downstream purification process.

18. Cost and Manufacturability Drivers for Smart Decision-making in Bioprocess Development and Facility Fit

**Suzanne Farid, UCL, United Kingdom*

Understanding cost and performance drivers is critical for effective decision-making across the biopharmaceutical process development pathway. UCL's Decisional Tools team have developed advanced decision-support tools that effectively integrate concepts from bioprocess economics, dynamic simulation, risk analysis, combinatorial optimisation and advanced multivariate analysis to

Poster Abstracts

address such challenges. This presentation will show practical applications of such models to enable "smart bioprocessing" for industrially-relevant problems related to facility fit, implementation of novel technologies such as integrated continuous bioprocessing and manufacturability assessment for monoclonal antibodies (mAbs). The first case study focuses on facility fit challenges in legacy facilities exposed to higher titres as well as batch-to-batch variability in cell culture titres, step yields and chromatography eluate volumes. Insights from the systematic use of advanced multivariate analysis techniques will be presented that illustrate critical combinations of factors that lead to undesirable mass loss levels and reveal the root causes of bottlenecks. A comparison of different debottlenecking solutions will be presented in term of their impact on mass output, cost of goods and processing time, as well as consideration of extra capital investment and space requirements. The second case study focuses on facility fit challenges in multiproduct facilities catering for both low and high concentration formulations. The work explores the capability of a particular TFF system to reach high concentration product formulations. Multiobjective optimisation is used to find the optimal final UF/DF design for different target product concentrations with both maximum annual product output and minimum cost of goods (COG). The third case study explores the economic feasibility of different configurations of continuous chromatography for clinical and commercial manufacture. It will address questions such as: How does the feasibility of continuous chromatography combined with pre-packed disposable columns change across different feed characteristics, resin properties, development phases and commercial production scales? What is the optimal design of integrated continuous downstream process configurations? Finally, the fourth case study presents a novel framework for deriving a set of manufacturability indices related to viscosity and thermostability to rank high-concentration mAb formulation conditions in terms of their ease of manufacture in the final UF/DF step. The indices were used to identify the optimal formulation conditions that minimize the potential for both viscosity and aggregation issues during UF/DF.

19. Acoustic Wave Separation – A Scalable Disruptive Technology for Continuous Clarification of Fed Batch Cell Culture Prior to Capture Chromatography

**Peter Levison, Pall Life Sciences, United Kingdom*
Ron Farkash, Pall Life Sciences, USA
Mike Collins, Pall Life Sciences, USA

With advances in fed batch cell culture leading to higher cell densities and higher product titers there is a drive to improve the efficiency and speed of the cell harvest and clarification stage to generate Harvested Cell Culture Fluid (HCCF) for capture chromatography and subsequent downstream processing. This is further driven by the evolution of continuous processes where there is a preference for a continuous feed of HCCF available for direct load to the continuous multicolumn capture chromatography step. Existing cell culture clarification using either centrifugation or depth filtration are typically operated in batch mode and require bulk storage of feed or HCCF during the process. In the present work we report on a novel disruptive and scalable single-use technology for cell culture clarification based on an acoustophoretic separation. Acoustic Wave Separation (AWS) technology involves the use of low frequency acoustic forces to generate a 3 dimensional standing wave across a flow channel. Cell culture from a fed batch bioreactor enters the flow channel and as the cells pass through the 3D standing wave they are trapped by the acoustic forces. The trapped cells migrate to the nodes and clump till such time as their buoyancy decreases and they settle out of the suspension by gravity. This yields a partially clarified HCCF which can be polished using a small area depth filter. We have not seen any demonstrable adverse effects on the quality of the HCCF or the cell viability following AWS clarification. We report the continuous clarification of fed batch culture of a CHO-S based cell line expressing a humanised IgG1 MAb. At process development (PD) scale we demonstrate the ability to clarify CHO cell culture at cell densities of 30 – 100 million cells/mL, in a continuous manner at flow rates of up to 3.6 L/h. Furthermore we have shown the technology to be scalable and using prototype systems have demonstrated clarification flow rates of 50 L/h that when configured in parallel enables the technology to be applicable for 2000L bioreactors. This enables the AWS technology to be positioned for clinical manufacture. The partially clarified HCCF is polished in a continuous mode using depth filtration but typically requires 3-5x less depth filter area than used for a traditional depth filtration process. This offers economic benefits in terms of footprint and depth filter costs, but also significant reductions in the volume of Water for Injection (WFI) used in depth media conditioning and post-harvest buffer wash following clarification as well as reduced waste disposal costs and significant set-up time savings. The economic benefits of the AWS approach will be discussed in more detail. AWS technology enables the continuous clarification of cell culture from bed batch bioreactors in a single-use operation. The technology has been shown to perform well at cell densities of up to 100 million cell/mL so is well positioned to meet the clarification demands of emerging higher cell density fed batch processes currently in development as well as perfusion applications that are gaining momentum in the biotech space.

20. Performance of a Novel, Truly Single-Use Adsorber Technology in mAb Purification

**John Boyle, MilliporeSigma, United States*
Benjamin Roman, MilliporeSigma, USA
Debola Banerjee, Roche Genentech, USA

In recent years there has been a very significant focus from both suppliers and drug manufacturers on developing and implementing single-use technologies. Drivers for the shift to single-use technologies can depend on production scale, existing installed capital base, and other factors. Single-use technologies have the potential to reduce costs (both capital costs and COGs). Single-use chromatography media can improve bioburden control and eliminate rare (but costly) packed bed integrity issues. This presentation will show the performance of a Quaternary Ammonium version of a truly single-use chromatography media. The new technology is based on an entirely new stationary phase support material – a novel synthetic fiber. The stationary phase provides both high capacity and rapid mass transfer. The performance of this technology is presented here versus several benchmark technologies (bead-based resin and membrane adsorbers). Data on HCP, DNA, and viral clearance in industrially relevant feed streams will be discussed under several different conditions (flow rates, conductivity, feeds, pH, etc.). A brief overview of the economics around this media will be presented as well.

21. Custom Affinity Chromatography – a Novel Platform for Rapid Development of Affinity Media Displaying DNA Aptamer Based Ligands

Aaron Ang, University of British Columbia, Canada
Eric Ouellet, University of British Columbia, Canada
Mark Snyder, Bio-Rad Laboratories, United States
**Charles Haynes, University of British Columbia, Canada*

Time-to-market pressures for recombinant protein therapeutics generally demand rapid design of product purification protocols. This need for speed challenges the time-consuming strategies generally employed to optimize the economics and performance of a purification train, including selection of a stationary phase for product capture. The problem is especially acute for biologic orphan drugs, for which production per annum is often a few kg to a few tens of kg, and for which proven downstream processing platforms are generally not available. This rapidly growing products class could benefit from creation of an integrated set of products and decision-making tools that enable one to efficiently define an affinity capture chromatography step that preserves yield and product integrity while limiting further downstream processing demands, and thus cost. We will describe our progress towards development of a new pipeline for efficient creation of robust affinity chromatography media, based on DNA aptamers as ligands, that have been custom designed to achieve specific performance metrics when applied to a particular product and feedstock. Central to this concept is the creation of a method to rapidly select candidate affinity ligands for product capture from a highly diverse starting aptamer library comprised of more than 1014 unique sequences. Our novel Hi-Fi SELEX protocol provides this critical capability, and we will describe in detail how it is used as the pipeline front-end to discover and characterize DNA aptamers offering high affinity and specificity for a target biologic. Methods for chemically stabilizing aptamer ligands will also be described, along with specific end-group chemistries encoded within or appended to the aptamers to enable orientation-specific ligand immobilization to Bio-Rad UNOsphere Epoxide or UNOsphere diol media. Panels of scaled-down candidate columns constructed using this pipeline may then be screened using standard liquid-handling robotics, and we will describe how this may be done if time permits.

22. An Integrated, High Throughput and Rational Design Platform for the Development of Affinity Peptide Resins for Biological Product Purification

Divya Chandra, Rensselaer Polytechnic Institute, United States
Steven Timmick, Rensselaer Polytechnic Institute, United States
Chaz Goodwine, Rensselaer Polytechnic Institute, United States
Nicholas Vecchiarello, Rensselaer Polytechnic Institute, United States
**Pankaj Karande, Rensselaer Polytechnic Institute, United States*
Steven Cramer, RPI, United States

While many strategies have been employed for the discovery and design of affinity peptides, in a broad sense most fall under the categories of either brute force screening of large libraries or targeted, rational design and evaluation of a small number of candidates. Here we present an integrated platform process for the rapid

Poster Abstracts

development of molecule-specific, affinity peptide resins for the purification of biological products. Our platform utilizes a synergistic combination of peptide design strategies (rational as well as brute force) along with novel, high throughput screening approaches geared towards identifying peptides which meet criteria necessary for efficient downstream purification. Specifically, these criteria include high affinity, capacity, selectivity, elutability, and regenerability. In this platform, two approaches are first used to generate a library of peptide candidates. High throughput phage display is employed to screen large peptide libraries, while simultaneously, known binding partners are utilized to rationally generate peptide candidates from epitope mapping and loop stitching. High throughput microarray screenings of these designed peptide libraries are then performed to generate leads that span a range of binding affinities for the target protein. Top candidates are selected for validation and characterization of binding via fluorescence polarization under various bioprocess-relevant conditions. Lead sequences are then docked in silico and subjected to affinity maturation to improve binding capacity and selectivity as well as to facilitate elution under a desired condition, such as the addition of histidines for low pH elution. Top peptide candidates from these screening efforts are synthesized on chromatographic resin and evaluated for purification performance in batch. Strategic sampling of the competitive isotherm space allows for the rapid, orthogonal assessment of peptide resin performance for the key metrics of affinity, elutability, and selectivity. Batch results are then used to inform peptide refinement in an iterative process until optimum candidates are identified. Finally, column-scale experiments are performed to select a single peptide resin for further optimization with regards to ligand density, process conditions, and resin regenerability. Results from the development of affinity peptide resins for two molecules, human Growth Hormone and Interferon Alpha 2b, will be discussed to illustrate the successful application of this platform process. This platform represents a novel approach for the rapid identification and development of affinity peptide resins for a range of new classes of biological products, which has important implications for simplifying and streamlining future downstream process development.

23. Polyacid Based Precipitation as an Alternative to Cold-ethanol Fractionation of Plasma Proteins: Alignment with Existing Downstream Processing Methods

*Karl McCann, CSL Behring Australia, Australia
Jose Martinez, CSL Behring Australia, Australia
James Van Alstine, Royal Institute of Technology, Sweden
Joseph Bertolini, CSL Behring Australia, Australia

Plasma is the source of many important biotherapeutic products such as albumin, immunoglobulins, coagulation factors and inhibitors. The fractionation of plasma is still predominantly based on cold-ethanol precipitation for initial processing, which involves precipitation of proteins through manipulation of pH and ethanol concentration at subzero temperatures. The requirement for low temperatures and large amounts of ethanol is a major disadvantage of this procedure. Polyacrylic acid (PAA) fractionation shows potential as an alternative to cold ethanol fractionation of plasma proteins, especially given that the precipitation steps can be conducted at room temperature. Previous studies have shown that polyacrylic acid (PAA) specific precipitation of fibrinogen-, immunoglobulin- and albumin-rich fractions was achieved at PAA concentrations of 7, 12 and 20% w/w, respectively with yields of greater than 80%. The current study explored whether established downstream processing methods used in the plasma fractionation industry could be applied to albumin and

Poster Abstracts

IgG precipitates derived from PAA fractionation. Downstream processing of the IgG-rich precipitate using a combination of caprylic acid precipitation and anion exchange (MacroPrep HQ) polishing successfully depleted the major impurity proteins of IgA, IgM and α-macroglobulin, resulting in a final product purity over 99%. The albumin-rich precipitate could be further processed using either cold-ethanol precipitation or a two-step ion exchange (DEAE and CM Sepharose-FF) polishing method to generate final product with a purity of 98.6% or 99.6%, respectively. The successful demonstration that the albumin and IgG intermediates derived from PAA fractionation could be purified using established downstream processing methods suggests that PAA fractionation of plasma could be a viable alternative to current cold-ethanol fractionation methods. PAA fractionation has the potential to reduce complexity, captial investment and cost of goods related to the manufacture of plasma derived biotherapeutic proteins.

24. A "Rheo-Chip" Platform for QbD Approach to Bioprocessing

**Alfredo Lanzaro, School of Chemical Engineering, the University of Manchester, UK*
Robin Curtis, School of Chemical Engineering, the University of Manchester, UK
Alain Pluen, School of Pharmacy, the University of Manchester, UK

Future trends in treating various chronic diseases with recombinantly-produced therapeutic proteins, like human serum albumin and monoclonal antibodies, require frequent and high dose of active protein ingredient in a small volume of liquid (c= 100 g/L or higher). Understanding the link between formulation, rheology, stability and aggregation propensity of concentrated protein medicines is of paramount importance for a successful implementation of the Quality by Digital Design paradigm to bioprocessing industry. We address such fundamental challenges by means of our "Rheo-Chip" technology*. It consists of a series of rigid and transparent microfluidic devices equipped with replaceable pressure sensors and with a series of flow actuation devices (syringe and pressure pumps, piezo stacks) with which every possible fundamental flow (steady and oscillatory shear, extensional, flow with a stagnation point) can be imposed to the tested fluid. The available data include steady shear and extensional viscosities measured over a range of deformation rates (102-105 s-1) typical of bioprocessing operations, the mechanical spectroscopy measured up to frequencies as high as 100 Hz, and micro-injection forces studied by means of a novel microfluidic "syringe-on-chip" device. Moreover, by coupling microfluidic chips having a functionalized inner surface (hydrophobic or hydrophilic) with fluorescence measurements, we are able to understand the effects of different interfaces as well as of specific flow conditions on protein aggregation. It is envisaged that this research will lead to improved design of bioprocess operations (vial filling, syringe injection, tangential flow filtration etc.), as well as to an insightful understanding
of the mechanisms leading to formation of protein aggregates under flow. *: The University of Manchester, Patent Application No: PCT/GB2011/051476

25. Robust Multi-objective Process Design

Lars Freier, Research Center Jülich, Germany
**Eric von Lieres, Research Center Jülich, Germany*

Separation processes are routinely designed and optimized using parallel high-throughput experiments and/or serial lab experiments, depending on the available equipment and current state of knowledge. Well characterized

Poster Abstracts

processes can further be optimized using mechanistic models. In all these cases – serial/parallel experiments and modeling – iterative strategies are customarily applied for planning novel experiments/simulations based on the previously acquired knowledge. Process optimization is typically complicated by conflicting design targets, such as productivity and yield, and by process variations, in particular fluctuating feed composition. We address all these issues by combining advanced statistical regression models with most recent methods for uncertainty quantification in multi-objective optimization. The methods are demonstrated by simultaneously optimizing elution gradient and pooling strategy for separating a three-component system subject to varying feed composition with respect to purity, yield, and processing time. Gaussian process regression (Rasmussen, 2006) is applied for estimating the system behavior from performance indicators (purity, yield, time) observed at systematically varied operating conditions (gradient, fractionation) with random fluctuations (feed). These predictions are provided with sound confidence estimates, reflecting the given data quantity, data quality, and process fluctuations. The statistical concept of expected improvement (Emmerich, 2006) is used for determining which additional data would supplement current knowledge in the best way, maximizing process performance and selectively minimizing prediction uncertainty in the most promising regions of the parameter space. The method supports optimal design of both serial and parallel experiments. Two and more conflicting objectives are treated using the latest algorithms (Hupkens, 2015) for efficiently computing expected hypervolume improvement. Recent methods (Binois, 2015) are also required for calculating the uncertainty propagated from process fluctuations to the Pareto front, which provides important and useful information for robust process design.
Rasmussen et al.: Gaussian processes for machine learning, MIT Press, 2006. Emmerich et al.: Single- and multiobjective evolutionary optimization assisted by Gaussian random field metamodels, IEEE Transactions on Evolutionary Computation 10 (2006): 421–439. Hupkens et al.: Faster exact algorithms for computing expected hypervolume improvement, EMO 2015, LNCS 9019, pp. 65–79, Springer, 2015. Binois et al.: Quantifying uncertainty on Pareto fronts with Gaussian process conditional simulations, Eur. J. Op. Res. 243 (2015): 386–394.

26. Straight Through Processing Using Integrated Chromatography Column Sequences

**Bernt Nilsson, Lund University, Sweden*
Niklas Andersson, Lund University, Sweden
Anders Holmqvist, Lund University, Sweden
Anton Sellberg, Lund University, Sweden
Peter Tiainen, Novo Nordisk A/S, Denmark
Arne Staby, Novo Nordisk A/S, Denmerk

Smart downstream processing can be performed with a sequence of integrated purification steps, which minimize the number of storage tanks and reduce hold-up time. The result is an integrated chromatography column sequence that performs straight through processing of the target protein, with minimal time from expression to formulation. This downstream processing technique is well suited to be connected to a continuous upstream process based on perfusion. To introduce this technique it has to be scalable from desktop lab-scale to full size production scale. This paper present a methodology for design and control of integrated column sequences. In straight through processing the eluting pool is directly loaded on to the next column. The consequence is that the next step has to have proper capacity to handle the loaded pool without losses. An overall design procedure of integrated column sequences is discussed based on its performance and limitations, together with requirements on each individual step. Design of integrated column sequences in different scales is discussed and exemplified on lab-scale. The propagation of disturbances in connected column sequences forces an open-loop control strategy to be unnecessary conservative with limited performance. Three issues on the individual step design will be discussed. First the design procedure of each individual step is modified to also handle disturbances which make it possible to find robust performance. Second, for some separation problems it is attractive to introduce local recycles over a step to enhance the yield of the target protein. Third, in many cases it is possible to increase performance and yield by modifications of the elution profile. A completely new case study is presented which uses a multiple step elution gradient. The procedure is illustrated both using computer simulations and experimentally on an industrially relevant case.

27. Design of a Continuous Virus Inactivation System for Clinical and Commercial Scale

**Raquel Orozco, Boehringer Ingelheim Fremont, Inc., United States*
Scott Godfrey, Boehringer Ingelheim Fremont, Inc., United States
Cameron Bardliving, Keck Graduate Institute, USA
Lindsay Hernandez , Keck Graduate Institute, USA
Linus Amarikwa , Keck Graduate Institute, United States
Shashidhar Hoskatti , Keck Graduate Institute, United States
Jinkeng Asong , Keck Graduate Institute, United States
Jeffrey Salm, Pfizer, United States
Jon Coffman, Boehringer Ingelheim Fremont, Inc., United States

Low pH virus inactivation lends itself to continuous processing. Continuous virus inactivation (cVI) allows a smaller process footprint and a more tightly integrated downstream. It also can eliminate the need for cycle-to-cycle cleaning validation, or disposable change over between cycles in a disposable plant. In this work we explore alternative options to incubate a stream of product for the desired incubation time of 30-60min. We have designed incubation chambers that meet the following criteria 1.The incubation chambers axial dispersion is such that the residence time distribution is sufficiently narrow, and empirically demonstrated. -<10ppm of tracer exits the chamber before the validated incubation time -Max residence time cannot be longer than 2hrs for more than 1% of the product 2.The chambers must not generate more than a 5 psi of pressure, thus plug flow is not feasible. 3.The flow inside the chamber must be well characterized such that chambers at different flow rates can be readily generated and can meet conditions 1 and 2 stated above. In this work, we describe incubation chambers that meet these requirements. These chambers are sufficient to support production for 25kg/year from a 100L perfusion bioreactor. These solutions should work for a wide range of flow rates that can potentially be adopted for other continuous/periodic downstream processes.

28. United Against the Bioburden Threat

**Anna Gronberg, GE Healthcare, Sweden*
Tomas Bjorkman, GE Healthcare, Sweden
Elin Monie, GE Healthcare, Sweden
Magnus Wetterhall, GE Healthcare, Sweden
Johan Avallin, GE Healthcare, Sweden

Bioburden can enter into the biopharmaceutical process through different routes e.g. via air, water, consumables, raw materials, and operators. Bacteria and their by-products will negatively affect the safety and potency of the biopharmaceutical drug. Therefore, regulatory authorities put increasing demands on biopharmaceutical producers. To be successful in the accomplishment of a bioburden free process, biopharmaceutical companies and their suppliers must fight this battle side by side. Bioburden must be attacked from different angles. It begins with the supplier developing products and operational routines that enable aseptic procedures in the biopharmaceutical process. Furthermore, the biopharmaceutical producers must develop control strategies and implement aseptic procedures to prevent bioburden entering their processes. This paper will focus on the commitment from suppliers and will describe the continuous improvement in delivery of aseptic chromatography media, further chromatography media development for sustainability at harsh sanitization conditions and investigation of new sporicidal agents for sanitization. Chromatography media suppliers can deliver products absent from microorganisms by using controlled manufacturing procedures. Pre-sanitization of media will even further minimize the risk of bioburden. Pre-sanitized media are available in pre-packed single use formats predominantly used for pilot scale. With modern high capacity media and improved formats, single use might be a reality also for future manufacturing of biopharmaceutical drugs. The potential for use of supplier pre-sanitized bulk media in a regulated GMP environment with large on-site packed columns will be discussed in conjunction with equipment enabling closed processing and aseptic procedures for chromatography media slurry preparation, column packing and column handling. The chromatography media can also be improved and stabilized to tolerate harsher conditions, which facilitates pre-sanitization and sanitization integrated into the biopharmaceutical process. Development of alkali stabilized protein A ligands tolerating up to 0.5 M NaOH has improved the ability to clean and sanitize these media over the last decade. However, resistant spore forming bacteria is still a challenge. Protein A ligand development is underway towards ligands that tolerate even sporicidal concentrations of NaOH, which will reach the market in the near future. In the continuous search for new and more efficient sanitization agents, oxidizing chemicals showed promising results for affinity media, sensitive to harsh cleaning conditions. Analytical methods such as Surface plasmon resonance, liquid chromatography-mass spectrometry, and a battery of chromatographic methods were used for evaluation of the ligand and base matrix compatibility with oxidizing agent. Bacterial challenge studies were performed using design of experiments to define concentrations and contact times for efficient reduction of bacterial spores. Furthermore, the effect on the media was assessed in functional lifetime studies where ligand leakage, product yield and impurity clearance were evaluated.

