Program, Abstracts & Conference Information

EXPLORING THE BOUNDARIES OF RECOVERY SCIENCE

RXVI

ROSTOCK 2014

27 July - 31 July 2014

Yachthafenresidenz Hohe Düne Rostock -Warnemünde Germany

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TOSOH BIOSCIENCE

RECOVERY OF BIOLOGICAL PRODUCTS XVI

YACHTHAFENRESIDENZ HOHE DÜNE
ROSTOCK-WARNEMÜNDE
GERMANY

27 JULY – 31 JULY 2014

An International Conference

Sponsored by:

The American Chemical Society
Division of Biochemical Technology

Conference Management Provided by:

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USA

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CONFERENCE CHAIRS

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Jörg Thömmes, Biogen Idec, United States

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William Wang, MedImmune, LLC, United States

POSTER SESSION CHAIRS

Charles Haynes, University of British Columbia, Canada
Brian Kelley, Genentech, Inc., United States
Abraham Lenhoff, University of Delaware, United States

A WARM WELCOME FROM THE CONFERENCE CO-CHAIRS

Dear Colleagues,

A very warm welcome to Recovery XVI! We are thoroughly excited to spend the next five days with you, taking you on a thrilling journey through the science and practice of Recovery of Biological Products. Under the motto "EXPLORING THE BOUNDARIES OF RECOVERY SCIENCES," we invite you to join us in deep scientific discussions, forward-looking debate, productive networking, and a great deal of fun, continuing the traditions of this great conference series.

Merriam Webster's dictionary defines EXPLORING as:

- to look at (something) in a careful way to learn more about it
- to talk or think about (something) in a thoughtful and detailed way
- to learn about (something) by trying it,

whereas BOUNDARY is defined as

- something that shows where an area ends and another area begins.

Together with the session chairs, we developed a scientific program that will examine Recovery Sciences from many different perspectives and try out new forms of scientific discussions. We will cross many boundaries by looking at Recovery from the viewpoint of neighboring scientific fields and by looking far into the future to understand what's next for our field.

As with previous Recovery conferences, the program mixes the tried and true with a few experiments. We introduced the concept of a daily theme with daily keynotes to give each day a specific focus:

- On Sunday, we will begin with a look into the future direction of Recovery Sciences, highlighted through a keynote by Dr. Ling.
- Monday's theme is charting the course with a focus on data, design, and predictions and a keynote by Dr. Iyer.
- On Tuesday, we will explore new molecular modalities and process concepts, and we will hear from Dr. Trounson about the exciting field of regenerative medicine.
- The boundary to drug delivery will be Wednesday's theme, and Dr. Peppas will outline future directions in Drug Delivery in his keynote.
- Last but not least, we will keep the ship of purification process development steady, the theme on Thursday, summarized by Dr. Atkinson's presentation on the critical role of case studies in our learning.

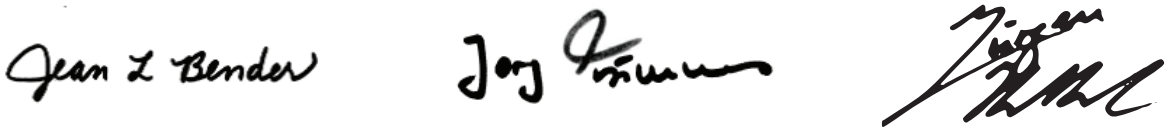
Workshops and poster sessions have been and will continue to be key elements of a Recovery conference. This year, we experimented with the workshop format by adding an expert panel to stimulate deep discussions. A highlight of the program, the poster session will be held in two dedicated evening sessions.

In addition to the exciting scientific program, Recovery conferences are also known for offering many opportunities to make new friends, reconnect with old ones, and engage in deep conversations about recovery sciences. This meeting will be no different. Both our location at Hotel Hohe Düne and the program schedule were chosen with these informal encounters in mind. The Baltic Sea and the historic town of Rostock will be a great backdrop for activities to join in with your colleagues and friends. We encourage you to participate in the activities offered on Monday and Wednesday. Finally, what would a scientific conference be without the continuation of debate after sessions at the bar? Hohe Dune's Kamin Bar will be our Recovery Lounge, the place to get together with colleagues and friends to "geek off" or just chat.

We would like to express our sincere thanks to the session, workshop, and poster chairs, all of whose contributions were invaluable to this year's program. Sponsorship by generous donors allows us to support the participation of colleagues from academia. We would like to extend a huge thank you to all sponsors. Your support is greatly appreciated.

A final word: the success of our conference depends on you! We encourage you to be activist in your participation, whether session chair, presenter or attendee. Discuss, question, debate, ideate, network, and forge collaborations and partnerships. Help create for this sixteenth time the collegial and inquisitive environment that makes this conference series so special.

All our best,



Conference Information

GENERAL CONFERENCE INFORMATION

Speaker Ready Area
Location: Congress Center, Salon 24, 2nd Floor
There will be a speaker ready area available. Speakers may preview and load their presentations onto a memory stick during the Conference. Check with the Conference staff at the conference registration desk to gain access to the area. You should have received a card in your registration packet with information for your specific presentation.

Poster Presentations
Location: Sonnendeck Gala
Posters should be in place no later than 08:00, Monday, 28 July and removed by 12:00, Thursday, 31 July. Posters remaining after 10:00, Friday 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 workshops 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, 27 July from 12:00-18:00 in Ballsaal Foyer on the ground floor of the Congress Center. The Conference Staff is here to assist you with anything you need. Please do not hesitate to contact someone if you have a question regarding transportation, schedule, activities, attire or any other aspect of the program.

Hotel Direction Information
Most sessions and meals will take place in the Congress Center and Main Building. Some meals will take place in other areas on property, but there will 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 the Ballsaal Foyer room 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 for this time of year are 71 to 75 (°F)/high 20s (°C). Typical low temperatures are in the mid-50s (°F)/low teens (°C). Please bring sunscreen and hats so that you may safely enjoy the beautiful shore.

Hotel Check-Out and Payment
Hotel accommodations from Sunday, 27 July – Thursday, 31 July are included in the 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 along with the credit card you provided for the additional night (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 charges, Internet access fees, bar bills, laundry, 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.

ACTIVITY INFORMATION

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

Activity Voucher
The Conference Chairs will be providing each participant with a credit worth 100 € to apply towards the RXVI activities. If you have pre-registered for an activity(s) prior to the conference, your credit card was charged the total amount minus the 100 € credit. If you haven't pre-registered, please visit the Conference Registration desk in Ballsaal Foyer sign up for an activity(s) of your choice. In addition to the voucher, the Conference Chairs will be providing the beach package, amber rush, and guided seal tour at no cost to the participant.

MONDAY, 28 JULY 2014

All activities depart from the Main Lobby. You will be returned to the resort with time to prepare for dinner.

PAPERBOAT COMPETITION
Price: Complimentary
Time: 13:30 - 17:30

With guidance from a seasoned instructor, six teams of five attendees each will construct boats made out of cardboard, paper, and duct tape starting on Monday, 28 July. Throughout the conference, teams will have the opportunity to craft their vessel. The instructor will offer assistance on the 28th and the 30th. On the 31st, teams will compete in a race. Choose your teams and leaders wisely, as this is bound to be an exciting activity.

BEACH PACKAGE
Price: Complimentary
Time: 13:30 - 17:30

Soak up some rays of sunshine as you relax on the beach by the Baltic Sea. A beach chair, sunscreen, beach towel, and hospitality area will be set up for you so that you can enjoy the natural beauty of Warnemünde Beach.

AMBER RUSH
Price: Complimentary
Time: 13:30 - 15:30

Stroll with an amber expert and learn of the Baltic Sea amber. At the end of the short stroll, you will be divided into teams and given tools to dig for real amber. After you discover your amber, give it to the expert to test if it is really "gold of the sea". Whether it is real or not, it is yours to keep!

GUIDED SEAL TOUR
Price: Complimentary
Times: 13:30 - 14:30
15:00 - 16:00

Explore the scientific life of marine species from the sundeck of the Lichtenberg, a stationed ship located in the Marina of Hohe Düne that investigates marine life. After learning about marine mammals and octopods, you will head over to the Marine Science Center. Here you will explore the fascinating world of seals and the current research program.

ROKKAKU: KITE FIGHT
Price: 70,00 € per person
Time: 13:30 - 17:30

The Rokkaku is traditional Japanese fighting kite. Like the kite, the Rokkaku kite battle originated in Japan. The idea of the kite fight is to force your opponent's kite to the ground. For this activity, you will team up with one other person to control your kite. The team that stays in the sky the longest will be declared the winner. Are you ready for this honorable battle?

KAYAKING FROM THE BEACH
Skill level: Intermediate
Price: 58,00 € per person
Time: 14:00 - 15:00

Enjoy the natural beauty of the Baltic Sea. Your guide will give you a short introduction to kayaking on the Baltic Sea. From there, you will head straight to the water to explore the magnificent sea for a one hour tour. This is sure to be an experience you'll share and recount for years to come.

STAND UP PADDLEBOARDING

Skill level: Intermediate
Price: 32,00 € per person
Time: 14:00 - 16:00

Stand up paddling is the new trend in the world of water sports. Stand up paddle boarding will challenge you to a full body workout. After an introduction to paddle boarding and the Baltic Sea on the beach, you will head into the water to enjoy a relaxing tour of the Baltic Sea.

PEDELEC TOUR

Price: 54,00 € per person
Time: 13:15 - 17:30

The "E-Bikes" or electrical bicycles support every push you make to the pedal with a certain amount of energy. You can choose several levels of support. The tour will begin with a short walk to the ferry and crossing of the Warnow River. After crossing the river, you will begin your bike ride. After the exhilarating ride, you can stroll through Warnemünde before heading back to the hotel. This activity is fun, fast, and safe!

GPS HUNT

Price: 84,00 €
Time: 13:00 - 16:30

Discover Warnemünde in a fun way! Go on a treasure hunt with your team and a GPS. The GPS will give you coordinates that will lead you all around Warnemünde, including the lighthouse and local history museum. At each new location, you will have to solve riddles and do activities. This is an activity that you will never forget!

WEDNESDAY, 30 July 2014

All activities depart from the Main Lobby. You will be returned to the resort with time to prepare for dinner, which begins at 18:30.

PADDLE-CYCLING

Skill Level: Intermediate
Price: 161,00 € per person
Time: 13:00 - 18:30

This activity will start on the peninsula of Fischland-Darβ-Zingst, about a 45 minute ride from the hotel. During the ride to the peninsula, an introduction of the tour will be given. After getting off the bus, the tour will start with a canoe ride in the shallow waters on the south end of the peninsula. After two hours of paddling, you will come ashore. Here you will take a bike ride through the old forest and beaches in a breathtaking national park. Park rangers will educate you on this special area located on the Baltic Sea. The tour will end with a picnic and some free time on the beach before taking the bus back to the hotel.

HISTORICAL CITY TOUR – LÜBECK

Price: 60,00 € per person
Time: 13:00 - 18:30

On this guided tour, you will take a bus to the historic city of Lübeck. The city is a UNESCO world heritage site, and has many landmarks including the Niederegger marzipan factory, the Holstentor, which is the only remaining city gate as an entrance to the old city, and the Thomas Mann house. Thomas Mann is one of Germany's most renowned authors and won a Nobel prize for his book "The Buddenbrooks".

WARNEMUNDE TOUR

Price: 18,00 €
Time: 13:30 - 17:00

Visit the former fishing village on the Baltic Sea coast with one of the most beautiful bathing resorts, Warnemünde. The town sits right on the beach where the Warnow river flows into the Baltic Sea, the reason behind the name Warnemünde. Explore the beach, the boutiques and stores, and some landmarks such as the local history museum, the church square, and the lighthouse. After, you will take a ferry and walk around the seaside resort.

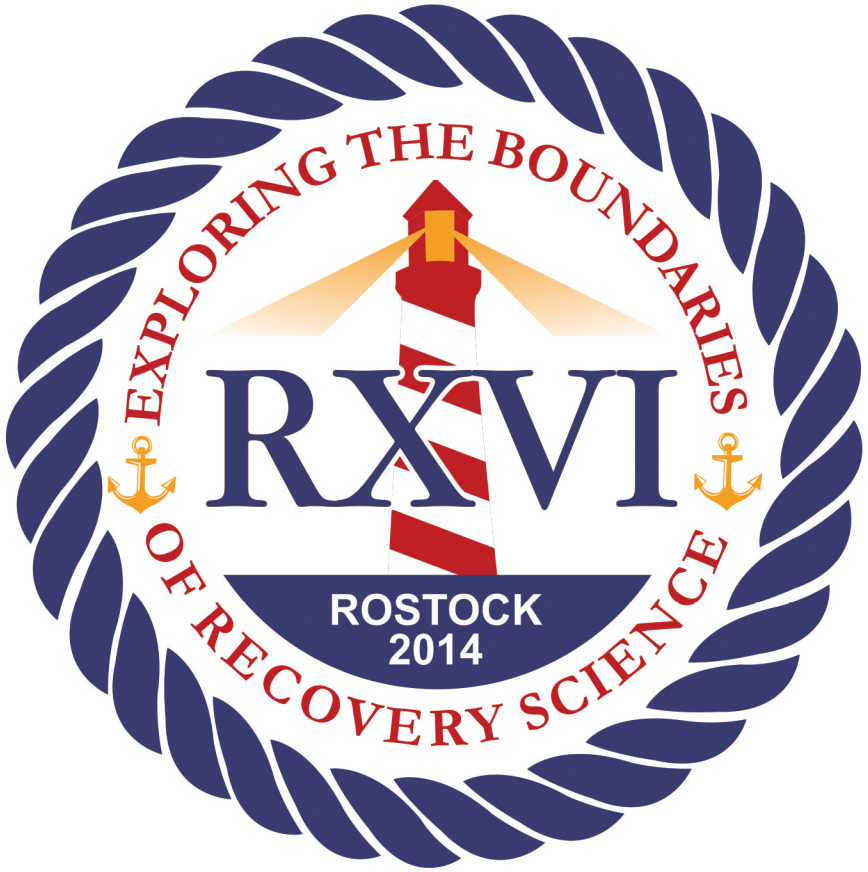
GPS HUNT

Price: 84,00 €

Time: 13:00 - 16:30

Discover Warnemünde in a fun way! Go on a treasure hunt with your team and a GPS. The GPS will give you coordinates that will lead you all around Warnemünde, including the lighthouse and local history museum. At each new location, you will have to solve riddles and do activities. This is an activity that you will never forget!

Schedule


EXPLORING THE BOUNDARIES
OF RECOVERY SCIENCE
RXVI
ROSTOCK
2014

SCHEDULE AT A GLANCE

Sun. 27/2	Mon. 28/7	Tues. 29/7	Wed. 30/7	Thurs. 31/7	Fri. 1/8
The Next Frontier	Charting the Course	Exploring New Modalities and Concepts	Exploring the Boundaries to Drug Delivery	Keeping the Ship Steady	
	7:00 - 8:00 *Breakfast Restaurant Brasserie*	**7:00 - 8:00** *Breakfast Restaurant Brasserie*	**7:00 - 8:00** *Breakfast Restaurant Brasserie*	**7:00 - 8:00** *Breakfast Restaurant Brasserie*	**7:00 - 10:00** *Breakfast Restaurant Brasserie*
	8:00 - 10:00 *Predicting Purification Success* *Ballsaal A&B*	**8:00 - 10:00** *Purifying New Molecular Modalities I* *Ballsaal A&B*	**8:00 - 10:00** *Phase Behavior & Rheology* *Ballsaal A&B*	**8:00 - 10:00** *Next Generation UnitOps* *Ballsaal A&B*	*Conference concludes*
	10:00 - 10:15 *Refreshments* *Ballsaal Foyer*	**10:00 - 10:15** *Refreshments* *Ballsaal Foyer*	**10:00 - 10:15** *Refreshments* *Ballsaal Foyer*	**10:00 - 10:15** *Refreshments* *Ballsaal Foyer*	
	10:15 - 11:15 *Keynote Address* *Harish Iyer, Shantha Biotechnics* *Ballsaal A&B*	**10:15 - 11:15** *Keynote Address Professor Alan Trounson, Monash University* *Ballsaal A&B*	**10:15 - 11:15** *Keynote Address* *Nicholas Peppas, University of Texas* *Ballsaal A&B*	**10:15 - 11:15** *Keynote Address* *E. Morrey Atkinson, PhD, Bristol-Myers Squibb* *Ballsaal A&B*	
12:00 *Registration Opens* *Ballsaal Foyer*	**11:15 - 12:45** *Data Mining and Modeling* *Ballsaal A&B*	**11:15 - 12:45** *Purifying New Molecular Modalities II* *Ballsaal A&B*	**11:15 - 12:45** *Recovery Science 2050 – Visions in Academia* *Ballsaal A&B*	**11:15 - 12:45** *New Stationary Phases* *Ballsaal A&B*	**12:00** *Check Out*
Poster Set-up *Sonnendeck gesamt*	**12:45 - 13:30** *Box Lunch*	**12:45 - 14:00** *Luncheon* *Pavillion Restaurants*	**12:45 - 13:30** *Box Lunch*	**12:45 - 14:00** *Luncheon* *Pavillion Restaurants*	
14:30 *Welcome Hospitality* *Brasserie*	**13:30 - 18:30** *Activities (optional)*	**14:00 - 15:30** *Break*	**13:30 - 18:30** *Activities (optional)*	**14:00 - 15:00** *Paper Boat Race* *Marina*	
15:30 *Opening Remarks* *Ballsaal A&B*		**15:30 - 17:30** *How Pure is Pure Enough?* *Ballsaal A&B*		**15:15 - 16:45** *Scale Up / Scale Down* *Ballsaal A&B*	
15:45 - 17:30 *Future Strategies* *Ballsaal A&B*		**15:30 - 17:30** *Muddling Though the Morass: Making Sense of Data and Models at Different Scales* *Salon Rotunde*		**16:45 - 17:00** *Refreshments* *Ballsaal Foyer*	
		15:30 - 17:30 *Partnership* *Salon 25-26*		**17:00 - 18:30** *Tricky Issues Case Study, Ballsaal A&B*	
17:30 - 19:00 *Cocktails & Entertainment* *Restaurant Brasserie*		**15:30 - 17:30** *Comparability* *Salon 19*			
19:15 - 20:30 *Welcome Dinner* *Restaurant Brasserie*	**18:45 - 19:45** *Dinner* *Bootshalle*	**18:45 - 19:30** *Dinner* *Restaurant Brasserie*	**18:45 - 19:45** *Dinner* *Restaurant Brasserie*	**19:00 - 22:30** *Closing Banquet* *Bootshalle*	
20:45 - 22:00 *Keynote Address* *Geoffrey Ling, DARPA* *Ballsaal A&B*	**20:00 - 22:00** *Poster & Dessert* *Sonnendeck gesamt*	**20:00 - 22:00** *Production Concepts* *Ballsaal A&B*	**20:00 - 22:00** *Poster & Dessert* *Sonnendeck gesamt*		

Sunday, 27 July 2014
The Next Frontier

12:00
Registration Opens
Ballsaal Foyer

Poster Set-up
Sonnendeck gesamt

14:30
Welcome Hospitality
Brasserie

15:30
Opening Remarks
Ballsaal A&B

15:45 - 17:30
Future Strategies
Ballsaal A&B

17:30 - 19:00
Cocktails & Entertainment
Restaurant Brasserie

19:15 - 20:30
Welcome Dinner
Restaurant Brasserie

20:45 - 22:00
Keynote Address
Geoffrey Ling, DARPA
Ballsaal A&B

Monday, 28 July 2014

Charting the Course

7:00 - 8:00
Breakfast
Restaurant Brasserie

8:00 - 10:00
Predicting Purification Success
Ballsaal A&B

10:00 - 10:15
Refreshments
Ballsaal Foyer

10:15 - 11:15
Keynote Address
Harish Iyer, Shantha Biotechnics
Ballsaal A&B

11:15 - 12:45
Data Mining and Modeling
Ballsaal A&B

12:45 - 13:30
Box Lunch

13:30 - 18:30
Activities (optional)

18:45 - 19:45
Dinner
Bootshalle

20:00 - 22:00
Poster & Dessert
Sonnendeck

Tuesday, 29 July 2014

Exploring New Modalities and Concepts

7:00 - 8:00
Breakfast
Restaurant Brasserie

8:00 - 10:00
Purifying New Molecular Modalities I
Ballsaal A&B

10:00 - 10:15
Refreshments
Ballsaal Foyer

10:15 - 11:15
Keynote Address - Professor Alan Trounson, Monash University
Ballsaal A&B

11:15 - 12:45
Purifying New Molecular Modalities II
Ballsaal A&B

12:45 - 14:00
Luncheon
Pavillion Restaurants

14:00 - 15:30
Break

15:30 - 17:30
How Pure is Pure Enough?
Ballsaal A&B

15:30 - 17:30
Muddling Though the Morass: Making Sense of Data and Models at Different Scales
Salon Rotunde

15:30 - 17:30
Partnership
Salon 25-26

15:30 - 17:30
Comparability
Salon 19

18:45 - 19:30
Dinner
Restaurant Brasserie

20:00 - 22:00
Production Concepts
Ballsaal A&B

Wednesday, 30 July 2014

Exploring the Boundaries to Drug Delivery

7:00 - 8:00
Breakfast
Restaurant Brasserie

8:00 - 10:00
Phase Behavior & Rheology
Ballsaal A&B

10:00 - 10:15
Refreshments
Ballsaal Foyer

10:15 - 11:15
Keynote Address
Nicholas Peppas, University of Texas
Ballsaal A&B

11:15 - 12:45
Recovery Science 2050 – Visions in Academia
Ballsaal A&B

12:45 - 13:30
Box Lunch

13:30 - 18:30
Activities (optional)

18:45 - 19:45
Dinner
Restaurant Brasserie

20:00 - 22:00
Poster & Dessert
Sonnendeck

Thursday, 31 July 2014

Keeping the Ship Steady

7:00 - 8:00
Breakfast
Restaurant Brasserie

8:00 - 10:00
Next Generation UnitOps
Ballsaal A&B

10:00 - 10:15
Refreshments
Ballsaal Foyer

10:15 - 11:15
Keynote Address
E. Morrey Atkinson, PhD, Bristol-Myers Squibb
Ballsaal A&B

11:15 - 12:45
New Stationary Phases
Ballsaal A&B

12:45 - 14:00
Luncheon
Pavillion Restaurants

14:00 - 15:00
Paper Boat Race
Marina

15:15 - 16:45
Scale Up / Scale Down
Ballsaal A&B

16:45 - 17:00
Refreshments
Location

17:00 - 18:30
Tricky Issues Case Study
Ballsaal A&B

19:00 - 22:30
Closing Banquet
Bootshalle

Friday, 1 August 2014

7:00 - 10:00
Breakfast
Restaurant Brasserie

Keynote Speakers

KEYNOTES

Dr. Geoffrey Ling
Director of the Biological Technologies Office, Defense Advanced Research Projects Agency
Sunday, 27 July, 20:45 – 22:00
Ballsaal A&B

Harish Iyer, Ph.D.
Managing Director and CEO, Shantha Biotechnics
Monday, 28 July, 10:15 – 11:15
Ballsaal A&B

Alan Trounson, Ph.D.
Founding Director, Monash Immunology and Stem Cell Laboratories, Monash University
Tuesday, 29 July, 10:15 – 11:15
Ballsaal A&B

Nicholas Peppas, Ph.D.
Department Chair and Professor, Biomedical Engineering, University of Texas at Austin
Wednesday, 30 July, 10:15 – 11:15
Ballsaal A&B

E. Morrey Atkinson, Ph.D.
Vice President, Process Sciences, Bristol-Myers Squibb Company
Thursday, 31 July, 10:15 – 11:15
Ballsaal A&B

Dr. Geoffrey Ling

DARPA

Biography

Dr. Geoffrey Ling is the Director of the Biological Technologies Office at the Defense Advanced Research Projects Agency (DARPA) and attending Neuro Critical Care physician at Johns Hopkins Hospital. He retired from the US Army in 2012 after serving as a military intensive care physician with multiple deployments to Iraq and Afghanistan. He formerly served in the Science Division at the White House Office of Science and Technology Policy. He received his Ph.D. in pharmacology from Cornell University, and M.D. from Georgetown University. Dr. Ling is board certified in both Neurology and Neuro Critical Care.

Dr. Geoffrey Ling

DARPA

DARPA's Biologically-derived Medicines on Demand: a new paradigm for manufacturing protein-based therapeutics

The current paradigm for manufacturing protein therapeutics to respond to medical emergencies often relies on mass production of a single biologic drug, a process that is time consuming (weeks or months) and requires complex logistics, including dependence on a 'cold chain' to accommodate the short shelf life. This model has severe consequences on the delivery of therapies to patients in challenging or remote environments and is non responsive to emerging threats. The Defense Advanced Research Projects Agency (DARPA) is now challenging this traditional scheme with a novel approach to generating biologics. The Biologically-derived Medicines on Demand (Bio-MOD) program seeks to develop a flexible and portable production platform that will allow for the selective synthesis, purification, and formulation of single dosage levels of multiple biologic drugs on site, in a timeframe of 24 hours. Research teams funded by DARPA are developing novel, flexible methodologies in genetic engineering to modify cellular and cell-free systems into highly efficient and selective protein production machineries. Novel purification methods are being developed to be integrated into modular platforms that incorporate novel on-line process analytical technologies for end-to-end manufacturing and real time release of therapeutic proteins.

Harish Iyer, Ph.D.

Shantha Biotechnics Ltd.

Biography

Harish Iyer is Managing Director and CEO of Shantha Biotechnics Ltd. since June 2011. He is responsible for operations and long term strategy at Shantha. Shantha's mission is the development of affordable, high quality injectibles for the developing world. Our pipeline is focused primarily on vaccines, and includes pediatric combinations, viral and other recombinant sub-unit vaccines. Harish has extensive experience in the biotechnology industry and was previously Vice-President and Head of Research & Development, Biocon (2001-2011). With a team of 300+ scientists, Harish worked on numerous projects at Biocon including biosimilars, novel discovery projects and other first-in-class biologics in the Biocon R&D pipeline. Prior to joining Biocon, Harish worked in Process Sciences at Genentech, Inc. (1995-1998), South San Francisco and Biogen IDEC, San Diego (1998-2001). He graduated in 1995 with a Ph.D. in Chemical Engineering from the Rensselaer Polytechnic Institute, Troy, New York, and in 1990, with a B. Tech. in Chemical Engineering from the Indian Institute of Technology, Madras. In 2010, Harish also participated in and completed an executive education course "Leadership & Strategy in Pharmaceuticals & Biotechnology" from Harvard Business School in Cambridge, Massachusetts.

Harish Iyer, Ph.D.

Shantha Biotechnics Ltd.

Abstract

Recent studies have shown that while global burden of disease is shifting from communicable to non-communicable disease, significant numbers of people in the world still do not have access to high quality, affordable medicine. Among the most vulnerable populations are babies, who still continue to die under the age of 5 in large numbers in some parts of the world (6.3 million babies died in 2013 under the age of 5). These large numbers are impacted by many factors, among which are requirements to provide high quality medicines and vaccines in sufficient quantities at affordable prices; additionally, these products have to be relevant to the landscapes they serve. Process and product design are critical aspects of ensuring that we increase access to these interventions to reduce these mortality numbers. In this talk, I speak on various theoretical and practical aspects of process and product design that can increase delivery of medicines and vaccines to vulnerable populations.

Alan Trounson, Ph.D.

Monash University

Biography

Alan was President of Californian Institute for Regenerative Medicine (2007-2014) the Californian state's $3 billion stem cell agency driving research in stem cell biology and facilitating the translation of stem cell discoveries into clinical therapies. He was the founding Director of the Monash Immunology and Stem Cell Laboratories at Monash University (2004-07). Alan founded seven for-profit life science companies and the National Biotechnology Centre of Excellence - 'Australian Stem Cell Centre' (2002-03). He held a Chair in Paediatrics/Obstetrics and Gynaecology, and also a Chair in Stem Cell Science at Monash University. He was Director of the Monash Centre for Early Human Development 1985-2002 and founding Deputy Director/Director of the Institute for Reproductive Biology 1990- 2002. Alan was a pioneer of human in vitro fertilisation (IVF), introducing fertility drugs for controlling ovulation, embryo freezing techniques, egg and embryo donation methods, early sperm microinjection methods, initiated embryo biopsy, developing in vitro oocyte maturation methods and the vitrification of eggs and embryos. He led the Australian team for the discovery of human embryonic stem cells in the late 1990's. Alan founded, with colleagues, the not-for-profit foundations Low Cost IVF and Friends of Low Cost IVF to enable wider access to ART and fertility education for all people across the globe.

Alan Trounson, Ph.D.

Monash University

Stem Cells and Regenerative Medicine: A Growing New Product Pipeline with Opportunities and Challenges

The field of regenerative medicine has advanced from the first generation of cell therapies involving bone marrow cells (including hematopoietic stem cells – HSCs) transplants to include many other cell types (e.g. mesenchymal stem cells, adipose cells, umbilical cord blood cells) to a second generation of genetically modified HSCs, and progenitor neural stem cells and pluripotent embryonic stem cells (ESCc). Transcription factors may be used for transducing adult human blood or skin cells to interrogate complex human diseases and this also provides for the development of patient specific immune compatible induced pluripotent stem cells (iPSCs) for cell therapy with the expansion derivative properties similar to ESCs, are enabling a whole new approach to regenerative medicine. These IPSCs are a powerful tool to study human disease. Further evolution has taken the field to directly convert support cells of tissues s to cell types of regenerative interest, using specific transcription factors, potentially avoiding the need for cell transplantation.

The Californian Institute for Regenerative Medicine (CIRM) has more than 90 research projects in translation to clinical trials and expects this to increase. The studies range from those in phase I/II clinical trials with FDA approval to those in early translation that are identifying a candidate product using assays in vitro and animal models in vivo. The studies in clinical trial include; a shRNA approach to interfere with the HIV co-receptor on HSCs and T cells for prevention of HIV/AIDS, cardiac derived allogeneic cell therapy for repair of heart muscle and correction of β Thalassemia by genetic engineering HSCs. Others expected to be in clinical trials within the next 12 months include; ESC derived pigmented retinal epithelial cells for macular degeneration and retinitis pigmentosa, ESC derived pancreatic β-Islet cells in a subcutaneous capsule for correction of juvenile diabetes, and several approaches to wipe out cancer stem cells with specific monoclonal antibodies, cytotoxic drugs and chimeric antigen technology. The research teams consist of a blend of academic and company personnel and are supported by CIRM management and an experienced CIRM external advisory panel. These teams are showing a high conversion from preclinical potential to clinical trial readiness.

Nicholas Peppas, Ph.D.

