17th PEACe

Protein Expression in Animal Cells Conference

September 14-18, 2025

Bonaventure Hotel • Montreal, Canada

www.peace-conference.org

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PEACe - Secretariat
+ 1 418 522-8182 or / ou 1 800 618-8182
peace2025@agora.events

PROGRAM

Sunday, September 14, 2025

17:30-20:30 | Registration

18:30-20:30 | Welcome Reception

Monday, September 15, 2025

08:00-19:00 | Registration

08:00-09:00 | Breakfast - Room : St Laurent 1-2

09:00-09:10 | Opening Words - Yves Durocher - Montreal 1/3

09:10-10:30 | Expression Systems 1 - Room : Montreal 1/3

09:10 - 09:40 **J. Christopher Love,** Koch Institute at MIT, U.S (Invited speaker)
09:40 - 09:50 **Linda King,** Oxford Expression technologies, U.K (Flash)
09:50 - 10:00 **Mark Elvin,** Sygnature Discovery, U.K (Flash)
10:00 - 10:20 **Kitty Agarwal,** Merck, U.S (Oral)
10:20 - 10:30 **Rena Mizrahi,** Grifols, U.S (Flash)

10:00 | Exhibition open - Room : St Laurent 1-2

10:30-11:00 | Coffee Break - Room : St Laurent 1-2

11:00-12:00 | Expression Systems 2 - Room : Montreal 1/3

11:00 - 11:20 **Claes Gustafsson,** ATUM, U.S (Oral)
11:20 - 11:40 **Matthew Reaney,** University of Manchester, U.K (Oral)
11:40 - 11:50 **Kakoli Bose,** ACTREC, Tata Memorial Center, India (Flash)
11:50 - 12:00 **Michael Anbar,** Lonza, U.K (Flash)

12:00-13:00 | Keynote lecture, Samuel Sternberg, Columbia University (USA) - Room : Montreal 1/3

13:00-14:00 | Lunch - Room : St Laurent 1-2

14:00-14:15 | Introduction - Yves Durocher - Room : Montreal 1/3

14:15-14:30 | Industrial Workshop ; Thomson, Sam Ellis - Room : Montreal 1/3

14:30 - 16:00 | Cell Engineering 1 - Room : Montreal 1/3

14:30 - 15:00 **Camil Diaz**, Genentech (Invited speaker)
15:00 - 15:20 **Hooman Hefzi**, Hooman Hefzi, Technical University of Denmark (Oral)
15:20 - 15:40 **Devika Kalsi,** Fujifilm, U.K (Oral)
15:40 - 16:00 **Matthew Stuible,** National Research Council of Canada (NRC), Canada (Oral)

16:00-16:30 | Coffee Break - Room : St Laurent 1-2

16:30 - 18:00 | Cell Engineering 2 - Room : Montreal 1/3

16:30 - 16:50 **Marzia Rahimi,** Technical University of Denmark, Denmark (Oral)
16:50 - 17:10 **Nikolas Zeh,** ASIMOV, U.S(Oral)
17:10 - 17:30 **Janis Marzluf,** University of Ulm, Germany(Oral)
17:30 - 17:40 **Javier Bravo-Venegas**, Pontificia Universidad Católica de Valparaíso, Chile (Flash)
17:40 - 17:50 **Emely Walker**, Biberach University, Germany (Flash)
17:50 - 18:00 **Manuel Reithofer,** Institute of Molecular Biotechnology, BOKU University, Austria (Flash)

18:00-18:15 | Break

18:15-20:15 | Poster Session 1 & Cocktail - Room : St Laurent 1-2

20:15 | Exhibition close - Room : St Laurent 1-2

Tuesday, September 16, 2025

08:00-17:30 | Registration

08:00-09:00 | Breakfast - Room : St Laurent 1-2

08:15 | Exhibition open - Room : St Laurent 1-2

09:00 - 10:30 | Multispecifics and protein engineering 1 - Room : Montreal 1/3

09:00 - 09:30 **René Hubert for Bram Estes,** Amgen, U.S (Invited speaker)
09:30 - 09:50 **Stephan Schmidt,** Evitria, Swizerland (Oral)
09:50 - 10:10 **Nicolas Doucet,** National Research Council of Canada (NRC), Canada (Oral)
10:10 - 10:30 **Jack Scarcelli,** Sanofi, U.S (Oral)

10:30-11:00 | Coffee Break - Room : St Laurent 1-2

11:00 - 12:00 | Multispecifics and protein engineering 2 - Room : Montreal 1/3

11:00 - 11:20 **Alison Young,** Fujifilm, U.K (Oral)
11:20 - 11:30 **Katja Rüger**, Rondo Therapeutics, U.S (Flash)
11:30 - 12:00 **Nathan Lewis,** Augment Biologics, U.S (Oral)

12:00-13:00 | Lunch - Room : St Laurent 1-2

13:00-14:30 | Product Quality 1 - Room : Montreal 1/3

13:00 - 13:30 **Jeff Smith**, Carleton University, Canada (Invited speaker)
13:30 - 13:50 **Will Johnson,** Asimov, U.S (Oral)
13:50 - 14:10 **Elisabeth Giudovacz**, BOKU, Austria (Oral)
14:10 - 14:30 **Linus Weiß,** Biberach University, Germany (Oral)

14:30-15:00 | Coffee Break - Room : St Laurent 1-2

15:00-16:30 | Product Quality 2- Room : Montreal 1/3

15:00 - 15:10 **Parastoo Azadi,** University of Georgia, U.S (Flash)
15:10 - 15:20 **Tilia Zinnecker,** Max Planck Institute for Dynamics of Complex Technical Systems, Germany (Flash)
15:20 - 15:30 **Ilona Metayer**, Polytechnique Montreal, Canada (Flash)
15:30 - 15:40 **Francisca Torres-Garcia,** Pontificia Universidad Católica de Valparaiso, Chile (Flash)
15:40 - 15:50 **Zach Demorest,** Evonik, U.S (Flash)
15:50 - 16:00 **Sheryl Lim**, University College Dublin, Irland (Flash)

16:00-16:30 | Industrial Workshop ; Thermo Fisher Scientific, Jon Zmuda - Room : Montreal 1/3

16:30 | Exhibition close - Room : St Laurent 1-2

16:30-17:15 | Free time

17:15-22:00 | Walking Tour & Cocktail at Montréal Big Wheel

Wednesday, September 17, 2025

08:30-19:00 | Registration

08:30-09:30 | Breakfast - Room : St Laurent 1-2

08:45 | Exhibition open - Room : St Laurent 1-2

09:30-10:30 | Keynote Lecture, Christopher Corbeil - Room : Montreal 1/3

10:30-11:00 | Coffee Break - Room : St Laurent 1-2

11:00-12:30 | Therapeutic viral and non-viral particles 1 - Room : Montreal 1/3

11:00 - 11:30 **Laura A. Palomares**, Universidad Nacional Autonoma de México, Mexico (Invited speaker)
11:30 - 11:50 **Margarida Queluz Rodrigues**, iBET- Instituto de Biologia Experimental e Tecnológica, Portugal (Oral)
11:50 - 12:10 **Johan Rockberg,** KTH, Sweden (Oral)
12:10 - 12:30 **Lennart Jacobtorweihe**, Max Planck Institute Magdeburg, Germany (Oral)