29. Novel Chromatographic Adsorbents Based on Dendritic Molecules for HIC

Marco Mata-Gomez, Tecnologico de Monterrey, Mexico
Sena Yaman, Izmir Institute of Technology, Tirkey
Jose Gonzalez-Valdez, Tecnologico de Monterrey, Mexico
Jesus Valencia-Gallegos, Tecnologico de Monterrey, Mexico
Canan Tari, Izmir Institute of Technology, Turkey
**Marco Rito-Palomares, Tecnologico de Monterrey, Mexico*

A major goal in the biotechnological industry is the purification of bioproducts such as proteins from complex mixtures. Since this represents up to an 80% of the total production costs, innovation on downstream processing strategies is required. In this work, we introduce the use of dendrons branched molecules with well-defined structures for the synthesis of chromatographic adsorbents for hydrophobic interaction chromatography (HIC). The adsorbents were synthesized by covalently attaching the amino cores of polyester dendrons, of two different branching degrees i.e. generations 3 and 5 (dG3 and dG5, respectively), with hydroxyl group (-OH) ends to NHS-activated chromatographic media. Dendronized resins were then functionalized by incorporation of organic carboxylic acid, containing a four carbon end as an hydrophobic ligand, by reaction with the available –OH groups. This resulted in two dendronized resins with clusters of hydrophobic ligands on the periphery. The aim of this work was to characterize and evaluate the performance of the dendronized HIC adsorbents. UV-Vis spectra and FTIR analyses of the modified resins confirmed the presence of the dendrons and their ligands on the resins. Modification of these supports resulted in an increased ligand density along the packed columns. Regarding adsorption capacity, the unmodified resin did not adsorb the protein, while modified resins were able to adsorb ~60 mg BSA/g resin. Interestingly, the resin modified with dG3 dendrons exhibited a better affinity than that one modified with dG5 as indicated by the affinity constant calculated from the adsorption isotherms. Dendronized resins were also tested for their performance on separating PEGylated proteins biomolecules with chains of PEG attached to their structure—. As expected, resins modified with dG5 exhibited a stronger hydrophobic interaction with PEGylated proteins, as these were not eluted into the gradient unless isopropanol was added into the mobile phase. On the other hand, PEGylated proteins were eluted from the adsorbent modified with dG3 into the gradient without needing isopropanol suggesting a weaker interaction. This innovative dendronized support opens a window to new generation chromatographic supports to develop novel downstream processing strategies.

30. Nanofibres as a True Single-use Chromatography Platform Achieved Through High-Productivity Product Capture

**Oliver Hardick, Puridify / UCL, United Kingdom*
Will Lewis, GlaxoSmithKline, United Kingdom
Daniel G. Bracewell, University College London, United Kingdom

Cellulosic nanofibres have been employed as an alternative to traditional chromatographic media for industrial product capture purification for a monoclonal antibody platform process. The nanofibres are fabricated to provide a combination of high flowrates and high capacities that enables productivity in terms of grams of product purified per litre of adsorbent, per hour to be increased >10 fold with a chromatographic cycle time of <4 minutes, a 100x reduction when compared to traditional packed bed operation. In practice, this means for an equivalent

Poster Abstracts

bioreactor harvest, a significantly smaller adsorbent cartridge can be used to purify a batch through rapid cycling. The output is a unit which can be sized such that its lifetime can be exhausted over the batch resulting in single-use operation with the goal of increasing facility flexibility, plant productivity, and reducing validation costs. Nanofibres exhibit a surface area to volume ratio in the 10's m2/g range which yields a binding capacity in the range of those expressed by traditional porous bead resins. In contrast to beads however, this surface area is immediately accessible for biomolecule interaction rather than requiring diffusional mass transfer into a porous structure. With an open macro porous structure this capacity enables high productivity separations achievable with second or sub-second residence times at low operating pressures. This is demonstrated in the work presented here where Protein A nanofibre adsorbents were employed from high-throughput screening scale through Tecan systems at µL scale to pilot scale purifying 30L of therapeutic mAb from a CHO cell harvest selected to be representative of current mAb platform systems at a titre of 0.88g/L. Chromatography productivity has been demonstrated to be above 130g/L/h, a 10 fold increase from our control packed bed system, without the need for multicolumn chromatography rigs, with a purity equivalent to that achieved with platform resins. The adsorbent lifetime was demonstrated at >100 cycles without significant loss in capacity or increase in column pressure. Key metrics of host cell protein content and Protein A leaching were comparable to a resin based process benchmark run using the same feed material. CEX nanofibre units demonstrated aggregate removal, from a feed of 4%, to below the limit of detection (using SEC-HPLC) with 96% recovery in a run time of <3minutes at pilot scale highlighting a resolution not typically associated to convective flow adsorbents. Finally a nanofibre AEX unit was employed for HCP clearance and performed to the required 3 log reduction. This work demonstrates feasibility and scalability for industrially relevant single-use product capture purification by employing existing chromatographic functionalities and processes through a chromatographic support structure that enables the utilisation of that powerful functionality at a much greater throughput and therefore productivity. This single-use enabling technology has clear advantages in some processing scenarios such as early clinical supply, stratified medicine supply, and localized manufacturing opportunities but offers promise to many existing facilities through providing a greater flexibility in processing strategy.

31. Design of Protein A Resins for Cost Effective Purification of MABS

**Hans Johansson, Purolite Life Sciences, Sweden*
Patrick Gilbert, Purolite Life Sciences, UK
Caroline Tinsley, Purolite Life Sciences, UK
Mark Hicks, Purolite Life Sciences, UK

The high cost of Protein A affinity resins has been an object of discussion for many years. The contribution to cost of goods in regular production, where resins are typically used for over one hundred cycles, is generally accepted because it only adds up to a small fraction of the manufacturing cost. However in clinical production, where resins are typically used for only a few cycles, the high resin cost becomes very significant. One way to address this would be to use a less expensive resin for early clinical production and switch to an alkaline stable Protein A when (if) the drug candidate is entering Phase III and regular production. Looking at the purification performance of Protein A resins, differences are mainly a function of the type of base matrix or immobilization chemistry used, rather than the type of Protein A. In this study we have compared native Protein A (rSPA), with a Protein A modified for improved alkaline stability. Both Protein A ligands have been immobilized to the same type of agarose matrix, using the same type of chemistry, and amount of Protein A. The idea was to design an inexpensive efficient resin for early clinical manufacturing where columns are rarely used for more than 10 cycles and where the inherent alkaline stability of rSPA is sufficient for efficient cleaning and sanitization using 0.1-0.5 M NaOH for up to 20 cycles. The second, alkaline stable protein A resin, is designed for regular manufacturing and will withstand use for several hundreds of purification cycles. The achieved dynamic binding capacities range from 40-50 mg hIgG/ml resin at residence times of 4-6 minutes. Purification data such as host cell protein content, DNA levels, Protein A leakage, aggregate, and fragment levels has been assayed to compare the two, agarose based, Protein A resins.

32. A New Platform for Affinity Chromatography Using Epoxy-Phage-Cryogels

Deckmyn Hans, KU Leuven KULAK, Belgium
**Noppe Wim, KU Leuven KULAK, Belgium*

The aim of the present study is to develop a new affinity chromatographic method to by using supermacroporous polyacrylamide (pAam) cryogel monoliths as chromatographic matrix in combination with peptide displaying phages as affinity ligands. 6%-pAam-epoxy cryogels were produced and optimized for binding capacity. Epoxy groups for coupling were introduced in the cryogel at varying molar ratios of Aam/Allyl Glycidyl Ether (AGE). Both the structural integrity of the columns and the ligand binding capacity were investigated. A decrease of the structural integrity was observed from a molar ratio Aam/AGE<7,5 onwards. A molar ratio=10 did provide excellent structural stability and with a sufficient number of epoxy groups for coupling. With higher molar ratio's, the lower number of available epoxy-groups did lead to a lower binding capacity. Next the use of peptide displaying phages as ligand for affinity chromatography was investigated. To provide "proof of principle", M13-filamentous phages with binding affinity for human lactoferrin were selected from 6-mer p3-phage display libraries and used as affinity ligand and coupled onto the cryogel. Due to the physical and structural properties (pores up to 100μm) of the cryogel, phages can be used for coupling as affinity ligand. Also crude samples such as milk, fermentation broth, cell cultures,.. can be applied on the cryogel without any flow/pressure restrictions. Proof of principle was provided by processing whole human milk on epoxy-phage-cryogels. Human lactoferrin was captured and recovered with a high purity (>95%) in a one-step chromatographic run. As however multiple epoxy coupling sites can be occupied by a single phage, this may lead to a decrease of target binding capacity. By introducing spacers between the epoxy-matrix and the phage ligand, indeed an increase of binding capacity is observed. Several spacers, linear and branched, and phages were tested. Up to a 20-fold increase in binding capacity was observed when using a phage from a cyclic 6-mer peptide library in combination with a linear spacer at a molar ratio spacer/AGE=20. A branched spacer combined with the same phage clone as above at a molar ratio spacer/AGE=10 resulted in a 15 fold increase of target binding capacity. The recovered lactoferrin showed again a high purity (>95%) in a one-step chromatographic run. These results show that the new platform combining epoxy-cryogels as matrix and phages as affinity ligand has great potential. Proof of principle was provided using phages selected against a proteineous target, but as phages can also be selected against other materials, such as metals, toxins, PCB's,….. the platform can be used for major applications, not only for purification but also for fast screening and analysis. For direct fast screening or analysis cheap, robust and easy handling chromatographic matrices should be available. In this respect, epoxy-phage cryogels present an ideal choice as a chromatographic set-up to deal with these demands.

33. CaptoCore700 Chromatography Media for Purification of Polysaccharide-Protein Conjugates

Sheng-Ching Wang, Merck & Co., Inc., United States
Anders Ljunglof, GE Healthcare, Sweden
John Daicic, GE Healthcare, Sweden
Marc Wenger, Merck & Co., Inc., United States
Joseph Joyce, Merck & Co., Inc., United States
**Michael Winters, Merck & Co., Inc., United States*

CaptoCore700 is designed for intermediate and polishing purification large biomolecules, such as viruses and virus-like particles. This bead technology with an inactive, porous outer shell and a multimodal, octylamine ligand-containing core gives CaptoCore700 dual functionality, with both size- and adsorption-based separation mechanism. In this study we describe the novel application of CaptoCore700 media for purification of polysaccharide-protein conjugates. Polysaccharide-protein conjugates can be large molecules (> 2000 kD) depending on the conjugation chemistry used and are therefore potentially well-suited for purification via CaptoCore700. Conventionally, tangential flow filtration (TFF) unit operation has been utilized to separate undesired unconjugated polysaccharide or unconjugated carrier protein from polysaccharide-protein conjugates. However, due to the limitation of TFF membrane sieving, the TFF process step usually results in poor clearance of unconjugated polysaccharide. To characterize CaptoCore700 for this application, binding isotherms for different molecular weight polysaccharides and carrier protein were performed under various solution conditions (pH, ionic strength). Competitive binding isotherms with polysaccharide and/or carrier protein with polysaccharide-protein conjugates were also generated. In addition, confocal microscopy studies were performed to visualize the adsorption of low and high molecular weight polysaccharide and conjugates. The results show that unconjugated polysaccharide and protein can be selectively adsorbed and cleared from conjugates. The separation efficiency between the unconjugated polysaccharide and polysaccharide-protein conjugate is significantly dependent on the relative sizes of the molecules. In general, the CaptoCore700 chromatography purification step enables better process performance than traditional TFF for clearance of unconjugated protein and polysaccharide.

34. Use of Magnetic Sulfated Cellulose Beads Allows Fast Purification and Blending of Potent Influenza Vaccines In Mice

**Michael Wolff, Max Planck Institute for Dynamics of Complex Technical Systems, Germany*
Michael Pieler, Max Planck Institute for Complex Technical Systems, Germany
Sara Fenzel, Otto von Guericke University, Germany
Lisa Fichtmüller, Max Planck Institute for Dynamics of Complex Technical Systems,, Germany
Anja Bastian, Max Planck Institute for Dynamics of Complex Technical Systems, , Germany
Dunja Bruder, Otto von Guericke University, Germany
Udo Reichl, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Influenza is a global, highly contagious viral disease of the respiratory tract occurring in humans and various animal species. The most effective means of controlling seasonal influenza outbreaks is prophylactic vaccination. Current inactivated influenza vaccines contain either viral membrane components or whole influenza viruses produced in embryonated chicken eggs or animal cells. As for other biological products, downstream processing

Poster Abstracts

(DSP) is of outmost importance for manufacturing of potent and safe products. Within this project an alternative to the elaborate chromatographic purification and formulation of virus harvests was investigated. In particular, magnetic sulfated cellulose particles (MSCPs) were used to capture and isolate cell culture-derived virus particles, and the virus loaded MSCPs were directly used to immunize mice. Therefore, influenza A/PR/8/34 (H1N1) virus was propagated in Madin Darby Canine Kidney (MDCK) suspension cells, and then clarified, inactivated, concentrated and diafiltered against an adsorption buffer. Subsequently, virus particles were either bound to the MSCPs via a short incubation in this buffer solution or purified by sulfated cellulose membrane adsorption (SCMA) chromatography. Four groups of C57BL/6J mice were immunized twice intraperitoneally (i.p.) with (1) virus bound to MSCPs (1 µg HA/dose), (2) virus purified by SCMA (1 µg HA/dose; positive control), (3) the latter and MSCPs (w/o virus; contralateral i.p. injection), and (4) the generally applied adjuvant solution (negative control). Fourteen days after the booster immunization the mice were intranasally challenged by a lethal dose of the same influenza virus strain. After the challenge, the mice were weight daily for six days, sacrificed, and their lungs extracted for subsequent influenza virus RNA analysis. Prior to the immunizations and challenge, the antibody titer was determined by a specific ELISA, which indicated that all groups except the negative control developed an immune response against the influenza virus. The successful immunization was confirmed by weight monitoring, and a qPCR quantification of the pulmonary viral load. Only the negative control group showed substantial weight losses and up to 400-fold higher viral load in the lung tissue. Between the other groups no significant differences could be determined. Hence, virus particles bound onto MSCPs or unbound purified virus co-injected with MSCPs induced a comparable immune response to chromatographically purified virus in mice. Therefore, time-consuming chromatographic purification steps can be substituted by isolation via MSCPs. Furthermore, it is anticipated that virus pretreatment prior to binding the virus onto MSCPs could be completely omitted, simplifying the DSP process even further. For human applications, however, the DSP needs to be expanded, e.g. by a nuclease treatment, to comply with regulatory limits. Furthermore, prior injection the virus might be released from the MSCPs by increasing the ionic strength of the formulation solution to avoid, if necessary, injection of nanoparticles. Overall, considering the broad range of virus species binding to sulfated cellulose matrices, MSCPs can be envisioned as a highly efficient and simple DSP platform technology for production of vaccines and viral vectors.

35. Negative Chromatography of Virus-like Particle Using Adsorbent Grafted With Thermoresponsive Polymer

**Micky Fu Xiang Lee, Mr, Malaysia*
**Beng Ti Tey, Monash University Malaysia, Malaysia*

Virus-like particle (VLP) is a large biomolecule with a valuable immunogenic property. However, the multivalent binding site requirement and limitation in the diffusion of large biomolecules have reduced the binding capacity of most of the commercially available adsorbent to VLP particles. Hence, the downstream purification of VLP encounters bottleneck, and the conventional bind and elute type of chromatography is not suitable for VLP purification. In negative chromatography, impurities are retained by the adsorbent whereas the VLPs are allowed to flow through the column. As a result, the limitation in adsorption capacity of VLP can be avoided. Thermoresponsive polymer, poly[oligo(ethylene glycol) methacrylate] (POEGMA), is excellent in repelling the protein from binding to the surface. Hence, in our work, POEGMA was grafted on the cationized adsorbents to repel the large size recombinant hepatitis B VLP (HB-VLP) from the binding site of adsorbent, while allowing the small size host cell protein (HCP) adsorbed onto the adsorbent. When a feed contained mixture of proteins [HB-VLP ~ 4 MDa (large), BSA ~ 66 kDa (medium), insulin ~ 5.8 kDa (small)] was loaded to the packed column of POEGMA grafted adsorbents, the large size HB-VLP was mostly (88%) excluded from the column along with 79% of medium size BSA, while the small size insulin was retained in the column. Further increase in temperature above the lower critical solution temperature (LCST) led to the collapse of the POEGMA chain, thus increased the adsorption of medium size BSA, while still retaining the exclusion of HB-VLP. We have further accessed on the effect of the chain length of grafted POEGMA on the HB-VLP exclusion and the adsorption of impurities. Two POEGMA grafted adsorbents were fabricated, namely SQ (shorter chain length) and LQ (longer chain length). The feedstock used was clarified E. coli lysate containing recombinant HB-VLP. A longer chain length of POEGMA (LQ) restricted the penetration of impurities (from E. coli HCPs) to access the cationic ligands which were embedded beneath the POEGMA chains. On the other hand, a shorter chain length of POEGMA (SQ) allowed a better penetration of impurities through the POEGMA layer. Furthermore, the exclusion of HB-VLP from the surface of SQ allowed more impurities to adsorb which resulted in 86% removal of impurities. The single step negative chromatography of HB-VLP has shown better performance compared to previous studies of anion exchange chromatography of HB-VLP. SQ was further benchmarked against inert layer coated adsorbents (InertShell and InertLayer 1000), which were prototypes for negative chromatography adsorbents. InertShell has a thicker inert shell which resulted in the thorough flow-through of the proteins while the thinner inert shell of InertLayer 1000 allowed 60% recovery of HB-VLP with 43.7% of purity. On the other hand, the purity obtained under similar operation condition for SQ (62%) was much higher than these two inert layer coated adsorbents. Using a lower feed concentration has improved the purity of flow-through HB-VLP from InertLayer 1000 by 51.5% but has no significant effect over SQ. Therefore, SQ is more capable in handling higher feed concentration compared to InertLayer 1000. Yet, SQ has not fully excluded the HB-VLP and a portion of impurities still remained in the flow-through pool of HB-VLP. Meanwhile, the impurities that having a size larger than HB-VLP will co-flowthrough together with HB-VLP that reduced the purity of flowthrough HB-VLP. Future work is recommended to further improve the shielding of HB-VLP of POEGMA without affecting the adsorption of the impurities from E. coli host cells.

36. Mixed Polyelectrolyte Brush

Surfaces Display 'Chameleon-like' Protein Binding And Elution Properties

**Owen R.T. Thomas, University of Birmingham, Edgbaston, United Kingdom*
Thomas C. Willett, University of Birmingham, United Kingdom
Thantawat Theeranan, University of Birmingham, United Kingdom
Zhenyu J. Zhang, University of Birmingham, United Kingdom
Eirini Theodosiou, Aston University, United Kingdom

Chromatographic matrices today are infinitely superior to their ancestors, and yet more than 60 years on the basic blueprint remains largely the same, with most continuing to perform just a single function. Against the backdrop of rising product titres, increasing size and complexity

Poster Abstracts

of emerging bioproducts, escalating waste generation, and mounting competition from alternative techniques/formats the continued incremental improvement of existing media represents an increasingly untenable approach to the development of new chromatographic materials for the biopharma industry. The past few years have seen a marked surge in research into 'smart' chromatography adsorbents, largely driven by the realization that 'smart materials', especially 'smart' polymers, afford potential solutions to some of the aforementioned problems. By combining chromatographic supports modified with ligand-bearing temperature-sensitive copolymers with a novel purpose-built device, the Travelling Cooling Zone Reactor (TCZR), we recently demonstrated continuous thermally mediated bioseparation of proteins without any alteration in mobile phase composition (i.e. under normal binding conditions), and also presented the first ever demonstration of single column continuous chromatography [1,2]. Here we describe some of our work with pH responsive polymers. By tethering two oppositely charged smart polymer chains, poly(2-vinyl pyridine) and poly(methacrylic acid), adjacent to one another on the support at high grafting density, we have succeeded in generating high capacity adsorbents, which in response to discrete changes in environmental pH have the 'Chameleon-like' ability to reversibly transform between anion exchange, hydrophobic mixed mode and cation exchange functionalities. In this presentation we shall: (i) briefly explain the rationale behind the mixed polyelectrolyte (PEL) brush concept and what's known about pH induced changes that occur in mixed PEL layers [3]; (ii) describe three manufacturing approaches we have used to create well-characterized mixed PEL brush modified adsorbents; (iii) illustrate the effects of pH, brush composition, polymer chain length and inter-graft spacing on the selectivity of binding of anionic, neutral and basic proteins out of a four protein mix (Ovalbumin, beta-Lactoglobulin, Carbonic Anhydrase, Lysozyme); and finally (iv) show the effectiveness of 'pH shift only' mediated elution of bound protein species from mixed PEL adsorbents.
[1] Müller, T.K.H. et al. (2013) J. Chromatog. A, 1285: 97 –109. [2] Cao, P. et al. (2015) J. Chromatogr. A, 1403: 118 – 131. [3] Hinrichs, K. et al. (2009) Langmuir 25: 10987 – 10991.