University of Texas

Biography

Nicholas A. Peppas is the Fletcher Stuckey Pratt Chair in Engineering; Professor of Chemical Engineering, Biomedical Engineering and Pharmacy; Chairman of the Department of Biomedical Engineering, and Director of the Center for Biomaterials, Drug Delivery and Bionanotechnology of the University of Texas at Austin. He is known for his pioneering research in biomaterials, polymer physics, drug delivery, bionanotechnology and medical devices. He is the inventor of numerous medical products including contact and intraocular lenses, artificial kidney membranes, cartilage, devices for oral delivery of insulin for treatment of diabetics, calcitonin for osteoporosis and interferon beta for multiple sclerosis, and recognitive delivery systems. He is the 2012 Founders Award recipient of the National Academy of Engineering (NAE). Peppas is an elected member of the National Academy of Engineering (NAE), the Institute of Medicine of the National Academy, the National Academy of France, the Royal Academy of Spain, the Academy of Athens (Greece) and the Texas Academy of Medicine, Engineering and Sciences. In 2008, AIChE named him on of the One Hundred Chemical Engineers of the Modern Era. He is President (2008-16) of the International Union of Societies of Biomaterials Science and Engineering (IUSBSE) and Chair (2014-15) of the Engineering Section of the American Association for the Advancement of Science (AAAS). Nicholas Peppas is a Fellow of the American Chemical Society (ACS), American Physical Society (APS), Materials Research Society (MRS), AAAS, AIChE, BMES, AIMBE, SFB, CRS, American Association of Pharmaceutical Scientists (AAPS), and the American Society for Engineering Education (ASEE). Peppas holds a Dipl. Eng., National Technical University of Athens (1971), a Sc.D. from MIT (1973), honorary doctorates from the Universities of Ghent (Belgium), University of Parma (Italy), University of Ljubljana (Slovenia) and University of Athens (Greece), and an honorary professorship from Sichuan University, China.

Nicholas Peppas, Ph.D.

University of Texas

Abstract

Recent developments in delivery of drugs, proteins and active agents have been directed towards the preparation of targeted formulations and products for delivery to specific sites, use of environmentally-responsive polymers to achieve pH- or temperature-triggered delivery, usually in modulated mode, and improvement of the behavior of their responsive behavior and cell recognition. We can now engineer the molecular design of intelligent biopolymers by controlling their recognition and specificity as the first step in coordinating and duplicating complex biological and physiological processes. We address design and synthesis characteristics of novel biopolymers capable of protein release as well as artificial molecular structures capable of specific molecular recognition of biological molecules. We address molecular imprinting and microimprinting techniques, as methods to create stereo-specific three-dimensional binding cavities based on a biological compound of interest and to preparation of biomimetic materials for intelligent drug delivery, drug targeting, and tissue engineering.

E. Morrey Atkinson, Ph.D.

Bristol-Myers Squibb Company

Biography

E. Morrey Atkinson, PhD, is currently the Vice President of Biologics Development at Bristol-Myers Squibb Company. Morrey is responsible for leading a team of scientists and engineers across five sites that is responsible for process development, analytical sciences, and manufacturing support for the company's biologics portfolio, directing the development of innovative manufacturing platforms and process improvements for clinical and commercial projects. Morrey holds a B.S. from Indiana University and a Ph.D. from Stanford University. Prior to BMS, Morrey's career has included various management roles at Eli Lilly and Company, Cook Pharmica and Targeted Genetics Corporation.

E. Morrey Atkinson, Ph.D.

Bristol-Myers Squibb Company

Abstract

In large-scale manufacturing of biologics, small perturbations can have profound effects. Our understanding of protein behavior is still quite limited, so when a change is detected, our ability to diagnose the problem and come up with a solution is critical to maintain supply of the drug. The talk will present case studies from an automated large-scale mammalian facility that show the interdependencies of downstream processing, biophysical attributes, and highly-regulated operational activities.

Oral Abstracts

ORAL SESSION ABSTRACTS

Future Strategies

Session Chairs:
Günther Jagschies, GE Healthcare, Sweden
Dorothee Ambrosius, Boehringer Ingelhim GmbH, Germany

Downstream Inventions for the Big Picture

Jonathan Coffman (Boehringer Ingelheim GmbH)
Nuno Fontes (Boehringer Ingelheim GmbH)
Jens Vogel (Boehringer Ingelheim GmbH)

The establishment of a mAb platform that is capable of delivering drug substance for less than $100/g has sparked a debate about the value of new technologies in purification of biopharmaceuticals. Except in a minority of cases,* commercial COGs for mAbs is not the most important factor in the industry. Even so, there is room for significant inventions downstream, especially if downstream scientists look at the broader picture. High drug costs are largely due to many failures in the clinic, which are, in turn, due to poor disease understanding. The R&D spending for each approved drug has been estimated to be between $3B and $11B and take between 10-15 years.** Can innovation in process development decrease the rate of failures in the clinic? Since process development is the gate between discovery and the patient, enabling a fast and smooth transition from discovery to the clinic may impact disease understanding, and therefore, the cost of a new drug. Key innovation areas for this transition include: In silico prediction of stability, concentration, and viscosity that is quantitatively predictive of actual formulated product in multiple formulations, In silico and HTS purification development of non-antibody products, High productivity methods for discovery team supply, and reg tox production that represent at-scale clinical manufacturing. The impact of such inventions will be discussed. Some examples will be provided.

* Two important exceptions: 1) the work that supports the hopes of the Bill and Melinda Gates Foundation, where significant innovation is required to achieve less than $10/g for perhaps as many as 10 million to 100 million patients. 2) Biosimilars, especially for emerging markets.
** Matthew Herper, Forbes, 2012 and 2013

Vaccine globalization: lessons learned from the bioprocess of development and biomanufacturing

Hari Pujar (Merck & Co.)

Vaccines are one of the oldest healthcare interventions of the biological kind, and have had a significant impact on global human health. Adoption of pediatric vaccines has reached fairly high levels in the upper income countries, but has been historically low in lower income countries, and to some extent in middle income countries. This picture has improved significantly over the last decade with the advent of new funding mechanisms, emergence of developing country vaccine manufacturing, as well as the pursuit of a global manufacturing strategy by the vaccine majors. Current global vaccine demand is dominated by the developing world due to its large birth cohort and disease burden. Significant manufacturing is now being conducted in the developing world, with one developing country vaccine manufacturer being responsible for more than half of global infant immunization. Despite these successes many challenges still remain for more widespread adoption of vaccines. In the biomanufacturing realm, the need to have simple, transferrable, and robust manufacturing processes is ever more important, particularly for the more complex vaccines (e.g. enveloped viruses, virus-like particles, multi-valent) and as the call for local manufacturing gets increasingly louder. In addition, vaccines still need to be low cost, thermo-stable, and amenable to multi-dose presentations. These present not only a rich menu of challenges and opportunities to the biological engineer/scientist, but could also hold lessons for other biological products.

Transforming Pharmaceutical Manufacturing Through Continuous Manufacturing

Charles Cooney (Massachusetts Institute of Technology)

As products, markets and needs in health care change, there is an opportunity to also change manufacturing strategy. Many industries have adapted to changing market demands by embracing continuous processing to provide efficiency, flexibility and competitive advantage. We examine here the evolution of an end-to-end continuous process for pharmaceuticals and translate the lessons learned to continuous manufacturing for biological products.

Predicting Purification Success

Session Chairs:
Xiangyang Wang, MedImmune, LLC, United States
John Erickson, GlaxoSmithKline, United States

A new rFVIII molecule: Combining molecular design and downstream processing to increase homogeneity

Haleh Ahmadian (Novo Nordisk A/S)
Johan Faber (Novo Nordisk A/S)
Ernst Hansen (Novo Nordisk A/S)
Johan Karlsson (Novo Nordisk A/S)
Lars Sejergaard (Novo Nordisk A/S)
Lars Thim (Novo Nordisk A/S)

A new rFVIII, turoctocog alfa, has been developed by Novo Nordisk A/S and has currently obtained marketing authorisation in US, EU, Japan and Australia. Here we describe development of downstream processing for turoctocog alfa together with a brief description of the rationale behind the molecular design of this new molecule. In turoctocog alfa, the 908 amino acid (aa) residue B-domain of full-length FVIII has been truncated, where the gene encodes for 21 aa of the original B-domain. During the design phase, the decision on the molecular structure of this future rFVIII was, on one hand, based on the developability and manufacturability concepts and on the other hand on maximising product homogeneity. Truncation of B-domain is shown to contribute to homogeneity as demonstrated by HPLC analysis of several rFVIII products with and without B-domain. A five step purification process is applied for turoctocog alfa starting by capture on multimodal resin followed by immunoaffinity, anion exchange (AIEX), virus filtration and size exclusion chromatography (SEC). Previously, we presented the specific site on turoctocog alfa interacting with the purification antibody, which has particular importance for removal of impurities structurally related to turoctocog alfa and thereby for final purity and homogeneity of the product. In this presentation we will discuss the role of the remaining steps to achieve the desired product quality. The final drug substance (DS) concentration is a critical quality attribute with regard to formulation of the product. The task of ensuring a sufficient DS concentration is challenged by the fact that in the final two steps, virus filtration and SEC, the protein solution is diluted and not concentrated. Dilution in the virus filter is primarily due to wash of the filter to promote displacement of the protein. In the SEC step, dilution occurs by dispersion as the loaded peak moves down the column. In order to increase the robustness of the process with regard to the final concentration, two "handles" were incorporated: For the AIEX

step, we derived a simple algorithm where the volume of the eluting gradient measured in CV was related to the load in g/L. This ensures a constant pool concentration when the amount loaded varies. For the SEC step the procedure for loading the column was changed from volume based (fixed CV) to mass based (load in g/L), so that a higher volume was loaded for dilute feed stocks. This reduced the variation of the pool concentration significantly. In both cases simulation using mechanistic models was applied in order to generate sufficient data to test different approaches. Data for final choice of SEC resin for manufacturing phase combined with results from mathematical modeling will be presented.

Ensuring Viral Clearance Success for Non-platform Processes

Lisa Connell-Crowley (Amgen Inc.)

Most companies develop monoclonal antibody processes using mature purification platforms, which include well-established steps to clear adventitious viruses. The emergence of more diverse products such as bispecific antibodies, Fab-based entities and non-mAb based proteins has required the use of non-platform steps to provide impurity removal and product stability. Given the desire to move products rapidly to the clinic, it can be challenging to ensure that such non-platform processes can meet viral clearance targets. This presentation will discuss a toolbox approach to viral clearance in which the mechanism and key parameters for virus inactivation or removal have been determined for a variety of steps, allowing for prediction of viral clearance during development, independent of product modality. For chromatography steps such as protein A, IEX, HIC and mixed mode, model virus behavior is studied using column or 96-well high throughput screening experiments. For low pH viral inactivation, key parameters and their impact on virus inactivation is assessed using a DOE approach. By understanding the behavior of model viruses on different steps, a process can be quickly developed to include both the desired product quality and viral clearance.

Computational Modeling: Insights into Molecular Biophysics to Advance and Improve Biologics Purification

David Roush (Merck & Co.)
Francis Insaidoo (Merck & Co.)
Thomas Linden (Merck & Co.)
Allison Ortigosa (Merck & Co.)
Michael Rauscher (Merck & Co.)

The increasing complexity and diversity of biologic agents challenge the current purification methodologies. To harness the full potential of biologics, there is a need to fully explore a broader range of purification techniques including non-chromatographic approaches. To achieve this goal, our current research focus is on understanding the biophysical properties of each specific biologic molecule *in silico*. Through exploiting advances in computational modeling we have developed a strategy to effectively study specific interactions and mechanisms that are important for biologics purification (e.g. interaction energies and conformational changes associated with binding) at an atomistic level of detail. This strategy includes the identification of ligands that specifically isolate biologics via a range of interactions (electrostatic, hydrophobic or mixed mode) and builds upon molecular design approaches from small molecule drug discovery. Insulin variants were used as a model system to screen ligands *in silico* that could improve the crystallization. Specific regions or motifs of insulins have been identified that could be used as targets for purification. The results of this computational modeling technique are supported by previously published empirical data and confirmatory experiments. Extending this mechanistic strategy to other biologics allows one to more efficiently develop a purification process with increased manufacturing productivity. Principles from this study can be readily applied to other areas, e.g. formulation where the issue of aggregation and concentration dependent oligomerization could be mechanistically modeled.

Implementation of QbD in Process Development of a Diverse Portfolio of Products

Joanne Beck (Shire)

One of the many components of the decision to advance a candidate molecule from the discovery to the development stage is the ability to manufacture product that meets the quality target product profile (QTPP). Process and analytical development and manufacturing should not be on the critical path of product development; therefore, a close collaboration between Discovery, Non-clinical and Process Development teams is required for the timely delivery of product that meets the QTPP. This paper will present examples of how we applied principles of QbD to early development of several drug candidates while meeting seemingly conflicting global development team goals for time and cost efficiency.

Data Mining and Modeling

Session Chairs:
John Pieracci, Biogen Idec, United States
Bernt Nilsson, Lund University, Sweden

Data Mining Full Scale Production Data for Continuing Process Validation

Victor Goetz (ImClone Systems)

Effective continuing process validation involves collecting commercial scale performance data that can be overlaid on process development data and models. In some respects this requires aligning two seemingly conflicting objectives – providing operators with clear execution instructions based on instrument displays and adjustments they can readily act upon versus the dreaded "experimentation at full scale on commercial batches." The key to effectively bridging this divide is to develop data systems that transparently collect the appropriate corollary data to be matched against a development database without distracting from routine batch record-driven operations. Data historians, LIMS systems and batch-record "scrubbing" software, with the help of data aggregators such as Discoverant, can all be fed into purpose-built database monitors running on platforms such as Spotfire to minimize the amount of manual data manipulation required to support continuing process validation protocols and effective ongoing process monitoring. Challenges such as keeping batch record limits in the realm of units displayed on instrumentation while normalizing monitoring data for ease of cross-scale comparison will be discussed along with the design principles behind various monitoring systems.

Managing large amounts of data coming from continuous biomanufacturing processes

Marc Bisschops (Tarpon Biosystems)
Jonathan Coffman (Boehringer Ingelheim GmbH)
Marcus Strawn (Boehringer Ingelheim GmbH)
Brian To (Boehringer Ingelheim GmbH)

The interest in continuous processing in the biopharmaceutical industries is growing rapidly. Particularly in the field of downstream processing, some breakthrough research and development has recently been presented at various conferences. One of the advantages of continuous bioprocessing is the amount of data that is being generated. In a batch manufacturing process, a protein A chromatography column may be used 2 or 3 cycles and in exceptional cases up to 5 or 6 cycles per batch. In continuous chromatography processes, the number of elution peaks in the protein A chromatography process can be as high as 50 or 60 per batch or 100 per day. This provides a lot of information to evaluate whether or not the process is operating within a stable situation or a steady-state. The downside of this is that there is also much more information and data to be interpreted and reviewed. Currently, assessing steady-state operation during development work mainly relies on visual comparison of the peaks. In the control software of the BioSMB technology, this is done by plotting the sensor readouts of subsequent cycles as an overlay, thereby allowing a direct visual comparison between subsequent cycles. This is a very valuable feature since it at least gives a qualitative impression on steady-state behavior. In this presentation, we will evaluate statistical tools to reduce the dataset into meaningful quantitative parameters. We will show results obtained by straightforward momentum analysis on the elution peaks as well as principal component analysis (PCA). The methodologies will be evaluated using datasets obtained during development work with continuous protein A chromatography and with continuous cation exchange chromatography of a monoclonal antibody, purified from CHO cell supernatant. The strategies will also be evaluated for implementation in real-time in order to be valuable for process control strategies during continuous biomanufacturing.

Whole-process evaluation of operating windows and critical process parameters

Ajoy Velayudhan (University College London)
Nicholas Field (University College London)
Spyridon Konstantinidis (University College London)

Critical quality attributes (CQAs) drive the selection of critical process parameters (CPPs) for each unit operation in a bioprocess. This talk outlines a method to identify predictively the CPPs and associated acceptance criteria (AC) for both individual steps and for the whole process. First, tractable and identifiable mathematical models are constructed for each step. Multi-objective optimisation is used to generate a robust operating window. Next, global sensitivity methods are used to identify the CPPs as the subset of the operating parameters that have the greatest impact on the CQAs. This analysis also provides the associated ACs, because the model quantitatively maps changes in CQAs to changes in CPPs. These ACs are *multivariate*, because interactions among CPPs are captured in the model. The critical individual-step models are then combined into a whole-bioprocess model (WBM). The same approach is taken as was described previously for individual steps: multi-objective optimisation identifies *global*, or process-wide, operating windows for each step, from which global CPPs and ACs are identified. These global results also quantify the extent of interaction among unit operations, and can be used to select steps that constitute a globally robust process. The approach is demonstrated through two case studies. In the first, high-throughput experimental data on a protein feed mixture are used to estimate the parameters in a WBM. The model then predicts the optimal robust operating windows for a process consisting of cell culture and two polishing columns, which can be compared to those obtained experimentally. Since no column data is used to estimate the parameters, this comparison of theoretical and experimental column behaviour involves no fitting, but is entirely predictive. Global worst-case evaluation is used to facilitate process robustness. A second case study examines the combination of two polishing columns run continuously in a simulated moving-bed format. Robust operating ranges for continuous operation show interesting differences from those for batch operation.

Purifying New Molecular Modalities I

Session Chairs:
Britt Sjøholm Novo Nordisk, Denmark
Todd Przybycien, Carnegie Mellon University, United States

The quest for cell based process for flu vaccine

Piotr Wnukowski (Crucell)
Ravinder Bhatia (Janssen R & D, LLC)
Patrick de Jong (Crucell)
Marcel de Vocht (Crucell)

More than 60 years after successful introduction, chicken embryo remains the dominant platform for production of the influenza vaccines. Inherent weaknesses of this technology are potential allergenic reactions associated with the use of animal derived material and limited production capacity, not sufficient to cope with rapid demand for antigen in case of the pandemic situation. These concerns have been main drivers for the industry to develop alternative routes for production of vaccine formulations with egg platform being replaced by cell cultures of mammalian origin. However, the transition from egg to cell based technologies have encountered formidable challenges, one of them being contamination of the Inactivated Virus Bulk with the host cell impurities. In this presentation, the case is presented where influenza virus is propagated in suspension cell culture of the PER.C6® cell line. The origin of the host cell impurities and their interaction with the product (virions) is discussed. It is explained how disintegration of the cell membrane of infected cells is responsible for the formation of the debris fragments in the size range of the virus particles. An important factor contributing to the disintegration of the cells has been identified to be lysis of the cells induced by mechanical shear exerted on the culture. Different clarification technologies are compared with selection criteria being: step recoveries, scalability under GMP regime and most importantly purity of the intermediate bulk clarified virus as well as virus fraction post-purification. Mechanism of the interaction between cell debris and virions is postulated, capable of explaining phenomenon of aggregation that is observed in the purification step. It is demonstrated that in order to meet quality specifications of the final product it is imperative to optimize the production process as a suite of interdependent steps rather than assembly of separate unit operations.

Building scalable and clinical-grade downstream strategies for human mesenchymal and stem cells

Margarida Serra (IBET/ITQB)
Paula Alves (IBET/ITQB)
Manuel Carrondo (IBET/ITQB)
Barbara Cunha (IBET/ITQB)
Cristina Peixoto (IBET/ITQB)
Marta Silva (IBET/ITQB)

Recent forecasts point towards a rapid growth of the cell therapy industry in the upcoming years, mainly due the promise of the stem cell use in therapies. Within this context, human mesenchymal stem cells (hMSCs) have gained special attention due to their immunomodulatory characteristics, as well as in secreting bioactive molecules with anti-inflammatory and regenerative features, which makes them attractive candidates for autologous and allogeneic therapies. Nonetheless, in order to face the high demand for this product (from 105 to 109 cells per patient) to be used in a clinical setting, the establishment of robust manufacturing platforms that can ensure the efficient production, purification and formulation of stem cell-based products is still a challenge. At this moment, extensive efforts are being focused on the development of scalable and robust new upstream technologies for the expansion of these cells, such as in microcarrier-based stirred culture systems, which will allow to increase the cell yields to a standard that can support their use in therapies. While these technologies mature to meet this need, the biomanufacturing bottlenecks are now shifting towards the downstream process (DSP). The industry is in a privileged position where it can learn from previous experience from protein and virus manufacturing, tackling the upcoming bottlenecks from the very beginning. Currently, the upstream is delivering high harvest volumes (tens to hundreds of litres) that need to be purified (ensuring efficient cell-bead separation), concentrated and washed, without compromising the product's quality attributes. This work shows our first insights to tackle such issues, where we aim to design clinical-grade, and integrated DSP methodologies (based on membrane technology) in a scalable and robust manner to support clinic therapies. Different filters, composed of different materials and mesh pore sizes, were evaluated and compared for their ability to remove the microcarrier support. Using tangential flow filtration, cells were concentrated and different process parameters (as initial cell concentration and cross-flow velocity) were optimized, in order to achieve maximum recovery. Our results show that we were able to ensure efficient removal of the major impurities of the cellular suspension (microcarriers), and the hMSCs could be successfully concentrated up to concentration factor of 10 with low product loss. At the end of the process, cells presented high viability (over 90%), remained metabolic active, maintained their immunophenotype, proliferation capacity and multilineage differentiation potential. Although further improvements are still required, this work contributes to the establishment of robust and clinical-grade bioprocesses for the purification of hMSCs to be integrated and applied on the biomanufacturing of cell-based therapies.

Multimodal Chromatography for Purification of Nucleic Acids

Leif Bulow (Lund University)

Recent advances in DNA-based vaccination and gene therapy have led to increasing demands for developing and characterizing robust separation materials for purification of DNA molecules. Such chromatographic resins should permit purification of nucleic acids in high amounts, often ranging up to milligrams of product. This challenge of purifying DNA at large-scale needs to be combined with careful control of contaminants, for which specifications are mainly guided by regulatory agencies, such as FDA or EMEA. Besides clinically oriented applications of DNA, there are several other areas in molecular biology where nucleic acids need to be purified effectively. This includes screening of DNA libraries, aptamers and purification of nucleic acids for labelling or PCR. In addition, also for other biological production systems, such as for DSP of mAbs, it is essential to follow the chromatographic behavior of a range of different nucleic acids. The chemical properties of DNA with a negatively charged backbone combined with a hydrophobic interior caused by the purine-pyrimidine base pairs, the large size of the molecules and the different conformations gives the molecule a complex behavior. The molecular recognition between nucleic acids and surrounding molecules is therefore multifaceted and may involve several different modalities which need to be taken into consideration when designing a purification process. Over the years, anion-exchange chromatography (AEX) and hydrophobic interaction chromatography (HIC) in particular have proved most versatile for DNA purification. In this study, we have examined multimodal chromatography (MMC) as an alternative tool for separations of nucleic acids. MMC embraces more than one kind of interaction between the chromatographic ligand and the target molecules, and it has previously been successfully applied for protein and antibody purifications. Some of these materials have originally been developed for removal of nucleic acids in downstream processing of monoclonal antibodies. The use of MMC allows the resin to combine hydrogen bonding, ionic and hydrophobic interactions and this opens up for possibilities to achieve a unique selectivity for nucleic acids compared to traditional anion exchangers. Single-stranded and double-stranded DNA molecules show differences in ionic and hydrophobic characteristics. In this study we have examined particularly the differences in chromatographic behavior between nucleotides, small single- and double-stranded DNAs, RNA and plasmid DNA. Due to the more hydrophobic nature of single-stranded DNA molecules they could be separated from double-stranded DNAs. Such differences of interaction between the ligand and different nucleic acids, can be explored for developing alternative and rapid purification strategies for many methods used in molecular biology, e.g. the purification of PCR products. The ability of MMC to separate different nucleic acids has thus allowed us to isolate amplified PCR products in a single chromatographic step.

Laboratory Scale Platforms to Accelerate the Development of Particle Conditioning Steps for Prokaryotically Expressed Vaccine Products

Alex Berrill (Pfizer Inc.)
John Cundy (Pfizer Inc.)
Sydney Hoeltzli (Pfizer Inc.)
Benjamin Huffman (Pfizer Inc.)
Tarit Mukhopadhyay (University College London)
Aaron Noyes (Pfizer Inc.)
Nick Russell (Pfizer Inc.)
Khurram Sunasara (Pfizer Inc.)

Particle conditioning steps such as precipitation and flocculation are routinely used to aid the recovery of microbial vaccine products during biopharmaceutical processes. The efficient development of unit operations involved with particle conditioning, flocculation and precipitation, have been constrained by lab-scale models that require large volumes and considerable time to evaluate. A modular approach to rapidly developing purification processes at the micro-scale would greatly enhance productivity, robustness and speed the development of such processes. In this presentation, we describe a scale-down system for high throughput particle conditioning (HTPC). This micro-scale process is comprised of a temperature-controlled microplate with magnetically driven stirrers, integrated with a Tecan liquid handling robot. With this system, 96 individual reaction conditions can be evaluated in parallel, including downstream centrifugal clarification. The associated high throughput analytics enable comprehensive analysis of product titer, product quality, impurity clearance, clarification efficiency, and particle size characterization. The scalability of micro-scale particle conditioning was evaluated between 1, 100, and 2000 mL scales for capsular polysaccharides. An engineering characterization of the reactors was performed, leading to the assessment of several published approaches for scaling particle conditioning processes. Product yield, impurity clearance, and product quality were comparable between scales. The HTPC approach was extended to microbial proteins and compared with manufacturing scale. Intrinsic to this work was a continuous assessment of the techniques developed along with the efforts made for further refinement. Establishment of a HTPC process at the micro-scale combined with evidence-based scaling metrics is a significant advance for purification process development. The full cycle of setup, processing, and analysis can be completed in a matter of days, leading to deeper process understanding. The rapid development of a particle conditioning unit operation is now possible by a single scientist using less than a liter of feed material.

Purifying New Molecular Modalities II

Session Chairs:
Pete Tessier, Rensselaer Polytechnic Institute, United States
Ganesh Vedantham, Amgen Inc., United States

The Challenges of Developing Processes for New Protein Formats

Kurt Lang (Roche)

The development and manufacturing of recombinant therapeutic antibodies is well established in the biopharmaceutical industry. High titer platform processes and methods exist for cell line development, cell culturing, purification and analytics. To overcome the therapeutic limitations of monoclonal antibodies, new tailor-made protein formats were designed and are now in preclinical or clinical development. New protein formats are usually generated by the fusion of protein domains to combine the biologic activity of different molecules and to achieve new superior therapeutic effects. The production of these synthetic multi-domain proteins may face several challenges: Some domains may not be suitable for high expression or susceptible to proteolytic cleavage, complex asymmetric molecules require the expression of more than two polypeptide chains, the natural chaperone repertoire of eukaryotic cells may not be able to assist and check the correct folding of the protein and the cell culture, downstream and analytical process steps and methods established for antibodies may not be suitable for new formats. We developed manufacturing processes for several new formats such as the Ang-2-VEGF-A cross-mAb or the tumor-targeted, engineered IL-2 variant (IL2v)-based immunocytokine. The establishment of high titer processes for these proteins with the required product purity for preclinical and clinical studies required an integrated approach considering the selection of a suitable format for development, generation of a cell line and a cell culture process with good and stable productivity and product quality and the development of an efficient scalable purification process with good removal of (product related) impurities and high yields.

Development and scale-up of a commercializable two chain immunotoxin fed batch refolding process

Thomas Linke (MedImmune, LLC)
Matthew Aspelund (MedImmune, LLC)
Alan Hunter (MedImmune, LLC)
William Wang (MedImmune, LLC)
Xiangyang Wang (MedImmune, LLC)

Expression of proteins as inclusion bodies (IB) in E. coli is a commonly used method for the production of therapeutic drug products that do not require post-translational modifications. The well understood genetics of E. coli and high expression yields make this an attractive feature for the biopharmaceutical industry. However, refolding of inclusion body proteins into bioactive form often results in low to moderate yields due to misfolded species and aggregates, which present a potential bottleneck for scale up, material supply and cost of goods manufactured (COGM). A case study on the development and scale-up of a commercializable two chain immunotoxin fed batch refolding process is presented. The clinical manufacturing process produced high quality material but required column fractionation and in-process testing leading to low yield and high variability. A systematic approach to commercial process development led to five-fold increase in yield to reduce COGM and to eliminate fractionation and in-process testing. The inclusion body recovery process was optimized to produce higher quality IBs with reduced number of washes for facility fit. The formation of an undesired deamidated species was controlled kinetically prior to refolding and eliminated the need for fractionation and in-process testing during purification. A switch from batch to fed batch dilution refolding and the use of a combination of urea and arginine for capture column cleaning further contributed to improvements in productivity and consistency. Scalability was demonstrated with data from 250L and 950L scale refolding processes. Compared to the prior generation clinical process, the improved commercializable process is more suitable for process validation and removes CMC activities from the critical path for clinical supply and COGM.

Development of a Purification Platform for Fully Human Bispecific Antibodies

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Benjamin Adams (Regeneron Pharmaceuticals)
Hanne Bak (Regeneron Pharmaceuticals)
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Dustin Kucko (Regeneron Pharmaceuticals)
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There is strong interest in the design of bispecific monoclonal antibodies that can simultaneously bind two distinct targets or epitopes to achieve novel mechanisms of action and efficacy. Multiple bispecific formats have been proposed and are currently under development. Regeneron's bispecific technology is based upon a standard IgG antibody in order to minimize immunogenicity and improve pharmacokinetic profile. A single common light chain and two distinct heavy chains combine to form the bispecific. One of the heavy chains contains a chimeric Fc sequence (called Fc*) that ablates binding to protein A via the constant region. As a result of co-expression of the two heavy chains and the common light chain, three products are created: two of which are homodimeric for the heavy chains and one that is the desired heterodimer bispecific product. The Fc* sequence allows selective purification of the Fc:Fc* bispecific product on protein A columns, due to intermediate binding affinity for protein A compared to the high avidity Fc:Fc heavy chain homodimer, or the weakly binding Fc*:Fc* homodimer. This platform requires the use of protein A chromatography in both a capture and polishing modality. Several challenges, including variable region protein A binding, resin selection, selective elution optimization, and impact upon subsequent non-affinity downstream unit operations, were addressed to create a robust, selective manufacturing process.

Production Concepts

Session Chairs:
Jens Vogel, Boehringer Ingelheim GmbH, United States
Suzanne Farid, University College London, Great Britain

Integrated and fully continuous processing of recombinant therapeutic proteins – from cell culture media to purified drug substance

Veena Warikoo (Genzyme Corporation)
Rahul Godawat (Genzyme Corporation)
Sujit Jain (Genzyme Corporation)
Konstantin Konstatinov (Genzyme Corporation)

Considering the implications of increasingly diverse product candidate pipelines, rapidly fluctuating market demands and growing competition from biosimilars, biotechnology companies should be motivated to develop innovative solutions for highly flexible and cost-effective biologics manufacturing. To address these challenges, we have developed an integrated, closed and fully continuous biologics processing platform. Our studies have focused on the integration of a perfusion bioreactor to a fully continuous downstream purification train using two four-column periodic counter-current chromatography (PCC1 and PCC2) systems. Running in an automated manner, these systems performed the protein drug capture, viral inactivation, and in-line buffer dilution along with intermediate and final polishing purification steps to generate the drug substance. To demonstrate proof of concept, we have run the process in an uninterrupted manner for 31 days without indications of time based system performance decline. The biologics product quality observed for the fully continuous process was comparable to that for a batch purification operation. Our data reveal that an integrated fully continuous process results in a dramatic increase in the process throughput (time in hours to produce a batch as compared to days), decrease in the equipment footprint, elimination of several non-value added unit operations, elimination of hold steps and reduced the number of unit operations to minimum. These findings demonstrate the potential of integrated fully continuous bioprocessing as a universal platform for the manufacture of various kinds of therapeutic proteins.

Continuous chromatography: the good, the bad, and the unexpected

Oliver Kaltenbrunner (Amgen Inc.)