12:30-13:30 | Lunch - Room : St Laurent 1-2

13:30-14:30 | Therapeutic viral and non-viral particles 2 - Room : Montreal 1/3

13:30 - 13:50 **Maria Toth,** BOKU University, Austria (Oral)
13:50 - 14:10 **Marc Garcia** Marc García, Universitat Autònoma de Barcelona, Spain (Oral)
14:10 - 14:20 **Zalma Sanchez** National Research Council Canada (NRC) / University of Montreal (UdeM), Canada (Flash)
14:20 - 14:30 **Jesus Levado Garcia** Technical University of Denmark, Denmark (Flash)

14:30-15:00 | Coffee Break - Room : St Laurent 1-2

15:00-15:45 | Industrial Workshops - Room : Montreal 1/3

15:00 - 15:15 **Asimov,** Raja Srinivas
15:15 - 15:30 **Atum,** Claes Gustafsson
15:30 - 15:45 **Sartorius**, Sandra Klausing

15:45-16:45 | Student-industry panel and networking - Room : Montreal 1/3

Thermo Fisher Scientific, Natasha Serzedello
Asimov, Raja Srinivas
Atum, Claes Gustafsson
Sartorius, Sandra Klausing

16:45-17:00 | Break - Room : St Laurent 1-2

17:00-19:00 | Poster Session 2 - Room : St Laurent 1-2

19:00 | Exhibition closed - Room : St Laurent 1-2

19:00-19:45 | Free time

19:45-23:30 | Gala Dinner & Awards - Room : Montreal 1/3

Thursday, September 18, 2025

08:30-13:00 | Registration

08:30-09:30 | Breakfast - Room : St Laurent 1-2

19:00 | Exhibition open - Room : St Laurent 1-2

09:30-11:00 | Omics, AI tools and intensification 1 - Room : Montreal 1/3

09:30 - 10:00 **Ioanna Tzani**, National Institute for Bioprocessing Research and Training, Ireland (Invited speaker)
10:00 - 10:20 **Jasmine Tat**, University of California, San Diego; Amgen Inc, US (Oral)
10:20 - 10:40 **Sarath Chandar**, Mila - Quebec AI Institute, Canada (Oral)
10:40 - 10:50 **Georg Smesnik**, BOKU University, Austria (Flash)
10:50 - 11:00 **Jimmy Gaudreault**, Universisté Laval, Canada (Flash)

15:30-16:00 | Coffee Break - Room : St Laurent 1-2

11:30-12:30 | Omics, AI tools and intensification 2 - Room : Montreal 1/3

11:30 - 11:50 **António Roldão**, iBET, Instituto de Biologia Experimental e Tecnológica, Portugal (Oral)
11:50 - 12:10 **Paula Meleady**, Dublin City University, Ireland (Oral)
12:10 - 12:20 **Jan Küchler**, MPI Magdeburg, Germany (Flash)
12:20 - 12:30 **David Latulippe**, McMaster University, Canada (Flash)

12:30-13:00 |Closing remarks - Yves Durocher - Room : Montreal 1/3

WELCOME MESSAGE

Dear Participants,

On behalf of the organizing committee, we welcome you to the 17th Protein Expression in Animal Cells (PEACe) Conference. Over the past 32 years, the conference has reflected changes in recombinant protein production and related areas for the biotechnology industry and academia. This year, the program includes six scientific sessions and industrial workshops on topics including expression systems, product quality, big data and AI applications in protein, cell line and process designs. The conference committee members have assembled an outstanding program highlighting leading-edge scientists whose recent and influential contributions are driving progress in the field. Session Chairs, drawn from the Organizing Committee and speakers, will facilitate scientific discussions during the conference. The program provides opportunities for early career scientists to present alongside keynote and invited speakers. All speakers and abstract submitters are acknowledged for their contributions. Approximately 70 poster presentations will be displayed, and participants are invited to review and discuss the work with their authors.

The Organizing Committee would especially like to thank our numerous sponsors. Their generosity is critical for us to hold this non-profit conference. Some funding initiatives have provided financial support for a number of graduate students to attend and present their research. Please take the time to visit their booths and displays and do not hesitate to engage discussion with them to learn about their latest innovations and gain from their expertise. I would also like to personally thank the Organizing Committee for undertaking all their assigned tasks in a timely manner despite their busy schedules and for working together so well. The Organizing Committee would like to highlight the expert guidance and organizational skills of Fanny Lagarrigue from Agora Opus who kept the committee on track.

Finally, the location, the scientific program, and the social events have been designed to encourage networking and enhance scientific discussion amongst participants. We hope that the oral and poster presentations in this stirring environment will generate new ideas and the initiation of fruitful novel research projects and collaborations. Your participation and engagement are what make this meeting truly special. Together, let’s ensure a memorable and successful conference.

We appreciate your participation and wish you a successful and engaging PEACe meeting in Montréal.

Yves Durocher,

Chair of the 17th PEACe Conference

THE ORGANIZING COMMITTEE

Yves Durocher, Chairman
PEACe Committee Chair
National Research Council Canada
Canada

Laura Cervera Garcia
PEACe Session Chair

Universitat Autònoma de Barcelona, Spain

Bram Estes
PEACe Session Chair

Amgen, USA

Simon Fisher
PEACe Session Chair

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PEACe Session Chair
Maynooth University, Ireland

Kerstin Otte
PEACe Session Chair
Hochschule Biberach, Germany

KEYNOTE SPEAKERS

Christopher Corbeil

Senior Research Officer, National Research Council Canada (Canada)

Dr. Christopher Corbeil is a Senior Research Officer at the National Research Council Canada (NRC) who specializes in the development and application of computational tools for biotherapeutic design and optimization. He is also an associate member of the McGill Biochemistry Department and teaches classes in Structure-Based Drug Design at McGill University. After receiving his PhD from McGill University, he joined the NRC as a Research Associate investigating the basics of protein-binding affinity. Following his time at the NRC he joined Chemical Computing Group as a Research Scientist developing tools for protein design, structure prediction, and binding affinity prediction. He then decided to leave private industry and rejoin NRC with a focus on antibody engineering. Dr. Corbeil has authored over 30 scientific articles and is the Lead developer of multiple software programs.