37. Development of a Novel and Efficient Cell Culture Flocculation Process Using a Stimulus Responsive Polymer to Streamline Antibody/Bispecific Antibody Purification Processes

**Kenneth Kang, Eli Lilly and Company, United States*
Richard Chen, Eli Lilly and Company, USA
Bryan Dravis, Eli Lilly and Company, USA
Dayue Chen, Eli Lilly and Company, USA
Michael Barry, Eli Lilly and Company, USA

Recent advances in mammalian cell culture processes have significantly increased product titers, but have also resulted in substantial increases in cell density and cellular debris as well as process and product-related impurities. As such, with improvements in titer, corresponding improvements in downstream processing are essential. In this study we have developed an alternative harvest process that incorporates flocculation using a novel stimulus responsive polymer, benzylated poly(allylamine). As tested on multiple antibodies including bispecific antibodies, this process demonstrates high process yield, improved clearance of cells and cell debris, and efficient reduction of aggregates, host cell proteins (HCP), DNA and viruses. A wide operating window was established for this novel flocculation process through design of experiments condition screening and optimization. Residual levels of impurities in the Protein A eluate were achieved that potentially meet requirements of drug substance thus alleviating the burden for additional impurity removal by subsequent chromatography steps.

Poster Abstracts

In addition, efficient clearance of residual polymer was demonstrated in the presence of a stimulus reagent. The mechanism of HCP and aggregate removal during flocculation was also explored. This novel and efficient process can be easily integrated into current mAb or bsAb purification platforms.

38. Accelerated Evolution: Advancing Antibody Affinity Technology to Meet the Needs of Next Generation Antibody Processes

Mats Ander, GE Healthcare Life Sciences, Sweden
Gustav Rodrigo, GE Healthcare Life Sciences, Sweden
Tomas Björkman, GE Healthcare Life Sciences, Sweden
Magnus Wetterhall, GE Healthcare Life Sciences, Sweden
Ronnie Palmgren, GE Healthcare Life Sciences, Sweden
**John Daicic, GE Healthcare Life Sciences, Sweden*

Native protein affinity ligands (e.g. Protein A, Protein L, Protein G, etc.) are the result of millions of years of evolution by bacteria as a protective mechanism against mammalian antibody immune response. Today, the needs of biological manufacturing are changing faster than ever, and an accelerated evolution is required to preserve the advantageous aspects of these ligands, while improving the less desirable characteristics. This case-study will describe how high throughput screening was used, in conjunction with surface plasmon resonance, to develop next generation protein ligands with improved alkali stability, capacity and elution characteristics while retaining the strong affinity for human antibodies. In total, over 400 modifications to antibody affinity ligands were investigated to evaluate their effect on key performance characteristics for industrial purification. These modifications were made in singular and in plural due to the codependent nature of amino acid substitutions and structural changes in affinity ligands. In some cases, up to 5X increases in alkali-stability were achieved. In other cases, novel ligands were developed to give milder elution pH, on average >0.5 pH units, when tested in a pH gradient. This increase resulted on average a greater than 30% increase in yield compared to traditional recombinant ligands. In one case, where an Fc fusion protein was tested, the yield was improved from 11% to 93%.. Capacity for antibodies and antibody fragments was investigated in combination with novel bead designs. Previous claims of a "steric barrier" to capacity were proven to be untrue, with dynamic binding capacity values on the order of 80-100 g/L being achieved using various process and product designs. Taken individually, each of these performance improvements are impressive, but combining them into a single design is an exercise in optimization. The design rationale chosen for various ligands will be discussed, as different applications require different prioritization. As a final point, these improvements will be analyzed in terms of their effect on process economy and the overall impact on antibody manufacturing costs.

39. The Origin of the Complex Hydrodynamic Behavior of Preparative Packed Chromatography Beds is Revealed by Discrete Particle Modeling

**Martin Dorn, Technical University of Munich, Germany*
Dariusch Hekmat, Technical University of Munich, Germany
Dirk Weuster-Botz, Technical University of Munich, Germany

Preparative packed-bed chromatography using polymer-based, compressible, porous resins is an essential separation method especially for macromolecular bioproducts. Due to a downstream processing bottleneck because of limited purification capacities,

Poster Abstracts

the chromatography equipment is often operated at its hydrodynamic limit leading to a complex, hysteretic, thus, history-dependent packed bed behavior [1]. The theoretical understanding of the causes is still limited. Therefore, a rigorous modeling approach of the chromatography column on the particle scale will be presented which takes into account the interparticle micromechanics and fluid-particle interactions for the first time. A three-dimensional deterministic model was developed by applying Computational Fluid Dynamics (CFD) coupled with the Discrete Element Method (DEM) [2]. The column packing behavior during either flow or mechanical compression was investigated in-silico as well as in laboratory experiments using a novel micro-chromatography column. The simulation satisfactorily reproduced the experimental observations regarding the complex packing compression behavior as well as the pressure-flow dependency. Pronounced axial compression-relaxation hystereses were identified that differed for both compression strategies. It will be shown for the first time that the direction of the hystereses switched depending on the hydrodynamic conditions. The individual study of flow and mechanical compression revealed distinct differences in the packing behavior which were related to wall support. The packed bed during flow compression exhibited nearly linear axial compression leading to a most compressed region at the column outlet. In contrast to this, mechanical compression led to an exponential compression profile with the most compressed region at the top of the packing. By applying a novel UV-microscopy method, still existing void spaces were detected in the packed bed even during high compression levels. It was assumed that these void spaces are surrounded by a particle force-chain network. Therefore, compaction of the chromatography packing is rather a result of particle rearrangement than particle deformation. Simulation results indicate that packing anisotropy as well as the packing dynamics are governed by particle-wall and interparticle friction effects. A novel modeling-based systematic strategy of combined flow and mechanical compression in a quantitative manner will be proposed in order to substantially improve packing homogeneity. References:

[1] Hekmat D, Mornhinweg R, Bloch G, Sun Y, et al. Macroscopic investigation of the transient hydrodynamic behavior of preparative packed chromatography beds. J. Chromatogr. A. 2010;1218:944-950. [2] Dorn M, Hekmat D. Simulation of the dynamic packing behavior of preparative chromatography columns via discrete particle modeling. Biotechnol. Prog. 2015. DOI:10.1002/btpr.2210.

40. Designing Efficient Separation Processes of Modified Proteins by Chromatography: PEGylated Proteins and Protein Aggregates

**Shuchi Yamamoto, Yamaguchi University, Japan*
Noriko Yoshimoto, Yamaguchi University, Japan

When native proteins are modified (intendedly or unintendedly), modified forms must be separated from the native protein and other unwanted contaminants. A typical intended protein modification is PEGylation, where PEG molecules are attached to a target protein. PEGylation reaction mixtures contain various unwanted species, such as PEGylated isoforms (positional isomers), multi-PEGylated proteins (PEGmers), the native protein, non-reacted PEGs and more. Electrostatic interaction based chromatography (ion-exchange chromatography, IEC) is known to be efficient for PEGylated protein separations. We have analyzed PEGylated protein separations by linear gradient elution (LGE) IEC based on our models. PEGylated proteins were much more weakly retained on the IEC columns. Weak retention of PEGylated proteins was to be due to a steric hindrance between the ion-exchange ligand and charges of PEGylated proteins. The shift of the retention volume of PEGylated proteins was well correlated with the calculated thickness of PEG layer around the protein molecule. PEGylated protein positional isomers were separated by IEC and the binding sites for mono-PEGylated proteins were similar to those for native proteins. Another important parameter is pore diffusion of PEGylated proteins. We measured molecular diffusivities of PEGylated proteins as well as native proteins and PEGs by the laminar flow based Taylor dispersion method. Pore diffusivities were determined by isocratic elution experiments at non-binding conditions and linear gradient elution experiments. Good correlations were established between the diffusivities and the hydrodynamic radius. Based on these data, it is possible to design efficient PEGylated protein separation processes. As an application, monolith disk IEC was designed, which can permit separation of 12 PEGylated protein isoforms and PEGmers within ca. 4 minutes while the pressure drop was below 1 MPa. Another unintended protein modification is protein aggregation. Dimer and aggregate removal is important for protein-drug separation processes. Since dimer and aggregates are more hydrophobic and have more charges, their retention volumes are larger in IEC. The binding site values as well as the peak salt concentrations of protein monomer and aggregates were examined for various different types of ion-exchange ligands. Among the ligands tested, a salt-tolerant polyamine anion exchange ligand showed quite high separation performance for the monomer-dimer separation. The binding site values were similar to those for conventional IEC. In order to understand the retention mechanism various different salts such as arginine chloride and sodium sulfate were employed as an additive or a gradient substance. Since no additives are needed this polyamine-based IEC is an attractive method for protein monomer separation. Finally, in addition to linear gradient elution (LGE) mode, flow-through chromatography (or weak-partitioning chromatography) operation was designed and tested based on our method using LGE experimental data and the pore diffusivity correlations.

41. 'ISep': A Pseudobioaffinity Adsorbent for the Purification Of Antibodies

*Sushmita Koley, DBT-ICT-CEB, Institute of Chemical Technology, India
Pamela Jha, DBT-ICT-CEB, India
Sandeep B. Kale, DBT-ICT-CEB, India

Polyclonal antibodies (pAbs) and monoclonal antibodies (mAbs) are an important class of biopharmaceuticals. The current rise of global mAbs and plasma biologics market is estimated to be up to \$58 billion and \$21 billion, respectively. Hence, it becomes pertinent to solve the underlying problems present in the manufacture of antibodies. Cohn plasma fractionation for purification of polyclonal IgG suffers from limitations such as poor process yield due to the use of ethanol and difficulty in process automation. Protein A/G affinity chromatography, widely used for purification of mAbs suffers from various drawbacks, including high cost (\$9000-\$12000/L), instability to strong base (1M NaOH), leaching (ligand is toxic) and elution at low pH (>3.5), which leads to IgG aggregates. The objective of the present work is to develop an affordable and effective pseudoaffinity adsorbent for purification of antibodies, followed by its use in the development of the antibody purification process. An indigenous pseudobioaffinity adsorbent was, therefore, designed by studying the X-ray crystallographic structure of the complex formed between B domain of Protein A and Fc portion of IgG. Based on this study, the potential ligands were selected and further assessed by molecular docking studies. The ligand with maximum dockScore and minimum internal energy was immobilized onto a rigid polymethacrylate base matrix (particle size 90 µm, pore size 400 A0). The novel pseudobioaffinity adsorbent, thus designed was named 'ISep'. The selectivity of 'ISep' was demonstrated by the purification of mAbs generated against glycated hemoglobin (Hb1Ac) and pAbs from CCS and human plasma, respectively, on a preparative scale. It was revealed that the elution of antibodies occurs at pH 7.0 for 'ISep', unlike low pH elution required by Protein A. The integrity and purity of the antibody were confirmed by SDS-PAGE and size exclusion chromatography (SEC). The centrifugal membranes of different porosity (30, 50, 100kDa) were screened for desalting and it was found that 50kDa performed best. The optimized protocol for 'ISep' chromatography gave the purity of >95% (mAbs and pAbs). In a similar study, the conventional adsorbent, Protein A was used to purify IgG1 and the results were comparable. Moreover, 'ISep' is found to be stable in 0.5M NaOH, the most common SIP agent. The inhouse adsorbent designing process of 'ISep' is simple, robust, reproducible, economical and can be scaled up. The critical parameters for indigenous purification process for both pAbs and mAbs using 'ISep' have been optimized and product characterization in terms of quality, safety and efficacy is underway. Hence, 'ISep' promises to serve as an alternative to the conventional platforms for the purification of pAbs and mAbs.

42. Mechanistic Study of Virus Particle Retention by Size Exclusion Membranes: The Interplay of Size Exclusion and Adsorptive Action Under Different Flow Regimes

Peter Kosiol, Sartorius Stedim Biotech , Germany
Catharina Kahrs, Clausthal University of Technology, Germany
Bjoern Hansmann, Sartorius Stedim Biotech , Germany
**Volkmar Thom, Sartorius Stedim Biotech, Germany*

Parvo virus removal by filter membranes is regarded as a robust and efficient unit operation. The retention of virus particles by virus filter membranes is predominantly based on size exclusion. However, recent results point to the fact, that virus particle retention can also be impacted by adsorptive interactions between virus particles and membranes. Furthermore, the impact of flow rate and flow interruptions on virus retention has been observed and possible mechanisms discussed. The goal of this investigation is to gain a holistic picture of the underlying mechanisms for virus particle retention in size exclusion membranes as a function of membrane structure and membrane surface properties, as well as flow- and solution conditions. Therefore, parvo virus retentive membranes with different pore sizes and different levels of surface hydrophilization were generated in order to systematically change the level of size exclusion and adsorptive action on particle retention. The respective membranes were characterized with regards to their permeability, non-specific protein adsorption, surface zeta potential and pore size distribution by using liquid-liquid displacement porometry (LLDP). Furthermore, an experimental space of solution conditions regarding pH and salt concentration was chosen, that impacts the adsorptive interaction between virus model particles and membrane surface. Bacteriophages (like PP7) were chosen as model particles, due to the established correlation to parvo virus retentive behavior and the related ease of handling. Filtration runs to determine the respective levels of retention were complemented by flow stop conditions and a set of permeate fractions for titer determination was taken at different process stages. This poster will introduce to the generated set of data and will draw first conclusions. Based on the DOE character of the experimental space and respective analysis, interactions between different factors can be determined. The results enable to differentiate size exclusion from adsorptive action with respect to virus particle retention within the given space of material, process and solution conditions, significantly sharpening our understanding of retention mechanisms in parvo virus filtration.

43. Scale-up of Filtration Methodologies for the Concentration of Human Mesenchymal Stem Cells: Comparison between Hollow Fiber and Flat Sheet Cassette Devices

Bárbara Cunha, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal, Portugal
Tiago Aguiar, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Sofia Carvalho, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Marta M. Silva, , iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Manuel J. T. Carrondo, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Margarida Serra, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Paula M. Alves, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
**Cristina Peixoto, IBET, Portugal*

Currently human mesenchymal stem cells (hMSC) are expanded using planar technologies or microcarrier-based stirred culture systems from one to hundreds of liters of culture volume, in order to guarantee the required cell numbers to be delivered to the clinic. To be applied in therapies low volumes for administration are required, and therefore downstream processing (DSP) workflow should comprise a concentration step without compromising cells' characteristics. Tangential flow filtration (TFF) is a well established technology that has been arising as an attractive solution for cell therapy DSP. Despite previous studies at lower scale (0.5 L), for TFF to be applied for processing the large expansion volumes (one to hundreds of liters) for autologous and allogeneic therapies, its scale-up and impact on hMSC's characteristics and quality needs assessment The aim of this work was to scale-up (0.25 to 2 L) already established TFF-based concentration process [1,2] based on process parameters assessed (load, shear rate and permeate flux). More specifically, we have compared the performance of hollow fiber and flat sheet cassette devices to concentrate hMSC. The impact of all methodologies on cells' quality (i.e. cell morphology, viability, identity and potency) and recovery yield was evaluated. The most promising conditions were validated with hMSC derived from bone marrow and adipose tissue. Results show that hMSC could be successfully concentrated up to a factor of 50, while maintaining their identity, potency and high cell viability using both hollow fiber and flat sheet cassette devices. Nonetheless, it was possible to observe that flat sheet cassettes allowed to recover more 10% of cells at the end of the concentration process in both processing scales (0.25 and 2 L). The device's fluid dynamics (more turbulent), coupled to the nature of the material of the membrane (more hydrophilic) could explain such results. However, unlike hollow fiber devices, pre-sterilization is a hurdle that needs to be circumvented by manufacturers, in order for these devices to be applied in the cell therapy industry. Overall, this work shows that TFF is a scalable and efficient methodology for the concentration of hMSC. It can be incorporated in the biomanufacturing workflow of cell-based therapies, having also applicability to other stem cell types (e.g. human pluripotent stem cells) relevant for the cell therapy industry. [1] B Cunha et al, J Membr Sci. 2015 478, 117-129. [2] B Cunha et al, J Biotechnol. 2015 213, 97-108.

44. Progress in Label-free Techniques for Scalable Downstream Processing of Cellular Therapies

*Nik Willoughby, Heriot-Watt University, United Kingdom
Helen Bridle, Heriot-Watt University, United Kingdom
Mike MacDonald, University of Dundee, United Kingdom

There are currently whole cell therapeutic treatments in development for a wide-range of "mass market" diseases. It is commercially inconceivable to consider an autologous model for indications affecting large populations; therefore a shift towards an allogeneic model is desirable. There is currently much debate regarding the regulatory landscape for cellular therapies, however we believe that the regulatory bodies are certain to require as stringent a demonstration of purity as is currently demanded for protein therapeutics. This will be especially the case for allogeneic therapies. Consequently, there is an urgent need to address the challenges associated with large-scale downstream processing of cell therapies This work will present results from within our group investigating several novel techniques to separate cells of different phenotypes and stage of differentiation based on physical and mechanical properties rather than the more currently used "gold standard" of biological surface markers. Specifically the research presented here will demonstrate promising results and progress made over the last two years in the areas of: • Cell-cell separation on the basis of elastic modulus using modified tangential flow filtration. Here, we identify differences in cell stiffness, expressed as cell elastic modulus (CEM), for hESC versus mesenchymal progenitors, osteoblast-like derivatives and fibroblasts using atomic force microscopy and data processing algorithms to characterize the stiffness of cell populations. Undifferentiated hESC exhibited CEMs three-fold lower than those of differentiated cells, information we exploited to develop a label-free separation device based on the principles of tangential flow filtration (TFF). With this device we segregated hESC mixed with fibroblasts and hESC-mesenchymal progenitors induced to undergo osteogenic differentiation. The device is easily scaleable, permitted a high throughput of cells, and achieved up to 50% removal of specific cell types per single pass. The level of enrichment soft, pluripotent hESC in the permeate was found to rise with increasing stiffness of the differentiating cells, suggesting CEM can serve as a major discriminator. • Cell-cell separation using inertial focusing devices to separate on a combined size/shape/density basis. Specifically we will present data regarding segregation of nucleated and enucleated cells at the latter stages of manufacture of red blood cells (RBCs) using a custom-designed inertial focusing device that can be stacked to enable scale-out to high throughputs. • Cell-cell separation in optical and acoustic energy landscapes where segregation of cells is achieved via focusing within the landscapes. Demonstration of 1-D cell separation using optical landscapes has been previously demonstrated however the work here combines this with ultrasonic acoustic focusing and translates into 2-D and 3-D landscapes to illustrate the scale-up potential of the technique. Our results demonstrate the principle of a scalable, label-free, solution for separation of heterogeneous cell populations deriving from human pluripotent stem cells through a number of approaches.

45. Retrospective Evaluation of Low pH Viral Inactivation and Viral Filtration Data from Multiple Company Collaboration

**Norbert Schuelke, Takeda Pharmaceuticals International Co., United States*
John Mattila, Regeneron, United States
Mike Clark, AbbVie, United States
Shengjiang Liu, Bayer, United States
John Pieracci, Biogen, United States
Thomas R. Gervais, BMS, United States
Xinfang Li, ImmunoGen, United States
David Roush, Merck, United States
Eileen Wilson & Olga Galperina, GSK, UK
Konstantin Zoeller, Novartis, Switzerland

Considerable resources are spent within the biopharmaceutical industry to perform viral clearance studies which are conducted for widely used unit operations that are known to have robust and effective retrovirus clearance capability. The collaborative analysis from the members of BioPhorum Development Group Viral Clearance Working Team considers two common virus reduction steps in biopharmaceutical processes: low pH viral inactivation and viral filtration. Analysis included eight parameters for viral inactivation and nine for viral filtration. The extensive data set presented in this paper provides the industry with a reference point for establishing robust processes in addition to other protocols available in the literature (ex. ASTM Std. E2888-12 for low pH inactivation). In addition, it identifies points of weakness in the existing data set and instructs the design and interpretation of future studies. Included is an abundance of data that would have been difficult to generate individually, but collectively will help support modular viral clearance claims.