Continuous downstream processing is considered by many to be the most productive and cost effective processing mode. While currently there is only limited adoption of continuous downstream processing in biopharmaceutical manufacturing, there is renewed interest in SMB type continuous production systems. A common objective is cost reduction of the protein A capture step in the production of monoclonal antibodies. This is motivated by the high cost of protein A resin and the promise of significant productivity gains from continuous column cycling operations. In this presentation we will report on the development of continuous column cycling operations within the framework of large scale biopharmaceutical manufacturing constraints. The same constraints are applied in a comprehensive and balanced comparison to traditional batch chromatography, showing that scenario and objective will determine the favorable processing mode. This analysis considers affected operational aspects including productivity, buffer volumes, column sizes, number of columns, number of column packs, footprint in the plant, skid complexity, and number of pools per day. We will demonstrate that there are many degrees of freedom in the design of a continuous downstream process and that the optimal balance of operational objectives depends on manufacturing scenario and requirements.

Column-free process, the future of downstream processing

Michael Dieterle (Boehringer Ingelheim GmbH)

Downstream processing of biopharmaceuticals is becoming more and more challenging since upstream technologies nowadays achieve very high product titers, whereas downstream processes have to deal with only moderate advances in throughput capacities. In order to process these huge amounts of proteins potential bottlenecks like column chromatography and tremendous increasing buffer volumes have to be eliminated. Innovative techniques like precipitation and aqueous two phase extraction might complete or even replace certain conventional purification steps. In the light of short development timelines BI developed a column-free process based on extraction, precipitation and membrane adsorber technology which covers cell separation, capture and polishing steps. This unique purification process is also feasible to handle new molecule formats and non-mAb proteins which lead to further challenges in capturing using conventional chromatography steps and affinity resins. This presentation will present case studies and give an insight into BIs column-free process that enables to handle the arising challenges and guarantees low process times and costs while maintaining a robust removal of contaminants like DNA, HCP and even aggregates. Furthermore the talk will deal with the evaluation of scale-up experiments up to 100 liter scale for the different unit operations (extractions, precipitation and membrane adsorbers). Different types of extraction systems (e.g. centrifugal extractor) have been tested for CHO cell reduction. Results will show cell and turbidity reduction within only minutes. Case studies will show how to combine extraction systems and precipitation technologies and how process conditions for filtration and washing steps, optimizing the precipitation step have been screened. Results for the application of Q and HIC adsorbers as polishing steps will complete the picture of BIs column-free process.

Evaluating new capture technologies within the context of manufacturability and the process economics

Alex Xenopoulos (Merck Millipore)

We evaluate new technologies for purification of recombinant proteins in a structured process that addresses both the technical feasibility and performance as well as aspects of manufacturability and process economics. Several examples will be presented from work in our laboratories, where acceptable performance and initially promising advantages were ultimately negated by practical considerations that we were able to concretely describe. Precipitation technologies were evaluated for both clarification (where impurities precipitate) and capture (where product precipitates) of monoclonal antibodies. In both cases, the additional equipment and process time needed for the precipitation step make this option less attractive. Performance improvements can compensate in the case of clarification and feasible paths to implement attractive processes can be described. The capture case is more complicated as the performance of protein A capture chromatography cannot be matched even under the best assumptions. Cation exchange chromatography was evaluated as a lower-cost option for capture of antibodies. Clear advantages in terms of resin cost and capacity suggest an obvious fit, yet the requirement to reduce the solution conductivity both before and after the unit operation makes its implementation unrealistic from a facility fit standpoint. Salt-tolerant cation exchange resins can be suggested to address the loading problem, while gradient pH elution could address the back end. In both examples, protein A chromatography continues to maintain its position as the workhorse of antibody purification. The success of protein A chromatography for antibodies has motivated active evaluation of affinity chromatography for non-antibody molecules. In parallel to experimental evaluations of affinity ligands, we have modeled entire processes where one novel affinity chromatography step replaces two or more ion exchange and/or hydrophobic interaction chromatography steps. Through the assumptions made during this exercise, we can define performance requirements for the affinity ligand in terms of expected overall benefits and exclude low performers early on.

Phase Behaviour and Rheology

Session Chairs:
Andy Ramelmeier, United States
Matt Westoby, Biogen Idec, United States

Predictive Approaches for Protein Phase Behavior

Sven Amrhein (Karlsruhe Institute of Technology)
Katharina Bauer (Karlsruhe Institute of Technology)
Lara Galm (Karlsruhe Institute of Technology)
Marie-Therese Schermeyer (Karlsruhe Institute of Technology)
Jürgen Hubbuch (Karlsruhe Institute of Technology)

As the relatively young biopharmaceutical industry continues to mature, the understanding of its products and product processing needs to grow. One key parameter in downstream processing and formulation is product stability. Current studies use protein phase diagrams providing information about phase transitions and thus stability. This methodology lacks molecular mechanistic understanding, hence specific and proactive manipulation of the processed solution is challenging. The thesis of this talk will show approaches for understanding protein solution behavior starting from molecular protein properties. The goal is to find solution characteristics capable of predicting its stability. Protein phase diagrams determined in a high throughput format and microbatch scale served as starting point for further investigation. To explain protein solution behavior under these tested conditions we like to present results from measurements of micro- and macroscopic perspective. On a microscopic level we investigated protein hydrophobicity and conformational stability. Protein hydrophobicity was correlated to surface tension and validated by measurements of solvatochromic dye absorption shifts. Surface tension was measured using a newly developed setup connected to a robotic liquid handling station. Conformational stability was examined by Fourier transform infrared spectroscopy to differ between native and non-native aggregation. On a macroscopic level dynamic light scattering and squeeze flow rheometry were used to characterize protein interactions in solution for protein concentrations up to 225 g/L. Dynamic light scattering measurements showed different hydrodynamic behavior dependent on the viscosity of the protein solution. These samples revealed viscoelastic behavior over a wide frequency range. The measured rheological parameters were sensitive concerning solution composition, protein concentration and solution inner structure. In summary, our results showed a qualitative correlation between micro- and macroscopic protein solution characteristics and phase transitions. The presented approaches are able to give a molecular mechanistic understanding of protein behavior in solutions. These might serve as predictive tool both for process control and formulation development.

Scattering and Microscopy Studies of the Microstructure of Amorphous Protein Dense Phases

Abraham Lenhoff (University of Delaware)
Daniel G. Greene (University of Delaware)
Yun Liu (University of Delaware)
Shannon Modla (Delaware Biotechnology Institute)
Stanley I. Sandler (University of Delaware)
Norman J. Wagner (University of Delaware)

Protein dense phases, such as precipitates, crystals, gels and aggregates, appear in many guises in downstream processing, formulation and delivery; in some cases their appearance is desirable and in others not. Several aspects of such dense phases are of interest, starting from the protein–protein interactions and thermodynamic properties that guide phase separation, which have been studied extensively using both experimental and modeling approaches. However, less attention has been paid to the detailed properties of the dense phases per se; these properties derive from the molecular structure and microstructure of the dense phase, which is known in exquisite detail in the case of crystals but is quite poorly understood for most amorphous dense phases. Such dense phases have been explored as candidate drug delivery vehicles, especially for monoclonal antibodies. Some structural insights have been obtained from simulations of colloidal systems, but the inherent anisotropy of protein molecules and their interactions makes direct experimental observations imperative. An improved understanding of the structure and of structure–function relations can facilitate methods for designing new formulations, ameliorating the effects of high molecular weight aggregates, and designing novel separation schemes. In this work we use scattering techniques and real-space imaging to explore a model protein system that has not been extensively investigated previously in order to understand better how protein interactions give rise to the observed phase behavior and to the microstructure of the resulting protein dense phases. The main system studied is a model one in which ovalbumin precipitates are formed in concentrated ammonium sulfate solutions. Small-angle neutron scattering (SANS) measurements show that macroscopically non-crystalline ovalbumin precipitate particles formed under high salt conditions (> 2.4 M ammonium sulfate) exhibit a microstructure at small length scales that can be described by a nanocrystalline cluster made up of a relatively small number of unit cells of ovalbumin. At longer length scales the SANS data can be described by a lamellar sheet. The model fits of the SANS data are supported by direct real-space imaging using electron microscopy and electron tomography, which together show evidence of protein clusters as well as a lamellar structure. In addition to the structural information, we find that the onset of the microstructure depends on the quench depth into the spinodal, with shallow quenches resulting in faster microstructure development. These kinetic observations can be related to results of simulations previously reported in the literature.

Engineering, characterizing and formulating aggregation-resistant antibodies

Peter Tessier (Rensselaer Polytechnic Institute)

Protein aggregation is not only one of the most important challenges in successfully developing therapeutic monoclonal antibodies (mAbs), but its significance continues to grow with increasing demands for high concentration antibody formulations. One key approach for preventing antibody aggregation is to use protein engineering methods to reduce the hydrophobicity of the antibody binding loops (complementarity determining regions, CDRs). We have developed a mutational strategy in which charged residues are inserted at the edges of hydrophobic CDRs (without removing any hydrophobic residues), and find that antibody solubility can be increased dramatically without reducing binding affinity. Interestingly, we find that positively and negatively charged CDR mutations have significantly different impacts on solubility, and that the optimal solubilizing mutations are dependent on the charge of the antibody scaffold. A second key approach for preventing antibody aggregation is to improve early assessment and selection of antibody candidates with high solubility. We have developed a screening method (affinity-capture self-interaction nanoparticle spectroscopy, AC-SINS) for assaying the self-association propensity of mAbs during early discovery. Our approach uses gold nanoparticles coated with polyclonal antibodies specific for human mAbs to selectively capture and concentrate mAbs around gold conjugates. Attractive self-interactions between immobilized mAbs lead to reduced interparticle distances and increased plasmon wavelengths (wavelengths of maximum absorbance) of the gold conjugates. Strengths of AC-SINS include its compatibility with unpurified and extremely dilute antibody samples (such as unpurified cell supernatants). We are currently optimizing AC-SINS for screening large antibody libraries during early antibody discovery as well as for screening diverse formulation conditions during early formulation development.

Towards a Platform, Continuous-ready, Precipitation-based Process for High Concentration Recombinant Protein Recovery

Todd Przybycien (Carnegie Mellon University)

Is it possible to construct a simple "platform" process for the recovery of a recombinant protein that doesn't rely on affinity media/affinity tagging? For target proteins secreted at high concentration, say greater than 5 to 10 g/L, the possibility of exploiting self-interactions via precipitation for recovery becomes attractive. This may make more engineering and economic sense than trying to adsorb so much target on chromatographic media in a bind/release operation. Precipitate phases may exhibit extended storage stability, permitting insertion of a hold step, and can be re-suspended at desired concentrations, provided re-suspension is facile. Subsequent polishing purification steps can operate in a subtractive, flow-through mode, making use of orthogonal ion exchange and hydrophobic convective media to remove remaining contaminants. And, precipitation, re-suspension and flow/through operations are readily amenable to continuous operation.

We have used zinc and polyethylene glycol to generate precipitates of several mock target proteins, spanning a range of molecular weights and physical properties that are present at high concentration in a background of mock contaminants such as yeast extract and fetal bovine serum. We have examined the apparent solubilities of both target and contaminants and find potential windows of operation. We have further used bioprocess design software to begin to estimate the economic and environmental sustainability metrics associated with a precipitation-centric downstream process.

Recovery Science 2050, Visions in Academia

Session Chairs:
Steve Cramer, Rensselaer Polytechnic Institute, United States
Nigel Titchener-Hooker, University College London, Great Britain

A Highly Integrated Microfluidic Platform for Discovery and Manufacturing of Therapeutic Antibodies

Charles Haynes (University of British Columbia)

Monoclonal antibodies (mAbs) are a major focus of the biotech industry, comprising nearly half of all therapeutic proteins in current development pipelines. In this presentation, I will describe a novel microfluidic platform recently developed in our laboratories that allows for functional screening and selection of monoclonal antibodies secreted by single plasma cells, as well as light and heavy chain amplification, isolation and sequencing on a single highly integrated device. By compartmentalizing single cells in microfluidic chambers over 1000X smaller in volume than conventional cell culture wells, each isolated cell can secrete its unique antibody at concentrations detectable by fluorescence microscopy. I will show how this can be linked to an on-chip fluorescence-based bead assay and RT-PCR to measure antibody-antigen binding kinetics/affinity and to determine the sequence of each candidate therapeutic antibody in a highly multiplexed and rapid manner when applied to screening primary antibody-producing plasma cells. As part of this platform, I will describe a low-pressure bead packing technique for the robust integration of high-performance chromatography columns in microfluidic devices made by multilayer soft lithography. A novel column geometry is used to achieve rapid packing of multiple high-quality columns that may be applied to achieve multiplexed clean-up of RT-PCR products. Time permitting, I will then briefly describe a related platform for multiplexed screening of cell growth and specific productivities to accelerate clonal selection among recombinant CHO cell populations. Application of the technology to the discovery of anti-influenza mAbs is described.

Purification 2050: The Single-Molecule Perspective

Richard Willson (University of Houston)
Jixin Chen (Rice University)
Wen-Hsiang Chen (University of Houston)
Sagar Dhamane (University of Houston)
Lydia Kisley (Rice University)
Katerina Kourentzi (Rice University)
Christy Landes (Rice University)
Andrea Mansur (Rice University)
Mohan-Vivekanandan Poongavanam (University of Houston)
Bo Shuang (Rice University)

The characterization and understanding of chromatographic processes and adsorbents has been steadily advanced by new methods of investigation. From isotherms and retention curves, through calorimetry and self-interaction chromatography, to scattering and confocal imaging, new tools have yielded new insights. This talk makes the prediction that by 2050 purification and adsorbent development will include a single-molecule perspective, and single-molecule data. This will encompass the characterization of single adsorption events on single sites, the heterogeneity of sites and their steric accessibility, and the distribution of dwell times on a given site, as well as single-molecule study of complex phenomena such as competition, aging, and trapping. These results will be used in the development of better adsorbents, and when such data are available they will support the use of the molecular-theoretic stochastic model of Giddings and Eyring as a more fundamental, predictive approach to the modeling of chromatographic separations. We report the use of super-resolution single-molecule imaging and fluorescence correlation spectroscopy to directly study adsorption of single protein molecules on ion exchange ligands on agarose. Additionally, we relate experimental results to the molecular-scale stochastic theory of chromatography. The combination of super-resolution spectroscopy, single adsorption site kinetic analysis, and statistical treatment allows us to establish the importance of charge clustering for stationary phase ligands, and to characterize the surprising heterogeneity of the individual kinetic properties even of adsorption sites created by immobilization of identical multiply-charged ligands.

Integrated and Scalable Cyto-Technology (InSCyT) Platform for Biopharmaceutical Manufacturing on Demand

J. Christopher Love (Massachusetts Institute of Technology)

The delivery of biologic drugs to patients can be challenging, if not impossible, in many regions of the world. Patients who could benefit from treatments may live in remote regions, under-resourced areas, or face challenging circumstances such as natural disasters that limit access to life-saving drugs. The state-of-the-art approaches to manufacture biopharmaceuticals are not compatible with on-site, rapid manufacturing of treatments on demand. This talk will update a new approach for producing biologics on demand called Integrated and Scalable Cyto-Technology (InSCyT). This platform emphasizes an integrated, milliliter-scale table-top system for (semi)continuous operation, consisting of a parallel set of micro bioreactors, filtration of cell debris from secreted product, innovative affinity-based purification, polishing, and finishing, as well as integrated on-line PAT and process control for QbD production and product qualification for release. Examples of the underlying technologies enabling this platform will be presented. Beyond the applications for rapid manufacturing of biologics, the technologies developed here should also address other areas of need in continuous manufacturing identified by large biopharmaceutical companies in academic workshops. This approach to manufacturing should supplement traditional processes to improve global access of drugs, and to accelerate drug development.

Next Generation Unit Operations

Session Chair:
Phil Lester, Genentech, Inc., United States
Frank Riske, BioProcess Technology Consultants, United States

Development and scale-up of the recovery and purification of a domain antibody Fc fusion protein- comparison of a two and three-step platform approach

Sibylle Herzer (Bristol-Myers Squibb Company)
Greg Barker (Bristol-Myers Squibb Company)
Atul Bhangale (Bristol-Myers Squibb Company)
Isha Chowdhary (Bristol-Myers Squibb Company)
Matthew Conover (Bristol-Myers Squibb Company)
Wallace Kaserer (Bristol-Myers Squibb Company)
Brian O'Mara (Bristol-Myers Squibb Company)
Nicole Payonk (Bristol-Myers Squibb Company)
Siegfried Rieble (Bristol-Myers Squibb Company)
Lily Tsang (Bristol-Myers Squibb Company)
Shiyy Wang (University of British Columbia)

Two different purification strategies were pursued during process development for a first in human (FIH) process of a domain antibody (dAB)-Fc fusion protein. A two-step process was compared to a three-step process. The two-step process leveraged additives to maximize impurity reduction either prior to, during or after affinity capture. Data quantity and quality was significantly improved by rigorous pursuit of high through put screening (HTS) and design of experiments (DOE). Medium length chain fatty acid, buffer and protein concentration, type of fatty acid and pH effects were evaluated. Use of sodium caprylate allowed reduction of HCP levels by 40-50% either pre, during or post capture chromatography by protein A. The design space of sodium caprylate for precipitation pre and post protein A purification proved to be limited. Use of caprylate was not deemed robust enough for scale-up due to significant curvature and steep drop offs in yield and commensurate increases in impurity levels at its boundaries. Robustness for a protein A wash step proved much higher as a fairly wide range of conditions was found to be acceptable. HCP clearance of 50% and yield above 90% were achieved. Use of the additives during affinity capture also simplifies manufacturing as feed stream manipulations are minimized. In addition, HCP clearance can be further improved as bound IgG can sustain a much wider range of pH/caprylate settings as compared to solution based caprylate addition. Analysis of protein A eluates indicated that HCP reduction is not due to residual caprylate carry over but most likely causes partial unfolding of HCP impurities during the

wash step which increases susceptibility to precipitation upon neutralization. Comparison of the impurity targets and overall process performance for the two and three step process indicated an improved overall impurity clearance for the three step process at slightly lower yield. Both process options met the preset acceptance criteria for the FIH process. Detailed data of the DOE assessments and scale up of optimized conditions will be presented.

Affinity Precipitation of mAbs Using Stimuli Responsive Smart Biopolymers: Methods Development and Process Considerations

Rahul Sheth (Rensselaer Polytechnic Institute)
Bharat Bhut (Bristol-Myers Squibb Company)
Wilfred Chen (University of Delaware)
Steven Cramer (Rensselaer Polytechnic Institute)
Mi Jin (Bristol-Myers Squibb Company)
Zhengjian Li (Bristol-Myers Squibb Company)

This work provides a detailed investigation into the development of a robust and scalable monoclonal antibody (mAb) affinity precipitation process using elastin-like polypeptides (ELPs) fused to the mAb binding Z domain. A multidimensional high-throughput screening (HTS) protocol was initially employed to determine initial capture and co-precipitation of pure model mAbs at high yields by the ELP-Z. MAb elution from the ELP-Z-mAb complex was subsequently determined using another HTS screen and mAb yields and aggregate content for the entire process were determined. High mAb yields with low aggregate content were obtained using mild elution conditions (pH 4.2) at room temperature. Findings from the HTS studies were then used to guide studies for mAb purification from a mAb harvest feed. The process resulted in more than 2 logs of HCP and more than 4 logs of DNA clearance from the harvest feed, which were comparable or superior to protein A chromatography for that mAb. Process performance was maintained for mAb final elution concentrations up to 20 g/l. Effective ELP-Z cleaning using NaOH and reusability over multiple purification cycles was also successfully demonstrated. Finally, process scalability was evaluated using scaled-down tangential flow microfiltration (TFF-MF) and dead-end filtration approaches that resulted in complete precipitate recoveries, high mAb yields and good product quality at high volumetric throughputs. This work demonstrates the potential of the ELP-Z based affinity precipitation approach as a next generation unit operation for industrial mAb bioprocessing.

Virus purification using osmolyte flocculation

Caryn Heldt (Michigan Technological University)
Eric Pearson (Michigan Technological University)
Maria Tafur (Michigan Technological University)

Vaccine technology has revolutionized the prevention of communicable diseases. In order to improve the speed and efficiency of viral therapeutic manufacturing, there needs to be an improvement in current virus purification processes. Chromatography resins are acceptable for proteins, but not for large biomolecules, such as virus particles. In tangential flow filtration, the capacity of the membrane can be affected by fouling of large biomolecules, leading to low flux and high transmembrane pressures. We have focused on virus flocculation in the presence of osmolytes, followed by microfiltration. Osmolytes are natural compounds that stabilize intracellular proteins against environmental stresses. We are currently working with a non-enveloped virus, porcine parvovirus (PPV), and an enveloped virus, Sindbis virus (SINV). We have discovered several protecting osmolytes that flocculate PPV and SINV and demonstrate a >80% removal with a 0.20 μm filter. This micropore filter is usually used to retain bacteria, not small virus particles. A 0.20 μm micropore filter improves the flux and reduces the fouling that is typically experienced with nanopore filters used for virus retention. We hypothesize that the high water binding capacity of the osmolyte flocculants preferentially structure water molecules. At high osmolyte concentrations, the osmolyte can remove the hydration layer from the virus surface and this results in the flocculation of the hydrophobic virus. Other work we have done demonstrates that PPV has a higher surface hydrophobicity than a panel of proteins tested. The highly hydrophobic virus surface prefers to flocculate under high osmolyte conditions as compared to less hydrophobic proteins. The flocculants that achieved the highest percent removal were tested for their ability to aggregate viruses at different pH, ionic strengths and temperatures, allowing optimal conditions within our design space to be determined. We were able to remove 97% of PPV in 3M glycine at a pH of 5 and 85% of SINV in 0.3M glycine at a pH of 6. Osmolyte flocculation is specific to virus particles, as these conditions have been tested with other proteins and they are not able to flocculate and remove the proteins. We have also been able to recover the virus from the filter, demonstrating that we can use this as a purification method. Virus flocculation followed by microfiltration has a high potential to purify virus from host cell proteins. Osmolytes are commonly used in protein therapeutic formulation, so complete removal may not be required for the final product. We propose to use virus flocculation in osmolytes, followed by microfiltration as a potential platform approach for virus purification.

A Unified Process Development Strategy for Batch and Continuous Chromatography

Karol M. Lacki (GE Healthcare)
Hans Blom (GE Healthcare)
Annika Forss (GE Healthcare)

Continuous processing has proven very successful in many industries, yet its implementation into the biopharmaceutical field is still relatively slow. While continuous upstream operations are considered as a viable option in commercial manufacturing, continuous downstream operations are still lagging behind. Among plausible reasons for this situation, a perception that a continuous downstream step will require more efforts to develop, characterize and control is most common. Questions related to steady state operation, variable feed concentration, and validation of virus clearance on a multicolumn system are often asked when a chromatography step operated in batch mode is to be converted to continuous operation. This presentation will discuss a unified process development and process characterization strategy that is applicable for both batch and continuous chromatography steps. The strategy is based on one set of experiments that leads to identification of key and critical process parameters for all types of chromatography operations namely batch, semi- and fully continuous. The strategy also allows development of process control strategies, and in its entirety forms an integrated part of life cycle management for either a single chromatography step operated in batch or continuous mode, or a sequence of chromatography steps operated in a connected mode. The presentation will include both the theoretical and practical perspective. Examples of use of the strategy as applied towards development and characterization of a single chromatography step and of a sequence of connected steps will be presented. Special emphasis will be placed on describing how the strategy reduces, or completely eliminates, a need for specific investigations that might seem necessary for investigating one or the other mode of operation separately.

New Stationary Phases

Session Chairs:
Conan Fee, University of Canterbury, Australia
Mike Phillips, EMD Millipore, United States

Virus capture using membrane chromatography: improving selectivity by matrix design

Louis Villain (Sartorius Stedim Biotech)
Manuel Carrondo (IBET/ITQB)
Joerg Mittelstaet (Sartorius Stedim Biotech)
Piergiuseppe Nestole (IBET/ITQB)
Cristina Peixoto (IBET/ITQB)
Udo Reichl (Max Planck Institute Magdaburg)
Florian Taft (Sartorius Stedim Biotech)
Michael Wolff (Max Planck Institute Magdaburg)

Because next generation cell-based influenza vaccines have to be produced faster and in greater quantities than traditional vaccines, future purification processes will require more efficient unit operations for their isolation and purification. Membrane chromatography has already demonstrated a number of positive characteristics for the bind and elute purification of viral particles >80 nm like adenoviruses or influenza viruses. The technology not only addresses the diffusion limitations of porous particle media but also offers dramatic advantages in binding capacity in a disposable format. The last remaining challenge for the easy adoption of this technology in the vaccine industry is selectivity and recovery. While the classical approach to improve selectivity aims at developing selective ligands, we present here a novel cellulose based stationary phase whose active specific surface area is designed for maximum virus accessibility. BET surface area measurements and characterization methods based on coated latex particles will be presented to highlight structural differences of the new matrix compared to commercial membrane adsorbers and to illustrate how binding-site competition between viral particles and process related contaminants, mainly DNA and HCP, can be sterically promoted. The resulting gain in selectivity and recovery but also in binding capacity will be demonstrated on ligands with low selectivity like strong quaternary amines for adenoviruses and on highly selective pseudo affinity ligands for influenza viruses respectively. Finally, the unique capabilities of these media do not only contribute to reduce the costs associated with the bind and elute purification of viruses but may also constitute one step forward in the development of pandemic ready platform process for the vaccine industry.

Potential Controlled Chromatography: Theory and Design of New Separation Modules

Matthias Franzreb (Karlsruhe Institute of Technology)
Sonja Berensmeier (Technical University of Munich)
Ellen Biegert (Karlsruhe Institute of Technology)
Dirk Holtmann (DECHEMA-Forschunginstitut)

Bioprocess chromatography suffers from several problems that compromise its economics and sustainability. The 'adsorption – desorption' processes currently involved are, for the most part, driven by changes in chemical composition of the mobile phases employed, resulting in the need for large volumes of buffer. In addition to chemical parameters physical ones such as temperature or electric potential, though rarely applied can also be exploited in bioseparations. The concept of 'Potential Controlled Chromatography' (abbreviated to PCC) derives from the observation that supplying a voltage to an electrically conductive stationary phase results in the adsorption of ions or charged molecules to it. In contrast to electrophoresis where molecule migration follows the electrical field between two external electrodes, in PCC molecules move towards the stationary phase, which itself serves as an electrode. Though 'proof-of-concept' demonstration of PCC for biomolecule separation has been documented previously, it is technologically immature and its true potential remains untapped. Addressing this requires much work on two broad fronts, (i) to advance the theoretical understanding of PCC processes; and (ii) to make future PCC competitive with conventional chromatographic methods (e.g. ion-exchange chromatography). Key to PCC's success will be the development of conductive matrices with substantially elevated sorption capacities (*cf.* previously reported values) and the associated equipment to handle them. Against the above, a tripartite cooperation project involving DECHEMA's Forschungsinstitut (DFI), the Technische Universität München (TUM), and Karlsruhe's Institute of Technology (KIT) was launched in 2012 specifically to significantly advance PCC theory and practice. While TUM and DFI's contributions in the project focus on the creation of improved stationary phases and classical column design, KIT's main aim is to develop new separation modules based on electrode stacks and process theory. In order to improve ion and protein binding capacity in the system PCC equipment must integrate conducting materials possessing very high inner surface areas with corrosion resistant electrode materials and thin chemically inert spacers serving dual roles of preventing electrical shortcuts and guiding the liquid flow. In this work the design of prototype modular flow cells composed of stacks of coated electrode sheets and the first results obtained with them are described. Various carbon materials (powdered activated carbon, carbon black, carbon sheets) were employed for the active stationary phase and electrodes, and the housing and spacers were CAD designed and fabricated in poly-acrylate by 3D printing. Using modeling it was shown that conducting stationary phases with specific surfaces of *ca.* 1500 m2/g can at applied voltages of as low as 1.2 V, deliver ion binding capacities comparable to those of commercial ion exchange adsorbents (0.3 mmol/g).

The Use of 3D Printing in the Study of Packed Bed Microstructures

Simone Dimartino (University of Canterbury)
Suhas Nawada (University of Canterbury)
Conan Fee (University of Canterbury)

What is superficially referred to as 'packing quality', a myriad of geometrical parameters governing the interrelations between pores, has only been measured post-hoc, in the form of separation efficiency. While several computational studies of chromatography bed microstructures have explored the effects of various packing parameters on dispersion, experimental replication of these in-silica studies has remained elusive. We propose the use of 3D printing as a tool to enable fundamental studies of packed bed microstructures.
The advent of 3D Printing offers the opportunity to create full columns with precise placement of individual beads in a manner that is structurally robust, versatile and repeatable. We can not only sidestep the problems associated with random packing by aligning beads in ordered lattices, we can also recreate random packings while systematically varying several geometrical factors such as micro- and macro-scale heterogeneity, extra-particle porosity and particle size distribution. These parameters were previously unintended by-products of the process of slurry packing, and often difficult to replicate. However, having precise and reproducible control over them should allow us to gain new insights into conventional packing behavior. In this study, we printed packed beds with deliberately introduced imperfections. Two types of defects were introduced into the printed columns: a 'line defect', a small cylindrical void that runs through the full length of the column and a 'cluster defect' where small spherical voids were created in the packing. We compared packed beds incorporating these imperfections within three bead arrangements: simple cubic, body-centered cubic and random close packing. Defect-less columns with identical bead configurations were also printed and compared. Reduced plate heights across a range of interstitial velocities were measured and compared with the corresponding computational results arrived at through the Lattice-Boltzmann method. The relative contributions of micro- and macro-structural packed bed heterogeneity to band broadening were thus compared.

Scale Up / Scale Down

Session Chairs:

Ranga Godavarti, Pfizer Inc., United States
Marcel Ottens, Delft University of Technology, Netherlands

Using CFD to Evaluate Chromatographic Performance in Process Scale Columns

Christopher Antoniou (Biogen Idec)
Venkatesh Natarajan (Biogen Idec)
Christopher Johnson (Biogen Idec)

Chromatography is an indispensable unit operation in the downstream processing of recombinant proteins. Scaling of chromatographic operations typically involves a significant increase in the column diameter and it is presumed that maintaining a constant length and linear velocity should result in scale-invariant performance. However, the flow distribution within a packed bed could be severely affected by the distributor design in process scale columns. The resultant effect on process performance and cleanability needs to be properly understood in order to prevent unpleasant surprises on scale-up. Computational Fluid Dynamics (CFD) provides a cost-effective means to explore the effect of various distributor designs on process scale performance. In this work, we present a CFD tool that was developed to compare the effect of two different header designs on column performance. The tool was validated against experimental dye traces and tracer injections. Results will be presented on the effect of the header designs on process performance and implications for scale-up/scale-down activities.

Challenges with particulate formation during process scale-up: Scale the unscalable?

Nihal Tugcu (Merck & Co.)
Valentyn (Merck & Co.)
Robin Ehrick (Merck & Co.)
Thomas Linden (Merck & Co.)
Mohammed Shameem (Merck & Co.)