Presentation on Wednesday, September 17 at 9.30 am : ''Beyond the Fold: Charting the AI-Driven Future of Antibody Discovery''

Samuel H. Stenberg

Associate Professor, Columbia University (USA)

Samuel H. Sternberg, PhD, runs a research laboratory in the Department of Biochemistry and Molecular Biophysics at Columbia University, where he is an Associate Professor and Investigator of the Howard Hughes Medical Institute. He received his B.A. in Biochemistry from Columbia University in 2007, graduating summa cum laude, and his Ph.D. in Chemistry from the University of California, Berkeley in 2014, where he worked with Nobel Laureate Dr. Jennifer Doudna. Sam's research focuses on CRISPR-Cas systems, RNA-guided enzymes, and genome engineering technologies. He is the recipient of the NIH Director's New Innovator Award and the NSF CAREER Award, and was previously a Sloan Fellow, Pew Biomedical Scholar, and Schaefer Research Scholar. In addition to publishing and speaking internationally, Sam remains involved in public outreach and discussions on the ethical issues surrounding genome editing. Together with Jennifer Doudna, he co-authored a popular science book about the discovery of CRISPR technology, titled A Crack in Creation, which The New York Review of Books called "required reading for every concerned citizen."

Presentation on Monday, September 15 at 12.00 pm : ''Discovery and development of CRISPR-associated transposases for RNA-guided gene insertion''

INVITED SPEAKERS

Camil Diaz

Technical Development Principal Scientist, Genentech (USA)

Camil Diaz is a Principal Scientist at Genentech. She joined the Media and Process Group in 2019, helping advance the globally aligned CHO cell culture platform currently in use for all new molecular entities at Genentech and Roche. She obtained her B.S. in Chemical Engineering from Stanford University in 2013 and her Ph.D. in Chemical and Biomolecular Engineering from the University of Delaware in 2019, training in the labs of Elizabeth Sattely and Maciek Antoniewicz, respectively. Her graduate work centered on the use of 13C-metabolic flux analysis as a tool for modeling and characterizing the core metabolism of an aerobic diazotroph. Her current interests are in mammalian metabolism, redox chemistry, and medium stability.

Bram Estes

Senior Principal Scientist, Amgen (USA)

Bram Estes is a Senior Principal Scientist at Amgen, Thousand Oaks. Bram received his B.S in Biochemistry and Cell Biology from the University of California in San Diego in 2001. Between high school and college, he spent seven years racing bicycles across the United States and Europe. He began his career studying agricultural plant and pathogen relations by reverse genetics at Novartis and Syngenta. He then shifted his focus to biopharmaceutical protein production at Xencor. In his 19 years at Amgen, Bram has had an assortment of roles, including developing high volume and automated high-throughput expression platforms, developing immunoglobulin scaffolds for monoclonal antibody and multi-specific therapeutics, and engineering therapeutic proteins for improved biophysical characteristics. He has also led numerous therapeutic projects through research and early development and leads the expression group in the Large Molecule Discovery department.

Christopher Love

Associate Professor, Carleton University (Canada)

J. Christopher Love is the Raymond A. (1921) and Helen E. St. Laurent Professor of Chemical Engineering and member of the Koch Institute for Integrative Cancer Research at MIT. In addition, Chris is an associate member at the Eli and Edythe L. Broad Institute, and at the Ragon Institute of MGH, MIT, and Harvard. Dr. Love received his Ph.D. in 2004 in physical chemistry at Harvard University. He extended his research into immunology at Harvard Medical School from 2004-2005, and at the Immune Disease Institute from 2005-2007. His research centers on using simple microsystems to monitor cells from clinical samples in chronic human diseases, and on developing new approaches to manufacturing biologic drugs and vaccines efficiently and affordably. Dr. Love was named a Dana Scholar for Human Immunology, a Keck Distinguished Young Scholar in

Medical Research in 2009, one of Popular Science's Brilliant 10 in 2010, and a Camille Dreyfus Teacher-Scholar. He served as a Distinguished Engineer in Residence at Biogen in 2015. Dr. Love is the founding director of the Alternative Host Research Consortium at MIT and current faculty director of the MIT Venture Mentoring Service (VMS). He has co-authored more than 150 peer-reviewed papers and is an inventor on multiple issued patents. Dr. Love has co-founded five companies for biopharmaceutical services and technologies, including Honeycomb Bio, OneCyte Bio, Sunflower Therapeutics, and Amplifyer Bio.

Laura A. Palomares

Professor, Universidad Nacional Autonoma de Mexico (Mexico)

Laura A. Palomares is a Biochemical Engineer from the Monterrey Institute of Technology, Mexico. She has a doctoral degree from the National Autonomous University of Mexico (UNAM) and pursued postdoctoral training at Cornell University. She is the Director of the Biotechnology Institute of UNAM, where she is also a Professor, Principal Investigator, and Group Leader. She is vice chair of the Scientific Advisory Committee and interim chair of the Portfolio Strategy and Management Board of CEPI, a scientific expert for COFEPRIS (Mexican regulatory agency), and a founding expert of the Mexican Pharmacopoeia, Biotechnological drugs section. Her research focuses on vaccine development, process development for vaccine production, and vaccine and protein characterization. Palomares collaborated with Protein Sciences on the development of Flublok, the first licensed recombinant influenza vaccine, and participated in the technology transfer of Flublok to Mexico and its licensure in the country. Her work has been recognized with the most prestigious awards for scientists in Mexico.

Jeff Smith

Associate Professor, Carleton University (Canada)

Dr. Jeff Smith is Full Professor in the Department of Chemistry and the Institute of Biochemistry at Carleton University. He received his BSc from Trent University in 2000 and earned his PhD at York University in 2005. After three years at the Ottawa Institute of Systems Biology at the University of Ottawa, he joined Carleton in 2008, and is currently the Director of the Carleton Mass Spectrometry Centre. He is the President of the Canadian Society for Mass Spectrometry and his research focusses on the use of mass spectrometry to investigate the biomolecular mechanisms of cellular life.

Ioanna Tzani

Senior Scientist, NIBRT (Ireland)

Ioanna Tzani is a senior scientist at NIBRT. She received her doctorate degree from University College Cork (Ireland) in 2017, where she studied protein synthesis regulation in cancer and identified previously uncharacterised proteoforms in the commonly mutated tumour suppressor PTEN. In 2017 she joined Dr Clarke's Systems Biology group in NIBRT. In her current role she studies the effect of bioprocessing relevant cell culture conditions on gene expression and protein synthesis. Her work aims at improving bioprocessing efficiency and safety of recombinant therapeutic proteins.

GENERAL INFORMATION

VENUE
Hotel Bonaventure
900 Rue De la Gauchetière O, Montréal, QC H5A 1E4

PARKING
Hotel Bonaventure has a large underground parking lot shared with Place Bonaventure. Accessible via Mansfield Street, the indoor parking extends over 6 floors and has a total of 750 spaces.

PUBLIC TRANSPORTATION

The hotel is directly accessible by STM bus or metro.

REGISTRATION DESK
All participants should sign up and collect their Conference Material at the Registration Desk located on the Conference level.