46. Moving Towards Platform Virus Particle Purification

**Caryn Heldt, Michigan Tech, United States*
Maria Gencoglu, Michigan Tech, United States
Ashish Saksule, Michigan Tech, United States

While antibody production has evolved into a standard platform purification technology, viral particle purification is still in its infancy. No one can yet predict if a platform technology can be used for such a wide array of products that have such a large size, pI, and surface charge distribution. One key goal of our lab is to explore methods that have the potential to become a platform technology for viral particles. Last Recovery meeting, we presented on the potential of osmolyte flocculation technology (OFT) to purify both enveloped and non-enveloped virus particles. Here, we will present a purification process that demonstrates purification for both types of viral particles, providing a potential platform process for viral particle purification. Osmolytes are natural compounds that stabilize intracellular proteins against environmental stresses. This is done by the ability of osmolytes to control water structure around a protein. We have capitalized on this osmolyte trait to induce flocculation in viral particles. This is likely due to the high hydrophobicity of viral particles. The high hydrophobicity makes viral particles flocculate in the presence of water structuring osmolytes, as compared to stabilizing the structure, as occurs in proteins. We have demonstrated that a non-enveloped virus, porcine parvovirus (PPV), and an enveloped virus, Sindbis virus (SINV) can be flocculated and removed with a micropore filter by a variety of osmolytes. The osmolytes were preferential to virus particles and not model proteins. We have now demonstrated a diafiltration process using mannitol as a common flocculant for both virus types. Infectious recoveries of >60% for both

Poster Abstracts

viruses was achieved with diafiltration using a micropore membrane. When an ultrafiltration membrane is used, >90% of infectious virus recovery can be obtained. There is a great protein reduction due to this operation, providing purification of the virus particles, while maintaining infectivity and activity. OFT has a high potential to become the key step in a platform virus purification technology that could revolutionize the production of viral particle therapeutics. Mannitol is commonly used in antibody formulation and is currently on the FDA inactive ingredient list for approved drugs for both powder and intravenous applications, reducing the need for stringent removal from the final product. Higher purity with a low cost, platform process would increase the safety and decrease process development time and production costs for future viral products.

47. Cost-effective Manufacturing of Viral Vaccines: Systematic Development of Inactivated Polio Vaccine Production Process

**Beckley Kungah Nfor, Janssen Vaccines, The Netherlands*

The production of safe and efficacious vaccines at low cost is essential to guarantee world-wide vaccine availability and affordability, especially in developing countries. Our proprietary PER.C6® cell line and PIN platform technologies have enabled the expression and production of viral vaccines at very high titers in cell cultures, thereby shifting the challenges and opportunities for further cost reduction to the downstream processing (DSP). Our DSP development strategy to deal with these challenges involves the use of our established purification platform, which was designed for treating high cell density/high titer cell culture harvests. In the presented case study, I will show how this strategy was applied at Janssen for fast development of a process for inactivated polio vaccine production from high cell density/high titer PER.C6® based cell culture harvests for which the use of existing polio virus purification technologies would be unsuitable. This was followed by optimization of bottleneck steps using statistical DoE and small scale experiments and finally process verification at pilot scale.

48. Mitigating Impurity Interactions with Protein A and Monoclonal Antibodies During Chromatography with PEGylated Ligands

**Justin Weinberg, Carnegie Mellon University, United States*
Shaojie Zhang, University of Virginia, United States
Giorgio Carta, University of Virginia, United States
Todd Przybycien, Carnegie Mellon University, United States

Protein A (PA) chromatography is a highly selective capture and separations technique in use throughout the biopharmaceutical industry for the downstream purification of monoclonal antibodies (mAbs). While PA is often responsible for removing ≥98% of mAb process impurities, the remaining small, but significant amounts of host cell proteins (HCPs), DNA, and product aggregates necessitate multiple subsequent polishing steps downstream. In this work, we aim to decrease the post-PA chromatography burden on mAb downstream processes by discouraging impurity interactions with PA media via chemical modification of the PA ligands with polyethylene glycol (PEGylation). Here, the main hypothesis is that PEG will impede non-specific binding of contaminant species to the ligand and to bound mAbs via steric hindrance as well as shield the ligand from proteolytic attack and fouling. In one study, we explore the interactions of a mAb and a model protein contaminant on unmodified and PEGylated PA resins using confocal laser scanning microscopy (CLSM). Experiments were conducted with both sequential and simultaneous loading of mAb and contaminant and on resins modified with both 5 and 20 kDa PEG chains. Through analysis of the CLSM images, we demonstrate that PEGylated PA is effective at reducing the interaction of the contaminant with mAbs that are bound to the affinity ligand; the magnitude of the reduction is dependent on the size of the PEG chain used. Additionally, we reveal that PEGylation reduces the amount of contaminant aggregates that bind to the outer surface of the resin particles. These promising results not only suggest that the PEGylated resins will have anti-fouling behavior, but also result in a lower level of product- associated impurities, a major source of impurity carryover in PA chromatography. In a follow up study, we test the selectivity of the PEGylated resins via separation of mAbs from Chinese hamster ovary harvest cell culture fluid (CHO HCCF). Here, we examine the relationships between the size and extent of the PEG modification used on the resin on product recovery, HCP eluate content, and aggregate eluate content. In a third and final study, we demonstrate that PEGylated PA resists proteolytic attack against chymotrypsin and host cell proteases by retaining a higher percentage of static binding capacity over the same digestion period compared to unmodified resin. While PEGylated PA offers multiple potential benefits, we confront the tradeoffs of simplified polishing operations and increased resin lifetime with reduced IgG dynamic binding capacity and increased resin costs via process simulation to make the case for commercial consideration.

49. Scale-down Tools to Assess the Impact of Cell Engineering by Co-expression of Staphylococcal Nuclease in E. coli to Improve Primary Recovery of Fab Fragments via Crossflow Microfiltration

**Andrea CME Rayat, University College London, United Kingdom*
Martina Micheletti, University College London, United Kingdom
Gary J Lye, University College London, United Kingdom

Viscosity is a key parameter that impacts the recovery and the overall manufacturability of biological materials. The release of DNA during homogenisation resulted in almost a 2-fold increase in viscosity of E.coli cells. We have shown that the homogenate viscosity could be reduced by over 3-fold through cell engineering of the E. coli strain via co-expression of Staphylococcus nuclease with antibody Fab fragments. We evaluated and compared the crossflow microfiltration performance of E. coli cell homogenate (Control) against the cell-engineered E.coli homogenate using a novel scale-down or microscale bioprocessing tool we created [1] which enabled linked process analysis of the impact of upstream operation, e.g. in situ DNA hydrolysis during homogenisation, on primary recovery operation via crossflow microfiltration. The novel device requires 10-fold smaller process volume and a 100-fold decrease in membrane area compared to available lab-scale crossflow microfiltration module. This approach of employing cell engineering to improve primary recovery of a biological product complements biomolecular modification

Poster Abstracts

for enhancing manufacturability. Very few articles in the literature reflect the inherent complexity in the crossflow microfiltration of biopharmaceutical feedstocks such as Fabs. Most focus on membrane fouling and flux decay studies, not on the effect of process parameters on steady state flux or product transmission which are important in bioprocessing and modelling studies. We demonstrated the utility of the novel microscale crossflow filtration device in manufacturability assessment of the E. coli homogenates. This was mainly due to the wider range of experimental data it enabled us to generate which allowed for a more in-depth analysis of crossflow microfiltration that described not only flux behaviour, but including transmission of the key product: antibody fragments, as well as transmission of impurities as reflected by the total protein and DNA. Our findings show that at 22kPa transmembrane pressure (TMP), viscosity reduction did not impact steady state permeate flux (12 LMH) but has improved %Fab transmission from 70% for the Control to 100% Fab transmission for the cell-engineered homogenate. At this TMP, total protein transmission from the Control is significantly lower (45% vs 80%). In contrast, DNA transmission is higher for the Control (45% vs 10%). Furthermore, we have demonstrated that flux and Fab transmission was found to be independent of TMP at the high cell concentration (90 gDCW L-1) used in the study. However, total protein and DNA transmissions were not TMP-independent. Results at the higher TMP (75 kPa) illustrated a decrease in %protein transmissions for both homogenates while for %DNA transmissions, only the Control homogenate showed lower transmission (20%) with no significant difference to the cell-engineered E.coli homogenate. Such rapid determination of key process information depicted the interaction of the linked process sequences of upstream operation, including cell engineering, and primary recovery operations such as homogenisation followed by crossflow microfiltration. This has ultimately informed larger scale crossflow microfiltration operation and the necessary design improvement of the cell engineered-strain. [1] Rayat, A.C.M.E., Lye, G.J., Micheletti, M. 2014. A novel microscale crossflow device for the rapid evaluation of microfiltration processes. Journal of Membrane Science Vol. 452, 284–293

50. Utilizing Mass Spectrometry and Mechanistic Modeling to Support Development of a Cation Exchange Chromatography Capture Step

**Stephen Hunt, KBI Biopharma, United States*
Mark Sleevi, KBI Biopharma, United States
Garo Dombourian, KBI Biopharma, United States
Lindsey Irish, KBI Biopharma, United States
Robert Todd, KBI Biopharma, United States

Although the ability to develop mechanistic models for protein-based chromatography has been demonstrated, the industrial application to process development remains limited. One of the challenges of mechanistic modeling for industrial applications is the complexity of the feed streams and therefore, the amount of analytical data required to estimate model parameters. In this case study, a combination of UPLC and mass spectrometry data are used to estimate binding isotherm parameters for a complex set of host related impurities and product related variants eluting during a cation-exchange capture step for an industrially relevant recombinant protein produced in E. coli. The challenges encountered in developing the model and the insights gained by applying the model during process development will be discussed.

51. Application of PAT in Chromatography: From Capture to Polishing

*Nina Brestrich, Karlsruhe Institute of Technology , Germany
Matthias Ruedt, Karlsruhe Institute of Technology, Germany
Laura Rolinger, Karlsruhe Institute of Technology, Germany
Juergen Hubbuch, Karlsruhe Institute of Technology, Germany

Since the publication of the PAT guidance by the FDA in 2004, the implementation of real-time monitoring of critical quality attributes and the real-time control of important process parameters in biopharmaceutical production have been discussed increasingly. In chromatography, the load volume in capture steps and the pooling decision in polishing steps are important process parameters. Due to a lack of analytical tools for selective inline protein quantification, both parameters are usually adapted between batches. This may lead to higher lot-to-lot variability and in the worst case to batch failure. In order to tackle this issue, a PAT tool based on inline UV absorption spectra and Partial Least Square Regression (PLS) modelling has been published recently [1-2]. The tool was successfully used for selective inline protein quantification and real-time pooling decisions in polishing steps under diluted conditions. In a first part of this presentation, an approach will be presented how the PAT tool can be applied for load control in capture steps. A PLS model is calibrated to selectively quantify mAb in the column effluent. The calibrated PLS model is subsequently applied for load control in Protein A chromatography under varying mAb and HCP concentrations in the feed. In a second part of the presentation, advances in PLS modelling for pooling control will be discussed. In contrast to the load control, where only the product is monitored, pooling decisions require the quantification of all co-eluting species. A difficulty regarding UV absorption measurements are the large concentration differences between product and contaminants. It is shown how UV absorption spectra measured at variable pathlengths overcome this issue. In combination with PLS modelling, the approach allows the selective quantification of both product and the co-eluting contaminants even in overloaded chromatographic conditions. In summary, PLS modelling with UV absorption spectra has a great potential for process control in chromatography and might simplify the transition from batch to continuous processing. Measurements at variable pathlengths can support this method by increasing the range of detectable concentrations. [1] Brestrich, N., Briskot, T. Osberghaus, A., Hubbuch, J. (2014). A tool for selective inline quantification of co-eluting proteins in chromatography using spectral analysis and partial least squares regression. Biotechnology and Bioengineering 111:1365-1373. [2] Brestrich, N., Sanden, A., Kraft, A., Hubbuch, J. (2015). Advances in inline quantification of co-eluting proteins in chromatography: Process-data-based model calibration and application towards real-life separation issues. Biotechnology and Bioengineering 112: 1406-1416.

52. Process Validation (Stage 1) Using Latin Hypercube Sampling: Chromatography Case Study

*Thomas Hansen, Novo Nordisk A/S, Denmark
Lars Sejergaard, Novo Nordisk A/S, Denmark
Ernst Broberg Hansen, Novo Nordisk A/S, Denmark

According to the process validation guideline the goal of stage 1 is to design a process suitable for routine commercial manufacturing that can consistently deliver a product that meets its quality attributes. Based on this statement one should validate likely combinations of the process parameters, i.e. the variation occurring on a routine basis, and focus on robustness rather than "worst case" conditions. In this work we present a practical example of an alternative approach to the classic DoE methodology used for process validation. The alternative approach is called Latin Hypercube Sampling (LHS). LHS can be considered an approximate Monte Carlo simulation and delivers a good approximation of the probability function even with a limited number of experiments. A big advantage of the LHS approach is that one can choose the number of experiments independent of the number of parameters to be investigated. Based on the distribution for each parameter (e.g. normal or uniform) the LHS approach will come up with a statistically relevant design leading to output data within the "routine manufacturing" range. An example of an actual validation study on a chromatography step is presented including experimental design and data interpretation in detail.

53. Process Validation (Stage 1) Using Latin Hypercube Sampling

*Lars Sejergaard, Novo Nordisk A/S, Denmark
Thomas Hansen, Novo Nordisk A/S, Denmark
Ernst Broberg Hansen, Novo Nordisk A/S, Denmark

To validate and document a robust process design, laboratory studies are often required. The widely accepted approach is to investigate the variation of the process parameters in a classic DoE set up. Since a process step often has 10 – 20 input parameters it is practically impossible to include all parameters. Therefore a pre-selection of the relevant process parameters is done using a risk based approach (e.g. FMEA type risk assessment). An inherent drawback of the DoE approach is that the number of experiments increases exponentially with the number of parameters. As a consequence, the risk assessment has to end up with a suitable low number of relevant parameters (4-6), and this can compromise the credibility of the risk assessment exercise. Since interactions in DoE are studied as a combination of end points, one for each parameter range, the likelihood of each combination occurring in an actual production scenario decreases exponentially with the number of parameters (assuming normal distributed ranges). Thus, a substantial number of experiments performed within the DoE study will cover conditions which will practically never occur during production. According to the process validation guideline the goal of stage 1 is to design a process suitable for routine commercial manufacturing that can consistently deliver a product that meets its quality attributes. Based on this statement one should investigate likely combinations of the process parameters, i.e. the variation occurring on a routine basis, and focus on robustness rather than "worst case" conditions. In this work we present an alternative to the DoE approach for process validation called Latin Hypercube Sampling (LHS). LHS is an extension of the classic Latin square concept to more than three dimensions (forming a hypercube) and can be considered an approximate Monte Carlo simulation. Using an algorithm, the parameter values are distributed in the hyperspace according to the stated probability distribution and even with a limited number of experiments we get a fairly good approximation to this distribution. In the LHS approach one chooses the number of experiments independent of the number of parameters. Based on the distribution for each parameter (e.g. normal or uniform) the LHS approach will come up with a statistically relevant design leading to output data within the "routine manufacturing" range. Thus, the LHS approach addresses the issues raised for DoE. In this presentation we discuss the advantages of the LHS approach compared to DoE based on both theoretical considerations and practical examples.

54. Antibody Higher Order Structure Analysis by Protein Conformational Array for In-depth Product and Process Understanding

**Deqiang Yu, Bristol-Myers Squibb, United States*
Yuanli Song, Bristol-Myers Squibb, United States
Elizabeth Schutsky, Bristol-Myers Squibb, United States
Mukeshkumar Mayani, Bristol-Myers Squibb, United States
Angela Lewandowski, Bristol-Myers Squibb, United States
Zhengjian Li, Bristol-Myers Squibb, United States

Higher order structure (HOS) is critical for product and process understanding in biopharmaceutical development. The clinical effect and biological functions of biologics are closely related to HOS which can be affected by processing, formulation and storage conditions. Therefore, HOS is important in determining structure-function relationships and in guiding process development for biologics. HOS determination is challenging for large proteins like antibodies. Different biophysical tools have different levels of resolution and throughput. Light Scattering for protein size and Circular Dichroism for secondary structure provide low resolution structural information. X-ray Crystallography and Nuclear Magnetic Resonance have high resolution but are not suitable for large proteins. Mass Spectrometry and Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS) are increasingly utilized to determine protein sequence and conformation but need special expertise and have low throughput. Protein Conformational Array (PCA) is a new technique to determine protein HOS. This ELISA or Luminex®-based method uses a panel of 34 antibodies to detect epitopes that cover the whole antibody and thus indicate the antibody 3-D structure. It is simple to perform with high throughput and can be a complementary technique to HDX-MS. It was initially used to determine biosimilarity for antibodies but is being further developed as a process development tool for novel antibodies. In this study, PCA is used to predict the structures of antibody aggregates, compare antibodies expressed from different cell culture conditions, and monitor antibody structural changes caused by the downstream process and different buffer conditions. The aggregate structure analysis by Protein Array found certain correlations with HDX-MS results. The antibody analysis for different processing conditions demonstrated the conformational changes along the process. The buffer conditions including pH and salt concentration showed significant impact on antibody structure, which correlate well with aggregate level and Thermal Shift results. Higher order structure analysis by Protein Conformational Array is very useful to provide in-depth product and process understanding.

55. Therapeutic Antibody Characterization During Process Development

Wei-Kuang Chi, Development Center for Biotechnology, Taiwan
Ai-Ning Irene Lin, Development Center for Biotechnology, Taiwan
Hui-Chun Li, Development Center for Biotechnology, Taiwan
Yi-Hsuan Pan, Development Center for Biotechnology, Taiwan
**San-Cher Chen, Development Center for Biotechnology, Taiwan*

Quality of therapeutic antibodies are deeply affected by the choice of host cells, expression vector systems, basal and feed media, fed-batch and perfusion process, downstream processes and bulk formulations. Appropriate analytical methods and bioassays are required to characterize the structure of both protein moieties and glycans attached. In this study analytical data will be presented to show that even for the same antibody, different cell clones will exhibit different aggregate levels and glycan compositions (Go, G1, G2 etc.). Also different processes using batch culture, fed-batch culture and concentrated fed-batch culture will generate different glycan distributions. A fast way to determine glycan structure distribution (G0, G1, G2) using LC/MS/MS were developed for the screening of culture conditions and media formulation designs leading to desired quality attributes such as CDC and ADCC. Comparison of qualities of monoclonal antibody produced by shake flask cultures, 2 – 5 liter and 20 liter bioreactor culture show that glycan profiles are quite different between batch culture and fed-batch culture, wheras that for concentrated fed-batch and batch cultures are quite similar, the glycan distributions are less affected during scale-up from 5 liter to 20 liter.

56. High Resolution Experimental Design to Study Antibody-Drug Conjugation Process Robustness

*Ben Sackett, Genentech, United States
Thanmaya Peram, Genentech, United States
Jayme Franklin, Genentech, United States
Jeff Gorrell, Genentech, United States

Antibody-drug conjugates (ADCs) are produced after antibody intermediate (AI) purification through a series of chemical reactions. To deliver ADCs of consistent product quality and robust manufacturing, an increase in process understanding of conjugation-specific parameters is required. In conjugation processes, kinetic parameters spanning multiple reaction steps can confound the identification of parameters impacting Critical Quality Attributes (CQA). A high resolution study was designed to increase understanding of ADC conjugation processes, mechanisms for product aggregation, and formation of minor Drug-to-Antibody Ratio (DAR) species. The study included 6 process parameters and allowed for detection of interactions across multiple reaction steps. Key outputs of the study demonstrated the overall robustness of the conjugation process for delivery of consistent product quality, but also provided specific observations, such as a correlation between formation of minor DAR species and aggregates which was not previously known. The study showed that these product quality outputs are driven by similar parameters, such as temperature, conjugation time, pH, and drug/Ab ratio, which may suggest formation through common mechanisms. In addition, conjugation reaction time and temperature were identified as process parameters contributing to vHMWS formation. The process design study enabled identification of key conjugation-specific parameters for robust control of product quality, delivering consistent manufacture for ADCs.

57. Use of DOE and Bottom-Up Mass Spectrometry Methodologies to Enable Rapid Process Characterization and Optimization of a Complex Glycoprotein Downstream Process

*John Cundy, Pfizer, USA
Emily Barr, Pfizer, USA
Paul Brown, Pfizer, USA
Brian X. Chen, Pfizer, USA
Keith Johnson, Pfizer, USA
Joseph P. Martin, Pfizer, USA
John Plihal, Pfizer, USA
Thomas W. Powell, Pfizer, USA
Nicholas Russell, Pfizer, USA

Poster Abstracts

A complex recombinant glycoprotein expressed in CHO is currently in late-phase development. To-date clinical manufacture had been performed at 1000-L scale. Commercial forecast necessitated a process upscale to 13,000-L scale. Given the aggressive pace of project progression, rapid facility fit experimentation and process characterization was required to enable an understanding of facility modifications required and a basis-of-design prior to start-up. To this end, we applied design of experiment (DOE) and heightened in-process analytical methodologies as appropriate to enable rapid process characterization and optimization for key unit operations to achieve our goals. Specifically, bottom-up mass spectrometry was used on key in-process samples and studies which enabled an enhanced understanding not achievable with the established analytical control strategy. We have identified proteases and other host cell proteins in mid-stream process samples, whose removal we can assure by appropriate modifications to key steps. DOE experimentation in conjunction with the mass spectrometry allowed for definition of an optimized operating space. Our approach not only delivered the required dataset to support manufacturing, but also provided key process understanding enabling improvement in overall process robustness, product quality, and control.