Challenges with particulate formation were encountered in the early development of a monoclonal antibody candidate during drug substance and drug product manufacturing. Changes in both visible and sub-visible particulate levels were observed. As part of the investigation a panel of monoclonal antibodies with different molecular properties was studied with regard to their sensitivity to shear and other stresses. While it was challenging to identify clear markers that would lead to particulates, multiple interacting factors were found. Upon data mining, drug substance storage conditions in addition to some unit operations related to drug substance and drug product manufacturing were identified as potential causes for particulate formation. The development of a true scale-down model was identified as the key challenge in support of this investigation. Multiple antibodies and their sensitivities to different unit operations with propensity to shear were investigated. To enhance our understanding for frozen DS storage, controlled freeze/thaw systems were utilized for the development of testing ranges and to evaluate the impact of formulation buffers on freezing time and stability. A set of experiments, including multiple monoclonal antibodies with different formulation and packaging components, was utilized to define an experimental template for evaluating the impact of storage temperature. The impact of thawing procedures on stability was also evaluated. As an outcome, the manufacturability assessment for our platform fit now includes specific measures to ensure the right equipment and operating conditions to be in place to mitigate the risk of particulate formation.

New Challenges for Scale-Down Model Qualifications

Annika Kleinjans (Roche)
Christian Hakemeyer (Roche)
Silke Werz (Roche)
Frank Zettl (Roche)

The scale-down model qualification is the fundamental basis for a state of the art process characterization and validation exercise (including the virus validation studies). In the past common approaches for scale-down model qualification was to perform a T- test or to assess the scale-down model results against the three fold standard deviation or the 95/99 tolerance intervals of the manufacturing scale. In the context with Quality by Design (QbD), the expectations of the health authorities increased significantly, as the scale-down model is the basis for the creation of a design space. It is expected that the scale-down model qualification is performed more rigorously, e.g. by assessing a broad panel of critical quality attributes and using statistical methods that prove equivalence (e.g. by performing a TOST). Here we show our strategy and important learnings from the qualification of scale down models for the purification unit operations of an FDA approved design space. The experimental approach as well as the TOST approach is outlined, and the results and the subsequent actions are discussed.

Tricky Issues Case Studies

Session Chairs:
Thomas Linden, Merck & Co., United States
Stefan Hepbildikler, Roche, Germany

An Intensified Refolding and Downstream Process for a Highly-Expressed Recombinant Protein in E.coli

Shuang Chen (Pfizer Inc.)
Scott Cook (Pfizer Inc.)
John Cundy (Pfizer Inc.)
Robert Fahrner (Pfizer Inc.)
Ratish Krishnan (Pfizer Inc.)
Joseph Martin (Pfizer Inc.)
Mathew Stork (Pfizer Inc.)
William Wellborn (Pfizer Inc.)

A typical recovery and downstream purification process for an *E.coli*-derived recombinant protein expressed as an inclusion body (IB) involves the following unit operations: cell harvest, homogenization, IB recovery, refolding, clarification, chromatography operations, and ultrafiltration. A significant increase in upstream productivity (higher fermentation titer) often leads to processing bottlenecks specific to the downstream operations. In the following case study, the fermentation titer was increased over tenfold, rendering the baseline refolding process, performed at <0.5 g/L (2x-fermentation volume equivalent, FVe), impractical on large scale due to the order of magnitude increase in refold volume. The development goal for an improved downstream process was to enable a high concentration refolding capable of being performed at 1 FVe. This would enable scale-up and process fitting within the existing manufacturing network. Additionally, a similar degree of downstream productivity improvement along with significant yield increase and comparable drug substance intermediate (DSI) critical quality attributes were needed to make the process commercially feasible. Along with further optimization of IB recovery, a high concentration refolding step was developed via the introduction of novel methods resulting in >30 fold increase in refolding concentration, eliminating the requirement for dilution and large tank volume. Additionally, an acid precipitation step was developed which significantly reduced impurities (HCP, DNA, and endotoxin), enabling a 3x load increase on the capture column. The chromatographic separation was improved as well, including elimination of a low-capacity, temperature-sensitive HIC step for enhanced process robustness. An overall, 40% DSP yield increase was achieved. In summary, we have transformed a scale-constrained and low-productivity downstream process into a fully-scalable and high-productivity process capable of generating the requisite supply of DSi within the existing Pfizer manufacturing network.

Racing Against Time: Novel Methodologies to Study Chromatography Resin Lifetime

Bruno Marques (GlaxoSmithKline)
Kent Goklen (GlaxoSmithKline)
Mark Lankford (GlaxoSmithKline)
Andrew Pike (GlaxoSmithKline)
Antonio Ubiera (GlaxoSmithKline)
Steve Weisser (GlaxoSmithKline)

This paper offers a case study on significant capture chromatography resin fouling identified during formal small-scale process characterization studies for a recombinant protein. Fouling was resolved with a combination of high-throughput screening of various cleaning agents and resin cleaning development studies, resulting in changes to the concentration of the cleaning solution, as well as to the flow direction and contact time during the cleaning. The effectiveness of this enhanced protocol (two-fold reduction of resin fouling rate) was confirmed with follow-up small-scale resin lifetime studies. In order to minimize the development time required to study effects on chromatography resin lifetime, we also developed a number of accelerated methodologies that mimic resin fouling and cleaning in extremely shallow chromatography beds. These techniques not only serve as good screening tools but also provide information on the mechanism of resin fouling through mass transfer models.

Do we streamline development or streamline manufacturing? Who says we can't do both?

David Robbins (MedImmune, LLC)

MedImmune, LLC has developed and successfully scaled up a 10+ g/L cell culture platform for antibody manufacturing. This platform provides many benefits throughout the product development lifecycle, such as accelerating availability of material for preclinical studies, and reducing time and resources needed for upstream process development. However, realizing the potential of high titer processes to drive commercial manufacturing productivity can be elusive, because of downstream process bottlenecks and the need to standardize the rhythm of batch run rate in the facility. The nature of the bottlenecks is usually facility-dependent, and not obvious to the purification scientist. Accelerated timelines and competing priorities for development resources can make it impractical to optimize each process individually to accommodate high titers. MedImmune's process development model includes an optimization stage to establish a commercial-ready process for manufacturing of Phase 3 clinical trial material. In order to proactively build process development capability to support MedImmune's rapidly growing late-stage pipeline, a variety of new tools, models, and platforms have been developed for the optimization stage of purification process development. Risk-based prioritization enables development activities and scientific innovation to be focused where it is most needed, driving greater efficiency in late stage development. Accurate computer models of MedImmune's commercial manufacturing facility fit and throughput constraints, combined with effective communication with the manufacturing group, have enabled the rapid optimization of efficient high-titer processes without sacrificing product quality or process robustness. The development of a commercial-ready platform process that incorporates and standardizes the solutions to common problems and constraints has streamlined resources and reduced risk in all stages of development. A case study will be provided to demonstrate how these tools were critical to a successful purification development to support a dramatic acceleration of the clinical program for a therapeutic monoclonal antibody. Within only six months available for process optimization, from planning to process lock, the effective productivity of the purification process was increased almost three-fold, while meeting all manufacturing requirements for control of product quality and process robustness.

Workshop Abstracts

WORKSHOP SESSION ABSTRACTS

How Pure is Pure Enough?

Session Chairs:
William Wang, MedImmune, LLC, United States
Josefine Persson, Genentech, Inc., United States
Jace Fogle, Eli Lilly and Company, United States

Identification of a free-drug impurity formed from an antibody trisulfide variant

Jayme Franklin (Genentech, Inc.)
Timothy Tully (Genentech, Inc.)

Antibody Drug Conjugates (ADCs) are a therapeutic agent in which cytotoxins or chemotherapeutic agents are chemically linked to monoclonal antibodies via cysteines, lysines, or other potential chemistries. In thiol-linked ADCs, antibody trisulfide bonds (RS-S-SR) are the major factor affecting the reduction stoichiometry during manufacture. Trisulfide levels ranging from 0 – 12% have been measured in preparations of unconjugated antibodies used as intermediates in ADC processes. The reduction variability due to antibody trisulfides is controlled by lot-to-lot titration experiments. Reduction of trisulfide bonds with TCEP uses additional TCEP that would be used to reduce mAb disulfide bonds and forms predominantly TCEP-S, which is easily removed by downstream TFF. With increased sensitivity of the free drug assay we discovered a previously undetected sulfide-linked free drug dimer species. By-products of the trisulfide reduction may be involved in the formation of the new free drug impurity, which is only partially cleared with traditional TFF operations. Process options to prevent the formation of the sulfide-linked free drug impurity have been evaluated. The impact of antibody trisulfides and the various unit operations which could be optimized to control the final drug purity will be discussed.

The Use of Single-Use MIX Vessels with Protein Solutions: Impact of Impeller Flow and Particle Shedding on Protein Turbidity and Aggregation

Bala Raghunath (Merck Millipore)

The increasing implementation of single-use equipment and assemblies in biopharmaceutical processes has resulted in more flexible facilities with faster batch-to-batch and product-to-product turnaround times. However, it has also resulted in new questions about how the materials of construction and system functionality might impact the drug products being produced. End-Users considering a move to single-use mixing systems for buffer preparation are interested in information about particulate count and generation and extractables/leachables identity and levels as well as guidance on an effective mix protocol to ensure robust dissolution of buffer components. But when considering these same mixing systems for protein pools, additional concerns are raised about the potential impact of the impellor action and the particulates on protein quality and stability. The current study examines the potential impact on protein quality during mixing in a single-use mix equipment and whether the change could be correlated to a mixing parameter. In addition, the study also looks at the potential impact on short-term protein stability due to the particulates that may be generated during mixing.

Proteomics-Based Approach For Assessing Host Cell Protein Impurities in a Complex Biological Product

Van Hoang (Merck & Co.)

Conventional approaches to measure host cell protein impurities involve the use of anti-host cell antiserum in a plate-based ELISA platform. Inherent to this approach is the need for a high quality immunoreagent with broad coverage against the host cell proteins and an appropriate reference standard. Generation of these reagents is not trivial and can require a long lead time. An alternate approach that relies on LC-MS is discussed. Identification of host cell impurities along with measurements of relative abundances can be determined using a proteomics approach. In contrast to traditional ELISA-based approaches, the proteomics-based method does not rely on either immunoreagents or a reference. A case study will be presented where this approach was used to characterize the host cell protein impurities from a complex vaccine candidate. Additionally, the approach was used by process development to further understand whether the process could be modified in order to increase the clearance of host cell proteins.

Use of Process Related Impurity Spike Challenge Studies to Inform Manufacturing Control Strategy

Roger A. Hart (Amgen Inc.)
Ryan Soderquist (Amgen Inc.)

As an Industry we control risk-to-patient associated with process related impurities such as nucleic acids and host cell proteins. In the case of platform processes producing monoclonal antibodies, the risk associated with host impurities may be low owing to process refinement over time. As an alternative to establishing measurement controls with acceptance criteria, ICH Q6B states "clearance studies, which could include spiking experiments at the laboratory scale" can be used to demonstrate removal of such impurities. This paper presents high resolution spike challenge studies designed to enrich relevant process-specific impurities and monitor their individual clearance by successive chromatography steps. Results provide greater understanding of purity identity, process step redundancy and, correspondingly, the necessity for in-process measurement controls.

A risk assessment framework for evaluating the impact of host cell protein(s) in biotechnology-derived products

Christina de Zafra (Genentech, Inc.)

Biotechnology-derived drugs, produced using engineered bacterial or mammalian cells, have been manufactured for over 30 years. These host cells contain an entire repertoire of proteins essential for their own function and survival, some of which may co-purify with the therapeutic protein and ultimately become a part of the final drug product. The thorough characterization of biotherapeutics includes the measurement of host cell protein (HCP) levels. A focus of the manufacturing process is the production of material with appropriate purity; a risk assessment framework that considers a number of important factors can help to inform decision-making about appropriate process development strategies designed to manage the levels of HCPs.

Muddling through the morass: making sense of data and models at different scales

Session Chairs:
Ajoy Velayudhan, Amgen Inc., Great Britain
Karol Lacki, GE Healthcare, Sweden
Victor Goetz, ImClone Systems, United States

Hybrid HTPD – Combining Models and Experiments for Rational Process Development

Marcel Ottens (Delft University of Technology)

High Throughput Screening (HTS) for process development is getting more and more established in the biopharmaceutical industry over the last decade. Several ways of using HTS can be distinguished. Design of Experiments (DoE) to find an optimal resin or optimal operational conditions for a chromatographic capture or purification step is most often used. Including mechanistic modeling may add another dimension to process development using HTS, which allows for *in-silico* process development. Mechanistic modeling requires model parameters that need to be obtained either via experimentation, literature correlations or from generated databases. In this presentation the High Throughput Process Development (HTPD) approach as developed in our lab in Delft will be outlined, which contains a hybrid miniaturized experimental and mechanistic modeling approach and the generation of a database for protein purification process development. The approach will be exemplified with several industrial cases.

SHARC – Software Integration for High Throughput Systems

Jan Griesbach (Roche)
Katharina Doninger (Roche)
Andreas Jux (Roche)
Alexander Kurtenbach (Roche)
Susanne Nath (Roche)

High Throughput (HT) Systems offer the great opportunity of parallelization and miniaturization, thus allowing to perform more experiments faster. Although this allows to significantly enhance the scope of experiments and the reliability of results, the large data sets which are generated increase the effort in data evaluation. The integration of components of the robotic platform and HPLC, UV-Photometers and the traceability represent major challenges. Here we present the software project SHARC (Software for High Throughput Applications using Robotics), which is designed to integrate all components of our robotic infrastructure at Roche's Technical Development and guide the user through the cycles of planning, executing, evaluating and reporting experiments and data. With this project we strive to decrease the workload and amount of user intervention, eliminating potential errors and making the entire system accessible to validation.

Addressing the Needs of End Users for Self-service Data Access, Aggregation, Contextualization, Analysis and Reporting of Process Data from Multiple Disparate Sources

Justin Neway (BIOVIA, a Division of Dassault Systemes)

The process and quality data stored in systems like LIMS, LES, EBR, ELN, Historians, ERP, etc., and on Paper Records is organized differently in each system to serve the needs of specialized users who focus on different portions of the process. This creates problems for users who need to perform data analysis on the process as a whole, to understand the sources of variability and design, develop, implement and trouble-shoot robust processes. These users need an automated way to access and contextualize all types of process-related data self-service from these multiple systems for analysis and reporting without making changes to the source systems or resorting to labor intensive, error prone spreadsheet methods. This includes on-line, real-time data from chromatography operations. This presentation will describe how biotech and pharmaceutical companies have overcome these problems by using a Manufacturing Informatics system that provides self-service, on-demand access and automated contextualization of data located in disparate source along with analysis and reporting capabilities in a validated environment. Examples of applications in the area of chromatography data analysis will be emphasized.

A Data Assimilation Platform to Drive Process Robustness and Improvement for Commercial Drug Substance Manufacturing

Canping Jiang (Biogen Idec)
Kyle Anderson (Biogen Idec)
Mark Byers (Biogen Idec)
Lilong Huang (Biogen Idec)
Haleh Valian (Biogen Idec)
Sarah Yuan (Biogen Idec)
Roland Zhou (Biogen Idec)

The development and improvement of a drug substance manufacturing process should continue over its lifecycle. A drug substance manufacturing process is developed and characterized primarily using laboratory scale models. Usually, limited full scale manufacturing experience is available before the product is commercialized. During commercial manufacturing, a process is likely to encounter sources of variation that were not previously detected or to which the process was not previously exposed. It is important to continuously accrue process knowledge from commercial manufacturing to improve process understanding, adjust control strategy, and ultimately ensure product quality. At Biogen Idec, a platform is being developed to collect, integrate and assimilate data from multiple sources, including continuous and discrete process data, raw material data, and product testing data. Data collection infrastructure, data analysis strategy, and implementation of process improvements are the key elements of this platform. Using a newly commercialized product as a case study, this presentation will illustrate the benefit of using this platform to integrate large volumes of process data from lab scale to two manufacturing sites of different scales as well as analyzing and transforming these data into process knowledge to drive process improvements. Examples will include process stability and capability analysis; understanding and mitigating product quality drift due to raw material variation; and enhanced understanding on the functional relationship between input process parameters and process performance. In addition, the body of process data and process deviations from the existing manufacturing batches were analyzed to refine and update the process parameter risk assessment. Improvements to manufacturing procedures and automation were implemented to mitigate risks identified and to improve process robustness.

Knowledge management in a CMO: mistakes, remedies and future state

Evi Dimitriadou (Lonza Group Ltd.)

For the last two decades, a vast amount of information has accumulated in paper records and "tribal knowledge" within the company. Data capture is gradually phasing into the LIMS system and high-throughput screening (HTS) data in early stage development are building up aggressively. Harvesting of process data and translating it into knowledge is an industry-wide challenge. This talk will outline the past, present and future state of data and knowledge management in Purification Development of Lonza Biologics, UK. Eleven years' worth of paper records were searched manually to populate an Impurity Clearance database; this has allowed us to evaluate different chemistries and steps in terms of their performance removing DNA, HCP, leached protein A and aggregates, and has guided the evolution of our platform. Trends arising from our Virus Clearance database have allowed us to identify steps that offer poor log virus reduction and thus eliminate them from Virus Validation stages. As a result we have been able to reduce Virus Validation prices by up to 30%, depending on the process. HTS data from early stage DSP development is stored and gradually being married with laboratory scale development and ultimately GMP scale; this is done by monitoring the drug's entire process lifetime. Results of a modeling study will be presented. Our future state is to integrate currently segmented databases and knowledge and our steps toward that end will be discussed.

Partnership

Session Chairs:
Uwe Gottschalk, Lonza Group Ltd., Basel
Joey Studts, Boehringer Ingelheim GmbH, Germany
Hanne Bak, Regeneron Pharmaceuticals, United States

It really does take a village- How a startup leveraged interactions with academia, suppliers, and an end-user to develop new technology for downstream process

Andrew Zydney (The Pennsylvania State University)
Oleg Shinkazh (Chromatin)

Purification is one of the remaining gaps for single-use technologies in downstream processing. It is a technically challenging and expensive operation, which may seem particularly daunting to innovators because of significant investment requirements, long adoption timelines, and high risk of implementation in GMP environments. In this talk we will discuss how a startup company's choices of key industrial and academic partnerships impacted the design and development of an innovative single-use continuous capture technology called Countercurrent Tangential Chromatography (CTC). Although there have been significant improvements in column chromatography, current technology providers have been largely unsuccessful at developing truly innovative / disruptive technologies for product capture, severely limiting the ability of manufacturers to fully exploit the opportunities for continuous or single-use processes. This presentation will examine the challenges and technical successes in Chromatan's efforts to develop the CTC system. All operations in CTC are conducted on a moving slurry that is continuously pumped through a cascade of static mixers (to achieve binding equilibration) and hollow fiber membrane modules (to separate the fluid phase from the resin particles). Contacting in the individual steps is performed in a countercurrent fashion using multiple stages to increase throughput, reduce buffer costs, and enhance product yield and purification. The development of CTC involved a partnership between Chromatan, a startup company, with academia (Penn State), two suppliers (Spectrum Laboratories and Life Technologies), and an end-user (Fujifilm Diosynth). This collaboration effectively combined the strengths of the individual partners, allowing the group to solve a wide range of technical challenges that have led to the successful development of this new downstream process technology.

Streamlining ADC development through partnering

John Liddell (Fujifilm Diosynth Biotechnologies)

The value of the ADC (antibody drug conjugate) market is estimated to grow to ca $9b within 10 years with two recent ADC approvals being Adcetris (Seattle Genetics, Inc.) and Kadcyla (Roche/ Genentech). Development of ADC's requires two quite different skill sets – biopharmaceutical development expertise together with conjugation chemistry expertise which is unlikely to be present in a single organisation. To facilitate development of ADC species, Fujifilm Diosynth Biotechnologies has operated a joint venture with Piramal Healthcare since 2012. The synergy obtained by integrated development of both components making up an ADC is significant giving compressed development timescales and early insights into ADC variants likely to give the best clinical performance.

Sharing in Implementing Best Practices when Entering Stage 2 Process Validation with a Partnership

Mark Teeters (Janssen R&D)
Marcelo Anderson (Biogen Idec)
Gene Schaefer (Janssen R&D)

Biogen Idec and Janssen have partnered in transferring several early and late phase clinical processes from Janssen Development into all scales of Biogen Idec Manufacturing over the last four years. To date, the success rate has been 97% with a reduction in overall tech transfer time and effort of >50%. With several of these processes now entering Stage 2 Process Validation, a focused team representing both companies collaborated in defining a standardized approach to Stage 2 and Stage 3 Process Validation activities for the partnership. This workshop presentation will discuss how we jointly defined a standardized approach, and share several different examples that contribute to our successes. Examples include: 1) leveraging site control procedures and document templates for PPQ batches by "mapping" Janssen control designations, 2) adopting site preferred filter trains and column storage procedures through simple process modifications, 3) leveraging leachable and extractable databases from the respective companies, 4) executing reduced-scale validation activities at the manufacturing site when preferred, and 5) jointly defining Stage 3 CPV procedures for a chromatography step. These examples will illustrate how both companies have benefitted from this collaboration through reduced process and operational risk and increased efficiency in Stage 2 and Stage 3 Process Validation activities.

Roche: Sartorius Stedim Biotech SRC Partnership

Annie Isaacson (Genentech, Inc.)
Rene Faber (Sartorius Stedim Biotech)

In June 2012, Roche launched a unique onsite global Supplier Relationship Center (SRC) at its Genentech site in South San Francisco, with the target to drive significant value creation and delivery from innovation through collaboration with five external partners. SRC innovation is created through a combination of dedicated people, processes, and environment. The custom built SRC facility includes leading edge technology and tools to facilitate the process. The SRC concept was born out of innovation and now supports the innovation of value. In a joint presentation, Roche and Sartorius Stedim Biotech will co-present their unique experience of partnership within the Alpha Class of the SRC.

Critical dimensions of the microchip: How partnerships and Moore's law defined the technology roadmap of semiconductor industry. Can there be lesson learned for the biopharmaceutical industry?

Marty Siwak (JSR Life Sciences)
Masayoshi Nagaya (JSR Life Sciences)

The average selling price of a transistor was $5.52 in 1955; today it is one billionth of a dollar. The remarkable advances were driven by the ever-shrinking critical dimensions of the integrated circuit. Technical progress was a key ingredient, but so was the confidence shared by industry players that Moore's law was possible, that it portrayed a roadmap, and that it would bring the expected result. This virtuous circle of transistor scaling, better performance/costs, market growth and investment to scaling again, was fueled by the semiconductor ecosystem. These partnerships crossed semiconductor companies, equipment and material providers, public and private research laboratories and institutes, and funding agencies. Several consortia evolved to develop the roadmap and the ground rules of industry partnership. These consortia and other partnerships will be reviewed with some clear examples of innovation and data-sharing towards common industry goals. What are the necessary conditions for industry-wide technical roadmap for Biopharmaceuticals? How extensive is our ecosystem? This review will hopefully bring some stimulating and contrasting discussions to the Recovery 2014 partnership workshop.

Comparability

Session Chairs:
Arne Staby, Novo Nordisk A/S, Denmark
Victor Vinci, Cook Pharmica, United States
Hari Pujar, Merck & Co., United States

One Molecule to Two Sites of Differing Scales

David Kahn (Eli Lilly and Company)

This case study highlights the challenges associated with transferring a monoclonal antibody purification process to two different sites within Eli Lilly's commercial manufacturing network. A cross-functional tech transfer team addressed significant differences in geographic location, scale, and equipment design while leaving the integrated control strategy (analytical methods, parametric parameters and raw materials) unchanged. Failure modes and effects analyses revealed challenges such as fixed versus portable equipment, a difficult to pack resin, a difficult to pack column, and volume constraints related to high concentration at the final TFF step. The volume constraints drove the design of a new TFF system which had to be integrated into the existing facility. In addition there were unexpected filtration challenges and analytical results that had the potential to impact comparability strategy. Ultimately the process at both locations was successfully validated.

Addressing Product Comparability Challenges in Second Generation Process Development

Mi Jin (Bristol-Myers Squibb Company)
Nicholas Abu-absi (Bristol-Myers Squibb Company)
Michael Borys (Bristol-Myers Squibb Company)
Zheng Jian Li (Bristol-Myers Squibb Company)
Siegfried Rieble (Bristol-Myers Squibb Company)

Achieving product comparability requires integrated effort of analytical characterization and process development for second generation process development. Detailed understanding of quality target product profile and CQAs, supported by consistent analytical methods to characterize these attributes and their variability ranges in the clinical and commercial manufacturing history, is a prerequisite for establishing the baseline for comparability. Upstream and downstream process changes can significantly impact multiple product quality attributes, which brings significant challenges for comparability. Here, we present a case study for a second generation process development of an Fc fusion protein with complex glycosylation pattern and multiple product related impurity control requirements. The challenges and approaches to comparability, with an emphasis on upstream and downstream process design and control, will be discussed.

Aggregation, Host Cell Protein and Facility Fit Challenges in the Development of a Multi-ton Monoclonal Antibody Production Process

Christopher Teske (Genentech, Inc.)
Michael Lee (Genentech, Inc.)
Mary Mallaney (Genentech, Inc.)
Atia Naim (Genentech, Inc.)
Maricel Rodriguez (Genentech, Inc.)
Stephen Woon (Genentech, Inc.)

This poster will describe development of a late-stage clinical / commercial purification process for a challenging high-mass demand monoclonal antibody. Development of a new high-producing cell line and cell culture production process warranted development of a new purification process. Early purification results using the existing purification process with the new cell culture process resulted in higher levels of aggregate and host cell proteins. Follow-up studies revealed increased baseline levels of aggregate in protein A pools as well as additional aggregate formation accelerated by low pH. Significant resin screening was conducted with both high throughput robotics and column experiments to develop downstream steps to reduce aggregate and host cell proteins to acceptable levels. An additional challenge emerged during process development when a specific host cell protein was detected in process pools. Initial robotic screening data to develop a method to separate the target protein and impurity protein did not appear promising. However, further column experiments using an unconventional mode of operation reduced the impurity to acceptable levels. Ultimately, a purification process was developed that met all product quality, impurity removal and productivity goals while enabling facility fit across our manufacturing network.

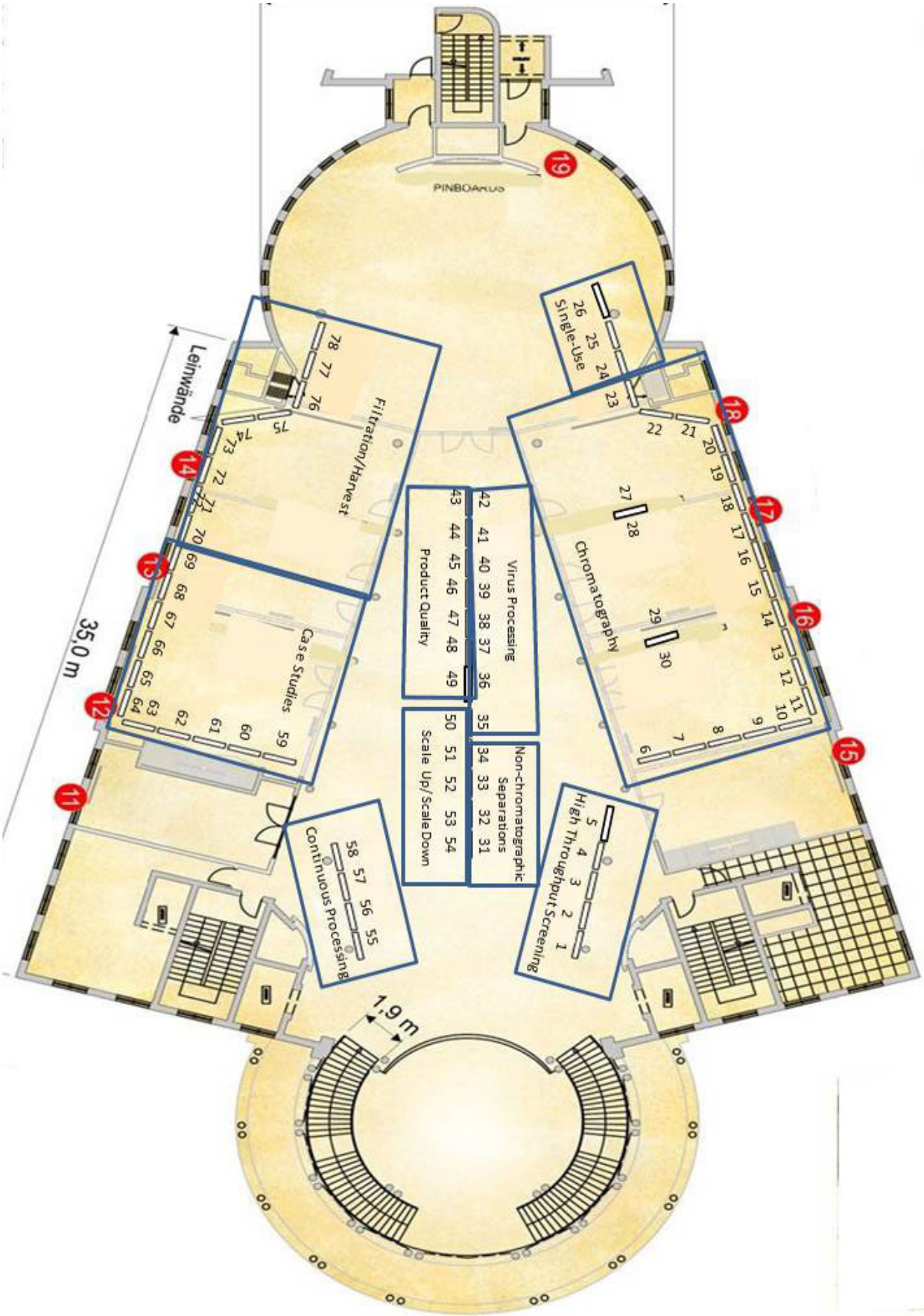
Hidden treasures in downstream process development

Mattias Ahnfelt (GE Healthcare)
Gunnar Malmquist (GE Healthcare)

Several factors need to be accounted for when designing new downstream processes, including variability in raw materials. However, it is typically realistic to use only a few lots of a raw material to test the potential impact of the material on process outcome. A better indicator of overall process robustness can be found by combining variation in raw materials and in process streams, but the effects on process outcome may go undetected if the inherent variability is not accounted for during process development. This may lead to serious challenges from a process life cycle perspective, including long term variability in raw materials and neglecting potential interactions between raw materials and product attributes. This may in some cases greatly increase the risk of lot failure even years after a successful regulatory filing. Illustrated by real life case studies, we will address some of these challenges from a chromatographic media raw material point of view, leveraging a proactive approach based on closer collaboration and data exchange between media vendor and end users using state of the art multivariate statistical methods. This approach will quantify, and subsequently significantly reduce, the long term process risks already in the process development phase. It will also enable combined life cycle management of both the chromatographic media and the protein purification processes.

Poster Abstracts

POSTER SESSION SITE MAP



POSTERS

1) Applying DoE and HTS for the selection of new versatile cation exchange mixed-mode prototype sorbents using new chromatography performance attributes

Rene Gantier (Pall Corporation)
R. Alexander Martino (Pall Corporation)
Mark Schofield (Pall Corporation)
Magali Toueille (Pall Corporation)
Audrey Uzel (Pall Corporation)

Mixed-mode (or multi-mode) chromatography is implemented in many process developments for the purification of monoclonal antibodies (mAbs) and recombinant proteins, and the existing library of commercial mixed-mode sorbents is expanding. The development of new mixed-mode sorbents therefore requires strict selection methods to offer unique benefits for the end-user. Different critical parameters (ligand chemistry, ligand density, bead size, base matrix pore size and porosity…) are generally reviewed to design a library of prototypes which can then add up to several hundred of sorbent candidates. It is then important to use rational methods to rigorously screen prototypes for the selection of the best and unique performance candidates. In the present study we have applied design of experiment (DoE) and high throughput screening (HTS) to select high performance cation exchange mixed-mode sorbents from a library of prototypes. Ligand chemistries were first screened using 96-well sorbent plates for a new performance attribute to measure their ability to do protein purification in both bind/elute and flow through modes for a wide range of protein iso-electric point and hydrophobicity. The selected ligand chemistries were then used to generate prototypes with different bead sizes, pore sizes and porosities. Those candidates were screened, using high throughput techniques, for standard performance attributes like protein static binding capacity (SBC), dynamic binding capacity (DBC), elution volume and yield of recovery. Candidates were further selected for their ability to separate model proteins in a pH gradient (protein selectivity). Finally, the best candidates were tested for protein DBC and regeneration studies using different real feedstocks. The HTS approach enabled a more complete understanding of the interactions of different ligand moieties with protein ranges and combined with the use of new quantified performance attributes allowed us to isolate a bank of ligands with unique and versatile cation exchange mixed-mode sorbent functionalities.