Opening hours:

Sunday, September 14	17:30-20:30
Monday, September 15	08:00-17:30
Tuesday, September 16	08:30-19:00
Wednesday, September 17	08:30-19:00
Thursday, September 18	08:30-13:00

EXHIBITION HALL
Room St Laurent

Monday, September 15	10:00-20:15
Tuesday, September 16	08:15-16:30
Wednesday, September 17	08:45-19:15
Thursday, September 18	08:45-11:30

LUNCHES AND COFFEE BREAKS
Room St Laurent
Lunches and coffee breaks are located in the exhibition hall.

INTERNET ACCESS

Free internet facilities are available to all participants in the conference venue.

Network : Conference Bonaventure

Password : hotel900

During the sessions, please turn off your mobile phone or set it to mute.

NAME BADGE

Name badge is the participant identification to access the sessions and exhibition and should be worn for all the conference and social events.

CERTIFICATE OF ATTENDANCE

An official Certificate of Attendance will be available on demand after the conference.

DISCLAIMER

The PEACe conference secretariat and organizers cannot assume liability for personal accidents, loss of or damage to private property of participants and accompanying persons, either during, or directly arising from the 17th PEACe. Participants should make their own arrangements with respect to health and travel insurance.

SECURITY & SAFETY

Please do not leave bags and luggage unattended at any time, whether inside or outside session rooms.

SOCIAL EVENTS

Social events held during PEACe are open to all registered participants

WELCOME RECEPTION

Sunday, September 14, 18:30-20:30
Ville Marie room

Cocktail Reception (Appetizers and Drinks)

Dress code : Business Casual

POSTER SESSION COCKTAIL

Monday, September 15, 18:15-20:15
St Laurent room

Posters and networking cocktail provide an opportunity for informal, interactive presentations and discussions.
Cocktail Reception (Station food & Drinks)

Dress code : Business Casual

TOUR AND OFF-SITE EVENT

Tuesday, September 16

Montreal Walking Tour, 17:15 - 18:45
Accompanied by a professional guide, explore the facets of Montreal's dynamic downtown and briefly immerse yourself in the atmosphere of Old Montreal during this fascinating 75 minutes tour.
Meet your guide in the lobby of the Bonaventure Hotel at 17:15.

La Grande Roue de Montréal (the Big Wheel) - Cocktail and Wheel ride, 18:45 - 21:45
The Committee is happy to invite you to a Cocktail dinner and a Wheel Tour. Enjoy the breathtaking panorama of the city, St Lawrence river and beyond from 60 metres above it all.
Cocktail Dinner and Drinks
Dress code : Casual with confortable shoes for the walking tour

GALA DINNER & AWARDS

Wednesday, September 17, 19:45-23:30
Montreal 1/3 room

Join us for a delightful seated dinner with live music and Awards Ceremony celebrating this year's winners. Keep the celebration going afterwards with a lively DL set and a cash bar - an evening not to be missed!
Dress code : Business casual

THANK YOU TO OUR PARTNERS!

The Organizing Committee of the 17th Conference on Protein Expression in Animal Cells would like to express its gratitude to and acknowledge the following partners for their generous support.

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ASIMOV

Asimov is developing a synthetic biology platform – from cells to software – to enable better design and manufacture of next-generation therapeutics. Asimov's CHO Edge System for biologic production minimizes the risk of cell line development by enabling exploration of vector design space – Asimov routinely achieve titers of 8–11 g/L across modalities.

Asimov is applying the same approach to improve cell & gene therapies – by developing stable lentiviral cell lines and a best-in-class AAV expression system, and enhancing safety profiles through the creation of synthetic tissue specific promoters. https://www.asimov.com/

ATUM

ATUM is a leading California-based Contract Research and Development Organization (CRDO) dedicated to providing integrated solutions that accelerate the development of biotherapeutic programs. Our cell line development services are built upon the foundation of the patented Leap-In Transposase® platform, a technology engineered to ensure the rapid, stable, and highly productive generation of cell lines. This innovative approach allows us to deliver high-quality materials and data that de-risk the journey from early discovery through to manufacturing. Our seamless workflow is a key differentiator, offering a comprehensive suite of services from initial gene synthesis and optimization to Research Cell Banking, all under one roof. We utilize our robust and well-characterized miCHO-GS and discoCHO cell lines, which are specifically designed to provide a predictable and consistent pathway to protein production. The discoCHO platform, for instance, offers robust transient expression with critical quality attributes (CQAs) representative of the final miCHO-GS manufacturing cell line, minimizing unpredictability and saving valuable time. ATUM's commitment to bioengineering and machine learning enables us to continuously enhance our platforms and deliver superior-performing biologics and cell lines. By leveraging these advanced technologies, we can control integration copy numbers, maintain the structural integrity of expression cassettes, and achieve a high degree of genetic stability. This integrated, data-driven approach not only accelerates your timelines but also enhances the overall quality and manufacturability of your biotherapeutics, ensuring a more confident path to clinical trials. https://www.atum.bio/

Evonik

Evonik offers high-quality cell culture solutions to pharmaceutical and biotech companies to help them boost cell culture performance in the manufacturing of therapeutic proteins like monoclonal antibodies, as well as in the production of viral vectors such as the adeno associated viral (AAV) vectors for gene therapy. Commercially proven for reliable media performance across countless biological programs, our cQrex® portfolio features a range of peptides and keto acids designed to address key issues in mammalian cell culture. The basis for such outcomes is our high-quality and non-animal derived amino acids, which have earned the industry's finest reputation for batch-to-batch reproducibility at any clinical or commercial scale. https://www.evonik.com/en.html

Hamilton

Hamilton Process Analytics is a division of Hamilton, a global leader in precision liquid handling and measurement solutions for research, education, and industry. At Hamilton, we strive to continuously enable the global life sciences community with cutting-edge sensor technologies and engineering innovations in process analytics solutions. Hamilton pioneers in-line sensor solutions for bioprocesses to enhance the understanding and control of critical process parameters and key performance indicators such as dissolved oxygen, pH & ORP, dissolved CO2, total and viable cell density, and conductivity. With Hamilton sensors, you can gain better process insights and control through real-time measurement. Additionally, intuitive tools like our ArcAir simplify the management of process sensors and ensure GMP compliance. All Hamilton sensor solutions are backed by our world-class customer service, support, on-site assistance, and training. https://www.hamiltoncompany.com/

Kuhner

Kuhner is a family-owned organization that has specialized in shaken technology since 1949. Headquartered in Basel, Switzerland, our team offers the highest level of quality and expertise to help provide you with solutions to improve your shaken processes. Kuhner manufactures world-renowned shakers and shaker incubators for life-science applications. From microplates to flasks and larger scales, Kuhner shakers are respected as the benchmark for quality, performance, flexibility and reliability (5-year standard warranty). In addition to shaker incubators, Kuhner also features a line of single-use, orbital shaken bioreactors for scales ranging from 10L to 2,500L, which is a low-shear alternative to wave and stirred-tank bioreactors. Kuhner also offers solutions for off-gas analysis in shake flasks (Kuhner TOM) and microtiter plates (Kuhner microTOM), as well as fed-batch control of nutrients in shake flasks and microtiter plates (Kuhner Feeding Technology), which can help reduce your time and analytical resource requirements. Our dedicated team of service engineers located around the world help ensure that our equipment continues to operate at the highest level of excellence and our scientific support team helps ensure that our customers are utilizing our equipment as effectively as possible. We view our customers as long-term partners, and we are honored to support their important work. Please come meet us at the Kuhner booth to let us know how we can help!
https://kuhner.com/en/index.php

RD-Biotech is a French contract research organization (CRO) with over 20 years of expertise providing custom biotechnology services, with a strong focus on expression vector design and recombinant protein production. Across Europe and North America, we support a wide range of clients, including academic institutions, biotech startups, and pharmaceutical companies.