58. QbD and Process Simulation for CIGB 550-E7, an Active Pharmaceutical Ingredient for a New HPV Vaccine

**Miladys Limonta, CIGB, Cuba*
Dayana Soler, CIGB, Cuba
Laura Varas, CIGB, Cuba
Maelys Miyares, CIGB, Cuba
Yasser Zárate, CIGB, Cuba
Aniurka Panfet, CIGB, Cuba
Alain Alfonso, CIGB, Cuba
Milaid Granadillo, CIGB, Cuba
Isis Torrens, CIGB, Cuba

The CIGB 550-E7 is a fusion protein comprising the HPV16 E7 antigen fused to a cell penetrating and immunostimulatory peptide which corresponds to the carboxy terminal region of LALF 32-51. Previously we demonstrate that CIGB 550-E7 induces a potent antitumoral response against E7 expressing tumors, therefore could become a promising vaccine candidate for the treatment of HPV 16 related malignancies. This work describes the process development to obtain an active pharmaceutical ingredient for a cancer vaccine candidate against HPV, based on the implementation of QbD. It helped us to build the knowledge space and get information about critical, non- critical attributes and process parameters; and also encompasses the design space and normal operating ranges as well as areas where the CIGB 550-E7 it is known that unacceptable product is produced. Finally the SuperPro Designer Software was used as a computer tool in order to consider the economic impact of some selected process parameters such as recovery, purity and lifetime of matrices on the recovery of CIGB 550-E7 downstream process. This methodology was found very convenient to identify where cost reduction can be achieved at early stage of the development project before the final stage of clinical trials will be conducted.

59. Approaches Enabling High Protein Concentration UV Sensing in Bioprocess Applications

*Hanno Ehring, GE Healthcare, Sweden

Increased titers in cell culture, increased capacity of chromatography resins and Ultrafiltration/Diafiltration operations with concentrations beyond 200 g/l in combination with stronger PAT requirements all put new demands on UV/Vis absorption sensing in bioprocess applications. Currently, results obtained often show elution peaks that are saturated or non-linear so that no information about fine structure or sample concentration can be extracted, which is not acceptable from a PAT perspective. To extend the dynamic range a wavelength where the protein extinction coefficient is lower is often used. For quantification, off-line sampling is utilized to estimate the final product yield. However these methods are cumbersome and not robust and the dynamic range is often not sufficient. In this study devices, concepts and results are presented that can overcome the limitations above. By combining different wavelengths and pathlengths including dual pathlength flow cells with very short pathlength down to 0.1 mm many different options are possible that can address multiple needs. The cells can be adapted to flow ranges from laboratory to large scale production equipment. Unit operations that currently see limitations regarding in-line sensing of high protein concentrations include sample loading, check for impurities in elution peaks, UV-based product pooling criteria, quantification of total product concentration, product concentration in ultrafiltration, straight-through processing and dynamic control in continuous chromatography applications. Data will be presented showing the usability of the concepts in different situations. Linearity of measurement up to 200 AU/cm corresponding to 150 g/l of a typical monoclonal antibody using a cell with 0.1 mm cell length is demonstrated as well as examples from some unit operations mentioned above. Requirements and possible limitations are discussed as well.

60. Purification of Recombinant Polyclonal Antibody (rpAb) Mixtures: Impact of Polishing Modality on Aggregate Selectivity and mAb Component Ratios

*Timothy Pabst, MedImmune, United States
Frank Bartnik, MedImmune, United States
Hongji Liu, MedImmune, United States
Jihong Wang, MedImmune, United States
Xiangyang Wang, MedImmune, United States
Alan Hunter, MedImmune, United States

Recombinant polyclonal antibodies (rpAbs) represent a novel class of biopharmaceuticals that enable targeting of multiple antigens. To reduce cost, it is anticipated that rpAbs may be manufactured in a single batch in which the individual component monoclonal antibodies (mAbs) are co-expressed in the same bioreactor and purified together. For mAbs, multimeric species are often the most challenging product related impurity to remove. Separation of multimers in mAb processes is frequently achieved using cation exchange chromatography (CEX), where monomer purity of the final antibody preparation can exceed 99%. For therapeutic IVIg preparations derived from human plasma, the level of multimers is often higher, likely due to the diverse nature of the material (i.e., different isoelectric points and IgG subclasses). As a result of this diversity, it is difficult to remove multimers without simultaneously separating monomers that differ based on characteristics such as charge. Similar to mAbs, it is desirable to control multimeric species at low levels for rpAbs. Dissimilar to mAbs, rpAb purification adds the additional constraint that the relative ratios of the individual component mAbs be well understood and controlled. For such a challenging separation, traditional approaches used for mAbs such as CEX may not be appropriate. In this work we examine purification options to be used in the manufacture of rpAb therapeutics, including hydrophobic interaction, hydroxyapatite, and multi-modal chromatography. Aggregate selectivity, yield and impact on component mAb ratios are discussed for each modality. Conceptual design of rpAb processes, purification strategies, and data will be presented.

61. Separation of IgG Subclasses and Glycoforms Using Fc Gamma Receptors as Affinity-based Chromatography Ligands

*Austin Boesch, Zepteon, Inc., United States
Ty Thach, Dartmouth College, United States
Alison Mahan, Ragon Institute of MGH, Harvard, MIT, United States
Galit Alter, Ragon Institute of MGH, Harvard, MIT, United States
Margaret Ackerman, Dartmouth College, United States

Human plasma is used as a manufacturing feedstock for a number of licensed IgG therapeutics in both immunity and infectious disease. Part of the mechanism of action for some of these therapies relies on the engagement of Fc gamma receptors (FcγRs) on immune cells, which in turn elicit effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis. Leveraging FcγRs as chromatographic affinity ligands, we were able to enrich in high affinity subclasses and glycoforms using human plasma derived IgG as a load material. Biophysical characterization of of both subclass and glycosylation using high-throughput techniques reveal receptor-specific speciation of IgG2, IgG3, nonfucosylated and bisected glycans. Additionally, the elution peaks were evaluated for their potentiation of both Natural Killer cell degranulation (ADCC surrogate) as well as Monocyte phagocytosis in in vitro cellular bioassays revealing FcgRIIIa-based chromatography most significantly increased effector function. This work demonstrates the use of FcγR-based chromatography to reformulate plasma IgG and directly its impact effector function and illustrates the potential use of this technology to develop new therapeutics from plasma IgG.

62. Use Cationic Mix Mode Chromatography (CMMC) to Purify Bi-functional Protein from E.coli Cell Lysate

*Honghao LI, Novo Nordisk Research Centre China, China
Hammad Shaikh, Novo Nordisk A/S, Demark
Wei Gong, Novo Nordisk Research Centre China, China

E.coli is one of the most wildly employed expression system for therapeutic protein production. One of the major expression formats is soluble expression in cytoplasm. Upon recovering soluble target protein from the cell, host cell proteins and chromosome DNA are released as well. If the target protein is acidic, capture will most likely be carried out using an anion exchange resin. As over 50% of E.coli host cell proteins are acidic in nature, they all compete with the target protein for the binding sites during capture. Moreover, both DNA and endotoxin, which are released during the recovery steps, are heavy negatively charged entities that bind aggressively and compete for the binding capacity with the target protein. Consequently, the binding capacity for the target protein is decreased dramatically and the CIP of the used anion exchange resin

Poster Abstracts

also often remains a challenge. To improve this situation, basic tags are designed and fused with the target protein, so that the pI of fusion protein can be increased for CIEX column. There have been successful cases reported. Further study indicates that the basic tag can form a distinct motif from target protein so that the fusion protein behaves as a 'bi-functional' protein with two pI domains instead of one average pI. With this character, the fusion protein can be captured by CIEX at pH base on pI of fusion tag but not average pI of fusion protein. With conventional cation exchanged chromatography purification at acidic condition(for example at pH 5-7), the removal of acidic HCP, endotoxin and DNA is enhanced, but the selectivity to the basic host cell proteins is not as efficient as that to acidic host cell proteins As the pKa of basic residues on the tag of the bi-functional molecule are much higher than 7, they can easily be protonated at neutral or even higher pH. So the positively charged tags can interact with the cationic groups on the resin. As CMMC can tolerate relative higher concentration of salt, which can enhance the hydrophobic interaction between the target protein (if the target protein exhibits medium or higher hydrophobicity) and hydrophobic group on the resin. The actual binding strength between the CMMC resin and the bi-functional protein is much higher than the simple sum up of independent cationic and hydrophobic interaction strength. As a result, the selectivity to the bi-functional molecule is much higher than to the basic host cell proteins, while the acidic host cell proteins, DNA and endotoxin are in flowthrough. The relatively higher conductivity would not be an issue as it would be when anion exchange chromatography was employed. Overall, the absorption shows not only very high selectivity but also high binding capacity and salt tolerance comparing with that of conventional chromatography techniques to normal proteins. The absorbed bi-functional proteins can be eluted out by relative higher pH and/or combination of proper concentration of salt, which compare with most of the affinity elution condition(low pH), is much milder. The testing purification cases show very nice selectivity and binding capacity, which indicates that the idea is worth to be further explored. In future it could be possible to become a generic protein production platform.

63. Demonstration of a mAb Chromatography Platform Process Operating in a Continuous and Integrated Mode: Flexibility for Facility Fit and Process Economics Optimization

Xhorxhi Gjoka, Pall Life Sciences, United States
Rene Gantier, Pall Life Sciences, United States
**Mark Schofield, Pall Life Sciences, United States*

The biopharmaceutical industry is adopting continuous processing to address the challenge of process intensification for monoclonal antibodies (mAbs). Continuous multi-column chromatography (MCC) is a key facilitative technology for the adoption of continuous downstream processing. The major focus so far has been on the implementation of MCC for the Protein A capture step, leading to significant reduction in cost through minimization of Protein A resin volume. In production scenarios, where resin cost is amortized over many cycles, additional benefits including facility utilization, buffer consumption and overall process cost can be achieved through the operation of a fully integrated and continuous chromatography purification process. In this study we establish a complete mAb chromatography purification platform, operating in a continuous and integrated mode, with optimized process productivity, yield of recovery and purity. The process, comprising a Protein A capture step followed by low pH viral inactivation and two polishing steps, anion exchange (AEX) and mixed-mode chromatography, is performed with minimal buffer adjustments enabling unit operations are integrated directly. To perform the four unit operations, only two BioSMB® MCC systems are employed: Capture on Protein A is performed onto a first system which is connected to the second system operating the two polishing steps with low pH viral inactivation step integrated in between. The simplification of the process, by combining unit operations, enables high productivity and throughput, and further reduction in footprint and process cost. The operation of this continuous and integrated platform allows processing the content of a 25L bioreactor, at 1mg/ml mAb titer, in under 8 hours by deploying a total volume of chromatographic media of less than 200 ml, leading to throughputs of up to 4g of purified mAb per hour. This operation is conducted using process development equipment requiring a total bench length of less than 20 feet. This represents a significant improvement in process time and facility usage compared to a traditional batch process where the 4 unit operations may normally be operated for at least 2 consecutive days using more than 1l of total chromatography media.

64. Assembly and Purification of a Bispecific Fab’2

Glen Giese, Genentech, United States

Recent advances in biologic drug development have led to creation of new formats of biologics to target diseases in new ways. A large and rapidly growing area of new biologic formats is bispecific and multispecific antibodies. A diverse set of bispecific formats and approaches for manufacturing them now exists. This talk will cover the development of a new format, a bispecific Fab’2. Different approaches can be taken to generate a Fab’2 bispecific. Enzymatic cleavage of a full-length bispecific can generate a bispecifc Fab’2. However, the cleavage site can introduce product heterogeneity and may pose an immunogenicity risk. Two Fab’s with hinge cysteines can be assembled into a Fab’2, but no structural preference for forming a heterodimer between two different Fab’s exists, which can lead to very low process yields. An improved process using a leaving group to promote heterodimerization of two Fab’s during an assembly reaction has been developed. The formation of a Fab’2 bispecific by the assembly of two distinct Fab’s in order to form a Fab-Fab heterodimer poses several unique challenges. The assembly mixture from combining the two Fab’s must be purified in order to remove unique assembly byproducts such as homodimers, free light chain and heavy chain, adducts, unreacted Fab’, leaving group, and others. Typical process-related impurities including host cell proteins, DNA and high molecular weight species must also be removed. Stability of the process intermediates must be investigated. Multiple novel unit operations were developed in order to achieve effective assembly and separation. High throughput screening was leveraged to rapidly develop a significantly off-platform purification process. Non-standard analytical methods were also implemented in order to support the purification process development.

65. Improved Aggregate Removal for Bispecific Antibodies Using a Three Column mAb-like Platform Process

**Wai Keen Chung, MedImmune, United States*
Cassia Andrade, MedImmune, United States
Matthew Aspelund, MedImmune, United States
Ronald Schoner, MedImmune, United States
Alan Hunter, MedImmune, United States

Full length IgG-like bispecific antibodies (BisAbs) are a novel class of therapeutics that allow for the binding of multiple targets or two distinct epitopes of the same target,

Poster Abstracts

potentially allowing mechanisms of action that may not be achievable with mAbs or mAb combinations. However, a common trend observed in many BisAb processes is a higher level of aggregates (10-20%) at the end of cell culture compared with mAbs. The increased aggregate burden at the start of purification exceeds the capabilities of a conventional mAb platform purification process, typically employing cation exchange chromatography (CEX) for aggregate removal. In addition, the high aggregate levels have been implicated in poor virus removal on anion exchange flowthrough unit operations. Alternative chromatography options (e.g. multimodal, hydroxyapatite, HIC) usually provide better aggregate clearance compared to CEX, but they each present distinct manufacturing challenges. Various methods of introducing improved aggregate removal capability into a commercial three column mAb-like platform purification process were evaluated. This included the use of i) pH gradient elution in the Protein A capture step; ii) calcium phosphate precipitation after harvest and/ or low pH viral inactivation; iii) High Performance Tangential Flow Filtration (HP-TFF). In all cases, the process modifications showed sufficient aggregate clearance to enable use of CEX polishing. The different process options were evaluated based on scalability, robustness, and platformability. High throughput development data as well complete process scale demonstration run data are provided. Results indicate that product quality and process performance obtained from precipitation and Protein A pH gradient processes are similar to that of a conventional mAb platform.

66. A New Fab–Fusion Protein Therapeutic

Michiel Ultee, Ulteemit BioConsulting, LLC, United States
Emily Schirmer, Catalent Pharma Solutions, United States
Dustin Armstrong, Valerion Therapeutics, Inc, USA

We describe the design, development and manufacture of a unique antibody-fusion protein consisting of a Fab-linked enzyme rather than the more typical Fc-linked fusion protein. VAL-1221, a preclinical product candidate for glycogen-storage diseases, contains a humanized anti-dsDNA Fab genetically linked to the acid alpha glucosidase enzyme (GAA). The Fab portion of this construct was derived from a murine antibody previously demonstrated as the Mab or single-chain fragment to possess cell-penetrating properties. These were dependent on its DNA-binding activity and membrane expression of a nucleoside transporter (ENT2), highly elevated in human skeletal muscle. The VAL-1221 fusion protein enables increased muscle delivery via its dual modes of both Fab-mediated and M6P-targeted (GAA) cellular uptake. This novel construct was initially produced in transient CHO cultures, followed by a stable CHO cell line based on the GPEx expression system. To substitute for the Fc-directed Protein-A capture step found in most antibody and Fc-protein downstream processes, we compared alternative affinity supports focused on the Fab, either on its CH1 or light-chain region. We selected a Protein L support rather than anti-CH1, since the fusion of the GAA reduced capacity of the anti-CH1 support for the Fab-GAA. A series of ion-exchange and hydrophobic-interaction chromatography polishing steps followed to remove both host-cell proteins (HCP) and excess light chain. Purification challenges included the sensitivity of the GAA to pH levels below pH 3.5 and above pH 7.0, a tendency for HCP to track with the fusion protein on some steps, and high levels of free and dimeric light chain in the Protein L eluate. The final process overcame these challenges to result in production of clinical material at the 250L scale.

67. Antibody Drug Conjugates: A New Platform of Protein Therapeutic Molecules

*Michel Eppink, Synthon Biopharmaceuticals BV, Netherlands
Guy de Roo, Synthon Biopharmaceuticals BV, Netherlands
Bram Kamps, Synthon Biopharmaceuticals BV, Netherlands
Ruud Verstegen, Synthon Biopharmaceuticals BV, Netherlands
Patrick Beusker, Synthon Biopharmaceuticals BV, Netherlands
Henri Spijker, Synthon Biopharmaceuticals BV, Netherlands
Ruud Coumans, Synthon Biopharmaceuticals BV, Netherlands

In the past years biopharmaceutical companies have searched for new biological entities (NBE's) as a follow-up of the very successful monoclonal antibodies. One of the most promising new lines of NBE's are the Antibody Drug Conjugates. Antibody Drug Conjugates (ADC's) are new biotherapeutic medicines consisting of a drug (chemotherapeutic agent), a (non-)cleavable linker and a monoclonal antibody. The mechanism of action consists of the recognition of a specific receptor on the cells by monoclonal antibodies, the complete ADC is internalized into the cell and the cytotoxic agent is released in the cell by cleaving the linker and the monoclonal antibody from the ADC so that the cytotoxic agent is able to block cellular processes followed by cell death. In the past few years the first new ADC variant brentuximab vedotin (Adcetris®) from Seattle Genetics received FDA approval as a new treatment for U.S. patients with a pair of rare lymphomas—Hodgkin's disease and anaplastic large-cell lymphoma. As second product T-DM1 (Kadcyla®) from Roche received FDA approval as improved treatment for HER2-breast cancer. We will show the current progress on the development of purification processes for Antibody Drug Conjugates, characterization of the ADC's with physicochemical/biochemical studies and the precautions taken to perform conjugations and purifications of the ADC's to final drug substance as cytotoxic agents are being used as drug.

68. Development of a Second-Generation Process for Andexanet Alfa, a Recombinant Protein for the Reversal of Anticoagulation by Factor Xa Inhibitors

*Mark Karbarz, Portola Pharmaceuticals, United States
Stuart Builder, Strategic BioDevelopment, United States
Kent Iverson, Consultant, Un
Kentaro Takeda, Portola Pharmaceuticals, United States
Genmin Lu, Portola Pharmaceuticals, United States
Pam Conley, Portola Pharmaceuticals, United States

It is estimated that by 2020, 30 million patients will be treated with a Factor Xa inhibitor for the prevention of atrial fibrillation, DVT or PE. Of these patients, an estimated 500,000 will be admitted to the hospital due to a life-threatening bleed, or because they require emergency surgery. There is no approved reversal agent for direct Factor Xa inhibitors. Andexanet alfa was developed as a fast acting, universal antidote for both direct and indirect Factor Xa inhibitors. It is a recombinant protein, derived from Factor Xa, with no pro- or anti-coagulant activity. Phase 3 trials demonstrated reversal of anticoagulation in older, healthy volunteers (Siegel et al., NEJM 2015). FDA granted breakthrough status and accelerated approval for Andexanet alfa. The company thus maintained an established 1st generation manufacturing process for earliest BLA submission. To meet all the expected demand, a 2nd generation manufacturing process was developed and scaled-up to 10,000L. This process is based on a novel affinity capture step, utilizing a naturally occurring protein ligand from soybean. The entire process is simpler, more robust, and increases overall yield by 4-fold, with half of the improvement coming from downstream operations. A case study will be presented detailing the issues that arose during development and the decisions that were made.

69. Purification of a 900 kDa Trimeric Glycoprotein: Marrying the Old and the New

**Anne Kantardjieff, Alexion Pharmaceuticals, United States*
Abe Friedman, Alexion Pharmaceuticals, United States

A process was developed to purify a 900 kDa heterotrimeric extracellular matrix (ECM) protein with a high propensity for polymerization. Low titers from cell culture, the presence of closely-related contaminating species, and close interactions between the target protein and several members of the heat shock protein family, posed significant challenges during development. A novel affinity-based capture step was developed which exploits the naturally occurring high affinity binding between the target protein and another ECM protein. An Fc-tagged version of both the full length and binding domain of the binding partner were generated and used as an affinity ligand in combination with Protein A. Elution conditions were identified that allowed for elution of the target protein, while keeping the Fc-tagged affinity ligand bound to Protein A. In addition, a refolding step was incorporated following elution of the target protein and re-use of the ligand was demonstrated out to 6 cycles. To increase binding capacity, the unit operation was optimized using a Protein A monolith, leading to an increase in resin load of more than ten-fold. The development of an affinity capture step was instrumental in achieving purity targets. Low pH precipitation was utilized as a key step for host cell protein removal. Conditions were identified that provided significant separation between the target protein and host cell protein impurities. Several members of the heat shock protein family were identified via mass spectrometry as persistent impurities throughout the purification process. However, a set of optimal precipitation conditions were identified that, when used in combination with the appropriate depth filter, could significantly reduce the abundance of these impurities. The combination of traditional precipitation techniques and a novel affinity-based capture step were instrumental in enabling the purification of a 900 kDa protein.