2) High throughput evaluation of chromatographic media selectivity by differential Kp screening

Thomas von Hirschheydt (Roche)

Novel antibody formats often come along with novel types of product related byproducts which may not be addressed by generic purification protocols. There is a need for enhanced/additional selectivity in chromatographic separation. Mixed mode phases turned out to be powerful separation tools on the one hand. Separation however is hard to predict and tedious to develop on the other hand. High throughput Kp screening is a common batch mode method to describe the binding and elution behavior during chromatography in (e. g.) a conductivity-pH-matrix. To get a quick insight into the separation potential of a certain stationary phase at an early stage of purification process development we developed the concept of differential Kp screening. The idea is to describe the binding-elution behavior of a crude mixture containing all byproducts of interest in one Kp screen and the characteristics of the pure product in another screen under the exactly same conditions. Both screens are then combined in a differential plot (by subtraction of the UV280 signals of corresponding plate positions) which indicates conditions where either the byproducts or the product selectively elute. Both screens can be performed on the same 96 well plate (48 data points for each screen) within half a day. Subsequently, the results have to be confirmed in a verification run under real chromatographic conditions. By applying this method we were able to rapidly identify two process alternatives for capturing and intermediate purification in a bispecific antibody case study.

3) A high throughput automated approach for studying precipitation and subsequent centrifugal isolation of therapeutic proteins

Chris Morris (University College London)
Paul Dalby (University College London)
Stefanos Grammatikos (UCB Pharma)
Edith Norrant (UCB Pharma)
Mark Pearce-Higgins (UCB Pharma)
Mariangela Spitali (UCB Pharma)
Nigel Titchener-Hooker (University College London)

With cell culture titres increasing to ever higher multi-gram concentrations, the shift of production costs from upstream to downstream processing operations is becoming more pronounced. Deviation from traditional chromatographic separation techniques to alternative solutions is being driven by process economics as bottlenecks are becoming further highlighted by resin capacities and processing times. Protein A chromatography is the primary capture step of choice by most antibody manufacturers but challenges remain over economic value, reusability and ligand leaching. The aim of this research was to investigate the application of precipitation to the primary purification of monoclonal antibodies in development. Precipitation can offer an old solution to a new challenge; it can struggle to compete with affinity techniques for selectivity, however comparable yields are achievable whilst offering faster processing times, lower cost unit operations, volume reduction and improved performance for higher titre feedstreams. This study demonstrates a micro-scale high throughput automated screening methodology for developing an optimised salt-driven precipitation process, taking into account centrifugal recovery and re-suspension of precipitates whilst monitoring product structure and function. Fully automated microscale techniques were demonstrated allowing for fast, precise and reproducible data generation with small material requirements. Precipitation, mixing, centrifugal separation and phase removal, re-suspension of protein pellet and assay preparations were all carried out in the 96 well format using a liquid handling platform. Screening on pure protein solutions allowed for rapid data generation investigating yield, feed, and processing conditions. This understanding was then translated successfully to mammalian cell culture fluid whereby yield and selectivity could optimised taking into account feed composition. A series of salts were investigated for their salting-out capability and optimised for the best yield and selectivity. Yields of >95% were exhibited by all, with purities ranging from 55-85% due to variation in ion-protein selectivities between the target protein and host cell proteins. Although a disruptive process, each salt precipitant under optimised conditions was shown not to affect the product quality, increase protein aggregation, or show variation in charge species. The method proved to be versatile, robust and well suited for characterizing the viability of precipitation as a purification method for therapeutic proteins. The automated approach provides a means for investigating novel precipitation approaches whilst building an understanding of how potential critical process parameters impact the resulting process design space.

4) Understanding & Disrupting Host-Cell Protein-Antibody Interactions Using High-Throughput Screens

Benjamin Tran (Genentech, Inc.)
Kelsey Dent (Genentech, Inc.)
Vanessa Grosskopf (Genentech, Inc.)
Paul McDonald (Genentech, Inc.)
Don Walker, Jr. (Genentech, Inc.)
Christopher Yu (Genentech, Inc.)

Purification processes for monoclonal antibodies typically exploit multiple and orthogonal chromatography steps in order to remove impurities such as host-cell proteins. While the majority of host-cell proteins are cleared through these steps, individual host-cell proteins can persist and are more challenging to remove. High-throughput screens using protein A chromatography can be used to understand the interaction of host-cell proteins with our antibody products and their co-purification in the protein A eluate. Spiking studies using either null harvested cell culture fluid or purified host cell proteins show that the level of host-cell protein interaction with our antibodies is dependent on the individual antibody as well as process conditions such as the use intermediate washes. Liquid chromatography-mass spectrometry analysis of elution pools from these studies further showed that the species of co-eluted host-cell proteins can vary between individual antibodies. We subsequently demonstrated that a high-throughput intermediate protein A chromatography wash screen can identify promising conditions for improved host-cell protein clearance that translate to improved clearance in packed-bed columns.

5) The application of high throughput screening to the chromatography development of bispecific antibodies

Paul McDonald (Genentech, Inc.)
Ben Tran (Genentech, Inc.)
Ambrose Williams (Genentech, Inc.)

High throughput screening using robotics is routinely used to develop the purification processes for monoclonal antibodies and antibody fragments. We are adapting these screens to meet the development needs of new format molecules such as bispecific antibodies. Bispecific antibodies present novel purification challenges compared to typical monoclonal antibodies. Starting feedstocks tends to be more heterogeneous placing a burden on downstream chromatography steps to reduce several different product variants, typically present at higher levels than in monoclonal antibodies. Case studies will be presented demonstrating how robotics screens in combination with high throughput analytics and data analysis tools can be used to rapidly identify chromatography conditions for the removal of multiple product variants, accelerating the process development for these next generation antibody therapeutics.

6) Mechanistic investigations of IgG adsorption onto high capacity Protein A resins

Egbert Müller (Tosoh Bioscience GmbH)
Judith Vajda (Tosoh Bioscience GmbH)
Angelika Wacker (Hochschule Mannheim)

The growing demand for monoclonal antibodies (mAb) is a major driver for the development of more efficient manufacturing technologies of these highly selective drugs. Since upstream technologies have been improved during the last decade, the main focus of potential process reforms is now put on downstream technologies. Recently, a new generation of protein A high capacity resins has been developed. Capacities higher than 65 g/L at a residence time of 5 min are possible. Miscellaneous protein A ligands are derived from different domains of the natural protein A of *S. aureus*. Differences in the elution pH and purity have been observed. A potential mechanism involving preferred VH3 domain binding has been described by Ghose et al. in 2005[1]. In the current study, we use a quartz crystal microbalance for a detailed investigation of the adsorption and desorption mechanism of a monoclonal antibody to a high capacity protein A ligand. Findings are correlated with mAb capacity and yield at a given pH. mAb purity and recovery were determined in a design of experiments based approach, employing robotic based parallel chromatography. Interestingly, yield is higher for the high capacity ligand than for a conventional ligand, both derived from the same domain of natural protein A.

[1] Ghose, S., Allen, M., Hubbard, B., Brooks, C., Cramer, S., Antibody Variable Region Interactions with protein A: Implications for the Development of Generic Purification Processes. Biotechnology and Bioengineering 2005, Vol. 92, No. 6, p 665-673.

7) Effects of Salt Induced Reversible Self-Association on the Elution Behavior of a Monoclonal Antibody in Cation Exchange Chromatography

Haibin Luo (MedImmune, LLC)
Yuling Li (MedImmune, LLC)
Nathaniel Macapagal (MedImmune, LLC)
Adrian Man (MedImmune, LLC)
Kelcy Newell (MedImmune, LLC)

Some monoclonal antibodies (mAbs) have been reported to display concentration-dependent reversible self-association (RSA). There are multiple studies that investigate the effect of RSA on mAb formulation such as viscosity, opalescence, phase separation and aggregation. This work reports and investigates the effects of RSA on a bind-and-elute mode cation exchange chromatography (CEX). We report a case study in which the RSA of an IgG2 (mAb X) resulted in significant peak splitting during salt gradient elution in CEX. Multiple factors were evaluated and demonstrated little effect on the peak splitting of mAb X including resin type, load challenge, residence time and gradient slope. It was determined that high NaCl concentrations in combination with high protein concentrations induced mAb X to form RSA species that bound more strongly to the column, resulting in a large second elution peak. The finding of NaCl-induced RSA suggested that lower NaCl elution concentrations and different types of salts could mitigate RSA and thus eliminate peak splitting. Different salts were tested, showing that chaotropic salts such as CaCl2 reduced the second elution peak by inducing less RSA. The addition of a positively charged amino acid (such as 50 mM histidine) into the CEX elution buffer resulted in elution at lower NaCl concentrations and also effectively reduced peak splitting. However, experiments that were intended to reduce salt concentration by increasing the elution buffer pH did not significantly mitigate peak splitting. This is likely because higher pH conditions also increase RSA. This work identifies salt-induced RSA as the cause of peak splitting of a mAb in CEX and also provides solutions to reduce the phenomenon.

8) Protein monomer separation by chromatography: Mobile phase and stationary phase properties that enhance the separation performance

Shuichi Yamamoto (Yamaguchi University)
Yu Isakari (Yamaguchi University)
Daisuke Itoh (Yamaguchi University)
Noriko Yoshimoto (Yamaguchi University)

Dimer and aggregate removal is important for protein separation processes. The separation can be done based on size, charge, hydrophobicity, and mixed-mode interaction. Size-based separation such as size exclusion chromatography (SEC) is possible only for small production size. Charge- or electrostatic interaction based separation such as ion-exchange chromatography (IEC) is most frequently employed. Mixed-mode or multi-mode chromatography including hydroxyapatite chromatography is also used. Since dimer and aggregates are more hydrophobic and have more charges, their retention volumes are larger in IEC. However, the resolution is not good enough without fine tuning of conditions such as operating parameters (flow-velocity, gradient slope) or mobile phase properties (pH, salt concentration). In this study, methods for improving monomer-dimer(aggregate) separation by IEC were developed. First, polyethylene glycol (PEG) was added into the mobile phase as a separation enhancer. The retention volume increases with increasing PEG molecular weight and/or PEG concentration. The shift of the retention volume became larger with increasing protein molecular weight. Consequently, almost baseline separation of a model protein separation system (BSA monomer-dimer) was possible by anion exchange chromatography (AIEC) with Q-Sepharose HP and QA-CIM monolith disk columns. This shift was considered to be due to "preferential exclusion". In order to understand the retention mechanism the binding site values were obtained over a wide range of mobile phase pH with and without PEG. We have also analyzed the separation efficiency based on the solubility curves of monomer and dimer and the peak spreading due to increased viscosities of the mobile phase. Secondly, the separation performance with a salt-tolerant polyamine anion exchange ligand was investigated. This AIEC 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 AIEC 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.

9) Understanding mAb monomer/aggregate separation: mechanistic analysis of cation exchange variants using CLSM and chromatography techniques

Matthias Joehnck (Merck KGaA)
Bernd Stanislawski (Merck KGaA)

Cation exchange chromatography (CEX) is widely used for the purification of post-protein A mAb feedstreams in downstream processing, aiming at efficient removal of dimers and higher molecular weight (HMW) impurities, host cell proteins and leached protein A. As cell culture processes are pushed to ever higher titres, the level of mAb aggregates has also increased. Aggregates of monoclonal antibodies have the potential to cause immune responses and therefore must be reduced to safe levels. Although a huge variety of different cation exchange resins are available on the market, the relation between the physicochemical characteristics of the resins and their respective separation efficiency in the mAb monomer/aggregate separation is not well understood. This presentation demonstrates, by combining confocal laser scanning microscopy (CLSM) and chromatographic techniques, how monomer and dimer binding and elution mechanisms vary as function of systematic variations of cation exchange physicochemical resin characteristics. For this work a variety of CEX resins varying in pore size, particle size, strong and weak cation exchange ligand density and different surface chemistries were synthesized, all exploiting the same polymeric base bead chemistry. The resins were then tested in the purification of post-protein A mAb feedstreams containing different levels of aggregates. The eluted fractions were analyzed with regard to content of monomer, dimer, higher molecular weight species. This testing provides a systematic examination of the relation between physicochemical resin characteristics and separation properties. To understand these findings mechanistically, CLSM studies were performed. mAb monomer and the corresponding dimer was purified and labeled with different fluorescence marker molecules, without significantly changing binding characteristics on cation exchange resins.

As the surface chemistry of the resin is varied, mAb dimers either bind to the outer surface of resins, or occupy binding sites within the porous systems of the bead, while monomer binding is typically more homogenous as function of the bead cross section. Depending on these binding positions, separation of monomer from dimer during elution is significantly altered. The study clearly reveals that the variation of resin characteristics has a huge effect on the separation of mAb monomer from HMW impurities. Combination of our chromatographic results with the CLSM studies provides the needed mechanistic understanding to enable development of significantly improved resin performance for mAb downstream processing. To the best of our knowledge, our results give for the first time a mechanistic view at CEX resin parameters which determine mAb separation efficiency with the main focus on aggregate removal.

10) A 21st century process for chromatography media design

Gunnar Malmquist (GE Healthcare)
Per-Mikael Aberg (GE Healthcare)
Mattias Ahnfelt (GE Healthcare)
Jesper Hansson (GE Healthcare)
Bengt Westerlund (GE Healthcare)
Susanne Westin (GE Healthcare)
Anna Åkerblom (GE Healthcare)

Coping with industry demands, the process for design of chromatographic media has moved from art to science. In the recent years we have not only leveraged the Design For Six Sigma framework, but also moved from deterministic models describing an ideal situation without any errors or deviations to a probabilistic view built on Monte Carlo simulations where realistic estimates of future process variability explicitly are accounted for in the process design. The next step forward is to apply the Quality by Design framework where the effect of chromatography media variability on quality attributes and process attributes is elucidated. This requires well designed experiments involving dedicated prototype media with variations in factors that may affect the performance according to a risk assessment. Combining the experimental design approach with characterization protocols focused on relevant quality attributes allows development of an adaptive process control strategy where identified process parameters can be used to increase process robustness in the light of inevitable raw material variability. The media design process described above will be illustrated by examples from the development of a new chromatography medium where the balance between process and quality attributes has been addressed from a user perspective.

11) Mixed Mode Resins and their Lot-to-Lot Variability in Acidic Peak Group Modulation

Hans Rogl (Boehringer Ingelheim GmbH)
Frederick Rudolph (Boehringer Ingelheim GmbH)

Antibodies are complex proteins that show a high degree of microheterogeneity including charge-, hydrophobicity-, glyco- and size-related variants. Charge heterogeneity can have diverse molecular origin and manifests in acidic and basic peak groups determined by analytical ion exchange chromatography. In this case study it is shown how a purification step was developed to modulate the acidic peak group content of the product in order to match a given quality target product profile which differed from the material produced in the cell culture process. Several polishing resins were assessed and a concept for comparing their selectivity was developed. As a mixed-mode resin showed best results, the lot-to-lot variability and its impact on critical quality attributes of the product were assessed for further characterization. The study outlines the contribution of the downstream process to hit a narrow product quality target, and how critical raw materials (resin) can be controlled to maintain high and excellent product quality.

12) Productivity comparison among different chromatographic stationary phases

Giulio Sarti (University of Bologna)
Cristiana Boi (University of Bologna)
Jouciane de Sousa Silva (University of Bologna)
Simone Dimartino (University of Canterbury)
Oman Herigstad (Abbott Laboratories)

The development of new convective stationary phases with improved binding capacity has greatly progressed in recent years; however the use of membrane adsorbers and monoliths for capture chromatography is still limited to niche applications as the workhorse of downstream processing is played by bead based chromatography. Conventional packed bead columns are preferred for their longer presence on the market and for their higher binding capacity, even if they suffer from several limitations such as high pressure drop, slow mass transfer through the diffusive pores and strong dependence of the binding capacity on flow rate. In all cases, however, a proper comparison with convective media columns, packed with membranes of monoliths, should be made in terms of productivity at the required purity. In this work a systematic comparison between the two processes based on bead columns and convective materials has been developed using an integrated approach that combines theoretical calculations and experiments. The performance of membrane adsorbers, monoliths and packed bed column has been studied experimentally in the same pilot scale chromatography system. Different affinity pairs have been characterized in detail using both BSA and IgG as target molecules, under a broad range of operating conditions to investigate the effects of operating parameters like feed concentration and flow rate on the separation performance. Material selectivity was evaluated using complex feed solutions like cell culture supernatant and sera. The results obtained have been compared in terms of binding capacity, flow velocity, selectivity, yield and productivity and a critical evaluation among the three chromatographic supports will be presented which demonstrates, in all the cases inspected, the superior productivity of convective media with respect to conventional beads. The productivity comparison among the different chromatographic media has been extended to a relatively broad range of operating conditions by using also the mathematical model for convective chromatography, which has been developed by our research group. The model accounts for the relevant chemical and physical mechanisms affecting the process in all the stages and has been validated with proper experimental data. Remarkably, describes very well all the stages of the process for complex mixtures, even though its parameters are determined through separate tests independent of the chromatographic process, apart from the binding/eluting kinetic constants which require only chromatography tests for pure solutions of the target biomolecule. Use of the model points out the effect of the convective media properties, elucidating the main operative differences between the membrane and monolithic columns for protein affinity chromatography. In particular, it is confirmed that the productivity difference becomes more marked as the flow-rate increases, in favor of the convective media.

13) Application of Mechanistic Chromatographic Models to Support Process Development and Characterization

John Moscariello (Amgen Inc.)
Yan Brodsky (Amgen Inc.)
Stephen Hunt (Amgen Inc.)
Trent Larson (Amgen Inc.)
Ashish Sharma (Amgen Inc.)
Ben Smith (Amgen Inc.)
Robert Todd (Amgen Inc.)
Kevin Tolley (Amgen Inc.)

Purification process development is often a trade-off between time and resources and process understanding. Recent advances in high throughput screening and the implementation of design of experiments (DoE) have resulted in a significant increase in process understanding; however these approaches result in empirical relationships between the subset of variables investigated with little mechanistic understanding of the separation. This presentation will discuss the use of computer simulations utilizing the general rate model for cation-exchange chromatography to predict chromatographic separations, specifically separation of monomer from soluble aggregates. Multiple case studies will be provided that will showcase the use of mechanistic models to identify the appropriate resin and operating conditions for process development and a thorough characterization of a defined process to support commercialization. Lastly, the presentation will conclude with a discussion of the benefits and limitations of mechanistic models relative to traditional use of high throughput screening and empirical models established through DoE.

14) Cationic Mixed Mode Resins: comparison of IgG1 binding and elution

Andreas Schaubmar (Roche)
Regina Reicherstorfer (University of Applied Sciences, Campus Vienna)

Electrostatic and hydrophobic interactions are the major forces used in separation processes for protein purification. New mixed-mode resins combine these two components in a single matrix and represent potential for new separation opportunities. We have compared commercially available cationic mixed-mode resins with regard to their binding and elution behavior for IgG1 antibodies. The binding of an antibody to eight resins under varying salt conditions was evaluated in batch binding experiments at pH 6.0. In a concentration range between 0mM and 1500mM, ammonium sulfate and potassium chloride were applied to differentiate the hydrophobic and electrostatic component of protein binding. Subsequently, linear gradient elution experiments with a mixture of two different antibodies were performed to characterize the separation capability of two resins, when using them either in a hydrophobic interaction or ion exchange mode. We could show that some cationic mixed-mode resins can be applied in an IEX and a HIC mode for antibody purification, whereas others did not display apparent hydrophobic interactions with the proteins under the conditions tested. In summary, on true cationic mixed-mode resins both ionic and hydrophobic conditions can be used either alone or in combination to achieve separation. The applied salt is of relevance as complete elution is only achieved if a discontinuous transition between hydrophobic and ionic interactions is enabled. These properties provide increased experimental space to solve challenging purification problems.

15) Partnership in Custom Affinity Chromatography Development for Novel Molecule Purification: Selectivity by Design

Xiangyang Wang (MedImmune, LLC)
Pim Hermans (Life Technologies)
Alan Hunter (MedImmune, LLC)

To bring innovative therapies to the clinic, research teams are exploring new and diverse classes of molecules such as recombinant toxins, enzymes and blood factors. Compared to mAbs, these novel products typically lack an affinity chromatography option. This leads to greater process complexity, longer development timelines, and limited or no platform opportunities. To date, affinity chromatography has been mostly reserved for separation of process related impurities such as HCP and DNA. Reports of affinity purification of closely related product variants and modified forms are much rarer. This presentation describes a partnership with BAC (part of Life Technologies®) in custom affinity chromatography development using Camelid VHH antibody fragments as "tunable" immunoaffinity ligands. One example demonstrates high selectivity for a recombinant immunotoxin where *no binding* was observed for the undesired deamidated species. Also discussed is affinity purification of a single chain fusion protein through specific recognition of fully carboxylated gamma-carboxy glutamic domains with no detectable cross binding towards inactive – or clipped forms of the protein.

16) Ensuring long term robustness of a CIEX chromatographic step for separation of charge variants with optimized yield

Eva Rosenberg (Roche)
Mattias Ahnfelt (GE Healthcare)
Eggert Brekkan (GE Healthcare)
Karin Haeringer (Roche)
Stefan Hepbildikler (Roche)
Karol Lacki (GE Healthcare)

To ensure lot-to-lot consistency, the control of raw materials such as chromatographic media is important, since their variation can directly affect both, the final biopharmaceutical product and the process. Since the final variance is the sum of variation from all steps involved, the process itself has to have "ability…to tolerate variability of materials and changes of the process and equipment without negative impact on quality" (according to ICH Q8/R2). Thus, the manufacturer needs to understand all critical attributes of raw materials and moreover how to control the variability to ensure consistent supplies. In this work, the preparative separation of charge variants of a monoclonal antibody by using cation exchange (CIEX) chromatography prove challenging, as the isoform pattern and the yield of the step presumably depends on a combination of properties of the chromatography medium and the process. Thus, data was collected from different scales by applying different media lots. Afterwards, a statistical model for assessing potential of different CIEX lots to separate the charge variants according to process specifications was developed. On the basis of that, potential critical attributes of the CIEX medium and the process responsible for the observed variations were investigated. The primary rationale is to achieve long term robustness of the CIEX step for separation of charge variants of this monoclonal antibodies by concomitantly optimizing the yield. In addition, a methodology for fast and advanced characterization of different chromatographic media lots to support a robust manufacturing process at large scale is aimed.

17) Isoelectric pH of Charged Surfaces Tethered with Proteins via Neutral Polymers

James Van Alstine (Royal Institute of Technology)
Kazunori Emoto (University of Alabama)

Biomaterial surfaces coated with neutral hydrophilic polymers (NHPs) are used to shield surface charge groups and reduce nonspecific protein adsorption. NHP coated surfaces to which ligands or proteins have been covalently tethered hold promise in regard to development of low fouling sensor or catalytic surfaces[1, 2]. Several commercial ion exchange resins are based on attaching specific charge groups, at relatively low substitution, to NHPs such as dextran or polyacrylamide in order to form charge modified NHP (CMNHP) coated resins[3]. Various published experimental and modeling studies suggest that hydrophilic proteins localized at an NHP or CMNHP coated surface may osmotically tend to be situated at or in the external region (brush) of surface localized polymers[1, 2, 4]. Such proteins are expected to contribute significantly to follow on protein adsorption, as well as surface potential, isoelectric pH, and electroosmotic flow. They may therefore affect the performance of sensors, biomaterials, and ion exchange resins. In the present study electroosmotic measurements conducted at pH 2 to 11 were used to characterize a series of organosilane and NHP [poly(ethylene glycol)] coated quartz surfaces to which a series of four proteins of pI 1 to 11 were grafted. The measurements provided quantitative information in regard to surface charge densities, NHP coating region thicknesses, and effect of protein tethering on surface isoelectric pH, i. e. bulk pH at zero electroosmosis (PZE). They may therefore provide insights to construction of novel bioanalytical and bioseparation surfaces. One interesting result is that changes in PZE varied linearly with protein pI, the most striking changes being seen for protein-tethered NHP-coated surfaces.

[1] Chemistry and Biological Applications of PEG Chemistry. J. M. Harris, S. Zalipsky (Eds.), ACS Symposium Series Vol. 680, American Chemical Society, Washington D. C, 1997.
[2] Malmsten, M., Emoto, K. and Van Alstine, J. M., J. Colloid Interf. Sci., 202 , 507-517, 1998.
[3] Lenhoff, A. M., Journal of Chromatography A, 218, 8748-8759, 2011.
[4] Johansson, H.-O., Van Alstine, J. M., Langmuir, 22, 8920-8930, 2006.

18) Insights into the nature of multimodal chromatographic selectivity using a designed library of Fab variants

Hanne Sophie Karkov (Rensselaer Polytechnic Institute)
Haleh Ahmadian (Novo Nordisk A/S)
Are Bogsnes (Novo Nordisk A/S)
Steven Cramer (Rensselaer Polytechnic Institute)
Berit Olsen Krogh (Novo Nordisk A/S)
Siddharth Parimal (Rensselaer Polytechnic Institute)
James Woo (Rensselaer Polytechnic Institute)

In this study, various protein surface property characterization tools were evaluated for their ability to predict multimodal chromatographic behavior and to improve our understanding of selectivity in these systems. We hypothesized that multimodal ligands, containing both hydrophobic and charged moieties, would interact strongly in protein surface regions where charged groups are in close proximity to hydrophobic patches. A series of antibody Fab fragments was designed *in silico* to generate different combinations of surface hydrophobicity and electrostatic potential and to explore the relative importance of local surface properties on protein retention. The Fab variants were generated by site-directed mutagenesis, expressed transiently in HEK293 cells and purified by affinity chromatography. Gradient column experiments were carried out with the resulting Fab variants in multimodal, ion-exchange and hydrophobic interaction chromatographic systems, examining the effect of ligand chemistry and ligand density on the selectivity of these materials. Retention behavior in all of these systems correlated well with predictions based on specific protein surface property evaluations. Importantly, this work sheds light on why different closely related Fab variants are able to be separated by different chromatographic modalities. This opens up the possibility of using *in silico* protein surface characterization techniques to aid in the design of new generation biologics for their biomanufacturability.

19) Protein A – Beyond Simple Capture

Nanying Bian (EMD Millipore)

With new antibodies and Fc-containing molecules being developed for better pharmacokinetics and specificity, there is an increased pressure on downstream purification, which may not always be addressed by the standard 3-step mAb purification used in the industry today. Protein A affinity chromatography has played a critical role in such downstream processes due to its specificity and efficiency in the removal of soluble impurities from clarified cell culture. However, the commonly established one-step elution protein A capture regimen might be under-utilizing protein A's purification potential, especially in the presence of antibody variants. Aggregate removal is a persistent challenge in mAb purification that is typically addressed using cation exchange chromatography. We have discovered that, with simple modification of elution conditions, protein A affinity chromatography not only removes aggregates and fragments from a target monomeric antibody, but also separates different variants of the Fc-containing molecules. Exploiting this property of a protein A resin could potentially improve downstream purification processes, increase process robustness and reduce the burden on the subsequent chromatography steps. In this study, a protein A affinity resin was investigated extensively for the removal of aggregates using several mAb molecules under different conditions. Aggregates were found in the early elution pools from the protein A resin in addition to the tail end as has been reported, leading to a greater percentage of total aggregate removal in this step as compared to that of the controls. Protein A elution pool purities and monomer yields are compared among different commercially available resins. The authors acknowledged that the implementation of a method to remove aggregates at the protein A step could require some process development due to differences in the mAb molecules and their aggregate content. We hereby propose a universal process development methodology to ensure that this application is amenable to scale-up and manufacturing settings. Further, preliminary understanding of the mechanism of protein A aggregate removal was attempted by studying protein-protein interactions with and without labels. The protein A resin has demonstrated effective aggregate removal, potentially alleviating the burden on the downstream chromatography steps. This leads to an even more robust aggregate removal purification process, especially when the suitable protein A chromatography step is combined with the appropriate choice of cation exchange resin.

20) The use of large experimental and simulation data sets for creating fundamental understanding and facilitating process development in multimodal chromatography

Steve Cramer (Rensselaer Polytechnic Institute)
Siddharth Parimal (Rensselaer Polytechnic Insitute)
Kartik Srinivasan (Rensselaer Polytechnic Institute)
James Woo (Rensselaer Polytechnic Institute)

In this presentation a protocol is presented for how to use large sets of chromatographic and biophysical data in concert with molecular and course grained simulations to provide both fundamental understanding of selectivity in multimodal chromatography and to significantly facilitate the development of selective and robust downstream processes. In multimodal (MM) chromatography interactions exist between four key components: proteins, MM ligand coated surfaces, fluid phase modifiers and water. The complex interplay of these interactions, multimodal or otherwise, can result in unique selectivities which can be very difficult to predict and control. All-atom explicit MD simulations are employed with a range of proteins, MM ligands, and fluid phase conditions to generate a wealth of data on the interactions of different MM ligands and surfaces with proteins. In order to use the large amount of data generated in these simulations, a spherical harmonic analysis is employed to enable the direct interrogation of the data to test various selectivity hypotheses derived from a large set of experimental chromatographic and biophysics data (QCM, NMR, SPR and ITC). The results of this analysis are then used to identify key surface properties for proteins and MM ligands which play an important role on their interactions in various MM systems. Coarse-grained protein surface characterization techniques are then employed to translate these key protein surface regions and properties into quantifiable physicochemical descriptors. The resulting sets of descriptors are then screened in several QSPR models and the optimal set are then used to classify and predict protein behavior in MM systems under a wide range of conditions. An important aspect of this work is the multiple feedback loops which identify the key characteristics relevant for different chromatographic systems and which guide the modifications of the descriptor algorithms for the refined models. The approach described in this presentation provides an example of how the knowledge base created at different scales of investigation can be combined and utilized to identify unique opportunities for selective, robust and efficient downstream bioprocesses.