Our team of seasoned scientists delivers expert, flexible support, from feasibility assessment to optimized protein expression strategies. We help you select the most appropriate expression system (CHO, HEK, prokaryotic, yeast or cell-free), design and optimize expression vectors, and define the best production workflow for your protein of interest.

Our integrated service offering includes:

- Custom vector design and optimization
- Protein production from small-scale R&D to larger-scale preclinical batches
- Process development and yield optimization
- Analytical characterization and quality control

We produce a wide range of protein types—including recombinant antibodies, enzymes, cytokines, and membrane proteins—for diverse applications such as discovery research, preclinical development, diagnostics, and biotherapeutic programs e.g. vaccines, gene therapy, cell therapy.

Whether you are looking to accelerate early-stage development or improve production of complex molecules, RD-Biotech offers custom, high-quality solutions built around your goals.

Meet us at our booth during PEACe—we look forward to learning more about your needs!

www.rd-biotech.com
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Repligen

Repligen, a global life sciences company, develops and commercializes innovative bioprocessing technologies that increase efficiencies and flexibility in biotherapeutic manufacturing. As leaders in filtration, chromatography systems, process analytics and fluid management, we are committed to inspiring advances in bioprocessing. We are a trusted partner in the production of monoclonal antibodies, recombinant proteins, vaccines as well as cell and gene therapies. Named one of the fastest growing biotech companies in the USA, the majority of our 18 manufacturing sites are located in the United States (California, Massachusetts, New Jersey, New Hampshire, New York and Texas). Outside the United States, we have manufacturing sites in Estonia, France, Germany, Ireland, the Netherlands, and Sweden. https://www.repligen.com/

Sartorius

Sartorius is a leading international partner of life sciences research and the biopharmaceutical industry. With innovative laboratory instruments and consumables, the Group's Lab Products & Services Division focuses on laboratories performing research and quality control at pharmaceutical and biopharmaceutical companies as well as academic research institutes. The Bioprocess Solutions Division, with its broad product portfolio focusing on single-use solutions, helps customers manufacture biotech medications, vaccines, and cell and gene therapies safely, rapidly, and sustainably. The company, based in Göttingen, Germany, has a strong global reach with around 60 production and sales sites worldwide. Sartorius regularly expands its portfolio through the acquisition of complementary technologies. In 2024, the company generated sales revenue of around 3.4 billion euros. Around 13,500 employees are working for customers around the globe.
https://www.sartorius.com/en

Thermo Fisher Scientific

Thermo Fisher Scientific is the world leader in serving science, providing innovative solutions to accelerate protein research and the discovery and development of therapeutics and vaccines. Our extensive portfolio spans the entire protein expression workflow, from cloning and gene synthesis to protein production, purification, and analysis. We ensure scientists have access to the most comprehensive and advanced tools and technologies available. Our protein expression technologies are unmatched, including fully optimized Gibco™ Expi transient expression systems designed for rapid, high-yield protein production across a wide variety of proteins. Additionally, our high-performing Gibco™ standalone media solutions and cell lines offer the flexibility needed to meet the diverse needs of the scientific community. We understand the current challenges faced by protein expression researchers and drug developers at companies of all sizes, including cost, speed, protein yield, and need for automation. We are committed to providing the tools, products, and services needed to overcome these obstacles, while our team of experts is dedicated to supporting your efforts and offering guidance at every step of the way. Thermo Fisher Scientific's commitment to innovation and excellence is reflected in our ongoing investment in research and development. We continuously strive to improve our products and services, ensuring that our customers have access to the latest advancements in protein expression technology. Partner with us to access high-quality products, services, and support needed to develop breakthrough drugs, accelerate scientific discovery, and make a meaningful impact on global health. https://www.thermofisher.com/ca/en/home.html

Thomson Instrument Company is a trusted leader in scientific innovation, delivering USA-made solutions for cell growth, clarification, and purification. By collaborating with researchers and leveraging technical expertise, we provide practical tools that enhance efficiency in cell culture, bioprocessing, and analysis, from small-scale to large-scale bioprocessing. Our products, developed with direct input from the research community, optimize cell expansion, protein and DNA purification, and cell clarification, ensuring reliable results across diverse applications. Our portfolio, including high-performance flasks, transfer caps, well plates, and filtration systems, is designed for flexibility, cost savings, and GMP compliance. Thomson's modular systems support researchers by addressing needs from small-scale experiments to large-scale bioprocessing, streamlining workflows and boosting productivity. Manufactured entirely in the USA, our solutions reflect precision, durability, and quality, meeting rigorous industry standards. Whether scaling up cell cultures, refining purification processes, or clarifying samples, Thomson's innovative tools save time and resources while delivering consistent, high-quality outcomes. Our commitment to user-friendly designs and customer collaboration ensures researchers have scalable, reliable solutions to advance their work. From mammalian and microbial cell culture to analytical sample preparation, Thomson empowers breakthroughs in bioprocessing. Connect with Thomson Instrument Company to discover how our cutting-edge solutions for cell growth, clarification, and purification can elevate your research, driving efficiency and success across all scales of bioprocessing. https://htslabs.com/

ORAL PRESENTATIONS

Monday, September 15th

EXPRESSION SYSTEMS 1

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Rene Hubert

Nikolas Zeh

09:40 - 09:50 SFC1B5 A NEW RHABDOVIRUS-FREE INSECT CELL LINE THAT IMPROVES THE YIELD OF SECRETED PROTEINS EXPRESSED USING THE BACULOVIRUS SYSTEM

Linda King, Oxford Expression Technologies Ltd, United Kingdom

Linda King, Adam Chambers, Mine Aksular, Mina Emamian, Ana Paula Pessoa Vilela, Robert Possee