70. Purification of Secretory Immunoglobulins

**Rainer Hahn, BOKU Vienna, Austria*
Hannah Engelmaier, BOKU Vienna, Austria
Gottfried Himmler, Angothera GesmbH, Austria
Alexander Matlschweiger, BOKU Vienna, Austria

Immunoglobulin A (IgA) is the most abundant antibody class in the human body. In its secretory form (SIgA) it is the main effector of the mucosal immune system. With its high therapeutic potential it is a promising candidate for prophylaxis or therapy against various diseases associated with the respiratory, gastrointestinal or urogenital tract. So far, medical application of SIgA has been hampered by difficulties in producing and purifying large quantities. We have studied the isolation of SIg from animal whey, which is a comparably rich source with SIg representing 4-5% of total protein. Besides SIgA, whey also contains considerable amounts of secretory immunoglobulin M (SIgM). In this work, the purification characteristics of these antibodies was studied on the basis of standard unit operations for protein purification, including membrane separations, precipitation and various chromatographic methods. Difficulties in the present study aroused from the high abundance of accompanying whey proteins. Due to the large molecular weight of SIg with 400 and 900 kDa, respectively, and also the low titer, ultra/diafiltration was the obvious first step in the purification sequence. Low transmembrane pressure was found to be most import operating parameter to achieve high purities of 60-70%, whereas the membrane cut-off was a less dominating factor. Polyethylen glycol at concentrations of 6-7% was very efficient in precipitating SIg out of solution but selectivity was poor with respect to removal of IgG, non-secretory IgA dimers and albumin. However, fractional precipitation at 3% was effective to remove around 50 % of large molecular weight fraction containing Ig oligomers. Furthermore, concentration of α-lactalbumin and β-lactoglobulin, which remained in the residual supernatant, was significantly reduced. In an optimized process, ultrafiltration was followed by fractional precipitation and the dissolved precipitate was then subjected to diafiltration resulting in a purity of ~ 75%. To obtain high purity of > 95% several chromatographic methods were investigated. Chromatography with a core bead technology (Capto core 700) was not efficient. In this case, a different exclusion limit of 300 or 500 kDa would be needed. A general problem of processing large molecules with chromatography is the restricted pore accessibility resulting in slow diffusion and/or low capacity. As has been shown previously, monoliths have the potential to overcome this problem due to their large channel diameter and convective transport but in the present case the application was hindered by a multi-component adsorption effect of SIg being displaced by oligomers leading to a capacity of only 3 mg/mL. High purity and good process performance could be obtained by a combination of two anion exchange steps. In the first step on Q-Sepharose FF, operating conditions were chosen, at which albumin and IgG were captured and SIg was collected in the flow through. This step utilized the low effective pore diffusion coefficient of SIg in the small-pore medium, an effect that was further enhanced when operated under conditions where the impurities were bound. The flow through was then supplemented with sodium chloride and directly loaded onto a POROS 50 HQ column. Under these conditions the residual IgG did not bind and SIg could be captured at capacities of 20 mg/mL. By applying a linear salt gradient, SIgA and SIgM could be separated during the elution step. The overall process yield for SIg was rather low at 15% when all steps were operated at conditions achieving maximal purity. In our case, this would not be a too serious problem since whey is an extremely cheap raw material available in practically unlimited amounts. However, results of this work can be of general interest and may go beyond the isolation of SIg from whey. The presented study highlights many of the critical steps and bottlenecks associated with purification of such a complex molecule and furthermore provides potential solutions and alternative processing options. Envisioning purification of recombinant monoclonal SIgA or SIgM, yield has certainly to be increased. This may be achieved by i) higher purity of the starting material and ii) better selectivity of the respective unit operations due to more homogenous characteristics of a monoclonal antibody.

71. Platform Filtration Process for Purification of Virus Like Particles

Sofia B. Carvalho, IBET, Portugal
Ricardo J. S. Silva, IBET, Portugal
Mafalda G. Moleirinho, IBET, Portugal
Paula M. Alves, IBET, Portugal
Manuel J.T. Carrondo, IBET, Portugal
Cristina Peixoto, IBET, Portugal
**Alex Xenopoulos, Merck Millipore, United States*

Virus-like particles (VLP) have become prime vaccine candidates because of their versatility, immunogenicity and safety profile. The diversity of surface epitopes contributes, however, to a variability in downstream purification, that could ultimately affect manufacturability. For baculovirus expression systems in particular, the similarity between residual baculovirus and VLP particles causes significant problems. For that purpose, we have undertaken an effort to develop platform processes for purification of VLPs. In one approach described here, we focus on size exclusion as the key mechanism of separation, with the ultimate goal of an all filtration purification process. The first step was to evaluate a legacy purification that was not robust or efficient and replace the ion exchange chromatography step with size exclusion chromatography. Performance of the SEC step will be described and the shortcomings of such a method for a scaled up, GMP process will be discussed. The proposed all-filtration process will then be described, employing either normal or tangential flow filtration for the clarification stage, followed by multiple ultrafiltration steps to achieve the needed concentration and diafiltration purity specifications. Efforts to clear nucleic acid without the use of an endonuclease digestion step will also be described. To show the potential for a universal, platform process, two insect cell systems producing two different VLPs were studies and preliminary results will be presented.

72. Assessment of the Impact of CHO Cell Culture Feeding Strategy on IgG Key Quality Attributes Throughout the Subsequent Downstream Process Steps and the Stability

*Nicola Roberts, UCB, United Kingdom
Laura Gimenez, UCB Pharma, Belgium
Mariangela Spitali, UCB Pharma, United Kingdom
Andrew Yates, UCB, UK
Stefanos Grammatikos, UCB Pharma, Belgium

Development of an efficient, commercially viable process to produce a therapeutic antibody involves many challenges, among them being finding the cell culture medium which provides the yields to meet growing therapy demands. Key to this however, is to consider the impact on product quality and process performance. The cell culture media plays a critical role in providing the primary source of the raw materials that eventually end up in the drug itself. Many of the amino acids making up the primary structure of the therapeutic protein are derived directly from the culture medium. Therefore determining the correct feeding strategy that meets all the process requirements, including the impact on downstream and product stability are central to meeting the demands for the requirements for consistent product quality profiles with little product variance. In this study, we describe the impact of changing the upstream feeding regime and varying the concentrations of the feed to the bioreactor on the subsequent downstream process steps and the effect on the product stability profiles. The feeding regime is shown to directly impact key product quality attributes, such as the level of monomer and charged species and therefore the yield achieved in the downstream process steps. Having then been processed to the drug substance the stability was evaluated to determine if following purification the stability profiles were affected by the differences in the feeding regimes.

73. Theoretical Analysis of the Benefits of Multi-step Loading for Affinity Chromatography Based on the Shrinking-Core Breakthrough Model

*Brian Bowes, Eli Lilly and Company, United States

As cell cultures titers have increased in recent years, so has the scrutiny placed on the productivity of the purification unit operations. For Fc-containing proteins, protein A chromatography is widely employed as an affinity capture step due to the purity it can deliver, often with minimal molecule-specific development. As such, protein A chromatography is a logical choice for productivity optimization efforts. Typically, protein A columns have been loaded at single flow rates, perhaps based on pressure/flow limitations. The product mass loading is often based on the dynamic binding capacity (DBC) at a given residence time, with an additional safety factor; the result could be loading to 80% of the 10% DBC, for example. While some productivity improvements may be possible through optimization of the single loading flow rate, it has been recognized that much greater improvement can be achieved via segmented loading at increasing residence times. The premise is that the initial loading can be executed at a short residence time where the DBC may be low, while the additional step(s) can be executed at increased residence time(s) where capacity is high, but the productivity would be low if used for the entire loading phase. Examples to date of successful implementation of 2+ loading segments have been somewhat anecdotal or empirically driven. In the cases where theoretical analyses have been applied, they have not necessarily been applied holistically and may require more time and/or expertise than is available to development scientists. In this work, the goal was to use the shrinking-core breakthrough model to determine the expected functional form of the relationship between the DBC and the residence time. This approach reveals a form of $DBC=q_{max}(1-\alpha\cdot[(RT)]^{\beta})$, where q_{max} is the equilibrium capacity of the column and RT is the residence time; reasonable data fit is achieved in many practical cases. In the limiting case of a constant pattern and negligible external mass transfer resistance, $\beta = -1$ and a physically meaningful value of α is obtained, providing some context for the more general case where α and β are fitted values. With the appropriate functional form for the DBC vs. residence time, one can seek to maximize productivity for one or more loadings steps, seeking to find how to select those steps. Here, the approach was to take a low threshold for the breakthrough percentage ($\leq 1\%$) in order to assume minimal yield loss during loading when taking the loading productivity as DBC/(load time + fixed time). This approach reveals that the optimal approach is for each increased residence time to be approximately a fixed multiple of the previous one (e.g., with three steps, the residence times might be 3, 3×1.5 = 4.5, and 3×1.52 = 6.75 minutes). With this approach, it is possible to make clear and fair comparisons of the potential productivity gains afforded by a certain number of loading segments, including the limiting case of a continuous flow ramp, allowing one to seek a balance with process simplicity or other priorities.

74. Competitive Binding of Antibody Monomer-Dimer Mixtures on CEX Resins: Equilibrium, Kinetics, and Separation by Frontal Analysis

Jason Reck, University of Virginia, USA
Timothy Pabst, MedImmune, United States
Alan Hunter, MedImmune, USA
Xiangyang Wang, MedImmune, USA
**Giorgio Carta, University of Virginia, United States*

The removal of soluble dimers from monoclonal antibodies (mAbs) is a significant downstream processing challenge. Despite the large difference in molecular mass, monomer-dimer resolution is often difficult, in part because of low selectivity and in part because of slow mass transfer. Although protein adsorption in cation exchange resins has been studied extensively for a variety of model systems, less is known, fundamentally, about the behavior of actual mAb monomer-dimer mixtures with respect to their competitive binding properties at high protein loadings. In this work, we have examined the single and two-component binding behavior on the cation exchange resin Nuvia HR-S (BioRad Laboratories) of a purified mAb monomer and of its dimer, which was isolated from a process feedstock using size-exclusion chromatography. The CEX resin used in this work contains relatively large pores allowing both monomer and dimer to diffuse freely within the particles. Single and two-component adsorption behaviors were studied both through macroscopic and microscopic (CLSM) means using fluorescently labeled forms of each protein. The selectivity was found to vary substantially with ionic strength, selectivity being lowest when conditions favor the strongest binding. The adsorption rates are strongly influenced by pore diffusion, although a kinetic resistance to the displacement of monomer by the dimer also exists, especially at low ionic strengths when protein binding is strongest. A model is developed to describe the competitive binding process accounting for both diffusion and kinetic effects, providing a tool to optimize column design and explain how process parameters such as feed composition and salt concentration affect resolution. The model is also used to examine the feasibility of separating monomer-dimer mixtures by fontal analysis. In such a process, the mixture is continuously fed to the column for conditions where selectivity favors binding of the dimer, yielding essentially pure monomer at the column outlet until breakthrough of the dimer occurs. Following dimer breakthrough under appropriate conditions, the column contains little bound monomer, resulting in a purification with high monomer recovery. Dimer species and any multimer present as trace components in the feed mixture can be recovered quantitatively with a high-salt strip. Compared to other chromatographic modes, frontal analysis maximizes the protein load, providing a very high utilization of the column binding capacity and yields the purified monomer in the same buffer in which it was fed to the column. For such a process to be successful, resins (such as Nuvia HR-S) that permit rapid diffusion as a result of larger pores and smaller particle size are needed to achieve the desired chromatographic efficiency.

75. Nanofiltration Optimisation Using Micro-titre Plates

**Alison Tang, MedImmune, United Kingdom*
Richard Turner, Medimmune, United Kingdom

A robust method to remove potential viral contaminants is a requirement for purification processes producing products from animal cell culture. A common technique and central to Medimmune's viral clearance strategy is Nanofiltration. This approach is typically very effective and generally agnostic to any product to product variation i.e. if the protein is smaller than the pore size then the protein will pass through the filter. Success of the step is measured in terms of the quantity of product to pass through an acceptable amount of membrane in a manageable period of time. Whilst still the exception rather than the rule it is becoming increasingly common that proteins such as antibody or antibody related proteins do not easily pass through nanofilter using standard nanofilter optimisation strategy. MedImmune has developed, in conjunction with Merck Millipore, a multi-well filter plate with an integral nanofilter membrane that has the potential, when used with the Tecan automation platform, to help simultaneously evaluate multiple parameters of nanofiltration, and determine optimal conditions rapidly with milligram quantities of product. In addition this technology can generate a 'fingerprint' of filtration performance as a function of operating conditions that provides a clear operating window for specific molecules. The standard approach to nanofiltration optimisation, the advantages of scaled-down nanofiltration device and data to support scale-up of the unit operation using the multi-well filter plates will be discussed.

Poster Abstracts

76. Optimization of a Conjugation Step Using Mathematical Modelling

*Charlotte S Hunneche, Novo Nordisk A/S, Denmark
Ernst Broberg Hansen, Novo Nordisk A/S, Denmark
Thomas Hansen, Novo Nordisk A/S, Denmark

Protein conjugation has become an increasingly important process step in the production of modern pharmaceuticals. This introduces new challenges both regarding cost (e.g. side chain) and further demand for purification. This study looks at a conjugation step consisting of reduction of a protected cysteine into a free cysteine followed by an alkylation step. The main challenges were product degradation during reduction as well as the amount of side chain and reducing agent used in the process. In order to optimize the process the kinetics of the reaction steps were described using a mathematical model. The model was used for describing a set-up of the reaction which resulted in increased yield, higher purity and decreased consumption of expensive starting materials such as the side chain and the reducing agent. The conjugation reaction was set up in a cross flow filtration system making it possible to increase the concentration of the protein for obtaining faster reactions. Additionally, reagents and unwanted side products could be removed by diafiltration after the first reaction step preparing for a better alkylation step. Finally, at the end of the reaction a final diafiltration step prepared the reaction mixture for the following purification step. Using this set-up of for the conjugation reaction allowed us to increase the yield from approximately 50 % to above 75 %. The side chain and the reducing agent consumption were halved. Additionally, the purity was increased significantly. The combination of the knowledge of chemistry and the use of mathematical modelling has proven to be efficient. Combining the tools resulted in a better process understanding and a better process.

77. Analysis of Residual Host Cell Protein During Protein A Chromatography Lifetime

Katherine Lintern, Department of Biochemical Engineering, University College London, Gordon Street, London WC1H 0AH, United Kingdom
Mili Pathak, Department of Chemical Engineering, IIT Delhi, Hauz Khas, New Delhi, India
Kevin Howland, School of Biosciences, University of Kent, Canterbury, Kent, CT2 7NJ, United Kingdom
C. Mark Smales, School of Biosciences, University of Kent, Canterbury, Kent, CT2 7NJ, United Kingdom
Anurag Rathore, Department of Chemical Engineering, IIT Delhi, Hauz Khas, New Delhi, India
*Daniel G. Bracewell, Department of Biochemical Engineering, University College London, Gordon Street, London WC1H 0AH, United Kingdom

Capacity reduction in protein A affinity chromatography with extended use during therapeutic antibody manufacture is well documented. These changes present a challenge in demonstrating process robustness at high cycle numbers. Identification of proteins remaining from previous cycles to understand their role in this ageing process, and any risk to product quality they might represent, is a significant metrological challenge. Here we combine scanning electron microscopy and liquid chromatography mass spectrometry to observe and map this phenomenon of protein carry-over. We show that there is a morphological change in the surface appearance of resin with increasing cycle number. Significantly, the composition of the protein cohort present on the resin is shown to shift with cycle number. Although an expected increase in the number of host cell proteins (HCPs) identifiable was observed with increasing cycle number, the functional class of predominant HCPs was also seen to change. This was all observed in the context of total HCP in the eluate remaining constant with cycle number.

Low cycle number resin contained greater proportions of residual mAb than did resin used for more cycles. A greater proportion of cytoskeletal components and proteins known to play a role in protein synthesis and metabolism were associated with resin from a higher number of cycles. These progressive changes raise the question as to what initiates this fouling process, three possible causes are suggested in the literature; histones, conformational changes to mAb, and proteases. To investigate this feedstreams varying in these components have been studied. This data is interpreted to give an insight into the dominant mechanism of resin fouling in the chromatographic process, and highlights those HCPs which are present at elevated levels as the resin ages and as a consequence may represent a risk to process robustness. This suggests that screening feedstocks for critical HCPs which are associated with these mechanisms so they can be moderated upstream may help to improve resin performance at extended cycle numbers.

78. Implementation of Mechanistic Modeling, DoE and QbD Principles into Process Characterization for an Fc-Fusion Protein

*Xuankuo Xu, Bristol-Myers Squibb, United States
Chao Huang, Bristol-Myers Squibb, United States
Zhiqiang Chen, Bristol-Myers Squibb, United States
Steven Traylor , Bristol-Myers Squibb, United States
Na Zhang, Bristol-Myers Squibb, United States
Amanda Lewis, Bristol-Myers Squibb, United States
Nicholas Abu-Absi, Bristol-Myers Squibb, United States
Michael Borys, Bristol-Myers Squibb, United States
Zhengjian Li, Bristol-Myers Squibb, United States

A significant improvement to the productivity and process robustness for manufacture of a legacy Fc-fusion protein with a complex and tightly-specified range of quality attributes was achieved through development of a second-generation manufacturing process. The primary challenge to the downstream process is the diverse population of product glycoforms generated in the cell culture step that require consistent control to ensure drug substance comparability, efficacy and patient safety. This work outlines two case studies where mechanistic modeling, DoE and QbD principles were incorporated into the characterization of the manufacturing process. The first example demonstrates how mechanistic modeling, with the fundamental parameters measured using conventional and high throughput screening (HTS) methods, and a DoE approach were integrated to establish the scale-down model of an anion exchange chromatography (AEX) step. Due probably to wall effects, small diameter AEX columns were unable to achieve the bed compression specified for manufacturing scale columns, resulting in practically higher protein loading per resin particle volume when the column loading was specified according to packed bed volume. A correction to the column loading based only on bed compression appeared inadequate to ensure equivalent column performance and to establish a representative scale-down model. To assess the impact of bed compression within the design space, a DoE framework was applied to a mechanistic column model describing glycoform separation in AEX using a minimal set of fundamental experimental measurements, which allowed for efficient identification of a representative operating condition for the scale-down model. The second example focuses on characterizing two polishing steps (AEX-HIC) in a single custom multivariate DoE study using QbD principles to achieve improved and

robust control of product glycoform distribution. Process parameters (PPs) were selected based on risk assessment and process knowledge to explore the optimal design space across these two successive unit operations using the output results of AEX as the input variables for HIC. With the predictive statistical models developed, an easily-implemented strategy was proposed, where the PPs of the AEX step could be adjusted according to the quality of the AEX input material to achieve the target drug substance specifications. The extensive process understanding would not be easily achievable if the two polishing steps were characterized independently. The knowledge gained in process characterization was finally used to define the control strategy for the drug substance manufacturing process of this fusion protein.

79. No Use for High-Throughput Batch Isotherms in Modelling? Why Column Experiments Yield More Accurate Information in Equal Time

Tobias Hahn, Karlsruhe Institute of Technology, Germany
**Thiemo Huuk, Karlsruhe Institute of Technology, Germany*
Gang Wang, Karlsruhe Institute of Technology, Germany
Juergen Hubbuch, Karlsruhe Institute of Technology, Germany

High-throughput screening (HTS) in batch format is often used in combination with Design-of-Experiments (DoE) and promises to characterize a large part of the process design space with low sample volume in short time. For model-based process development, only few experiments of a DoE campaign are of relevance, those that provide information for model calibration. In order to use batch data for model-based process development, the data quality must be high, and the parameters must be representative and transferable. This contribution discusses whether these challenges can be met by HTS and if it is more efficient than conventional column experiments. Quality: A reliable distribution of adsorber slurry in the 96-well plates must be guaranteed, pipetting errors must be minimized and UV measurement variations in the 96-well plates must be taken into account. Even if these sources of errors can be eliminated, isotherm fitting is not reliable per se. For model proteins on the HIC adsorber Capto phenyl, we obtained much larger parameter confidence intervals compared to column mode. Representativeness: Under weak binding conditions the influence of diffusion parameters can be as important as the isotherm parameters themselves. Muca et al. (J Chromatogr A, 2009) measured an exponential influence of salt concentration on peak broadening such that four different diffusion parameters must be determined for each involved protein to model HIC and mixed-mode chromatography accurately. This is impossible in batch mode. Transferability: For low salt concentrations, e.g. when using IEX, parameter transferability is the main issue. To cope with this, we developed a method to determine the ionic capacities in column and batch mode, based on the adsorption/desorption of UV-detectable histidine that allows to quantify the adsorber amount in each well and, thus, the static binding capacity. The results show that the equivalent column volume for a batch experiment is highly resin dependent and that the protein-specific difference in static and dynamic binding still remains as source of error. While HTS is faced with these challenges, targeted model calibration in column mode using Optimal Experimental Design allows to determine the parameters with highest statistical quality using the least number of experiments. Hence, column experiments become on a par with typical DoE campaigns in terms of sample volume, time and labor.