21) Evaluation of novel affinity bio-beads for use in the production of plasma derived albumin

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Sara Ladd (CSL Limited)
Jose Martinez (CSL Limited)
Karl McCann (CSL Limited)
Sergio Pagliazzi (CSL Limited)
Tracy Thompson (CSL Limited)

The purification of albumin at CSL Limited from human plasma involves a combination of chromatographic steps. The final gel filtration polishing step has limitations and constitutes a major process bottleneck. The aim of the project was to find an alternative to gel filtration. An affinity chromatography process involving the use of a new biological resin - bio-beads, was evaluated. Bio-beads are produced in bacteria and can be made to incorporate specific ligands onto the polyester backbone. As the main contaminants of the in-process crude albumin intermediate are IgG, IgA and IgM, the utility of two types of bio-beads to remove impurities were examined. These were Polybind-Z, with a specific affinity for IgG and Polybind-L which is specific for the kappa light chain and able to interact with IgG, IgA and IgM. The results obtained showed that Polybind-Z had high binding capacity and specificity for IgG, but low specificity for IgA. Polybind-L had bound IgM and to a lesser extent IgA and IgG. Given the small size and structure of the bio-beads compared to typical chromatographic resins, they cannot be used in chromatographic column mode. Therefore alternative batch mode approaches were examined for the use and removal of bio-beads, with potential for use at large scale. The use of depth filtration coupled with a filter aid was found to be a successful means of removing bio-beads from a treated solution and resulted in no residual resin detected by microscopic examination in the resultant filtrate. This process could be readily scaled up and optimised for surface area back pressure and through put. This study shows that novel bio-beads with specific ligands have the capability of removing significant amounts of trace immunoglobulin contaminants from a predominantly albumin matrix and suggests that they have potential to be used in an alternative low cost method for polishing an in-process albumin intermediate at large scale.

22) Mixed Mode Separations – Can we do without them in Bioseparations?

Milton Hearn (Monash University)

This presentation examines the need for a paradigm shift in the bio-separation and bio-recovery of bio-macromolecules, and proteins in particular. Underpinning the described work has been a series of experimental studies that address several fundamental questions related to what forces are involved for water, or alternatively an organic solvent or an ionic liquid, at a liquid-solid interface to make proteins shrink, expand or stay the same size as found in their solid (crystalline) state and secondly how do different ions or buffer species in water, or alternatively in an organic solvent or an ionic liquid, modulate the physical or chemical properties of a solid stationary phase surface that contains a dominant hydrophilic or hydroxylic backbone with attached or pendant chemical ligands of different mixed mode functionality and structure? Because of their relevance to practical separation outcomes, answers to these fundamental questions have challenged scientists and engineers over the past decade, with a number of key aspects yet to be fully elucidated. The availability of additional characterisation tools, including zeta potential (ZP) measurements, energy-dispersive X-ray spectroscopy (EDX) analysis, 2H NMR studies to differentiate between surface 'frozen' and 'unfrozen' solvent at different temperatures, isothermal titration microcalorimetry, ATR-FTIR analysis, SAXS investigations and phase transition monitoring, has in particular opened up new avenues to address the above questions. In these studies, our objective was to deploy a set of accessible experimental tools to more precisely characterise the nature of the interactive surface of existing and new types of chromatographic adsorbents and secondly to provide insight into the fundamental mechanisms involved in protein-chromatographic resin interactions, which lead to a successful separation or alternatively to aggregation or denaturation. Importantly, this knowledge has been found to assist in the more rational evaluation of the impact of eluents of non-traditional composition, and to better classify the behaviour of mixed mode adsorbents in terms of their selectivity and application utility. Arising from these findings, an approach has been established for the design and development of new types of adsorbent materials able to undergo phase transitions upon application of an external stimulus, not only with water rich eluents but also in water depleted environments. This utility suggests that a paradigm shift in bioseparations is attainable through the development of generic mixed mode adsorbents that can respond to such stimuli. Examples of this utility are documented for the recovery and purification of commodity proteins derived from industrial biotechnological process.

23) Model-based scale-up in membrane chromatography

Eric von Lieres (Research Center Jülich)
Pranay Ghosh (Research Center Jülich)

Membrane chromatography (MC) systems are increasingly used in the downstream processing of biopharmaceuticals due to high operational flow-rates and unique mass-transfer characteristics. With improvements in surface chemistries, binding capacities of MC systems have become comparable with conventional packed bed chromatography, in particular for large biomolecules. MC capsules are provided by several vendors at a variety of flow configurations and sizes ranging from microliter to liter scales. Scale-up of MC systems can be tricky due to varying contributions of non-ideal binding and non-ideal flow to band broadening in lab and preparative scale capsules, even when both contain the same membrane. However, this potential drawback can be overcome by model-based approaches for analyzing scaled-down units and predicting the performance of preparative scale units. To permit scale-up, models must account for specific device geometries and flow profiles within both units and quantitatively decouple flow and binding related non-idealities. Two such modeling approaches are presented, computational fluid dynamics (CFD) and the zonal rate model (ZRM). Novel results for such model-based scale-up are shown for MC capsules at scales ranging from 80 microliters to 1.2 liters. Non-invasive MRI measurements provide important insights on membrane arrangements and holdup volumes within fully assembled MC systems. Based on this information, CFD simulations reveal internal flow patterns and predict non-binding chromatograms without any parameter estimation required. Several binding models are compared with respect to physical consistency and predictiveness. Though the studied small-scale units were not specifically designed as scaled-down models of their larger counterparts, we demonstrate that they can be used for obtaining all data required for quantitatively predicting the performance at preparative scale, minimizing sample requirements. Lately, highly accurate predictions were achieved for extreme scale-up factors of up to 15,000. The presented modeling approaches, CFD and ZRM, complement each other in terms of the required data and compute resources. However, both approaches are shown to permit equally accurate predictions over a range of industrially relevant systems and operating conditions.

24) Disposable Custom Affinity Media

Tracy Thompson (PolyBatics)

Monoclonal antibodies have emerged as one of the most successful drug classes in part due to a consistent manufacturing methodology. This is enabled in large part due to incorporation of protein A in the capture step, which, through its specificity for the Fc domain, provides unrivaled purification. But what can other therapeutic molecules hope for in terms of uniform manufacturing processes exhibiting similar specificity without being cost prohibitive? Insoluble polyester inclusions that display a wide range of custom ligands on their surface have been produced in microbial hosts that demonstrate high specificity similar to other affinity ligands i.e. protein A. *In vivo* production of ligands on these polyester supports simplifies the process whereby custom chromatography media can be produced. Use of these novel materials has been demonstrated both for the capture of target molecules or in a polishing step for the removal of impurities. Here we discuss incorporation of the ligand displaying beads into, and onto, support matrices demonstrating their potential as a customizable disposable affinity chromatography media.

25) Advancements in Large Scale Pre-Packed Chromatography Columns

Dana Pentia (Repligen Corporation)
James Peyser (Repligen Corporation)
Travis Ward (Repligen Corporation)
William Wilde (Repligen Corporation)

Single use technologies have been adopted by biopharmaceutical industry as a mean to faster product changeover, favorable economics, and improved safety. While single-use technologies are prevalent in many areas within upstream and downstream processing, there has been very limited broadly applicable solution for chromatography steps. The need for scalability within the same column system is critical for ensuring consistency, ease, and reliability in transitioning from lab/development scale to production scale of the purification process. Knowing that the columns characteristics and performance are maintained for different column diameters makes for an easy and reliable transition from lab/development scale to production scale. A case study of a process that was scaled up to a 45 cm diameter pre-packed column will be presented. Because pre-packed columns are packed at a different site than the use site, transportation studies were performed, demonstrating maintenance of column characteristics after shipping. Mechanical testing was also performed on the 45 cm diameter column to demonstrate durability of the columns. Although pre-packed columns are designed primarily as campaign-use/disposable technology, biological and chemical compatibility of the hardware makes them suitable for multiple uses. Leachables and extractables levels were determined for the large scale columns, and shown to be minimal, and well within the acceptable ranges for biological molecules purification. The use of pre-packed columns for multiple purification runs needs to meet the requirements of good cleanability of small molecules, and of microbial and endotoxin removal. A study on cleanability of the 45 cm columns will be presented, demonstrating the ease of removal of any small molecule, and microbial contaminants.

26) Modular Chromatography for Flexible Bio-Manufacturing

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Kajsa Stridsberg-Fridén (GE Healthcare)
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Chromatography is today the most important separation technique in Biopharmaceutical downstream processing. Standard adsorption media are produced as beaded particles filled/packed into a column to facilitate the classical 'fixed bed' unit operation of chromatography. The technologies for producing and tailoring beaded chromatography media have been refined over the last 50 years to meet the increasing industrial demands. At the same time chromatography column hardware has significantly developed during recent years, hereby reducing overall cost and complexity in traditional stainless steel factories by improved fluid management and fluid distribution, ease of use and robustness in packing and maintenance. Traditional column hardware formats may, however, be limited for conceptual, economical and practical reasons when looking for integration into new processes and facility concepts requiring highest levels of flexibility. For example, short bed height (pancake like) column configurations desirable in high throughput flow through applications are difficult to accommodate with traditional column hardware. This represents a significant bottleneck, in particular as the chromatography media itself is well suited for a scalable adaption to such new processing formats and high throughput formats, for example by use of beads with small particle sizes at short bed height. We present here a novel concept for a format that overcomes the limitations of currently available column hardware and allows the efficient use of chromatography media in beaded format in standard, but also modern high throughput and single-use applications. Pre-filled modular cartridge units have been designed and tested for assembly in a parallel or serial fashion, hereby allowing assembling of an integrated adjustable size chromatography unit at the point of use. The equivalent column diameter and bed height of the unit is determined by the number of modules and by the selected configuration of the integrated flow conduits. A single fluid inlet and fluid outlet are connecting the assembled unit with the chromatography system as with a traditional column. The units could be deployed and installed as self-contained disposable units with aseptic connectivity, however, they may also be cleaned and re-used in a more traditional fashion. Examples of chromatographic performance of different cartridge assemblies as applied for different chromatography steps will be presented. Also, a new packing technology for filling of the modules with standard beaded chromatography media will be described. The technology that has been specially developed to achieve uniformity in between the modules, which is a pre-requisite for hydrodynamic and chromatographic uniformity in parallel installations, delivers an improved overall cost efficiency of the modular concept, by addressing aspects related to process performance as well as storage and shipping.

27) Buffer Type dependence of HCP and Virus Removability by Weak Anion-Exchange (AEX) Membrane with Graft Chains

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Masashi Enzu (Asahi Kasei Bioprocess, Inc.)
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Membrane adsorbers are considered to be a powerful tool for impurities removal from bioprocess products. AEX membranes are especially widely used in flow-through mode to remove impurities and it is well known that HCP reduction is often seriously affected by the buffer type, i.e., using multivalent buffer such as citrate sometimes decrease the HCP removability significantly. In this report, firstly buffer type dependences of HCP removability are evaluated systematically to confirm the effect of multivalent buffer then virus removal evaluations are performed in order to clarify whether virus removability is also affected by the buffer type. Hollow fiber type weak AEX membrane (QyuSpeedTM D, Asahi Kasei Medical) is used in the experiments. HCP removability of QyuSpeedTM D was evaluated with several buffer types under the range of pH 6.0 - 8.5 and 0 - 0.3 M NaCl. 0.1 mg/mL HCP solution was loaded to the QyuSpeedTM D and HCP concentration in the flow through pools were evaluated by ELISA. In the other experiment, the solution containing 1 vol% Minute Virus of Mice (MVM) and 10 mg/mL human-IgG was loaded to AEX membrane and packed column resin and both flow through fractions were collected. The dependencies of buffer type (Tris-HCl, Phosphate-Na, Acetate-Tris, Citrate-Tris), salt concentration (0 - 0.2 M NaCl) and pH (6.9 - 7.9) on the MVM removability were evaluated. Virus titres in the flow through fractions were evaluated by TCID50 hemagglutination (HA) method. According to the HCP removability evaluation, the solution of the monovalent buffer such as Tris-HCl and Acetate-Tris showed the higher HCP removability. On the other hand, the solution of multivalent buffer such as Citrate-Tris showed lower HCP removability as expected. This tendency is also the case for MVM reduction. QyuSpeedTM D has a good MVM removability with Tris-HCl and Acetate-Tris buffer. However, one of the multivalent buffers, Phosphate showed less MVM reduction compared to other monovalent buffers. Another multivalent buffer, Citrate showed significant effect on the MVM reduction and almost no virus reduction was detected in the evaluation of the flow through fractions. Effect of the salt concentration on the virus reduction was also evaluated for Phosphate buffer and it clearly showed that higher salt concentration significantly decreases the MVM removability. It is confirmed that the buffer types significantly affect the impurity removability of AEX membrane in the flow through mode. Both HCP and MVM showed higher reduction when monovalent buffer solution was used. However, using multivalent buffer showed significant decrease in the reduction of HCP and MVM. This tendency is more significant for MVM reduction than HCP.

28) The ChromX software package for liquid chromatography: success stories

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Although model-based approaches are developed and used in academia, technology transfer to industry is still limited. The transition from traditional experimental procedures to applying model based tools seems cumbersome. From the authors' perspective, the main reason is the inability of novel tools to assimilate into laboratory work-flows. To integrate simulation tools successfully, the underlying models have to cover the usual operations, simplify parameter determination and optimisation from routine data collection while maintaining high flexibility. The developed tool box addresses these shortcomings by the use of parameter estimation techniques, exploiting simple scouting runs or already existing chromatographic data. By applying a multi-wavelength approach, detector saturation problems as often occurring in industrial applications are avoided. The model tool box currently supports IEX and HIC in axial and radial flow settings and can be easily extended to other modes due to the modular architecture. ChromX uses state of the art numerical concepts, such as stabilized finite elements, fractional-step-theta schemes and hardware-aware linear algebra. It provides interfaces to deterministic and heuristic optimization algorithms with highly adjustable objective functions. We present classical single column optimisation, as well as sequential and parallel multi-column set-ups. The case studies include optimal aggregate separation in industrial antibody purification using existing data and multi-objective optimisation of 3-column Periodic Counter-Current Chromatography (3C-PCCC).

29) Protein Processing Figures of Merit and Novel Ligand Chemistries Employing Capillary-Channeled Polymer (C-CP) Fiber Stationary Phases

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A "blank slate" approach to the development of new stationary phases for the separation of biologics would include aspects of column permeability, solute mass transfer rates, flow uniformity, and dynamic binding capacity. Beyond these "physical" characteristics, the ability to affect chemical selectivity in a cost-effective manner is key. Capillary-channeled polymer (C-CP) fiber phases have demonstrated a number of positive characteristics relevant to the downstream processing of proteins. Melt-extrusion of C-CP fibers from simple thermopolymers (polypropylene, polyester, and nylon) means that the primary materials costs are very low. These base polymers present very different surface chemistries as well as potential surface modifications. Regarding physical attributes, the longitudinally-aligned fibers in column structures provide very high permeability, while promoting very efficient mass transfer as interfiber separations are 1 – 5 μm. Extruded polymer fibers have very low internal porosities (rp = ~1.3 nm) as determined by inverse size exclusion chromatography (iSEC). As such, proteins having molecular weights of >10,000 Da experience no van Deemter C-term broadening. In practice, analytical-scale separations can be performed at linear velocities of 100 mm sec-1 without sacrifice of efficiency. While the lack of fiber porosity limits their specific surface area (1-5 m2g-1), and thus equilibrium binding capacities, very high throughput and yield can be realized. A preliminary assessment using nylon 6 C-CP fibers for the ion exchange processing of lysozyme produced throughputs of 2.6 mg min-1g-1 fiber mass and 0.4 mg min-1mL-1 bed volume, at a relatively low load concentration of 0.25 mg mL-1, with yield values of >95%. Current studies look to elucidate the roles of column packing density and solution linear velocity on dynamic binding capacities and recoveries. As suggested above, the base polymers from which C-CP fibers can be extruded presents a rich palette in terms of imparting chemical selectivity. As suggested above, nylon 6 is an excellent surface for weak ion exchange separations, as well as hydrophobic interaction chromatography (HIC). Additionally, the surface can be covalently modified using triazine chemistries. Polyester provides a more hydrophobic surface having aromatic character, which can also be used for weak cation exchange. Finally, polypropylene provides for solely hydrophobic interactions between solutes and the fiber surface. This interaction allows for very robust adsorption of capture ligands. This concept, and practical figures of merit, will be demonstrated through the simple adsorption of protein A to the fibers for the capture of IgG. Finally, a novel, yet powerful use of modified PEG-lipid ligands will be demonstrated. These commercially available phospholipids have a wide variety of head groups, including amines and carboxylic acids, succinyl/thionyl groups, polydentate metal ligands, and high-selectivity agents including biotin.

30) Challenges and Opportunities for 3D Printed Chromatography Columns and Solid Phases

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Additive manufacture, or 3D printing, offers the opportunity to manufacture porous media composed of micro-structural elements of different shapes and sizes, and to precisely locate and orient them within the bed. For example, spherical beads with a narrow size distribution can be constructed individually at desired locations within the bed, allowing the creation of specific packing arrangements, i.e. perfectly ordered lattices or random packing mimicking conventionally packed chromatography columns. Furthermore, non-spherical elements can be created and oriented in appropriate directions to align particular features or to optimize mobile phase flow. Taken one step further, geometric elements with different shapes or sizes can also be placed at individual locations within the same bed. Alternatively, the structural focus can shift from the solid-phase to the mobile phase, with the design of complex flow channels within a monolithic bed. These opportunities for precise control of bed morphology, combined with the simultaneous printing of the overall column walls, internal flow distributors and flow fittings, mean that entire, one-piece chromatography columns can potentially be created locally on demand, each tailored to exact specifications. The main challenges to this approach include achieving sufficient printing resolution to compete with current media in terms of theoretical plate height and developing materials that have appropriate internal porosity and surface functionalities to enable high binding capacity and specificity. Other challenges are as for conventional media, for example good swelling properties, low non-specific adsorption, and the absence of toxicity and leaching. Here, we show examples of progress made to date in creating 3D printed chromatography columns. These include micro-structural analyses of columns containing porous beds with a variety of lattice arrangements and channel structures, printed at a maximum current printing resolution of 16 mm; comparison of residence time distributions and flow characteristics for a range of columns, including several printed with different integrated flow distributors and column cross-sections; and demonstration of chromatographic separations of proteins using some commonly available printing materials. Finally, we outline the overall opportunities and challenges related to printing chromatography columns.

31) Process crystallization as a next generation unit operation?

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Process crystallization of small molecules is a long-established unit operation and has been refined in the past in order to obtain a desired product quality. On the contrary, process crystallization of biological macromolecules has a relatively young history and therefore, systematic knowledge is still lacking. Nevertheless, the interest of industry to apply process crystallization of macromolecules is high due to the prospect of creating a superb product quality on large scale while significantly reducing production costs. We present recent results concerning the identification of a new criterion for scale-up of stirred tank batch crystallization of the exemplary protein lysozyme[1]. It will be shown that process time can be reduced to few hours by applying an optimum stirrer speed. The maximum local energy dissipation was identified as a proper scale-up criterion. Other new results of stirred tank batch crystallization of lysozyme and a lipase will be presented[2]. It will be shown that the use of proper additives can lead to significantly enhanced yield and kinetics while maintaining a favorable protein crystal morphology and an appropriate crystal size distribution. The acquired experience from enzyme crystallization was applied to the crystallization of higher-valued proteins from mammalian cell cultures[3]. We will show that stirred tank batch crystallization of a purified therapeutic monoclonal antibody fragment was scalable and reproducible. Hence, an optimization was technically feasible. This was achieved by fed-batch operation of the stirred crystallizer via stepwise addition of the crystallization agent. Again, adequate crystal morphology and crystal size distribution were obtained. In another case study, process crystallization of a whole therapeutic monoclonal antibody from impure clarified CHO cell culture harvest was attempted for the first time[4]. It will be shown that fast, scalable, and reproducible stirred tank batch crystallization was possible after a simple pretreatment step. The antibody kept its high biological activity during crystallization, dissolving, and recrystallization steps. Our findings show that process crystallization has a strong potential to replace preparative chromatography steps and can easily be integrated into existing purification platforms. Preliminary molecular modeling gave some insight into possible general mechanisms which result in a high antibody crystallization propensity. As a result, the so far lacking transferability of proper crystallization conditions from one antibody to another could be facilitated in near future. Furthermore, process crystallization of newer non-antibody biotherapeutics should be pursued.

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32) Styrene Maleic Acid (SMA) copolymers: One solution to two DSP problems?

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Many membrane-associated and other hydrophobic proteins are extremely valuable targets for drug companies; the G-protein coupled receptor family of proteins, for example, play important roles in the development of small molecule therapeutics. This said, the notorious difficulties encountered in producing such proteins in active purified forms severely limits both their current utility for drug screening and discovery, and their future manufacture as therapeutic drugs in their own right. Clearly, the onus to remove obstacles to the efficient manufacture of membrane proteins is one that falls on the sector best equipped for the task, i.e. the DSP community. Another pressing task requiring the attention of DSPers is selective extraction of soluble biotherapeutic protein targets from *E. coli's* periplasmic space. This compartment is becoming the favored destination of an increasing number of biopharmaceuticals produced in *E. coli*, most notably antibody fragments, but the poor understanding and hence predictability of currently practiced periplasmic release processes, especially when applied to new production lines, are serious issues to resolve. At industrial scale, classical cold osmotic shock (OS) methods have proved difficult to control, and not all protein targets directed to this compartment are released by OS treatments. Several simple thermal release methods have also been developed and applied within the biopharmaceutical industry, supplementing classical OS; but while these appear effective for numerous antibodies, they are inappropriate for thermo-labile products, and hence do not constitute a generic solution to release of protein targets from the periplasm. At Birmingham we are pioneering radical solutions to the *purification of membrane proteins* and *extraction of target proteins from the periplasmic space of E. coli* centred on the common use of cheap water soluble low molecular weight copolymers of StyreneMaleic Acid (SMA) [1, 2]. SMA is employed widely in the plastics industry, and its amphipathic properties have previously been exploited in biology, e.g. in the delivery of hydrophobic drugs[3]. More recently, we have found that, in the presence of biological membranes, SMAs function as 'molecular cookie cutters'. Multiple SMA units auto-assemble within the membrane, forming homogeneous nanodisc-like structures containing a lipid interior surrounded by bracelets of SMA molecules. In this presentation we

shall show that these highly unusual reagents can be used to: (i) extract, stabilize and purify membrane proteins; and (ii) effect selective release of protein targets from the periplasm of *E. coli*.

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33) Development of a non-protein A mAb capture step based on selective precipitation combined with CEX

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Many current research initiatives are focused on finding alternatives to protein A chromatography for the purification of monoclonal antibodies (mAbs). Protein A, is expensive, has relatively low capacity compared to ion - exchange (IEX) resins, and does not tolerate cleaning agents, which limits the resin lifecycle. An initial purification step with the purification capability of protein A is required, but at lower cost and increased capacity.
In order to address this issue, we developed a process which uses selective precipitation combined with a novel cation exchange (CEX) resin as the initial purification step. After an initial evaluation, a low cost and non-toxic precipitation agent was identified which can be added directly to the mammalian cell culture broth and which allows removal of >75 % DNA and >30% HCPs after centrifugation and resulted in a significant reduction of depth filter are required for clarification. The culture filtrate could be directly loaded on a CEX resin. Several CEX resins were evaluated for binding capacity, selectivity and cleanability. The selected CEX resin had a significant increased capacity over protein A and data indicate a purity which is nearly equal to a typical protein A eluate. The initial data show that the combined use of selective precipitation and CEX are promising for future 'high titre' antibody purification processes.

34) A tubular reactor for continuous protein refolding and precipitation methods

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Process integration is a promising strategy to improve efficiency of downstream processing. Moreover, continuous downstream processing operations have gained interest for industrial applications. Motivated by these considerations, a tubular reactor for continuous processing of recombinant proteins was designed and developed in laboratory scale. Exemplary, a fully continuous process for an autoprotease fusion protein was established. The process comprised of inclusion body dissolution, refolding and autocleavage, followed by purification of the released target protein by acidic precipitation of the residual autoprotease. The process was shown to be robust over extended period of time whereat reaction rates of specific step operations were equal or even higher compared to batch processing. Most importantly, product quality and yield were also equal to batch processing. The simple design of a tubular reactor allows for easy change of process conditions by feeding of different process solutions in designated segments of the reactor. The high surface-to-volume ratio enables rapid and efficient temperature change. Alternative refolding strategies like pulsed or fed batch refolding and a temperature leap strategy were successfully implemented. In an advanced set-up, loop reactors were integrated to perform processing of proteins with long reaction times while at the same time keeping the reactor volume small. In another exemplary process, an oxidation reaction of a partially refolded protein was performed by aeration in a specially designed segment of the tubular reactor equipped with static mixers, sparging elements and air-traps. Additionally, process sensors were implemented into the tubular reactor set up for inline monitoring of critical process parameters like pH, conductivity, redox potential, turbidity and dissolved oxygen. Productivity calculations showed that an optimal refolding concentration to achieve highest productivity value is a balance between a rational reactor volume and a reasonable refolding time. Productivity in a tubular reactor is always higher as emptying and refilling times required for batch reactor decreases productivity. This productivity improvement is higher for a fast refolding protein than a slower one and can be up to 2-3 times as will be shown for model protein systems tested with the laboratory scale reactor. Although it is more complex to setup a tubular than a batch reactor, it offers faster mixing, higher productivity and better integration to other bioprocessing steps. With increasing interest of integrated continuous biomanufacturing, the use of tubular reactors in industrial settings offers clear advantages.

35) Investigation of virus aggregation on performance of downstream processing in influenza vaccine manufacturing

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The most important measure to control influenza virus outbreaks is vaccination, which is reflected in steadily increasing demands for vaccines. As a result, extensive efforts are being undertaken to optimize current and to establish new influenza vaccine production processes. Among many factors to be considered, virus aggregation is crucial for process productivity. In general, virus particles tend to form aggregates, resulting in potentially high losses during a wide range of unit operations, e.g. filtration, centrifugation, and chromatography. Understanding the mechanisms behind virus aggregation and implementing the gained knowledge to new processes will help to avoid unnecessary product losses and contribute to the robustness of production processes. In this work we investigate the aggregation behavior of cell culture-derived influenza virus particles. The main focus is on basic mechanisms of influenza virus aggregation, and process conditions that potentially effect aggregation behavior, i.e. buffer conditions (ionic strength, pH, etc.), virus concentration and the dynamics of virus aggregation. Virus aggregates are characterized by "differential centrifugal sedimentation" (DCS), "tunable resistive pulse sensing" (TRPS) and "dynamic light scattering" (DLS). The individual methods complement each other and allow an extensive picture of the virus aggregation process. Initial experiments confirm literature regarding the impact of ionic strength and pH on virus aggregation. Interestingly, significant differences were also detected between different virus strains and their aggregation dynamics. As expected, low pH values around 5 led to aggregation, which might be due to conformational changes of the hemagglutinin (HA), which is a major envelope protein of the influenza virus. Currently, the effects of specific ions, proteins, nucleic acids, and other process-related components are investigated in an extensive ongoing design-of-experiment (DOE) approach. From this we expect to get further insights on the virus aggregation mechanisms, process conditions initiating it, and the impact of product aggregation on the performance of unit operations used in downstream processing of influenza vaccines.

36) Development of a Purification Strategy for Enterovirus 71 Like Particles for Vaccine Production

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Enterovirus 71 (EV71) is one of the major causative agents of hand, food and mouth disease. In the past mainly mild cases of the disease and low case fatalities have been encountered. However, this has changed during the last years emphasizing the need for vaccine development. Within this project virus like particles (VLP) where chosen as vaccine agent. VLPs are empty viral capsids comprising major epitopes of viral proteins but lacking viral DNA or RNA. Thus, these particles can induce a strong immune response comparable to whole viral vaccines without the risk of virulent revertants. For the conducted experiments clarified and pre-concentrated EV71 VLPs produced by the baculovirus expression vector system (Sentinext Therapeutics Sdn Bhd) were used. One focus was to evaluate various membrane adsorbers (MA) for a downstream process suitable for vaccine manufacturing. Therefore, two anion exchange (AEX) MAs with different chemistries (Sartobind Q and Sartobind STIC PA, Sartorius Stedim Biotech) were investigated to optimize product yield and contaminant depletion. This was carried out by a design of experiment (DOE) approach. The second focus was to evaluate different types of cross flow membranes to concentrate VLPs and to deplete baculovirus. Results obtained for the two MAs indicated a good recovery of VLPs in flow through mode and acceptable depletion of DNA, whereas the level of contaminating proteins needs to be further reduced. The STIC MA bound DNA efficiently and DOE evaluation indicated a strong influence of the phosphate concentration and pH on VLP yield and purity. The ionic strength affected these parameters only at high salt concentrations and the ligand density had no significant impact. Depletion of baculovirus by crossflow filtration could be achieved up to 98% by a recovery of model VLP particles in a range of 70 to 100%. Overall, the conducted experiments showed encouraging results to develop a purification process for insect cell culture-derived VLPs primarily based on a combination of ultrafiltration and chromatography steps via different types of MA.

37) Understanding Virus Retention Behavior of Different Virus Filters Using Confocal Microscopy

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Although virus filtration is a well-established method for combating the inherent risk of viral contamination in the production of therapeutic proteins, there are still significant uncertainties regarding the underlying mechanisms controlling virus retention and the overall log removal rate. Previous studies have reported a significant decline in virus retention during constant pressure virus filtration with certain filters (but not others). Several studies showed that a temporary release in the transmembrane pressure can cause a significant transient increase in virus transmission. However, there is currently no detailed understanding of the factors controlling these phenomena or the underlying physical basis for the observed increase in transmission. The objective of this work was to obtain quantitative data for the log reduction value (LRV) during filtration of a model bacteriophage ΦX174 through different virus filters and in response to different types of "pressure release". In addition, confocal microscopy was employed to visualize the capture of fluorescently labeled bacteriophage within the membrane. Images were also obtained using phage that were labeled with different fluorescent dye before and after the pressure release to identify changes in phage capture in response to the change in operating conditions. Results with the DV20 filter showed a transient increase in bacteriophage transmission by approximately a factor of 10 (one log) immediately after the pressure release, due to the release of trapped phage and their subsequent migration further into the depth of the filter pore structure. The details of the virus retention were different with Viresolve membranes due to the very different underlying pore morphology. The combination of the LRV profiles and the confocal images provide important insights into the factors controlling virus retention during virus filtration as well as a framework for developing approaches to increase the overall effectiveness of the virus filtration step in downstream processing.

38) Inactivation of Viruses Using Novel Protein A Wash Buffers

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Ionela Iliescu (Biogen Idec)
Keith Selvitelli (Biogen Idec)

A low pH viral inactivation step is typically performed in the eluate pool following the protein A capture step during the manufacturing of monoclonal antibodies and Fc-fusion proteins. However exposure to acidic conditions has the potential to alter protein quality. This can be worse at large scale where it can be time consuming and difficult to manually acidify, mix, transfer, and then neutralize a large pool. The acidification and subsequent neutralization can increase pool conductivity, which can impact the subsequent ion exchange chromatography step. When performing simulated moving bed or continuous multi column chromatography, it is challenging to perform the low pH inactivation step on the many elution pools that are generated. To avoid these difficulties, novel protein A wash buffers capable of inactivating viruses while antibodies were bound to chromatographic resins were developed. By equilibrating the column in high salt buffer (2 M ammonium sulfate) after loading, the interactions between antibodies and protein A ligands were increased enough to prevent elution at pH 3. The ammonium sulfate was also found to cause binding of an antibody to a mixed mode anion exchange resin (Capto Adhere) at a pH value (3.5) that caused elution in a conventional anion exchange resin. This indicated that retention was likely due to enhanced hydrophobic interactions. The potential of the 2 M ammonium sulfate pH 3 buffer, a 1 M Arginine buffer, and a buffer containing the detergent (LDAO) to inactivate viruses when used as protein A wash buffers with a 1 hour contact time were studied. The high salt and detergent containing column wash buffers provided about five logs of removal, determined using PCR, and complete combined removal and inactivation (> 6 logs), determined by measuring infectivity. The arginine provided complete removal of xMuLV, as determined using PCR. The novel protein A washes could provide more rapid, automated viral inactivation steps with lower pool conductivities. In free solution, the 2 M ammonium sulfate pH 3 buffer did not completely inactivate xMuLV virus, probably due to viral aggregation, indicating that this buffer may be more effective when used during protein A chromatography.