09:50 - 10:00 HOW WE USE DIVERSE EXPRESSION PLATFORMS TO SOLVE PROBLEMS FOR "DIFFICULT-TO-EXPRESS" TARGETS

Mark Elvin, Sygnature Discovery Limited, United Kingdom

Mark Elvin

10:00 - 10:20 NON-CLONAL CHO CELL DERIVED MATERIAL FOR PRECLINICAL STUDIES OF COMPLEX MOLECULES

Kitty Agarwal, Merck & Co, United States of America

Kitty Agarwal, Jessica Pan

10:20 - 10:30 THE CHALLENGES IN BRINGING RECOMBINANT POLYCLONAL ANTIBODIES TO THE CLINIC

Rena Mizrahi, Grifols, United States of America

Rena Mizrahi

EXPRESSION SYSTEMS 2

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Rene Hubert

Nikolas Zeh

11:00 - 11:20 ENGINEERING OF CHO GENOME USING MULTIPLE ORTHOGONAL TRANSPOSASE/TRANSPOSON PAIRS TO INCREASE YIELD AND CONTROL OUTPUT

Claes Gustafsson, ATUM, United States of America

Claes Gustafsson, Mario Pereira, Ferenc Boldog, Jeremy Minshull

11:20 - 11:40 BENCHMARKING NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT), A NOVEL CHO METABOLIC SELECTION MARKER, AGAINST GLUTAMINE SYNTHETASE SELECTION FOR PRODUCTION OF RECOMBINANT PROTEINS IN CHINESE HAMSTER OVARY CELLS

Matthew Reaney, University Of Manchester, United Kingdom

Matthew Reaney, Zeynep Betts, Jon Dempsey, Alan Dickson

11:40 - 11:50 PURIFICATION AND CHARACTERIZATION OF HETERO-OLIGOMERIC VARIANTS OF PROAPOPTOTIC HTRA2 USING A MODIFIED TANDEM AFFINITY PURIFICATION APPROACH

Kakoli Bose, ACTREC, Tata Memorial Centre, India

Kakoli Bose

11:50 - 12:00 RAPID PRODUCTION OF BISPECIFIC ANTIBODIES USING A PRODUCTION-PHASE PROMOTER

Michael Anbar, Lonza Integrated Biologics, United Kingdom

Michael Anbar, Yusuf Johari

KEYNOTE LECTURE 1 - SAMUEL STERNBERG

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Yves Durocher

12:00 - 13:00 DISCOVERY AND DEVELOPMENT OF CRISPR-ASSOCIATED TRANSPOSASES FOR RNA-GUIDED GENE INSERTION

Samuel Sternberg, Columbia University, United States of America

Samuel Sternberg

INTRODUCTION - YVES DUROCHER

Chair:

14:00 - 14:15 INTRODUCTION - YVES DUROCHER

INDUSTRIAL WORKSHOP - THOMSON INSTRUMENT COMPANY

Chair: Yves Durocher

14:15 - 14:30 CONSISTENCY IN BIOMANUFACTURING FROM 4ML-50L WITH THOMSON TOOLS FOR PLASMID, CHO, AND HEK MANUFACTURING. SPEEDING UP TIME TO CLINIC AND MARKET

Sam Ellis, Thomson Instrument Company, United States of America

Sam Ellis

CELL ENGINEERING 1

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Simon Fischer

Kerstin Otte

14:30 - 15:00 MANIPULATING BRANCHED CHAIN AMINO ACID CATABOLISM TO IMPROVE CHO ANTIBODY TITERS: A COMBINED CELL LINE ENGINEERING AND MEDIUM OPTIMIZATION APPROACH

Camil Diaz, Genentech, United States of America

Camil Diaz, Cynthia Lam, Alyssa Getty (Sargon), Zijuan Lai, Dewakar Sangaraju, Inn Yuk, Gavin Barnard, Shahram Misaghi

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15:00 - 15:20 REIMAGINING CHO CELL METABOLISM

Hooman Hefzi, Technical University of Denmark, Denmark

Hooman Hefzi

15:20 - 15:40 DESIGNING THE NEXT GENERATION OF CHO CELLS THROUGH ENGINEERING BIOLOGY

Devika Kalsi, FUJIFILM Biotechnologies, United Kingdom

Leon Pybus, Devika Kalsi

15:40 - 16:00 ENGINEERING OF ENDOGENOUS RETROVIRUS-LIKE PARTICLE (RVLP)-DEFICIENT CHO CELLS BY CRISPR OR SHRNA IS FACILITATED BY ENRICHMENT METHODS BASED ON CELL-SURFACE EXPRESSION OF RETROVIRAL ENVELOPE PROTEIN

Matthew Stuible, National Research Council of Canada (NRC), Canada

Matthew Stuible, Sergio Alpuche-Lazcano, Christian Gervais, Manon Ouimet, Julie Lippens, Martine Page, Audrey Morasse, Anna Moraitis, Yves Durocher

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CELL ENGINEERING 2

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Simon Fischer

Kerstin Otte

16:30 - 16:50 CONSTITUTIVE AND INDUCIBLE UGCG OVEREXPRESSION: IMPACTS ON VIRAL VECTOR PRODUCTION IN A TARGETED INTEGRATION PLATFORM

Marzia Rahimi, Technical University of Denmark, Denmark

Marzia Rahimi, Lars Keld Nielsen, Jesús Lavado García

16:50 - 17:10 COMBINING COMPUTATIONALLY GUIDED PROTEIN ENGINEERING WITH MULTI OBJECTIVE CODON OPTIMIZATION (MOCO) FOR A NOVEL AND HIGHLY EFFICIENT TRANSPOSASE FOR CELL LINE DEVELOPMENT

Nikolas Zeh, Asimov Inc, United States of America

Nikolas Zeh, Mike Leonard, Sai Akash Gopaluni, Joe Collins, Kevin Smith, Scott Estes

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17:10 - 17:30 ACCELERATING DRUG DEVELOPMENT: LINKING CHO CELL GENOMICS TO RAPID GROWTH

Jannis Marzluf, Department of Gene Therapy, University of Ulm, Germany

Jannis Marzluf, Ann-Cathrin Leroux, Christoph Zehe

17:30 - 17:40 CHALLENGING CELL DEATH: NOVEL ANTI-APOPTOTIC TARGETS FOR EXTENDED FEDBATCH BIOMANUFACTURING

Javier Bravo-Venegas, Pontificia Universidad Católica de Valparaíso, Chile

Javier Bravo-Venegas, Camila Orellana, Mauro Torres, Mauricio Vergara, Alan Dickson, Marcela Hermoso, Julio Berrios, Claudia Altamirano

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17:40 - 17:50 PAVING THE WAY FOR A MINIMAL CHO GENOME: INVESTIGATING THE MOLECULAR MECHANISM DRIVING LARGE-SCALE DNA DELETIONS

Emely Walker, University of Applied Sciences Biberach, Germany

Emely Walker, Melina Bräuer, Stefan Schneider, Simon Fischer, Kerstin Otte

17:50 - 18:00 REMBAC - A RAPID EFFICIENT MANIFOLD BACULOVIRUS TRANSDUCTION PLATFORM FOR STABLE CELL LINE DEVELOPMENT

Manuel Reithofer, Institute of Molecular Biotechnology, BOKU University, Austria

Manuel Reithofer, Sophie Huber, Sandra Díaz Sánchez, Miriam Klausberger, Reingard Grabherr