Poster Abstracts

Participant List

Jeff Allen
PFENEX INC
10790 Roselle
San Diego, CA 92121
United States
jallen@pfenex.com

Ashraf Amanullah
ATYR PHARMA
3545 John Hopkins Court, Suite 250
San Diego, CA 92121
United States
aamanullah@atyrpharma.com

Dorothee Ambrosius
BOEHRINGER INGELHEIM
Biberach
Germany
dorothee.ambrosius@boehringer-ingelheim.com

Patrick Asplund
COOK PHARMICA, LLC
1300 South Patterson Drive
Bloomington, IN 47402-0970
United States
patrick.asplund@cookpharmica.com

Morrey Atkinson
BRISTOL-MYERS SQUIBB
311 Pennington Rocky-Hill Rd
Hopewell, NJ 8534
United States
morrey.atkinson@bms.com

Engin Ayturk
PALL CORPORATION
20 Walkup Drive
Westborough, MA 1581
United States
engin_ayturk@pall.com

Hanne Bak
REGENERON PHARMACEUTICALS
777 Old Saw Mill River Road
Tarrytown, NY 10591
United States
hanne.bak@regeneron.com

Gregory Barker
BRISTOL-MYERS SQUIBB
519 Route 173 West
Bloomsbury, NJ 8804
United States
gregory.barker@bms.com

Kristopher Barnthouse
JANSSEN / J&J
200 Great Valley Parkway
Malvern, PA 19355
United States
kbarntho@its.jnj.com

Ronald Bates
BMS
6000 Thompson Road
East Syracuse, NY 13057
United States
ronald.bates@bms.com

Pascal Baumann
KARLSRUHE INSTITUTE OF TECHNOLOGY (KIT)
Engler-Bunte-Ring 3
Karlsruhe, Baden-Wuerttemberg 76131
Germany
pascal.baumann@kit.edu

Kevin Beam
SEATTLE GENETICS
21823 30th Drive SE
Bothell, WA 98021
United States
kbeam@seagen.com

Lydia Beasley
GENENTECH
1 DNA Way
South San Francisco, CA 94080
United States
lydiab@gene.com

Christopher Belisle
BIO-RAD LABORATORIES
3210 Meadow View Ln
Walnut Creek, CA 94598
United States
christopher_belisle@bio-rad.com

Jean Bender
MEDIMMUNE
One Medimmune Way
Gaithersburg, MD 20878
United States
benderj@medimmune.com

Joseph Bertolini
CSL BEHRING
189-209 Camp Rd
Broadmeadows, Victoria 3047
Australia
joe.bertolini@cslbehring.com.au

Bharat Bhut
MERCK & CO.
2000 Galloping Hill Rd
Kenilworth, NJ 7033
United States
bharat.bhut@merck.com

Marc Bisschops
PALL LIFE SCIENCES
Nijverheidsweg 1
Medemblik, 1671 GC
Netherlands
marc_bisschops@pall.com

Austin Boesch
ZEPTEON, INC.
42 Chauncy St. STE 10A
Boston, MA 2111
United States
austin.boesch@zepteon.com

Glen Bolton
AMGEN
360 Binney St
Cambridge, MA 2141
United States
gbolton@amgen.com

Brian Bowes
ELI LILLY AND COMPANY
Lilly Corporate Center
Indianapolis, IN 46285
United States
bowes_brian_d@lilly.com

John Boyle
MILLIPORE SIGMA
80 Ashby Road
Bedford, MA 1730
United States
john.boyle@emdmillipore.com

Reinhard Braaz
ROCHE DIAGNISTICS GMBH
Nonnenwald 2
Penzberg, Bavaria 82377
Germany
reinhard.braaz@roche.com

Daniel Bracewell
UCL
Gordon Street
London, WC1H 0AH
United Kingdom
d.bracewell@ucl.ac.uk

Nina Brestrich
KARLSRUHE INSTITUTE OF TECHNOLOGY
Fritz-Haber-Weg 7
Karlsruhe, Baden Wuertemberg 76131
Germany
nina.brestrich@kit.edu

Ernst Broberg Hansen
NOVO NORDISK A/S
Smørmosevej 17
Bagsværd, 2880
Denmark
eha@novonordisk.com

Mark Brower
MERCK & CO., INC.
2000 Galloping Hill Road
Kenilworth, NJ 7033
United States
mark_brower@merck.com

Deirdre Buckley
ELI LILLY
Dunderrow
Cork,
Ireland
buckley_deirdre_a@lilly.com

Stuart Builder
STRATEGIC BIODEVELOPMENT
2827 Wemberly Dr.
Belmont, CA 94992
United States
sbuilder@ix.netcom.com

Steve Burton
PROMETIC BIOSCIENCES LTD
Horizon Park, Barton Road
Cambridge, CB23 7AJ
United Kingdom
s.burton@prometic.com

Michelle Butler
GENENTECH, INC.
1 DNA Way
South San Francisco, CA 94002
United States
butler.michelle@gene.com

Jordan Byrd
REGENERON PHARMACEUTICALS INC
81 Columbia Turnpike
Rensselaer, NY 12144
United States
jordan.byrd@regeneron.com

Ruben Carbonell
NORTH CAROLINA STATE UNIVERSITY
850 Oval Drive
Raleigh, NC 27695
United States
ruben@ncsu.edu

Giorgio Carta
UNIVERSITY OF VIRGINIA
102 Engineers Way
Charlottesville, VA 22904
United States
gc@virginia.edu

San-Cher Chen
DEVELOPMENT CENTER FOR BIOTECHNOLOGY
101, Lane 169, Kangning St., Xizhi Dist
New Taipei City, 22180
Taiwan
scchena@mail.dcb.org.tw

Shuang Chen
PFIZER INC
700 Chesterfield Parkway West
Cheterfield, MO 63017
United States
shuang.chen@pfizer.com

Qi Chen
GENENTECH, INC
1 DNA Way
South San Francisco, CA 94080
United States
qi@gene.com

Jie Chen
SHIRE
300 Shire Way
Lexington, MA 2421
United States
jchen@dyax.com

John Chickosky
NATRIX SEPARATIONS INC.
5295 John Davis Drive
Burlington, Ontario L7L 6A8
Canada
chickosky@natrixseparations.com

Wai Keen Chung
MEDIMMUNE
One MedImmune Way
Gaithersburg, MD 20878
United States
chungw@medimmune.com

Jonathan Coffman
BOEHRINGER INGELHEIM
Fremont
United States
jonathan.coffman@boehringer-ingelheim.com

Charles Cooney
MIT
MIT 56-469B
Cambridge, MA 2139
United States
ccooney@mit.edu

Myra Coufal
AMGEN
360 Binney St
Cambridge, MA 2142
United States
mcoufal@amgen.com

Steven Cramer
RPI
3211 CBIS, 110 8th St.
Troy, NY 12180
United States
crames@rpi.edu

John Cundy
PFIZER
700 Chesterfield Parkway West
Chesterfield, MO 63017
United States
john.cundy@pfizer.com

Bárbara Cunha
IBET / ITQB-NOVA
Apartado 12
Oeiras
Portugal
bcunha@itqb.unl.pt

John Curling
JCC AB
Swedenborgsgatan 5
Uppsala, 753 34
Sweden
john@consultcurling.se

John Daicic
GE HEALTHCARE LIFE SCIENCES
Björkgatan 30
Uppsala, 75184
Sweden
john.daicic@ge.com

Andy Davies
PROMETIC BIOSCIENCES
Horizon Park
Comberton, Cambridgeshire CB23 7AJ
United Kingdom
a.davies@prometic.com

Nuria de Mas
BRISTOL-MYERS SQUIBB
38 Jackson Rd
Devens, MA 1434
United States
nuria.demas@bms.com

Kumar Dhanasekharan
COOK PHARMICA
1300 S Patterson Dr.
Bloomington, IN 47402
United States
kumar.dhanasekharan@cookpharmica.com

Evi Dimitriadou
LONZA BIOLOGICS
224 Bath Road
Slough, Berkshire SL1 4DX
United Kingdom
evi.dimitriadou@lonza.com

Florian Dismer
NOVO NORDISK A/S
Novo Nordisk Park
Maalov, DK-2760
Denmark
fld@novonordisk.com

Mandar Dixit
SARTORIUS STEDIM BIOTECH
5 Orville Dr..
Bohemia, NY 11716
United States
mandar.dixit@sartorius-stedim.com

Martin Dorn
TECHNISCHE UNIVERSITÄT MÜNCHEN
Boltzmannstr. 15
Garching, Bavaria 85748
Germany
martin.dorn@tum.de

Chris Dowd
GENENTECH
1 DNA Way
South San Francisco, CA 94080
United States
cjdowd@gene.com

Andre Dumetz
GSK
709 Swedeland Road
King of Prussia, PA
United States
andre.c.dumetz@gsk.com

Michael Egholm
PALL CORPORATION
20 Walkup Drive
Westborough, MA 1581
United States
michael_egholm@pall.com

Hanno Ehring
GE HEALTHCARE
Björkgatan 30
Uppsala
Sweden
Hanno.Ehring@ge.com

Michel Eppink
SYNTHON BIOPHARMACEUTICALS BV
Microweg
Nijmegen, Nederland 6503 GN
Netherlands
michel.eppink@synthon.com

John Erickson
GSK
709 Swedeland Rd
King of Prussia, PA 19406
United States
john.c.erickson@gsk.com

Derek Ettie
GEA
17 Overidge Ln
Wilton, CT 6897
United States
derek.ettie@gea.com

Rob Fahrner
PFIZER
700 Chesterfield Pkwy W
Chesterfield, Missouri, MO 63017
United States
robert.fahrner@pfizer.com

Suzanne Farid
UCL
Gordon Street
London, WC1H 0AH
United Kingdom
s.farid@ucl.ac.uk

Conan Fee
UNIVERSITY OF CANTERBURY
Ilam Road
Christchurch, 8041
New Zealand
conan.fee@canterbury.ac.nz

Gisela Ferreira
MEDIMMUNE
1 MedImmune Way
Gaithersburg, MD 20877
United States
FerreiraG@medimmune.com

Stefan Fischer-Frühholz
SARTORIUS STEDIM BIOTECH GMBH
August-Spindler-Str. 11
Goettingen, 37079
Germany
stefan.fischerf@sartorius-stedim.com

Jace Fogle
ELI LILLY
Eli Lilly and Co. DC 35353
Indianapolis, IN 46285
United States
jace@lilly.com

Nuno Fontes
BOEHRINGER INGELHEIM
6701 Kaiser Drive
Fremont, California, 94555
United States
nuno.fontes@boehringer-ingelheim.com

Dan Freymeyer
AVITIDE, INC
PO Box 1774
Grantham, NH 3753
United States
dfreymey@gmail.com

Stuart Gallant
ANTHERA PHARMACEUTICALS
55 Hill Road, Suite 706
Belmont, MA 2478
United States
sgallant@outlook.com

Rene Gantier
PALL LIFE SCIENCES
20 Walkup Drive
Westborough, MA 1581
United States
rene_gantier@pall.com

George Georgiou
THE UNIVERSITY OF TEXAS AT AUSTIN
200 E. Dean Keeton St., C0400
Austin, TX 78712
United States
gg@che.utexas.edu

Sanchayita Ghose
BIOGEN
Research Triangle
Research Triangle, NC
United States
sanchayita.ghose@biogen.com

Glen Giese
GENENTECH
1 DNA Way
South San Francisco, CA 94080
United States
ggiese@gene.com

Ranga Godavarti
PFIZER
One Burtt Road
Andover, MA 1810
United States
ranga.godavarti@pfizer.com

Rahul Godawat
ALEXION PHARM
100 College St
New Haven, CT 6501
United States
godawatr@alxn.com

Victor Goetz
ELI LILLY AND COMPANY
33 ImClone Drive
Branchburg, NJ 8876
United States
victor.goetz@lilly.com

Rüdiger Göhmann
GEA
Werner-Habig-Str. 1
Oelde, NRW 59302
Germany
ruediger.goehmann@gea.com

Elizabeth Goodrich
MILLIPORESIGMA
900 Middlesex Turnpike
Billerica, MA 1821
United States
Elizabeth.goodrich@emdmillipore.com

Uwe Gottschalk
LONZA
Munchensteinerstrasse 38
Basel, 4002
Switzerland
uwe.gottschalk@lonza.com

Jan Griesbach
ROCHE
Nonnenwald 2
Penzberg, 82377
Germany
jan.griesbach@roche.com

Anna Gronberg
GE HEALTHCARE
Bjorkgatan 30
Uppsala, 75184
Sweden
anna.gronberg@ge.com

Robert Gronke
BIOGEN
225 Broadway
Cambridge, MA 2142
United States
rob.gronke@biogen.com

David Gruber
MEDIMMUNE
Milstein Building
Cambridge, CB21 6GH
United Kingdom
gruberd@medimmune.com

Qin Gu
CARNEGIE MELLON UNIVERSITY
Doherty Hall, 5000 Forbes Avenue
Pittsburgh, PA 15213
United States
qingu@andrew.cmu.edu

Xuejun Gu
LILLY
Lilly Research Lab
Indianapolis, IN
United States
sherrygu@lilly.com

Rainer Hahn
BOKU VIENNA
Muthgasse 18
Vienna, 1190
Austria
Rainer.Hahn@boku.ac.at

Tobias Hahn
KARLSRUHE INSTITUTE OF TECHNOLOGY
Engler-Bunte-Ring 3
Karlsruhe, BW 76135
Germany
tobias.hahn@kit.edu

Thomas Hansen
NOVO NORDISK A/S
Smørmosevej 17-19
Bagsværd, DK-2880
Denmark
tmhs@novonordisk.com

Oliver Hardick
PURIDIFY / UCL
Stevenage Bioscience Catalyst
London, SG1 2FX
United Kingdom
oliver@puridify.com

Roger Hart
AMGEN, INC.
360 Binney Street
Cambridge, MA 02142-1097
United States
rhart@amgen.com

Charles Haynes
UNIVERSITY OF BRITISH COLUMBIA
Vancouver
Canada
charles.haynes@ubc.ca

Xuemei He
BIO-RAD LABORATORIES
6000 James Watson Drive
Hercules, CA 94547
United States
Xuemei_He@biorad.com

Milton Hearn
MONASH UNIVERSITY
Building 23
Clayton, Victoria 3800
Australia
milton.hearn@monash.edu

Sarah Hedberg
IMPERIAL COLLEGE LONDON
Department of Chemical Engineering
South Kensington Campus
London, SW7 2BY
United Kingdom
s.hedberg13@ic.ac.uk

Caryn Heldt
MICHIGAN TECH
1400 Townsend Dr.
Houghton, MI 49931
United States
heldt@mtu.edu

Pim Hermans
THERMO FISHER SCIENTIFIC
J.H.Oortweg 21
Leiden, 2333 CH
Netherlands
pim.hermans@thermofisher.com

Naokatsu Hirotomi
ASAHI KASEI BIOPROCESS AMERICA
1855 Elmdale Avenue
Glenview, IL 60026
United States
naokatsu.hirotomi@ak-bio.com

Regina Holzhauser
TOSOH BIOSCIENCE GMBH
Im Leuschnerpark 4
Griesheim, 64347
Germany
regina.holzhauser@tosoh.com

Juergen Hubbuch
KARLSRUHE INSTITUTE
OF TECHNOLOGY
Engler-Bunte-Ring 3
Karlsruhe, 76131
Germany
Juergen.Hubbuch@kit.edu

Charlotte S Hunneche
NOVO NORDISK A/S
Hagedornsvej 1
Gentofte, 2820
Denmark
chun@novonordisk.com

Stephen Hunt
KBI BIOPHARMA
2500 Central Ave
Boulder, CO 80503
United States
shunt@kbibiopharma.com

Thiemo Huuk
KARLSRUHE INSTITUTE
OF TECHNOLOGY (KIT)
Engler-Bunte-Ring 3
Karlsruhe, 76131
Germany
thiemo.huuk@kit.edu

Michael Inglis
PFIZER BIOTECH
Grange Castle, Grange Castle Bus. Pk.
Dublin
Ireland
michael.inglis@pfizer.com

Günter Jagschies
GE HEALTHCARE LIFE SCIENCES
Björkgatan 30
Uppsala, 75184
Sweden
guenter.jagschies@ge.com

Canping Jiang
BIOGEN
250 Binney Street
Cambridge, MA 2142
United States
canping.jiang@biogen.com

Mi Jin
TEVA PHARMACEUTICALS
145 Brandywine Pkwy
West Chester, PA
United States
mi.jin@tevapharm.com

Hans Johansson
PUROLITE LIFE SCIENCES
Vårdkasvägen 7
Uppsala, 75655
Sweden
hans.johansson@purolite.com

David Kahn
MEDIMMUNE
1 MedImmune Way
Gaithersburg, MD 20878
United States
kahnd@medimmune.com

Anne Kantardjieff
ALEXION PHARMACEUTICALS
352 Knotter Drive
Cheshire, CT
United States
kantardjieffa@alxn.com

Pankaj Karande
RENSSELAER POLYTECHNIC
INSTITUTE
110 8th St.
Troy, NY 12180
United States
karanp@rpi.edu

Mark Karbarz
PORTOLA PHARMACEUTICALS
270 East Grand Avenue
South San Francisco, CA
United States
mkarbarz@portola.com

Brian Kelley
GENENTECH
1 DNA Way
South San Francisco, CA 94080
United States
kelley.brian@gene.com

Hans Kiefer
BIBERACH UNIVERSITY OF APPLIED SCIENCES
Karlstrasse 11
Biberach, 88400
Germany
kiefer@hochschule-bc.de

Annika Kleinjans
ROCHE DIAGNOSTICS GMBH
Nantesbucher Weg 5b
Iffeldorf
Germany
annika.kleinjans@roche.com

Sushmita Koley
DBT-ICT-CEB, INSTITUTE OF CHEMICAL TECHNOLOGY
Institute of Chemical Technology, N. P. Marg, Matunga(E)
Mumbai, Maharashtra 400019
India
sushsparky@gmail.com

Mikhail Kozlov
MILLIPORESIGMA
80 Ashby Rd.
Bedford, MA 1730
United States
mikhail.kozlov@emdmillipore.com

Wolfgang Kuhne
ROCHE DIAGNOSTICS GMBH, PHARMA BIOTECH PENZBERG
Nonnenwald 2
Penzberg, Bavaria 82377
Germany
wolfgang.kuhne@roche.com

Pawan Kumar
PORTOLA PHARMACEUTICALS INC.
270 E Grand Ave
South San Francisco, CA 94080
United States
pkumar@portola.com

Beckley Kungah Nfor
JANSSEN VACCINES
Archimedesweg 4-6
Leiden, 2333 CN
Netherlands
bkungahn@its.jnj.com

Karol Lacki
N-ONE
Sweden
k.lacki@icloud.com

Michael Ladisch
PURDUE UNIVERSITY
LORRE
W. Lafayette, IN 47906
United States
ladisch@purdue.edu

Asif Ladiwala
GENENTECH
1 DNA Way
South San Francisco, CA 94080
United States
ladiwala.asif@gene.com

Alfredo Lanzaro
THE UNIVERSITY OF MANCHESTER
131 Princess Street
Manchester, Greater Manchester M1 7DN
United Kingdom
alfredo.lanzaro@manchester.ac.uk

Michael Laska
MODERNA THERAPEUTICS
5th Floor
Cambridge, MA 2139
United States
michael_laska@live.com

Micky Fu Xiang Lee
MONASH UNIVERSITY
Jalan Lagoon Selatan
Sunway City, Selangor 47500
Malaysia
mickylfx@gmail.com

Steven Lehrer
CIPLA BIOTEC
1771 Ringling Blvd PH 203
Sarasota, Florida 34236-6872
United States
steven.lehrer@cipla.com

Thorsten Lemm
ROCHE DIAGNOSTICS GMBH
Nonnenwald 2
Penzberg, Bavaria 82377
Germany
thorsten.lemm@roche.com

Abraham Lenhoff
UNIVERSITY OF DELAWARE
150 Academy Street
Newark, DE 19716
United States
lenhoff@udel.edu

Philip Lester
GENENTECH
1 DNA Way
South San Francisco, CA 94080
United States
lesterp@gene.com

Peter Levison
PALL LIFE SCIENCES
5 Harbourgate Business Park
Portsmouth, PO6 4BQ
United Kingdom
peter_levison@pall.com

Paul Lewus
AMGEN, INC
11804 N. Beasly Rd
Longmont, 80504
United States
plewus@amgen.com

Honghao LI
NOVO NORDISK RESEARCH CENTRE CHINA
Building #2, 20 Life Science Park Road
Beijing 102206
China
hhli@novonordisk.com