39) Evaluation of alternative unit operations for the purification of virus-like particles

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Julia Seiler (Karlsruhe Institute of Technology)
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A new approach for responding to pathogens by vaccination is the design of virus-like particles (VLPs). VLPs are gaining increasing importance in vaccine technology, gene therapy and diagnostics. They carry no replicative genetic information and can be produced like other recombinant proteins in various expression systems. VLPs can either mimic the structure of the virus they derived from or present epitopes of foreign pathogens or oncoproteins on their surface. In recent years VLP expression in yeast or insect cells has been optimized to yield high product titers of,. Hepatitis-, Papilloma- or Influenza-VLPs at large scale. Currently process costs shift increasingly to the downstream part of a manufacturing process. There are no universal platform processes available for the purification of VLPs. Hence, each product and product subclass needs an adequate downstream process, generating high experimental effort, especially before and during clinical phases. The present work focuses on the evaluation of alternative unit operations for VLPs on a robotic liquid handling station: aqueous two phase extraction (ATPS), precipitation and membrane chromatography. All three unit operations represent flexible, time-saving and cost-effective separation techniques applicable for large biomolecules and disposable processing. ATPS and precipitation with polymers were optimized in micro scale for the recovery of VLPs from *Spodoptera frugiperda (SF9)* insect cell lysate. Studies were conducted in 96 well plates with a sample consumption of less than 10 mg VLP and experimental times of only 2-3 hours per screening. Fast quantification and characterization of VLPs was realized on a UHPLC system by reversed-phase and size-exclusion chromatography. The evaluation of anion-exchange membrane adsorbers was performed both by high-throughput experimentation in 96-well format and by *in silico* chromatography modeling. Lumped rate model incorporating radial flow geometry was used for simulating hydrodynamics and steric mass action (SMA) model for simulating thermodynamics. Process simulation enabled the design of an optimized, robust and scalable process with minimal experimental effort for a complex VLP feedstock. Finally the combination and process performance of all three unit operations were evaluated. Results demonstrate the high potential of high-throughput experimentation and *in silico* process simulation for developing downstream processes for VLPs and evaluating unit operations in short time with low sample consumption. Robotic liquid handling stations enable a scale down of whole VLP manufacturing processes to an automated microliter scale, which allows a fast assessment and adaptation of running processes for new and emerging vaccine candidates.

40) How can we streamline influenza virus purification?

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Rene Gantier (Pall Corporation)
Annelies (Pall Corporation)

Apart from their current application in viral vaccines, nanoparticle-based entities such as viruses and non-infectious virus-like particles are holding great promise in a myriad of clinical targets, including cancer, cystic fibrosis, Alzheimer's, Perkins's, hemophilia and HIV/AIDs. Virus production processes have seen a big evolution on the upstream side, moving from chicken eggs in more reliable and controlled cell lines. The recent approval of several cell culture based influenza vaccines is poised to further intensify virus production. The challenge is development of better virus purification processes to meet ever increasing regulatory expectations in regards to contaminant removal, including host cell DNA and HCP while mitigating high cost pressures. As a result, novel viral purification processes have to be of high yield and throughput, easy to scale-up, economical and have to allow for efficient contaminant removal. Chromatography is the best positioned purification technique to meet high purity requirements. Packed-bed or membrane chromatography processes are currently being evaluated in process development for several new viral products both in capture and flow-through mode. Achieving both a high yield and high purity can be difficult due to virus stability and challenging DNA and virus separation. Mitigation strategies including endonuclease facilitated DNA degradation during primary clarification have a limited success rate. Better purification solutions are required to move forward with nanoparticles for vaccine and gene therapy purposes. With viruses ranging in size from 20 nm to well over 150 nm, membrane chromatography has been proven to outperform packed-bed chromatography in terms of capacity and processing time. Because of the pre-packed format they typically are supplied in, they also enable to reduce hardware investments associated with chromatography and allow for a more flexible and nimble operation, even up so far that they can be single used, eliminating the need for cleaning and cleaning validation. Barriers to implementation of membrane chromatography include a general lack of experience with membrane chromatography linked to concerns about cost and yield. In this study we show how these barriers can be overcome by using a structured experimental design and a high throughput process development approach to define the scalable optimal operating conditions. In the context of the purification of influenza virus over Mustang Q membrane chromatography, the study demonstrates the predictive power of this methodology. The new insights obtained allow us to propose a simpler manufacturing platform for influenza virus. Economic modelling gave us the insight that the new platform can be more cost efficient than the conventional ultracentrifugation based one. The data confirm that membrane chromatography is a valuable alternative for the purification of influenza virus from clarified cell culture feedstock, allowing for faster, simpler and more cost efficient processing.

41) Viral vaccine production a low cost: reducing facility size by process optimization

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Ann-Marie de Villiers (Crucell)
Charles Hensgens (Crucell)

Despite the dramatic declines in vaccine-preventable diseases in the 20th century, such diseases persist, particularly in developing countries. In this "decade of vaccines", one of the challenges in the industry is to make vaccines available at an affordable price. The capital investments required can be huge and a major obstacle in making the vaccines available to the people in need. In Crucell a production process was developed that allows highly intensified manufacturing of viral vaccines. The base process required a 10,000 L production facility and 5 unit operations per process train to supply the world vaccine demand for high-volume vaccines. This platform was optimized by increasing the productivity in the PER.C6 infection system and implementing membrane absorbers for purification. The optimized process can supply the same amount and quality of vaccine from a 500L production facility and 4 unit operations. This platform, which was developed for Adenovirus production of different serotypes, could be easily adapted for Polio vaccine production. Further optimization was done on the harvest process. By implementing TFF for cell separation, the process time could be reduced by 70% and the number of filtration steps was reduced from 3 to 1. After optimization of the process parameters at 2 L scale the TFF process was successfully scaled-up to 50L scale. In the future we will explore the possibility to integrate the cell retention used during infection and the cell separation device during harvesting, further reducing the footprint required for the facility. The newly developed process requires significantly less investment for a commercial facility and makes the vaccines available at an affordable price.

42) Size matters: optimising clarification of feed streams when nanoparticles are the product

Michael Collins (Pall Corporation)

The clinical relevance of nanoparticle drugs and vaccines, based on virus or virus-like-particles, has never been so high. Not only are they still a successful approach to develop vaccines, but in addition they hold great promise in a myriad of therapeutic targets, including cancer, cystic fibrosis, Alzheimer's, Perkins's, hemophilia and HIV/AIDs., with the first gene therapy products finally reaching the market. Virus production processes have seen a big evolution on the upstream side, using reliable and controlled cell lines, growing on better defined culture media and using larger batch sizes. The recent approval of several cell culture based influenza vaccines is poised to further intensify virus production. However, because of their size and other properties, several of those nanoparticles bring a challenge to the downstream purification process, not least the primary purification step post bioreactor. An efficient clarification step separates the nanoparticles from cells, cell debris and many impurities, including insoluble precipitants, aggregates and other materials found in typical cell cultures. This step needs to combine high capacity for impurity removal, high product yield, ease of scale-up and to maximally protect any further downstream operation, making the overall process more efficient and economical. Cellulose based depth filters have proven to meet all these objectives for typical biotech feed streams and are hence a preferred solution used for many protein based drugs. With nanoparticles however, the standard cellulose based depth filters have sometimes performed poorly, with particularly low product yields. Our research hence focused on gaining a better understanding of the mechanisms responsible for retention and/or transmission of large viruses or VLP's, which were expected to include size-exclusion and adsorption. The outcome was used to a) select more efficient filter aids to enhance the performance of a depth filter solution whilst maintaining high product yield and b) optimise pre and post use buffer rinsing of the depth filter to enhance product yield. This combined approach was tested on a recombinant live influenza process against current benchmark technologies and demonstrated high product yield with high contaminant capacity that would provide for an economic depth filter solution for post bioreactor feed solutions up to typically 2000 liter scale.

43) Protein Glycosylation Heterogeneity in Downstream Processing

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Protein glycosylation is a crucial post-translational modification and serves a major role in stability, efficacy, pharmacodynamics, and pharmacokinetics of biotherapeutics. The glycosylation profiles such as sialylation level have become increasingly important to regulatory requirements for manufacture process consistency. Previous studies have been conducted to understand and control glycosylation through cellular engineering, cell line selection, cell culture media optimization, and bioreactor operation improvement. Relatively less known is how different glycoforms behave across unit operations during downstream processing, and to what extent they can be resolved and recovered. The heterogeneity resides in both the glycosylation sites in the backbone as well as the individual glycan components. The variations among sugar groups and sialic acids lead to variations in surface charge and surface hydrophobicity. Those local structural perturbations contribute to electrostatic and hydrophobic interactions, which are governing mechanisms in ion-exchange (IEX) and hydrophobic interaction (HIC) chromatography. A systematic understanding about resolving glycoforms in the typical downstream platform has not been fully established. Here we provide an overview about the potential and limitation of common purification techniques. We also present layers of experimental studies employing multiple Fc-fusion proteins to gain further knowledge in this topic. First we characterize the broad distribution in their glycosylation profile (e.g. total sialic acid, N-link domains, and charge variants) and explore how the pattern evolves across IEX and HIC. We then separate and regroup low and high sialic acid populations and characterize their biophysical properties, mass transfer and binding capacity in batch mode, and their resolution in column mode. In AEX, average sialic acid level is controlled by salt gradient where a trade-off between yield and desired sialylation level is present. In addition, pore diffusion of glycoproteins is likely affected by sialic acid level and competitive binding among charge variants is possible. In HIC, a correlation between sialic acid level and retention is observed for monomers. For high-molecular weight species (HMW), in addition to size and hydrophobicity their glycosylation profiles also differ. Therefore the HIC performance is affected by the load glycosylation profile where different monomer and HMW populations compete for binding sites. Taken together, there is interplay between AEX and HIC regarding the

yield and quality. Moreover, the cell culture harvest often displays a compromise among titer, sialylation level, and impurity profiles regarding conditions like harvest day. The combination of those attributes affect disproportionally on the downstream (and thus overall) process performance. Here we highlight how upstream decisions can impact downstream by illustrating several case studies. It becomes clear that the overall upstream and downstream process optimization must consider both the individual unit operation performance and their complex interactions. Such findings emphasize the importance to implement QbD framework across all parts of manufacture process together.

44) Separation of Nonfucosylated Antibodies

Austin Boesch (Dartmouth College)
Glen Bolton (Biogen Idec)

Post-translational modifications can dramatically impact protein activity, but identifying such structure: function relationships, as well as capitalizing on functionally enhanced variants, are significant challenges for biosimilars development. Here, affinity chromatography resins that contained immobilized FcγRIII receptors were used to enrich nonfucosylated antibodies 6- to 9-fold, offering what may be a tractable method for both the identification of post-translational modifications that affect function, as well as a means to enrich variants with enhanced activity.

45) Adapting manufacturability assessment to non-antibody proteins

Diana Woehle (Amgen Inc.)

Early stage manufacturability assessments of biopharmaceutical candidates have been developed to select molecules that best fit pre-determined product attribute targets and manufacturing processes rather than adapting the manufacturing processes to fix a molecule that has stability, solubility, productivity or other manufacturing issues. There are feedback loops built into the assessment process, so that experience from recent research molecules or development programs can be added to the assessment in an iterative fashion. Feedback can encompass steps like sequence engineering, expression, cell culture, purification, analytics and formulation. This has been employed successfully in the case of monoclonal antibodies (mAbs) and in this presentation we will discuss how the process can be adapted to non-mAbs. Typically purification and formulation can be most impacted by these non-mAb proteins. In the case of purification, the reliance shifts from mAb platform "plug-and-play" approaches to rapidly developed first draft tailored processes capable of assessing drug substance manufacturability and providing representative material for biophysical and biochemical testing. In addition, these first draft processes need to be developed for several related candidates at the same time. These processes are heavily reliant on product attributes like isoelectric point, Fc domain if present, sequence information, and any available stability or solubility information to help focus the high-throughput chromatographic screening techniques to reduce resource costs. One other technique that will be discussed is the use of sequence information to predict proximal chromatographic behavior capable of spotlighting operating regions that are most likely to deliver successful results.

46) Manufacturability index for ranking high-concentration monoclonal antibody formulations

Suzanne Farid (University College London)
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Yang Yang (Imperial College London)

The need for high concentration formulations for subcutaneous delivery of therapeutic monoclonal antibodies (mAb) presents viscosity and hence manufacturability challenges. As a result it is critical to be able to choose the optimal formulation for both efficacy and manufacturability in terms of being able to process the material in the final ultrafiltration/diafiltration (UF/DF) step. This work presents a set of manufacturability indices (e.g. aggregation, viscosity) as early predictors to select the most promising proteins from panels of candidates as well as the optimal formulation designs for selected candidates. On the viscosity front, this work uses advanced multivariate analysis techniques to analyse published experimental DoE data from industry that explores the influence of different formulation conditions (pH, ions and excipients) on the solution viscosity and mAb thermostability. A decision tree classification method, CART (Classification and Regression Tree) is used to identify the critical drivers that influence the viscosity and thermostability. Multivariate regression techniques were used to transform the DoE data into a set of viscosity and thermostability stress maps as a function of the formulation conditions. Viscosity indices are derived from analysis of the stress maps and the protein concentrations experienced in the final UF/DF step. The indices are used to identify the optimal formulation buffer conditions that minimise the potential for viscosity issues while meeting the thermostability requirement. This approach can be used early in development to rank formulation conditions in terms of their ease of manufacture.

47) Model-Assisted Chromatography Process Development to Control Glycosylation Profiles and High-Molecular Weight (HMW) Species for Fc-Fusion Proteins

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Fc-fusion proteins produced in cell culture processes usually possess complex glycosylation patterns (e.g., sialylation) and product-related impurities such as high-molecular weight (HMW) species. Due to their potential influence on product stability and biological activity, glycosylated product variants and HMW species need to be properly controlled in manufacturing purification processes to consistently meet drug substance requirements. Ion-exchange chromatography (IEX) is often employed to adjust glycosylation profile via selective removal of product charge variants of certain glycosylation properties (e.g., sialylation) using process conditions (e.g., wash and elution) with tight range to achieve adequate chromatographic resolution. On the other hand, hydrophobic interaction chromatography (HIC) is the workhorse to remove those HMW species for which analytical characterization and design of purification conditions can be very challenging due to glycosylation heterogeneity. Currently, IEX and HIC process development and characterization are still largely based on empirical statistical approach, leading to difficulties in interpreting data generated at various scales and limited model predictability outside design space. This work explores adsorption and mass transfer properties of product charge variants at different sialylation levels to study subtle differences in their chromatographic behaviors. Mechanistically-based chromatography models are applied to data analysis, gaining improved process understanding as well as correlating analytical properties of product-related impurities and their downstream separation. The presentation will focus on the use of an appropriate combination of data and chromatography modeling as a scientific basis for developing robust and efficient chromatography purification processes.

48) Experimental investigation, modeling and prevention of aggregate formation in downstream processing

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Fabian Bickel (Biberach University of Applied Sciences)
Martina Merg (Biberach University of Applied Sciences)
Olubukayo Oyetayo (Biberach University of Applied Sciences)

Protein aggregation is a common phenomenon in DSP with well acknowledged negative therapeutic, and economic consequences. Therefore research on protein aggregation mechanisms as well as approaches to mitigating this instability have surged in the past years. We are developing experimental systems that simulate stress conditions, which result in aggregate formation during mAb downstream processing. Currently we focus on shifts in pH and ionic strength as well as exposure to UV radiation. Aggregation kinetics are obtained from turbidity measurement, while thermal stability as well as secondary structure changes are investigated using DSF and ATR-FTIR. The data allow constructing phase diagrams for different aggregate types and modeling aggregation kinetics. For aggregation induced by pH shift, a nucleation / growth / refolding model has been established, which can be fitted to the data to extract the relevant kinetic constants. The model is predictive for other starting protein concentrations and relevant from an application perspective, as it simulates the neutralization step subsequent to mAb elution from a protein A column. On a next level, we use this experimental system to investigate the effect of various additives both on thermal stability as well as on nucleation, growth and refolding rate constants. Currently, a large set of molecules from the amino acid, methyl amine and polyhydroxy compound classes have been selected based on chemical diversity, and their effect on aggregation parameters is screened in a parallel format. Data will be analyzed using a QSPR approach with the goal to understand which chemical properties enable additives to act as stabilizers. The method will allow separating additive effects on thermal and kinetic stability of mAbs. Ultimately, we hope to identify novel additives with enhanced stabilization potency.

49) Challenges during DSP of highly concentrated antibody solutions

Annette Berg (Sanofi)
Hendrik Flato (Sanofi)
Stefan Schleper (Sanofi)
Tamara Uthe (Sanofi)

High antibody concentrations are often a pre-requisite for products where high dosing and self-administration is one of the targets. Besides the work done on the formulation also downstream processing has to adapt "standard low concentration procedures" for steps like ultra-/diafiltration, virus and sterile filtration to sometimes challenging antibody properties with increasing concentration. We would like to present data on our optimization procedure for the ultra-/diafiltration step using an AKTA cross-flow system for cross-flow parameter optimization and membrane screening. Furthermore data on virus filter performance using higher antibody concentrations will be summarized for different nanofilter types. A last short section will deal with a route cause analysis performed to find a link between ultra-/diafiltration parameters and differences in drug product opalescence.

50) Scale up from µ scale: Power Input and Energy Dissipation in Microtiter Plates

Astrid Duerauer (Austrian Centre of Industrial Biotechnology)
Stefanie Hobiger (Austrian Centre of Industrial Biotechnology)
Alois Jungbauer (University of Natural Resources)

High throughput screening systems are established for many production steps in up and downstream processing to reduce material and cost for process design. Microtiter plates are state of the art for high throughput screening. It is a challenge to transfer processes from *µ*-scale to laboratory and industrial scale. Differences of applied mixing techniques come into play. While microtiter plates are preferentially processed on orbital shakers, on laboratory and industrial scale solutions are stirred. Many reactions depend on efficiency of mixing by which the energy dissipation is controlled. Therefore mixing is one of the most crucial parameters in scale up. So far no technique to determine power input or energy dissipation on µ-scale has been available. Temperature change is a measure for the effective power input, too. Mixing leads to an increase of a system`s entropy which is related to the specific heat capacity, the mass of the liquid and the time. The effective power input is direct proportional to temperature difference in the system. This is the underlying concept for micro-calorimetry. In an agitated system of defined geometry the maximal energy dissipation em is a measure for the shear / particle stress. Therefore determination of em is an essential step for characterization of an agitated system. Three model particle systems are described for that purpose, the clay / polymer flocculation, the enzyme resin, and the silicone oil / water droplet method. Their destruction kinetics is a measure for particles stress and the shear forces these are exposed to and consequently a measure for energy dissipation in a system of arbitrary geometry. In the present study an experimental setup enabling the determination of the temperature change related to the power input by mixing in microtiter plates and laboratory scale stirred tank reactors is established. The particle model systems have been adapted for the µ-scale too. The applicability of temperature change and model particle systems to measure the effective power input and energy dissipation in microtiter plates is shown and compared to a laboratory scale reactor. Temperature curves and destruction kinetics have been determined for several mixing conditions in both agitated systems. The characteristic dimensionless process numbers for agitated systems *Ph, Re,* and *Fr* are correlated to the determined values. The effective power input of the system determined by these orthogonal methods enables the correction of the calculation of *Ne* for the agitated µ-scale systems, which has so far been carried out based on empirical data only available for stirred reactors. The established methods allow the engineering based scale up of mixed systems from µ-scale to pilot scale.

51) Understanding Manufacturing Capabilities and Facility Fit for Late Stage Purification Process Development: A High Titer / High Aggregate Monoclonal Antibody Case Study

Mary Switzer (Pfizer Inc.)
Alice Furgeson (Pfizer Inc.)
Christopher Gallo (Pfizer Inc.)
Prashant Ganji (Pfizer Inc.)
Ranga Godavarti (Pfizer Inc.)
Wendy Piacenza (Pfizer Inc.)
Paul Thoday (Pfizer Inc.)

Development of efficient and cost effective commercial manufacturing processes requires an understanding of large scale manufacturing capabilities. In recent years, increasing cell culture titers in monoclonal antibody processes have required downstream process solutions that alleviate bottlenecks during large scale manufacture. Even so, downstream bottlenecks can still arise when these high titer processes result in higher than typical levels of product- and/or process-related impurities such as high molecular weight aggregate and/or host cell proteins. For optimal removal of these impurities, the load challenge to downstream process steps is reduced to enable high selectivity of product from impurity while maintaining maximum recovery of product. These processes present unique issues in established manufacturing facilities including increased column sizes, limited tank capacity, increased column and filter cycling, and buffer volume handling issues. In this presentation we will discuss a monoclonal antibody production process case study where the cell culture process delivered product titers > 5 grams/liter but contained high levels of high molecular weight aggregate. The development of downstream process steps with an eye on the fit and capabilities of an existing large scale manufacturing facility is considered and will be discussed. The development and optimization of several process steps including anion and cation exchange chromatography to maximize load challenge while maintaining selectivity for aggregate will be presented. In addition, parallel development efforts to mitigate the aggregate level via molecular design in the cell line and through cell culture process optimization will be presented. The impact of these efforts on the downstream process fit will be discussed.

52) Improving Manufacturing Productivity by the use of Established Technologies in other Industries and Newer Concepts

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Alex Brinkman (Biogen Idec)
Lynn Conley (Biogen Idec)
Matthew Westoby (Biogen Idec)
ennifer Zhang (Biogen Idec)

With a maturing product pipeline and higher dose (and thereby production) requirements for many protein therapeutics, internal manufacturing capacity will be exceeded in the coming years, requiring additional manufacturing facilities and/or outsourcing production to CMOs. Improved manufacturing processes are being developed using a combination of technologies established in other industries, such as continuous processing, and newer concepts to improve manufacturing network productivity and enable process fit into existing plants. In addition, advancements in cell line and culture technologies have led to higher titers (>5 g/L) for monoclonal antibodies coupled with shorter bioreactor duration. This has necessitated the development of higher throughput downstream processes to prevent purification from being the throughput bottleneck in the manufacturing process. The primary bottlenecks reside in the initial purification (capture) step using protein A affinity chromatography with relatively low antibody binding capacity, and in the intermediate product pools and buffer volumes exceeding the capacity of existing production vessels throughout the manufacturing process. Several technologies were evaluated to address these bottlenecks and help improve productivity including (i) newer generation protein A resins with higher binding capacity (ii) a semi-continuous mode of chromatography (sequential multi-column chromatography) for antibody capture on protein A, and (iii) single pass tangential flow filtration coupled to purification steps to enable in-line concentration of intermediate product streams. This presentation will show how integration of these process improvements into the purification process can facilitate debottlenecking and improve facility fit. Ultimately, these process improvements are expected to extend the lifespan of current manufacturing capacity, minimizing the need to retrofit existing facilities or to build additional manufacturing facilities in the future.

53) Suitability of scale-down models for bioprocess characterization: merging scientific understanding with statistical analysis

Gisela Ferreira (MedImmune, LLC)
Sanjeev Ahuja (MedImmune, LLC)
Tizita Mammo (MedImmune, LLC)
Guillermo Miro-Quesada (MedImmune, LLC)
David Robbins (MedImmune, LLC)

Scale-down models of biopharmaceutical processes are widely used by industry to predict the impacts of process parameters on product quality and process performance, at commercial scale. Regulatory agencies expect the inclusion of data reflecting the predictive validity of these models. Recently, some companies have shifted toward statistical methodologies such as the use of the Equivalence Test to qualify scale-down models. For this purpose, quantitative acceptance criteria are defined prospectively for product quality and other process outputs. The strategy can be somewhat arbitrary because the definition and justification of these criteria is user-based and not universally accepted. MedImmune, LLC has developed a modified approach to scale-down model verification, derived from the traditional Equivalence Test. A Mixed Effect model is used to analyze data and resolve the observed mean differences due to scale (fixed effects), from random effects (e.g., batch to batch variability). Confidence intervals from the statistical analysis are used to establish model suitability instead of using prospective acceptance criteria. These ranges provide the mathematical context to justify the suitability of the scale-down model data based on process, product or analytical knowledge. This reflects a paradigm shift, from a pass/fail approach to scale-down model suitability to another focused on understanding the impact of scale differences on model predictions. The poster will explore the suitability of this type of analysis for chromatography operations, from miniaturized columns through production scale. Scenarios will be presented to illustrate situations when the range suggested by the confidence interval i) can be explained by normal process and/or analytical variability, ii) cannot be fully understood but represent a worst case scenario for the scale-down model prediction; iii) do not have practical significance; or iv) requires an offset in the predictability of process performance.

54) The paradigm change in process validation – How small scale studies enhance process performance qualification and impact continued process verification

Susanne Richter (Sandoz)

With the paradigm change in process validation, the importance of the process design phase increases rapidly. Small scale studies of different downstream unit operations such as chromatography and ultrafiltration/diafiltration are used to predict process performance at manufacturing scale and to support the definition of the control strategy and the continued process verification program. In this poster the value of small scale studies for classification of process parameters, definition of resin reuse or hold time are highlighted. The opportunities and limitations of different scale-down models will be illustrated. In this context different scale-down models for intermediate hold studies will be compared. Further impact of intermediate hold on subsequent unit operations such as filtration or chromatography will be elaborated. The predictive power of the studies for large scale process performance and product quality will be discussed and consequences on the continued process verification program outlined. Essential lessons learnt from the process performance qualification of one biosimilar product will be presented including risk-based approaches for definition of the testing program and rationales for the number of process performance qualification batches. Based on process knowledge gained in the process design phase and expanded during process consistency batches, recommendations for continued process verification will be provided.

55) Comparison of Simulated Moving Bed Chromatography and Batch Chromatography using Affinity, Mixed Mode and Ion Exchange Chromatography and Different Biological Feedstreams

Peter Levison (Pall Corporation)
Rene Gantier (Pall Corporation)

With advances in cell culture and improved expression systems the bottleneck in manufacture of biologicals is fast being pushed down downstream. In order to optimise throughput and productivity, continuous bioprocessing provides an opportunity for the future and as part of this overall approach continuous chromatographic processes are under evaluation. There have been several investigations particularly in antibody processing where continuous approaches have been evaluated and reported in the literature. However there is limited systematic comparison of batch versus continuous chromatography or a rigorous assessment of loading conditions, flow rates and column number especially over a wider range of chromatographic applications. In the present work we have studied 3 chromatographic separations namely, monoclonal antibody purification from a clarified CHO supernatant using protein A affinity chromatography, Fab capture from E.coli periplasmic extract by mixed mode chromatography using MEP HyperCel and Fab capture from periplasmic E. coli extract using membrane chromatography on Mustang S. In each application we optimised a batch chromatographic process and then used this as a benchmark for continuous operation. We evaluated the influence of feedstock concentration, flow rate (residence time), and column number under continuous operations in order to assess the process economics of each condition. We report the comparison of continuous processes using Simulated Moving Bed (SMB) Technology for each of these 3 separations. Product purity profiles were consistent between SMB and batch runs in each case. For the mAb purification using protein A we demonstrated up to 3-fold productivity improvement (g/L/h) in SMB mode compared to batch. Dependent on the mAb titer the number of columns required ranged from 3-6 each operating at a DBC up to 3-fold higher than that obtained in batch operation. For the Fab purification using MEP HyperCel we demonstrated a productivity increase of up to 10-fold in SMB mode using 3-5 columns with a significant improvement in sorbent DBC. Use of Mustang S membrane chromatography gave high productivity in batch conditions and did not generate significant improvements under SMB using 3 membrane devices. We attribute this to the diffusion properties of the Mustang S membrane. We have demonstrated the utility of SMB for different protein containing feedstocks using affinity, mixed mode and ion exchange adsorbents using bead and membrane formats. These data will be presented and analysed in detail.

56) Economic continuous chromatography case analysis in three different application modes

Romas Skudas (Merck KGaA)
Sven Andrecht (Merck KGaA)
Christopher Gillespie (EMD Millipore)
Michael Phillips (EMD Millipore)

In pursuit of continuous biopharmaceutical molecule purification, we evaluated continuous chromatography technology in real case studies, exploring the potential of economic efficiency in affinity, ion exchange and reversed phase chromatography modes. The studies were performed comparing production cost of standard batch operation and continuous chromatography approach. With straightforward continuous chromatography technique, we were able to show >10 x productivity increase in antibody (mAb) capture applications using various affinity resins and mAb titers from 0,5 to 5 g/L expressed in several cell lines. The obtained cost savings were 30-50% for this unit operation accounting full costs. In addition, the robustness of this technology was proven for the variety of ion exchange chromatography steps implementing Eshmuno® S resins, where >7 x productivity increase was obtained, enabling 10-40% production savings. Therefore the reliability of this continuous approach encouraged us to expand the scope of the technology. It was integrated for peptide polishing steps using reversed phase PharmPrep® P 100 RP-18e resins in insulin purification. Our experimental data confirms not only increased productivity in each investigated case, but reduced processing costs as well. Additionally, our results indicate enhanced resin utilization enabling smaller purification unit footprint and column size. Maintained critical product quality attributes enable to adapt this technology in standard purification templates as well as in new ones in pursuit of economically efficient continuous biopharmaceutical molecule purification.

57) Towards a protein refinery: Automated perfusion antibody purification

Mark Brower (Merck & Co.)
Ying Hou (Merck & Co.)
David Pollard (Merck & Co.)

In recent years, continuous chromatography methods for protein purification have been developed using classical modalities such as ion exchange and affinity adsorbents. These methods have demonstrated large increases in specific productivity (3-10x) as well as reduced buffer demand (10-20%) of the step compared to batch based processes. The same continuous chromatography techniques can be applied to facilitate longer term protein purification campaigns with a continuous supply of feed material. In this presentation, the development and demonstration of a laboratory-scale continuous mAb primary recovery will be discussed where perfusion bioreactor permeate from a disposable filtration assembly is fed directly to a BioSMB continuous chromatography skid for protein A affinity purification. The protein A effluent is then carried forward through continuous viral inactivation and continuous anion exchange membrane chromatography in an entirely automated and single-use purification scheme with minimal human interaction. In this system, product quality and process residuals of the perfusion runs are maintained at consistent levels over time. Critical aspects of systems automation as well as the economic justification of the automated perfusion/continuous purification process for stable proteins will be discussed in the context of supporting implementation of disruptive technologies and a departure from traditional facility designs concepts.