Tuesday, September 16th

MULTISPECIFICS AND PROTEIN ENGINEERING 1

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Rene Hubert

Nathan Lewis

09:00 - 09:30 TRANSFORMING THERAPEUTIC PROTEIN ENGINEERING

Rene Hubert, Amgen, United States of America

Bram Estes, Carolyn Shomin, Marissa Mock, Suzanne Edavettal, Rene Hubert

09:30 - 09:50 TRANS-SPLICING MEDIATED RECOMBINATION TO GENERATE MULTI-SPECIFIC ANTIBODIES

Stefan Schmidt, evitria AG, Switzerland

Stefan Schmidt, Bastian Kohl

09:50 - 10:10 SPASE: A WEB-BASED TOOL FOR STREAMLINING PROTEIN ENGINEERING

Nicolas Doucet, Institut National de la Recherche Scientifique (INRS) - Université du Québec, Canada

Nicolas Doucet, Alex Paré, Sacha T. Larda

10:10 - 10:30 FLOW CYTOMETRIC APPROACHES TO CONTROLLING PRODUCT QUALITY DURING CELL LINE GENERATION

Jack Scarcelli, Sanofi, United States of America

Jack Scarcelli

MULTISPECIFICS AND PROTEIN ENGINEERING 2

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Rene Hubert

Nathan Lewis

11:00 - 11:20 ENHANCING THE APOLLOX PROCESS DEVELOPMENT TOOLBOX: STRATEGIES FOR NAVIGATING COMPLEX MODALITIES

Alison Young, Fujifilm biotechnologies, United Kingdom

Devika Kalsi, Samuel Walker, Caitlin Morter, Carmen Heeran, Jessica Kane-Fidgeon, Jodie Symington, Kelly Gibson, Fay Saunders, Leon Pybus, Alison Young

11:20 - 11:30 DEVELOPABILITY ASSESSMENT OF A CD28 X NECTIN-4 CO-STIMULATORY BISPECIFIC FOR THE TREATMENT OF BLADDER CANCER

Katja Rüger, Rondo Therapeutics, United States of America

Katja Rüger, Starlynn Clarke, Manpreet Kaur, Sebastian Moreno Arteaga, Soumili Chattopadhyay, Elaine Chen, Ruth Chu, Laura Davison, Jacqueline Morgan, Cynthia Nguyen, Udaya Rangaswamy, Imani Smith, Katherine Harris, Shelley Force Aldred, Nathan Trinklein

11:30 - 11:50 PROTEIN STRUCTURE, A GENETIC ENCODING FOR GLYCOSYLATION

Nathan Lewis, University of Georgia, United States of America

Ben Kellman, Nathan Lewis

PRODUCT QUALITY 1

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Laura A. Palomares

Anne Tolstrup

13:00 - 13:30 ENHANCING OUR UNDERSTANDING OF VIROCEUTICALS USING AN OPTIMIZED MASS SPECTROMETRY-BASED LIPIDOMICS APPROACH

Jeff Smith, Carleton Mass Spectrometry Centre, Department of Chemistry, Carleton University, Canada

Jocelyn Menard, Tilia Zinnecker, Joshua Roberts, Elena Godbout, Rozanne Arulanandam, Andrew Chen, Anne Landry, Christopher Boddy, Udo Reichl, Jean-Simon Diallo, Yvonne Genzel, Jeff Smith

13:30 - 13:50 MULTI-OBJECTIVE OPTIMIZATION OF MONOCLONAL ANTIBODY TITER AND PRODUCT QUALITY USING DATA-DRIVEN AND HYBRID MODELS

Will Johnson, Asimov, Inc, United States of America

Will Johnson, Kate Dray, Sai Akash Gopaluni, Ally Kotopoulos, Nikolas Zeh, Ta-Chun Hang

13:50 - 14:10 GLYCOSYLATION SITE ASN168 IS IMPORTANT FOR SLOW IN VIVO CLEARANCE OF RECOMBINANT HUMAN DIAMINE OXIDASE HEPARIN-BINDING MOTIF MUTANTS

Elisabeth Gludovacz, Department of Biotechnology, BOKU University, Austria

Elisabeth Gludovacz, Marlene Rager-Resch, Kornelia Schuetzenberger, Karin Petroczi, Daniel Maresch, Stefan Hofbauer, Bernd Jilma, Nicole Borth, Thomas Boehm

14:10 - 14:30 WITHOUT A TRACE: IDENTIFICATION, CHARACTERIZATION AND 9X KNOCKOUT OF CHO HYDROLASES TO TACKLE THE POLYSORBATE DEGRADATION CHALLENGE

Linus Weiß, University of Applied Sciences Biberach, Germany

Linus Weiß, Nikolas Zeh, Melanie Maier, Daniel Lakatos, Kerstin Otte, Simon Fischer

PRODUCT QUALITY 2

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Laura A. Palomares

Anne Tolstrup

15:00 - 15:10 GLYCOMICS AND GLYCOPROTEOMICS METHODS FOR APPLICATIONS IN BIOTHERAPEUTIC PRODUCTS AND ENDOGENOUS BIOMARKERS

Parastoo Azadi, University of Georgia, United States of America

Parastoo Azadi, Bhoj Kumar

15:10 - 15:20 IMPLEMENTATION OF QUALITY BY DESIGN PRINCIPLES FOR INFLUENZA A VIRUS PRODUCTION

Tilia Zinnecker, Max Planck Institute for Dynamics of Complex Technical Systems, Germany

Tilia Zinnecker, Kristin Thiele, Timo Schmidberger, Yvonne Genzel, Udo Reichl

15:20 - 15:30 A SURFACE PLASMON RESONANCE-BASED INTEGRATED ASSAY FOR QUANTIFICATION AND GLYCOSYLATION CHARACTERIZATION OF MONOCLONAL ANTIBODIES IN CRUDE HETEROGENEOUS SAMPLES

Ilona Metayer, Polytechnique Montréal / CNRC, Canada

Ilona Metayer, Catherine Forest-Nault, Julie Guimond, Simon Joubert, Olivier Henry, Yves Durocher, Grégory De Crescenzo, Jimmy Gaudreault

15:30 - 15:40 SUPPLEMENTATION OF CHEMICAL ADDITIVES AS A STRATEGY TO IMPROVE NOVEL ANTI-SST2 IGG IN CHO CELLS SYSTEM

Francisca Torres-García, Pontificia Universidad Católica de Valparaiso, Chile

Francisca Torres-García, Javier Bravo-Venegas, Mauricio Vergara-Castro, Isis Araya-Cuello, Oscar Latorre, Gonzalo Vásquez, Jose Alejandro Rodriguez-Siza, Claudia Altamirano