Zhengjian Li
BRISTOL-MYERS SQUIBB
Devens, MA 1434
United States
zhengjian.li@bms.com

Miladys Limonta
CIGB
Ave 31 e/ 158 y 190
La Habana
Cuba
miladys.limonta@cigb.edu.cu

Naichi Liu
BIOMARIN
105 Digital Drive
Novato, CA 94949
United States
Naichi.liu@bmrn.com

Lotta Ljungqvist
GE HEALTHCARE LIFE SCIENCES
Björkgatan 30
Uppsala
Sweden
lotta.ljungqvist@ge.com

Tim Lowery
JSR MICRO
1280 North Mathilda Avenue
Sunnyvale, CA 94089
United States
tlowery@jsrmicro.com

Mats Lundgren
GE HEALTHCARE LIFE SCIENCES
Bjorkgatan 30
Uppsala, 75184
Sweden
matslundgren@ge.com

Herb Lutz
MILLIPORE SIGMA
6220 Pacific Ave.
Playa del rey, CA 90293
United States
herb.lutz@emdmillipore.com

Chris Major
PUROLITE
Unit D
Wales
United Kingdom
chris.major@purolite.com

Jan Makela
GE HEALTHCARE
Little Chalfont
Amersham, Buckinghamshire HP7 9LL
United Kingdom
jan.makela@ge.com

Gunnar Malmquist
GE HEALTHCARE
Bjorkgatan 30
Uppsala, SE-751 84
Sweden
gunnar.malmquist@ge.com

Christian Manzke
SARTORIUS STEDIM BIOTECH GMBH
August-Spindler-Strasse 11
Goettingen, 37120
Germany
christian.manzke@
sartorius-stedim.com

Bruno Marques
GLAXOSMITHKLINE
709 Swedeland Road
King of Prussia, PA 19406
United States
bruno.f.marques@gsk.com

Tiago Matos
NOVO NORDISK
Novo Nordisk Park
Malov
Denmark
tgmm@novonordisk.com

Karl McCann
CSL BEHRING AUSTRALIA
189 – 209 Camp Road
Broadmeadows, Victoria 3047
Australia
karl.mccann@cslbehring.com.au

Amit Mehta
GENENTECH
1 DNA Way
South San Francisco, CA 94080
United States
mehta@gene.com

Lee Mermelstein
PORTOLA PHARMACEUTICALS
270 East Grand Avenue
South San Francisco, CA 94080
United States
lmermelstein@portola.com

Atul Mohindra
LONZA BIOLOGICS
224 Bath Road
Slough, Berkshire SL1 4DX
United Kingdom
atul.mohinDr.a@lonza.com

Massimo Morbidelli
ETH ZURICH
Vladimir-Prelog-Weg 1 / HCI F131
Zurich
Switzerland
nadine.widmer@chem.ethz.ch

John Moscariello
CMC BIOLOGICS
22021 20th Ave SE
Bothell, WA 98133
United States
jmoscariello@cmcbio.com

Morten Munk
NNE PHARMAPLAN
Nybrovej 80
GENTOFTE, 2820
Denmark
mbmn@nnepharmaplan.com

Christopher Murray
INSTITUTE FOR HEALTH METRICS
AND EVALUATION
2301 5th Ave.
Seattle, WA 98121
United States
cjlm@uw.edu

Jill Myers
FORTRESS BIOTECH
95 Sawyer Rd.
Waltham, MA 2453
United States
jmyers@fortressbiotech.com

Amith Naik
NORTH CAROLINA STATE UNIVERSITY
1791 Varsity Drive
RALEIGH, NC 27695
United States
adnaik2@ncsu.edu

Alpana Naresh
JSR LIFE SCIENCES
1280 N Mathilda Ave
Sunnyvale, CA 94089
United States
anaresh@jsrmicro.com

Darren N. Nesbeth
UNIVERSITY COLLEGE LONDON
Bernard Katz Building, Gordon Street
London, WC1H 0AH
United Kingdom
d.nesbeth@ucl.ac.uk

Sara Nilsson
GSK
Medicines Research Centre (5G104)
Stevenage, Hertfordshire SG1 2NY
United Kingdom
sara.x.nilsson@gsk.com

Wim Noppe
KU LEUVEN CAMPUS KORTRIJK
E. Sabbelaan 53
Kortrijk, B-8500
Belgium
wim.noppe@kuleuven-kulak.be

J. Kevin O'Donnell
TOSOH BIOSCIENCE LLC
3604 Horizon Drive Suite 100
King of Prussia, PA 19406
United States
kevin.odonnell@tosoh.com

Stefan Oelmeier
BOEHRINGER INGELHEIM PHARMA GMBH & CO. KG
Birkendorfer Straße 65
Biberach, 88397
Germany
stefan.oelmeier@boehringer-ingelheim.com

Brian O'Mara
BRISTOL-MYERS SQUIBB
1201 Eastlake Ave E
Seattle, WA 98102
United States
brian.omara@bms.com

Raquel Orozco
BOEHRINGER INGELHEIM FREMONT, INC.
6701 Kaiser Drive
Fremont, CA 94555
United States
raquel.orozco@boehringer-ingelheim.com

Marcel Ottens
DELFT UNIVERSITY OF TECHNOLOGY
Julianalaan 67
Delft, Zuid Holland 2628 BC
Netherlands
m.ottens@tudelft.nl

Timothy Pabst
MEDIMMUNE
1 MedImmune Way
Gaithersburg, MD 20872
United States
pabstt@medimmune.com

Olga Paley
TAKEDA PHARMACEUTICAL COMPANY
35 Landsdowne
Cambridge, MA 2139
United States
olga.paley@takeda.com

Lars Pampel
NOVARTIS PHARMA
WKL-693.1.139.14
Basel, 4002
Switzerland
lars.pampel@novartis.com

David Paolella
GLAXOSMITHKLINE
709 Swedeland Rd
King of Prussia, 19406
United States
david.n.paolella@gsk.com

Siddharth Parimal
BIOGEN
5000 Davis Drive
Durham, NC 27709
United States
siddharth.parimal@biogen.com

Shelly Parra
THERMO FISHER SCIENTIFIC
27 Windsong Road
Cumberland, RI 2864
United States
shelly.parra@thermofisher.com

Cristina Peixoto
IBET
Apartado 12
Oeiras, 2781-901 Oeiras
Portugal
peixoto@ibet.pt

Dana Pentia
REPLIGEN CORPORATION
41 Seyon St
Waltham, MA 02453
United States
dpentia@repligen.com

James Peyser
REPLIGEN CORP
41 Seyon Street
Waltham, MA 2453
United States
jpeyser@repligen.com

Michael Phillips
MILLIPORESIGMA
80 Ashby Road
Bedford, MA 1730
United States
michael.phillips@emdmillipore.com

John Pieracci
BIOGEN CORPORATION
125 Broadway
Cambridge, MA 1890
United States
john.pieracci@biogen.com

Nuno Pinto
MERCK & CO., INC.
Room K15-B412C
Kenilworth, NJ 7033
United States
nuno.pinto@merck.com

Jennifer Pollard
MERCK & CO., INC.
2000 Galloping Hill Rd
Kenilworth, NJ 7033
United States
jennifer_pollard@merck.com

Todd Przybycien
CARNEGIE MELLON UNIVERSITY
5000 Forbes Avenue
Pittsburgh, PA 15213
United States
todd@andrew.cmu.edu

Maen Qadan
LILLY
Lilly Corporate Center
Indianapolis, IN 46285
United States
qadan_maen@lilly.com

Natraj Ram
ABBVIE INC.
100 Research Drive
Worcester, MA 1581
United States
natraj.ram@abbvie.com

LP Raman
3M
400 Research Parkway
Meriden, CT 6450
United States
lraman@mmm.com

Andrew Ramelmeier
PORTOLA PHARMACEUTICALS
270 East Grand Avenue
South San Francisco, CA 94080
United States
aramelmeier@portola.com

Andrea Rayat
UNIVERSITY COLLEGE LONDON
Department of Biochemical
Engineering
LONDON, London WC1H 0AH
United Kingdom
andrea.rayat@ucl.ac.uk

Marco Rito-Palomares
TECNOLOGICO DE MONTERREY
Ave Eugenio garza sada 2501
Monterrey,
Mexico
mrito@itesm.mx

David Robbins
MEDIMMUNE
One MedImmune Way
Gaithersburg, MD 20878
United States
RobbinsD@MedImmune.com

Nicola Roberts
UCB
638 Ajax Avenue
Slough, Berkshire SL1 4BG
United Kingdom
nicola.roberts@ucb.com

Julie Robinson
RENSSELAER POLYTECHNIC
INSTITUTE
110 8th Street
Troy, NY 12180
United States
robinj6@rpi.edu

David Roush
MERCK & CO., INC.
2015 Galloping Hill Road, Mailstop
K15-2-H206
Kenilworth, NJ 7033
United States
david_roush@merck.com

Frederik Rudolph
BOEHRINGER INGELHEIM PHARMA
GMBH & CO KG
Birkendorfer Strasse 65
Biberach an der Riss, 88397
Germany
frederik.rudolph@boehringer-
ingelheim.com

Ben Sackett
GENENTECH
1 DNA Way
South San Francisco, CA 94080
United States
sackettb@gene.com

Jeffrey Salm
PFIZER
1 Burtt Rd
Andover, 1810
United States
jeff.salm@pfizer.com

Mark Saltzman
YALE UNIVERSITY
55 Prospect Street
New Haven, CT 6520
United States
mark.saltzman@yale.edu

Nooshafarin Sanaie
GILEAD SCIENCES
5582 Renaissance Ave
San Diego, CA
United States
nooshies@yahoo.com

Kimo Sanderson
ASAHI KASEI BIOPROCESS
AMERICA, INC.
1855 Elmdale Ave.
Glenview, IL 60026
United States
kimo.sanderson@ak-bio.com

Lalit Saxena
CIPLA BIOTEC PVT.LTD.
L147/ B
Goa, Goa 403722
India
lalit.saxena@ciplabiotec.com

Pierre Schelling
MILLIPORESIGMA
Merck & Cie
8200 Schaffhausen,
Switzerland
pierre.schelling@merckgroup.com

Emily Schirmer
CATALENT PHARMA SOLUTIONS
726 Heartland Trail
Madison, WI 53717
United States
emily.schirmer@catalent.com

Christine Bruun Schjødt
NOVO NORDISK A/S
Hagedornsvej 1
Gentofte, 2820
Denmark
chbs@novonordisk.com

Stefan Schmidt
RENTSCHLER BIOTECHNOLOGY
Erwin Rentschler Sr. 21
Laupheim, BW 88471
Germany
stefan.schmidt@rentschler.de

Mark Schofield
PALL LIFE SCIENCES
20 Walkup Dr.
Westborough, MA 1581
United States
Mark_Schofield@pall.com

Tim Schroeder
ATOLL GMBH
Ettishofer Str. 10
Weingarten, 88250
Germany
tschroeder@repligen.com

Norbert Schuelke
TAKEDA PHARMACEUTICALS
INTERNATIONAL CO.
35 Landsdowne Street
Cambridge, MA 2139
United States
norbert.schuelke@takeda.com

Lars Sejergaard
NOVO NORDISK A/S
Smørmosevej 17-19
Bagsværd
Denmark
lsej@novonordisk.com

Shinya Sekine
ASAHIKASEI AMR.ICA
525 Middlefield Road, Suite 110
Menlo Park, CA 94025
United States
sekine.sd@om.asahi-kasei.co.jp

Ian Sellick
PALL LIFE SCIENCES
20 Walkup Drive
Westborough, MA 1581
United States
ian_sellick@pall.com

Hammad Shaikh
NOVONORDISK
Smørmosevej 17-19 , 6BC2.59,1
2880, Copenhagen Bagsværd
Denmark
hams@novonordisk.com

Rahul Sheth
BIOMARIN PHARMACEUTICAL INC.
105 Digital Drive
Novato, CA 94949
United States
rsheth@bmrn.com

Eric Shierly
REGENERON PHARMACEUTICALS
81 Columbia Turnpike
Rensselaer, NY 12144
United States
eric.shierly@regeneron.com

Joseph Shiloach
NIDDK/NIH
Bldg 14 A Room 173
Bethesda, Maryland 20852
United States
Josephs@niddk.nih.gov

Russell Shpritzer
PFIZER
1 Burtt Road
Andover, MA 1810
United States
russell.shpritzer@pfizer.com

Abhinav Shukla
KBI BIOPHARMA INC.
2 Triangle Drive
Durham, NC 27704
United States
ashukla@kbibiopharma.com

Joseph Shultz
NOVARTIS PHARMA AG
Klybeckstrasse 141
Basel, 4058
Switzerland
joseph.shultz@novartis.com

Nripen Singh
BRISTOL MYERS SQUIBB
38 Jackson Road
Devens, MA 1748
United States
nripen.singh@bms.com

Marty Siwak
JSR LIFE SCIENCE
1280 N Mathilda Ave
Sunnyvale, CA
United States
msiwak@jsrmicro.com

Mark Snyder
BIO-RAD LABORATORIES
6000 James Watson Drive
Hercules, CA 94547
United States
mark_snyder@bio-rad.com

Mariangela Spitali
UCB PHARMA
638 Ajax Avenue
Slough,
United Kingdom
Mari.Spitali@ucb.com

Rick St. John
GENENTECH
1 DNA Way
South San Francisco, 94030
United States
st-john.richard@gene.com

Arne Staby
NOVO NORDISK A/S
Smørmosevej 17
Bagsværd, 2880
Denmark
ast@novonordisk.com

Sofie Stille
GE HEALTHCARE LIFE SCIENCES
Björkgatan 30
Uppsala, 75184
Sweden
sofie.stille@ge.com

Daniel Strauss
ASAHI KASEI BIOPROCESS AMERICA
1855 Elmdale Ave
Glenview, IL 60026
United States
daniel.strauss@ak-bio.com

Alison Tang
MEDIMMUNE
Aaron Klug building
Cambridge, CB21 6GH
United Kingdom
tanga@medimmune.com

Peter Tessier
RENSSELAER POLYTECHNIC INSTITUTE
Center for Biotech & Interdisciplinary Studies
Troy, NY 12180
United States
tessier@rpi.edu

Volkmar Thom
SARTORIUS STEDIM BIOTECH
August Spindler Str. 11
Göttingen, 37083
Germany
volkmar.thom@sartorius-stedim.com

Owen R.T. Thomas
UNIVERSITY OF BIRMINGHAM, EDGBASTON
School of Chemical Engineering, College of Engineering and Physical Sciences
Birmingham, UK, West Midlands B15 2TT
United Kingdom
o.r.t.thomas@bham.ac.uk

Jorg Thommes
BIOGEN
250 Binney Street
Cambridge, MA 2142
United States
jorg.thommes@biogen.com

Peter Tiainen
NOVO NORDISK A/S
Novo Nordisk A/S
Måløv, 2760
Denmark
ptii@novonordisk.com

Ben Tillotson
AMGEN
One Amgen Center Drive
Thousand Oaks, CA 91320
United States
bent@amgen.com

Steven Timmick
RENSSELAER POLYTECHNIC INSTITUTE
110 8th Street
Troy, NY 12180
United States
timmis@rpi.edu

Nigel Titchener-Hooker
UCL
Faculty of Engineering Sciences
London, WC1E 7JE
United Kingdom
nigelth@ucl.ac.uk

Alejandro Toro
AMGEN
PO Box 4060
Juncos, Puerto Rico 00777-4060
atoro@amgen.com

Benjamin Tran
GENENTECH, INC.
1 DNA Way
San Francisco, CA 94080
United States
benjampt@gene.com

Nihal Tugçu
MERCK
2000 Galloping Hill Road
Kenilworth, NJ 7033
United States
nihal_tugcu@merck.com

Andrew Tustian
REGENERON PHARMACEUTICALS
777 Old Saw Mill River Road
Tarrytown, NY 10591
United States
andrew.tustian@regeneron.com

Michiel Ultee
ULTEEMIT BIOCONSULTING, LLC
800 Atkinson Circle
Hillsborough, NJ 8844
United States
mike@ulteemitbio.com

James Van Alstine
ROYAL INSTITUTE OF TECHNOLOGY
JMVA Biotech
Stockholm, 11254
Sweden
jim.vanalstine@telia.com

Ganesh Vedantham
AMGEN
Amgen Drive
Cambridge, MA
United States
vedanthg@amgen.com

Ajoy Velayudhan
UCL
Bernard Katz Building
London WC1E 7JE
United Kingdom
a.velayudhan@ucl.ac.uk

Louis Villain
SARTORIUS STEDIM BIOTECH
August-Spindler Str. 11
Göttingen, 37097
Germany
louis.villain@sartorius-stedim.com

Monchois Vincent
NOVASEP PROCESS
81 Boulevard de la Moselle
POMPEY, 54340
France
vincent.monchois@novasep.com

Alexei Voloshin
3M COMPANY
3M Center
St. Paul, MN 55144
United States
amvoloshin@mmm.com

Thomas von Hirschheydt
ROCHE INNOVATION CENTER PENZBERG
Roche Diagnostics GmbH
Penzberg, 82377
Germany
thomas.von_hirschheydt@roche.com

Eric von Lieres
RESEARCH CENTER JÜLICH
Wilhelm-Johnen-Strasse 1
Jülich, 52425
Germany
e.von.lieres@fz-juelich.de

Suresh Vunnum
AMGEN
One Amgen Center Drive
Thousand Oaks, CA 91320
United States
vvunnum@amgen.com

Cornelia Walther
BOKU & BOEHRINGER-INGELHEIM-RCV
Muthgasse 18
Vienna, 1190
Austria
cornelia.walther@boku.ac.at

Sheng-Ching Wang
MERCK & CO., INC.
770 Sumneytown Pike
West Point, PA 19486
United States
sheng-ching_wang@merck.com

Chen Wang
ABBVIE
100 Research Drive
Worcester, MA
United States
c.wang@abbvie.com

Yong Wang
SHIRE
200 Shire Way
Lexington, MA 2421
United States
ywang@shire.com

Veena Warikoo
SANOFI
31 New York ave
Framingham, MA 1701
United States
veena.warikoo@sanofi.com

Michaela Wendeler
MEDIMMUNE
One MedImmune Way
Gaithersburg, MD 20878
United States
WendelerM@medimmune.com

Marc Wenger
MERCK & CO., INC.
PO Box 4
West Point, PA
United States
marc_wenger@merck.com

Matthew Westoby
BIOGEN
5000 Davis Drive
Chapel Hill, NC 27709
United States
mattwestoby@gmail.com

Catherine White
BIOMARIN PHARMACEUTICAL INC
105 Digital Drive
Novato, CA 94949
United States
cwhite@bmrn.com

Matthias Wiendahl
NOVO NORDISK A/S
Hagedornsvej 1
Gentofte, 2820
Denmark
mbsc@novonordisk.com

Gavin Wild
UCB
638 Ajax Avenue
Slough
United Kingdom
gavin.wild@ucb.com

Chris Williams
GENENTECH
1 DNA Way
South San Francisco, CA 94080
United States
christrw@gene.com

Nik Willoughby
HERIOT-WATT UNIVERSITY
School of Engineering and Physical Sciences
Edinburgh, EH14 4AS
United Kingdom
N.A.Willoughby@hw.ac.uk

Richard Willson
U OF HOUSTON
UH CHBE 4004
Houston, TX 77004
United States
willson@uh.edu

Michael Wolff
MAX PLANCK INSTITUTE FOR DYNAMICS OF COMPLEX TECHNICAL SYSTEMS
Sandtorstrasse 1
Magdeburg,
Germany
mwolff@mpi-magdeburg.mpg.de

James Woo
GILEAD SCIENCES
4049 Avenida De La Plata
Oceanside, CA 92056
United States
james.woo@gilead.com

David Wood
OHIO STATE UNIVERSITY
151 W. Woodruff Ave
Columbus, OH 43210
United States
wood.750@osu.edu

Michael Xenelis
MOMENTA PHARMACEUTICALS
675 West Kendall Street
Cambridge, MA 2142
United States
mxenelis@momentapharma.com

Alex Xenopoulos
MERCK MILLIPORE
80 Ashby Road
Bedford, MA 1730
United States
Alex.Xenopoulos@EMDMillipore.com

Xuankuo Xu
BRISTOL-MYERS SQUIBB
38 Jackson Rd
Devens, MA 1434
United States
xuankuo.xu@bms.com

Shuchi Yamamoto
YAMAGUCHI UNIVERSITY
Tokiwadai
Ube, 755-8611
Japan
shuichi@yamaguchi-u.ac.jp

Deqiang Yu
BRISTOL-MYERS SQUIBB
38 Jackson Road
Devens, MA 1434
United States
deqiang.yu@bms.com

Nick Zecherle
BIOMARIN PHARMACEUTICAL INC
105 Digital Drive
Novato, CA 94945
United States
nzecherle@bmrn.com

Min Zhu
MEDIMMUNE
1 Medimmune Way
Gaithersburg, MD 20878
United States
zhum@medimmune.com

Andrew Zydney
PENN STATE UNIVERSITY
118B Fenske Lab
University Park, PA 16802
United States
zydney@engr.psu.edu

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