58) Centrifugal partition chromatography for the recovery of biological products from complex mixtures

Gerhard Schembecker (Technical University of Dormund)

Centrifugal partition chromatography (CPC) is process in which the separation mechanism is based on different distribution of components between two immiscible liquid phases. As an advantage compared to other chromatographic devices no supporting solid phase is used in CPC. Thus, CPC provides gentle separation conditions (e.g. no irreversible adsorption) and a high amount of stationary phase (60-80% of the total column volume) accessible to the sample solutes providing high capacity. To immobilize one liquid phase, the column of a CPC consists of a cascade of chambers connected by ducts and aligned around a central axis of rotation. Caused by the rotation, a centrifugal force is generated which is used to immobilize the liquid (stationary) phase in each chamber, while the mobile phase is pumped through the stationary one along the whole chamber cascade. Due to different distribution behavior between the two phases, the components of a mixture injected at the beginning of the cascade will elute at different times. E.g., a component that distributes more to the stationary phase will be retained for a longer time in the rotor. For the operation in CPC basically all liquid systems forming two immiscible liquid phases can be processed. Aqueous organic systems are common for the separation of natural products. Aqueous two phase systems (ATPS), however, are relatively new as phase system for CPC operation. Here, both phases are mainly composed of water enriched with phase forming components (two incompatible polymers, one polymer and one salt or two different salts). ATPS provide gentle conditions for the separation of biomolecules (e.g. proteins). However, due to their physical properties (higher viscosities, lower density difference and lower interfacial tension when compared to common aqueous organic systems) the efficient operation in CPC is difficult and up to now it has not been well investigated. The poster will introduce the CPC technology as alternative separation technology for the recovery of biological products. Important impact factors on the design and operation will be discussed. Moreover, the separation efficiency of CPC for small molecules, like secondary metabolites, in organic-aqueous systems, and for proteins in ATPS will be presented.

Literature:

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59) Addressing Challenges with Non-Platform Purification Behavior for a Monoclonal Antibody

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Brandon Doyle (Eli Lilly and Company)
Agatha Feltus (Eli Lilly and Company)
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The Lilly mAb purification platform includes detergent treatment of the clarified CHO cell culture harvest as a simple step for robust inactivation of retrovirus. Recent experience with an early phase IgG1 demonstrated that protein A affinity chromatography may not always provide robust clearance of the detergent. In this case, Triton X-100 formed a specific interaction with the mAb at a molar ratio of approximately 2:1. Further, the level of residual detergent remained unacceptably high even after additional downstream polishing operations. This non-platform behavior triggered significant efforts both to remediate the downstream process, as well as to understand the unusual behavior that was observed. Investigations into options for detergent removal by orthogonal modes of chromatography were unsuccessful. Ultimately, a decision was made to delete the detergent step from the process, which resulted in a reevaluation of the viral safety strategy. Deletion of detergent from the process also reduced the capability of the downstream process to remove host cell proteins. The downstream purification scheme was optimized to address both of these challenges, and the new process was used successfully to produce the clinical drug substance that met release specifications. This case study highlights the fact that diversity in clinical candidates occasionally leads to non-platform behavior that necessitates a sponsor remain vigilant and nimble. Optimization of the platform process or fully customized solutions may be needed to deliver a robust manufacturing process with minimal impact to timelines.

60) Purification of a single chain antibody fragment produced in bacteria, yeast and tobacco plants using a simple and robust chromatographic process

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Driss Elhanafi (North Carolina State University)
Gary Gilleskie (North Carolina State University
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Amith Naik (North Carolina State University)

Antibody fragment-based drugs are being widely considered as the next generation biopharmaceuticals. They have the high specificity of whole antibody but offer better tissue penetration and less immunogenicity. Moreover, they can be produced in microbial systems such as bacteria and yeast at high titres leading to lower manufacturing costs. However, the lack of the constant Fc region means that the protein A based platform purification process cannot be employed for antibody-fragments. Therefore, there is an impetus for the downstream processing scientists to develop efficient, robust and low-cost purification process for antibody fragments. In this work we present the development of a chromatographic step for capture and purification of a single chain antibody fragment (scFv) from three different sources: bacteria (*E. coli*), yeast (*Pichia pastoris)* and a transgenic plant, (tobacco). Based on the differences in physicochemical properties of scFv and host cell proteins we developed an ion exchange chromatographic step for capture and purification of scFv from *E. coli* lysate. This process was then used for purification of scFv from *Pichia pastoris* supernatant. The recovery and purity obtained in both cases was 90 % and 95 % respectively, and the dynamic binding capacity was above 100 g/L. Finally, the scFv was purified from tobacco plant extract free from plant proteins and phenolic compounds.

61) Investigation of methods for the recovery of PEGylated proteins from human serum

Nicola Roberts (UCB Pharma)
Mariangela Spitali (UCB Pharma)

In early drug development vital information on stability and efficacy is determined from analysis of candidate antibody therapeutics in a range of standard buffers and storage conditions. More recently there has been evidence on the influence of physiological conditions on stability of antibody therapeutics and therefore analyses following studies in serum have been documented [1]. Serum composition typically contains large numbers of proteins including: albumin, IgG, IgA and IgM; this complex environment presents a challenge for selective recovery of an antibody therapeutic. Conjugation of proteins to polyethylene glycol (PEG), termed PEGylation, is known to improve product stability, increasing the circulating half-life *in-vivo* [2]. Charged variants of PEGylated proteins are known to occur *via* two pathways: deamidation of asparagine, a common modification seen in antibody therapeutic proteins [3], and succinimide ring opening leading to PEG linker hydrolysis. Presented here are the recovery data for the isolation of PEGylated proteins following incubation in physiological conditions. Various methodologies were investigated to recover product of suitable purity for analysis. Method investigated have included selective recovery (immuno-affinity), semi-selective (albumin binders), affinity chromatography, and product labelling (biotinylation). Once the purification strategy was established and the recovery process was shown to not affect the product, time course studies were performed to generate data enabling product analysis under physiological conditions. Overall a purification route was achieved with greater than 90% step recovery, with minimal product manipulation and effective resolution of the product to sufficient purity for the analyses required.

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Therapeutic antibody fragments with prolonged in vivo half-lives. Nat. Biotechnol. 17, 780-783 (1999). Harris JM, Chess RB. Effect of pegylation on pharmaceuticals. Nat. Rev. Drug Discov. 2003; 2:214-221.

62) Taming the beast – purification process development for a novel, high yielding recombinant von Willebrand factor fusion protein

Magnus Schroeder (CSL Limited)
Aytac Unal (CSL Limited)
Guy Harris (CSL Limited)
Ingo Brand (CSL Limited)
Deirdre O'Sullivan (CSL Limited)

Von Willebrand factor (vWF) is the largest plasma protein and consists of multimers of its dimer up to a size of >20,000 kDa. Furthermore, it has a flexible structure that is known to respond to external shear stress and has strong self-association properties. One of its functions is to bind and protect Factor VIII from inactivation in the bloodstream and it is important in platelet adhesion at wound sites. Absent or dysfunctional vWF causes von Willebrand disease, a form of hemophilia. CSL is developing a novel, recombinant vWF human serum albumin fusion protein expressed in mammalian cell culture. The fusion protein presents unique challenges for purification development due to its multimer distribution properties and maximum size. Furthermore, its sensitivity to shear stress questions the utility of conventional unit operations. Development, optimization and scale-up of a capture step will be discussed, including the applicability and use of high throughput process development tools to select and optimize purification steps. Challenges and potential solutions in the use of conventional and novel unit operations will also be discussed.

63) A Revisit of Purification Unit Operations – Coming Back with Just Simple

Michelle (Jue) Wang (Bristol-Myers Squibb Company)
Reb Russell (Bristol-Myers Squibb Company)

With improvements in upstream productivity, downstream purification faces challenges of up-scaling without increasing the cost. To tackle that, we have thoroughly examined several unit operations in monoclonal antibody purification, with the understanding of what the critical quality attributes to control in each of them. By adding simple solutions to some of the unit operations, the purification steps are reduced and the process becomes more robust. For instance, protein A affinity capture chromatography is improved by adding novel wash steps to reduce Chinese Hamster Ovary (CHO) host cell proteins (HCP) by several logs to less than 100 ppm in the elution in just one single step. With the novel washes, the CHO HCP is 1-2 logs lower in the elution compared to the conditions without these washes. The viral inactivation and neutralization step is also optimized to further reduce the CHO HCP & DNA to less than 10 ppm and 10 ppb, respectively. With the improvement of the capture and viral inactivation steps, the major process related contaminants of CHO host cell line are controlled well below the final drug substance acceptance criteria with just one column chromatography and pH titration. These conditions also allow product related contaminants (e.g. aggregate) to be controlled at below 1%. In addition to the control of process and product related contaminants removal, viral clearance control is the other important aspect to be considered. The robustness of viral clearance is built into the process by adding a single-use anion exchange membrane adsorber as the polishing step. The overall viral clearance provides 8-10 log reduction value (LRV) safety of A-MuLV virus by a purification process consisting of the protein A affinity, viral inactivation, anion-exchange membrane and parvo viral filtration steps. The above purification process reduces the processing time by approximately 20% and yields high downstream productivity of more than 80%. Furthermore, precipitation using n-octanoic acid at a cleaner stage after capture has also been evaluated as an additional tool for process contaminants removal and viral clearance. Seamless transition from step to step is included in the design to avoid additional processing. High-throughput technology (HTP) is utilized during the development for screening. Coupled with automated HTP in-process analytical assays the development time is significantly reduced with less manpower. A case study will be presented with the application of HTP during process development. The developed process eventually leads to a clinical manufacturing in a single-use facility with a one column plus one membrane purification. Ultimately, manufacturing time and costs are reduced by incorporating less steps and generating high yields, and this helps move pipeline forward faster.

64) Downstream process development of an Apo-A1 fusion protein expressed in E. coli

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Martin Bader (Roche)
Claudia Giessel (Roche)
Adelbert Grossmann (Roche)
Thorsten Lemm (Roche)
Maria Laura Magri (Roche)
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Silke Mohl (Roche)
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Bernhard Spensberger (Roche)

Artificial elevation of HDL levels (high density lipoprotein) was identified for its potential in the treatment of acute cardiovascular events in the early 1980s. However the major hurdle in developing HDL compounds is the large amount of drug needed for late stage development and market coverage. Whereas other therapeutic proteins are potent at low doses, effective drug amounts per patient for apolipoprotein-based therapeutics (reconstituted HDL) tend to be in the gram range. Thus a yearly production of greater than 1 ton would be needed. To complicate matters further, apolipoproteins, the major protein constituents of HDL particles, have a very low solubility under native conditions and a strong tendency to bind endotoxins. One obvious solution to resolve these difficulties during purification is the use of denaturants such as guanidinium chloride (GuaCl) and urea. However working under denaturing conditions limits process scalability and makes mass production impossible due to the unmanageable amounts of chaotropic salts required. To date there are no publications reporting the purification of apolipoproteins under native-like conditions. The goal of this study was to develop an innovative manufacturing process for reconstituted HDL particles which allows scalability to a final yield of many kg per batch. Such a process would reduce the use of denaturants and limit the number of process steps in order to be suitable as a scalable and cost-effective market process.

65) Establishing and maintaining a design space in downstream processing

Frank Zettl (Roche)
Nadja Alt (Roche)
Christian Hakemeyer (Roche)
Marion Hueckel (Roche)
Feliz Kepert (Roche)
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The poster outlines the challenges and solutions when establishing a design space in downstream processing. It shows how a risk based approach was applied to explore and control the ranges of process parameters in qualified scale down models. It describes key success factors that lead to the FDA approval of a process wide design space for a monoclonal antibody. The systematic procedure that was applied consisted of risk assessments as well as several rounds of experimental studies, including response surface DoE studies and studies to examine the linkage of the unit operations. The filing also included a post approval commitment to verify the design space based on the results of the process characterization and validation studies.

66) Applying the Industrial Internet to increase the process comfort zone

Per-Mikael Aberg *(GE Healthcare)*
Mattias Ahnfelt (GE Healthcare)
Rhett Alden (GE Healthcare)
Anders Nygård (GE Healthcare)

A cloud-based framework for comprehensive bioprocess data mining and visualization has been developed by leveraging the Industrial Internet for ingestion of disparate data from process skids and other sources as well as "Big Data" concepts to provide aggregation and discovery services. The framework utilizes a platform developed for high data integrity in other industries, e.g. for compartmentalized analytics of jet engine usage data from competing airlines and for data mining of sensitive patient data. It is based on a generalized service based architecture that can be easily extended with additional modules, e.g. from the open source statistical packages or proprietary analytic services. The current implementation focuses on life time optimization and process consistency monitoring for individual downstream unit operations in manufacturing settings. The framework relies on a data matrix that can be visualized using interactive graphing tools and analyzed by multivariate data analysis, e.g. Principal Component Analysis or dedicated big data analytics. Results from the analysis can be traced back to original data and drilled down to perform root cause analysis. The use of the framework will be illustrated by examples from chromatography life time studies and troubleshooting of process deviations where the ability to correlate chromatographic signatures extracted from chromatography run data to off-line quality attributes and raw material properties can be used to increase process understanding and lay a foundation for Quality by Design and process excellence.

67) Strategies for overcoming difficulties in protein production

Iris Asen (Diarect AG)

Production of recombinant proteins is usually a straightforward process. Optimized up- and downstream procedures result in a stable production process that paves the way for an up-scaled production platform. However, proteins with special features require specific conditions and establishing a reliable production process is a challenging task. Proteins which have regions of unusual amino acid sequences, e.g. arginine-rich repetitions, hydrophobic patches or specific posttranslational modifications tend to appear mostly as an unusable recombinant product as a result of translational breakdown, accumulation of insoluble and/or inactive products or protein degradation. We successfully adopted several strategies to enhance and optimize the protein expression to enable the purification of problematic recombinant proteins for use in diagnostic applications: Codon optimization was used to increase the expression level of a protein with repetitive sequences containing a high degree of arginine-residues. Site-specific mutagenesis of the active site facilitated a stable production process of an autocatalytic transferase. Expression of a halogenase fusion protein enabled the solubility of a membrane-associated protein and resulted in a simplified purification process. Co-infection strategies implemented in the baculovirus-insect cell expression system led to important selective and specific posttranslational modifications. Integration of novel strategies for protein design, expression and purification can enable the efficient production of challenging recombinant proteins to obtain large amounts of high quality products.

68) Evaluation of a 'Limited Design Space' for Biotherapeutics

Amit Mehta (Genentech, Inc.)

Quality by design (QbD) principles are increasingly being employed during design and development of manufacturing processes to gain enhanced product and process understanding and to ensure that the commercial manufacturing process delivers product with a predefined quality. Implementation of QbD principles requires defining acceptable ranges for product's critical quality attributes (CQAs) and performing process characterization studies to determine acceptable process parameter ranges that ensure that the CQA acceptable ranges are met. The acceptable process parameter ranges are implemented using 'process-wide design space' which is defined by ICH guidance Q8(R2) as "the multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality". While a process wide design space, can provide significant flexibility for manufacturing site-transfers and operational improvements, it may not be feasible for bioprocesses where small changes in process parameters can significantly affect purity and product quality. This poster will focus on a 'limited design space' claim for a process having narrow multivariate acceptable ranges (MARs). The limited design space with a small set of process parameters can provide significant manufacturing value with minimal additional experimental investment over a traditional regulatory filing and can simplify health authority review and approval.

69) Monoclonal Antibody Production Platform Development and Optimization for A Rapid Clinical Phase Enabling Manufacturing Process

Yi Liu (Bayer Healthcare)
Steve Garger (Bayer Healthcare)
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Margarette Mariano (Bayer Healthcare)

The rapid growth and high demand for monoclonal antibodies (mAbs) in preclinical and clinical studies justify the resources and time needed to standardize the production process from cell culture to downstream purification and the corresponding analytics. Platform processes use standard sets of unit operations, conditions and methods which allow for rapid development of pipeline molecules to clinical manufacturing. The platform approach employs similar purification processes in the recovery of different products with minimal process and equipment alterations. Here, we present the development of a phase I enabling process using a platform for mAb purification, as well as the optimization of the platform for clinical manufacturing. Impurities, such as host cell protein, DNA, putative adventitious and endogenous viruses, endotoxin, aggregates and other species must be removed through purification process while an acceptable yield is maintained. Our process uses three chromatography steps and several filtration steps. The process comprises affinity chromatography for capture step followed by different combinations of ion-exchange chromatography for intermediate and polishing steps. We present case studies to show the implementation of the platform process for the purification of several pipeline therapeutic IgG molecules. The platform approach has facilitated process development resulting in the rapid achievement of scalable, clinical manufacturing processes.

70) A Robust Development Strategy for Primary Recovery of High Cell Density Mammalian Cell Cultures by Tangential Flow Microfiltration Options

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Suzanne Farid (University College London)
Dave Pain (Lonza Group Ltd.)
Adam Sonier (Lonza Group Ltd.)
Nigel Titchener-Hooker (University College London)

Advances in cell culture have led to significant increases in cell densities and titres, but also associated increases in solids and impurity loads to primary recovery and purification operations. These pose an additional challenge for the formulation of efficient and cost effective strategies for the future. We have previously demonstrated a methodology for screening and selecting future primary recovery technologies. This identified tangential flow filtration technologies as potential candidates for efficient processing of high cell density mammalian cell broths. In this new work we have explored further the effects of key cell culture variables on tangential flow microfiltration performance in terms of yield, impurity removal capability as well as economic considerations, ultimately aiding the decision making involved in new technology implementation and strategy formation. Cell culture test material methodology developed previously has been used to generate a specific range of high cell densities (20-100x106 cells/mL) with varying titre (5-20 g/L) and impurity (5,000-20,000 µg/mL) concentrations. A central composite DoE design approach was used to explore the effects of cell density, cell viability, titre and host cell protein load (HCP) on throughput, product, HCP and DNA transmission achieved when using the selected hollow fibre modules (Bio-Optimal MF-SL and QuySpeed D, Asahi Kasei, Japan). The uncharged Bio-Optimal MF-SL module has been previously shown to achieve high throughputs when processing high cell density feed materials, whereas the QuySpeed D was shown to have a high capacity for impurity removal. The experimental data collected allowed us to derive empirical correlations so as to evaluate the future performance of this technology and to identify the likely cell culture conditions at which a technology switch from current platforms would be most effective. A detailed economic model was developed using the experimentally derived correlations and was applied to a range of scale scenarios: <2,000L and 2,000-20,000L. Cost of goods outputs, encompassing indirect (equipment depreciation) and direct (materials, labour and utility) costs, were compared. Facility and economic constraints were then applied based on current example processes using centrifugation and depth filtration stages. Finally, a performance benefit criteria was set to identify the areas where implementation of the filtration technology became economically viable, as well as to indicate the most suitable technology choice for each set of conditions.

71) Membrane filtration can substitute chromatographic purification steps for plant-derived and ELP-tagged biopharmaceutical proteins

Johannes Buyel (RWTH Aachen)
Rainer Fischer (RWTH Aachen)
Hannah Gruchow (RWTH Aachen)
Patrick Opdensteinen (RWTH Aachen)

Chromatographic techniques are most frequently used for the purification of biopharmaceutical proteins. However, identifying an effective capture step can be a challenging task for non-antibody target proteins, especially if these are expressed at low levels or contaminated by a large number of host cell proteins (HCPs) as it is often the case for plant-derived products. In such cases, several HCPs can bind to the capture resin and reduce the effective binding capacity resulting in the necessity for large column dimensions and increasing downstream processing (DSP) costs. A selective removal of HCPs prior to the initial capture step may help to circumvent this problem and reduce costs in DSP. Here we present how membrane based pre-capture purification strategies can simplify DSP and replace chromatographic purification steps for various proteins. On the one hand, we highlight how a design of experiments approach can be used to identify conditions and membrane cut offs for the separation of plant HCPs and target proteins of different molecular masses. On the other hand, we show how membrane inverse transition cycling can be implemented into a scalable production of ELP-tagged target proteins. Both techniques can be easily adapted to other expression systems and thus may be of interested to other colleagues from academia as well as from industry. This is especially true for those working with novel non-antibody products of next generation biopharmaceuticals like enzymes and vaccine candidates.

72) Potential of Depth Filtration Steps within an Established DSP Sequence to Ensure Appropriate Product Quality of mAb Products with Respect to Virus Safety

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Sabine Faust (Rentschler Biotechnologie GmbH)
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Sophie Winterfeld (3M)

In the past robust platform purification processes were developed for monoclonal antibodies and antibody-related molecules. Certain platform variations were caused by facility needs and different DSP strategies of the companies. Most of them include an affinity step like protein A and two bind/elute or flow through polishing steps. As many platforms were improved over time, one polishing step might be sufficient for adequate process robustness and appropriate product quality of the Drug Substance. The demonstration of virus clearance is part of ensuring safety of biopharmaceutical drugs and has still to be guaranteed with a reduced number of purification steps. In this way anion exchange chromatography in flow through mode is commonly used as unit operation beside by virus filtration, virus inactivation at low pH and protein A chromatography and maintained in most purification processes. Depth filtration is a widely used unit operation in state-of-the-art platform purification processes for clearance of precipitates and process–related impurities like DNA and HCP. It could also become attractive for use as virus removal step based on its adsorption capacity caused by its positively charged surface. This would unchain the remaining polishing step from virus removal needs and it could be optimized for product quality demands. The first part of this poster outlines case studies of two chromatographic step purification processes. In the second part virus removal data for two model viruses (X-MuLV and MMV) with two commercial depth filters and a prototype hybrid purifier at various conditions will be shown supporting orthogonal virus removal mechanisms of classical virus filtration and depth filtration steps.

73) Boosting microorganism retention or productivity by smart selection of filtration parameters

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Gerhard Haake (Sartorius Stedim Biotech)
Alexander Helling (Slovak University of Technology)
Jörg Hosch (Sartorius Stedim Biotech)
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Paula Sanchez (George August University Göttingen)
Iwan Schaap (George August University Göttingen)

Size exclusion is the predominant mechanism for particle retention in most membrane based filtration processes. This is mostly true for colloidal particles (e.g. viruses) and very often true for microscopic particles (e.g. bacteria). This work demonstrates how operating parameters like applied trans-membrane-pressure or temperature can significantly impact the retentive performance of a filter membrane. We have collected a quite unique set of data describing the level of retention for a panel of 7 different bacteria and mycoplasma species as a function of trans-membrane-pressure and filtration temperature. In these filtration studies, we used single layer flat sheet model membranes, exhibiting levels of retention that can easily be detected and differentiated. In addition, we have characterized the physical properties of the respective microorganisms with different direct characterization techniques, like SEM and DLS for particle size distribution as well as AFM for ductility measurements. To further characterize particle properties, filter cakes were generated for purified mycoplasma and similarly sized latex beads and the respective hydrodynamic cake resistance as a function of operating pressure evaluated. The results show that the observed retention data can be rationalized and correlated to the ductility of the target particles. Furthermore, for a particular particle cake, hydrodynamic resistance increases significantly at the same operating pressure range at which particle retention starts to decay in independent filtration trials. Strong correlations to particle retention were seen with respect to the influence of operating temperature. Based on these results, operating parameters can be chosen that boost microorganism retention. Alternatively, when higher levels of retention are not required, overall filtration performance can be significantly increased by using more open membranes at selected operating parameters.

74) Filtration Technology Miniaturization for Rapid Biopharmaceutical Process Development

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Jurgen Hannemann (Biberach University of Applied Sciences)
Hans Kiefer (Biberach University of Applied Sciences)
Shuo Sui (East China University of Science & Technology)

In search for more optimized processes and their variables, conventional biotechnology process development usually entails tens or even hundreds of laboratory scale experimentations at timings and for durations determined by the resources available. This approach for developing biosimilar processes obviously is not pragmatic, as there will be stringent time pressure for entering into clinical trial phases. Furthermore, without patent protection, the ultimate winners on the market for this industry have to be those achieving process intensification earlier rather than later. The total volume of feed materials and the number of trials for such time urgent tests often hamper a large number of initial stage tests due to a blend of prohibiting factors like cost and time. Given the abundant usages of membrane technology in downstream processing in biopharmaceutical manufacturing, the objective of the present work was to design, fabricate and test a novel miniaturized, low cost membrane filtration technology platform for the mAb industry. The platform would offer potential for conducting high throughput experiments within very short time spans for establishing competitive downstream processing and for meeting business goals of the manufacturers. We have designed and then fabricated a set of ultra-small parallel filtration devices for ultrafiltration, nanofiltration, microfiltration, and diafiltration, containing micro sensors, with a channel length being 5.6 cm, and the working volume being 0.17 ml. The design for flow channel is in plate form and channel heights are mostly 0.5-1.0mm. Compared with laboratory scale and even pilot plant scales, our new device for applications, typically for recovering monoclonal antibodies out of cultured mammalian cells, was tested and validated under like-to-like macroscopic operating conditions (primarily average pressure in the retentate and flow rate per unit membrane area or average linear velocity) for Computation Fluid Dynamics (CFD) experimentation for 3D pressure profile, supported by selective direct measurement; CFD experimentation for 3D velocity vector profile, coupled with macroscopically controlled retentate tangential flow rate; CFD simulation on shear force profile, supported by assessment on stability and folding of monoclonal antibodies; Diavolume time profile over the model buffer swap process demonstration; Protein separation and isolation processing demonstration using UF and nano membrane; Cell separation and isolation processing demonstration by micro-filtration; Protein and particle process demonstration for concentration processing. Such a developed new platform has potential (a) in handling very small sample volume per experiment, (b) for being very cost effective, used either as disposable or merely for a short duration, (c) for developing into a high-throughput commercial assembly to considerably increase process development speed.

75) Case Study: Implementing Pall's Single-pass TFF technology and hardware to improve yield and optimize process operations. From Challenge to Success; Development to Manufacturing

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Recent trends in biopharmaceutical manufacturing toward achieving high drug product concentrations for subcutaneous delivery have prompted the need for technologies and hardware that deliver high concentration product pools with high yield. High concentration monoclonal antibody (mAb) and intravenous immunoglobulin (IVIG) applications can target pool concentrations of 200 to >250 g/L. With conventional TFF, the exponentially increasing viscosity with increased product concentration can complicate the recovery of the product from the system. Pall's Cadence™ single-pass TFF technology enables high concentration of product pools with minimal product dilution and high yields. This work discusses the implementation of the Cadence single-pass TFF technology for the final product concentration step following an initial concentration and diafiltration using a conventional TFF operation. The existing TFF process suffered from poor product yield and the risk of foaming due to system limitations. The single-pass TFF process was developed at a 0.14 m2 scale to define the appropriate operation conditions to concentrate a mAb from ~55 (+/- 10) g/L to >230 g/L and to explore process robustness. The process was scaled up 270 fold to a 37.5 m2 operation in a GMP environment. During engineering runs, the system start-up conditions were defined and further process robustness studies were conducted. Four GMP runs were then performed on an approved commercial product, which demonstrated the ability to attain product pool concentrations of >230 g/L, with a much improved step yield compared to the previous conventional TFF process. Some of the challenges encountered from the development stage through scale-up to manufacturing, as well as the strategies to overcome them will be illustrated.

76) Characterization of post-centrifugation, CHO cell flocculation using an ultra scale-down mixing methodology

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The increase in demand for biologics has led to the need for a large global manufacturing capacity. Improvements to upstream processes over the past two decades have significantly increased this production capability, but often resulting in an increase of biomass from the cell culture broth that presents challenges to both clarification and purification operations. It is in this context that technologies such as flocculation are being revisited. Here we address the use of a novel ultra scale-down (USD) methodology for the characterization of flocculation processes. The methodology consists of a multiwell, magnetically agitated rotating disk system for operation with an automated liquid handling robot. This microwell system was used to mimic the flocculation performance of a pilot-scale vessel by matching the predominant mixing scale and applying average turbulent energy dissipation or impeller tip speed as the scale-up correlation. *Saccharomyces cerevisiae* clarified homogenate was first used to develop the technique as a representative high-solid-level fluid for the flocculation and removal of the residual solids present in disk-stack centrate. The applicability of the USD method in mammalian cell broths was then explored by flocculating the supernatant of a clarified high cell density, mAb producing CHO cell culture. The work presented covers a discussion of the importance of the predominant mixing scale (whether macromixing, mesomixing or micromixing) in determining the particle size distribution of the resulting flocs and shows how these mixing types can modulate the success of some of the scale-up correlations reviewed in the literature. The USD methodology is verified at pilot-scale, where the flocculation performance is mimicked depending upon the predominant mixing scale and the characteristics of the material being flocculated. The USD methodology was successful in scaling up flocculation processes from a 96-well microplate to a pilot-scale vessel representing a scale-up greater than three orders of magnitude between two non-geometrically matched systems. This method has the potential of performing 96 different flocculation conditions at the same time thus fulfilling the need for a high-throughput and automated platform required during the initial primary recovery development studies.

77) Expanded Bed Adsorption Processing for High-Density Mammalian Cultures in GMP: on the interface of Upstream and Downstream Processing.

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The RHOBUST® technology is the next generation Expanded Bed Adsorption chromatography (EBA), using cross linked agarose beads with Tungsten Carbide to increase the particle density. EBA is applied as direct capture step, without harvest clarification, for mammalian (e.g. XD®) and microbial harvest. Important aspects of EBA have been investigated to better understand flow distribution, behavior of the resin beads and binding properties of the protein A coated beads. A validated 30cm-diameter cGMP column will be presented. Data on resin bed expansion and flow distribution will be presented for different RHOBUST® EBA columns equipped with rotating fluid distributors (RFD) to demonstrate good scalability. Residence time distribution (RTD) measurement is a suitable method to investigate the stability of the expanded resin bed in manufacturing-scale and reduced-scale columns. Different methods, including the biomass pulse-response method and "ion" RTD test, were used to evaluate axial bead distribution and biomass influences in Expanded Bed using in-bed sampling on 2cm-diameter EBA columns. RTD data will show that flow distribution within the column (measured at 10 points along the expanded bed) remains unchanged and is not influenced by the presence of yeast cells. The importance of a good flow distribution will be made clear from process data. In order to understand the distribution of protein adsorption in the expanded bed, IgG adsorption along the bed height was measured with an on-line sampling method. 5 positions along the bed height were sampled, and UV detection was used to measure protein breakthrough at each position. Based on the data of local voidage distribution the settled bead volume at different zones was obtained, and then the IgG adsorption was calculated at each zone. Best adsorption efficiency was found at the bottom of the resin bed. For the first zone (0~7 cm bed height), the adsorption capacity of resin reached 57 mg IgG/mL resin. With the increase in bed height, the effective adsorption capacity decreased. The zone (17~27 cm bed height) at the middle of bed had the lowest adsorption capacity of 35 mg IgG/mL resin, while the zone at the top of the bed (37~47 cm bed height) had an adsorption capacity of 48 mg/IgG/mL resin. The average adsorption capacity for whole column was 43 mg IgG/mL resin. Influence of different process parameters relevant for the EBA process like pH, residence time, and temperature will be briefly addressed. A case study will be presented where product capture from cell culture using traditional methods (clarification and packed bed chromatography) and RHOBUST® EBA are compared. We will demonstrate that RHOBUST® EBA enables efficient biomass removal and product capture and results in high product purity, good DNA and HCP removal, less processing time and lower cost.

78) Novel and Efficient Cell Culture Flocculation Process Using a Stimulus Responsive Polymer to Streamline Antibody Purification Process

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Increasing cell culture densities and productivities during therapeutic protein (mAbs) production are placing a larger burden on downstream clarification and purification operations due to elevated levels of cell densities and cellular debris as well as process and product related impurities, necessitating improvements in downstream processing. In this study we have developed an alternative antibody harvest process that includes flocculation using a novel stimulus responsive flocculant, benzylated poly(allylamine), followed by depth filtration. The process demonstrates high process yield, improved clearance of cells and cell debris, and efficient reduction of aggregates, host cell proteins and DNA for multiple antibodies. This process has achieved residual levels of impurities in the protein A eluate that potentially meets the requirements of drug substance and thus alleviates the burdens for further impurities removal in the subsequent chromatography steps. This novel and efficient process can be easily integrated into current mAb purification platforms, and may improve current mAb purification processes by overcoming downstream processing challenges.

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