15:40 - 15:50 STREAMLINING BIOPROCESSES BY CONVERTING A DUAL-FEED INTO A SINGLE-FEED SYSTEM WITH PEPTIDES

Zach Demorest, Evonik, United States of America

Christina Jost, Tomislav Trescec, Zach Demorest, Jianfa Ou, Anne Benedikt, Stephan Brinkmann

15:50 - 16:00 TARGETED GENE INTEGRATION FOR ROBUST PERFORMANCE OF INDUCIBLE TRANSCRIPTIONAL CIRCUITS IN CHO CELLS

Sheryl Lim, University College Dublin, Ireland

Sheryl Lim, Jesús Lavado García, Lars Keld Nielsen, Ioscani Jiménez del Val

INDUSTRIAL WORKSHOP - THERMOFISHER

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair:

16:00 - 16:30 EXPI293TM PRO EXPRESSION SYSTEM: ACHIEVING HIGHER TITERS AND HIGHER THROUGHPUT TO SUPPORT THE EVER-EXPANDING NEEDS OF PROTEIN EXPRESSION SCIENTISTS

Jonathan Zmuda, Thermo Fisher Scientific, United States of America

Matt McKenna, Jonathan Zmuda

Wednesday, September 17th

KEYNOTE LECTURE 2 - CHRISTOPHER CORBEIL

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Yves Durocher

09:30 - 10:30 BEYOND THE FOLD: CHARTING THE AI-DRIVEN FUTURE OF ANTIBODY DISCOVERY

Christopher Corbeil, National Research Council Canada, Canada

Christopher Corbeil

THERAPEUTIC VIRAL AND NON-VIRAL PARTICLES 1

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Laura Cervera

Yves Durocher

11:00 - 11:30 FUNCIONALIZED VIRAL PROTEIN ASSEMBLIES AS SCAFFOLDS FOR NEURAL TISSUE

Laura A. Palomares, Universidad Nacional Autonoma de México, Mexico

Francisca Villanueva-Flores, Andrés Castro-Lugo, Laura A. Palomares

11:30 - 11:50 ENGINEERING FERRITIN NANOPARTICLES FOR PRECISE ANTIGEN DISPLAY

Margarida Queluz Rodrigues, iBET - Instituto de Biologia Experimental e Tecnológica, Portugal

Margarida Queluz Rodrigues, Inês Cardoso, Mónica Thomaz, Nádia Duarte, Paula Marques Alves, António Roldão

11:50 - 12:10 SYSTEMS BIOLOGY, CELL LINE AND PROTEIN ENGINEERING FOR INCREASED AAV PRODUCTION

Johan Rockberg, KTH, Sweden

Johan Rockberg

12:10 - 12:30 PROCESS INTENSIFICATION FOR HIGH CELL DENSITY ONCOLYTIC NEWCASTLE DISEASE VIRUS PRODUCTION

Lennart Jacobtorweihe, Max Planck Institute Magdeburg, Germany
Lennart Jacobtorweihe, Brice Madeline, Arnaud Léon, Yvonne Genzel, Udo Reichl

THERAPEUTIC VIRAL AND NON-VIRAL PARTICLES 2

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Laura Cervera

Yves Durocher

13:30 - 13:50 RECOMBINANT AAV PRODUCTION: INSIGHTS FROM STABLE CELL LINES AND ADENOVIRUS INFECTION

Maria Toth, BOKU University, Austria
Maria Toth, Manuel Reithofer, Astrid Dürauer, Reingard Grabherr

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13:50 - 14:10 FUNCTIONALIZED HIV-1 GAG VLPS FOR TARGETED DELIVERY TO CXCR4-POSITIVE CANCER CELLS

Marc García, Universitat Autònoma de Barcelona, Spain
Elianet Lorenzo Romero, Marc García, Ugutz Unzueta, Eric Voltà, Francesc Gòdia, Laura Cervera

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14:10 - 14:20 CHO CELL PRODUCTION OF A SINGLE ENVELOPED VLP VACCINE TARGETING SARS-COV-2, INFLUENZA A AND RSV

Zalma Vanesa Sanchez Martinez, National Research Council Canada (NRC) / University of Montreal (UdeM), Canada
Zalma Vanesa Sanchez Martinez, Matthew Stuible, Brian Cass, Simon Lord-Dufour, Anh Tran, Rohan Mahimkar, Sabahudin Hrapovic, Yves Durocher

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14:20 - 14:30 MIXTURE DESIGN AS A TOOL FOR IMPROVING FULL-TO-EMPTY PARTICLE RATIOS ACROSS VARIOUS GOIS IN RAAV

Jesús Lavado García, Technical University of Denmark, Denmark
Konstantina Tzimou, Pol Hulsbus I Andreu, Ece Bahar Yildirim, Lars Nielsen, Jesús Lavado García

INDUSTRIAL WORKSHOPS - ASIMOV, ATUM & SARTORIUS

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Rene Hubert

Nathan Lewis

15:00 - 15:15 MODEL-DRIVEN GENETIC DESIGN AND BIOPROCESS OPTIMIZATION ACROSS MODALITIES

Raja Srinivas, Asimov, United States of America
Imroz Ghangas, Raja Srinivas

15:15 - 15:30 LEAP-IN TRANSPOSASE AND DISCOCHO; TOOLS FOR RAPID, STABLE, SCALABLE BIOLOGICS DOWN SELECTING AND MANUFACTURING

Claes Gustafsson, ATUM, United States of America

Claes Gustafsson

15:30 - 15:45 THE POWER OF DATA - COMBINING ANALYTICS AND MODEL-DRIVEN APPROACHES TO OPTIMIZE CELL LINES, MEDIA AND PROCESSES

Sandra Klausing, Sartorius Xell GmbH, Germany

Sandra Klausing, Ali Safari, Kristin Thiele, Monika Zauner, Niklas Kraemer, Alyssa Buve, Kathrin Teschner, Vera Ortseifen, Mareike Schulze, Maren Lehmkuhl, Tim Steffens

STUDENT-INDUSTRY PANEL AND NETWORKING

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Rene Hubert

Nathan Lewis

15:45 - 17:45 STUDENT-INDUSTRY PANEL AND NETWORKING

Natasha Serzedello, Thermo fisher scientific, United States of America

Raja Srinivas, Asimov, United States of America

Claes Gustafsson, ATUM, United States of America

Sandra Klausing, Sartorius, Germany

Alberto Estevez, Thomson, United States of America

Thursday, September 18th

OMICS, AI TOOLS AND INTENSIFICATION 1

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Olivier Henry

Chris Corbeil

09:30 - 10:00 DETECTION OF HOST CELL MICROPROTEIN IMPURITIES IN ANTIBODY DRUG PRODUCTS

Ioanna Tzani, National Institute for Bioprocessing Research and Training, Ireland

Ioanna Tzani, Marina Castro Rivadeneyra, Paul Kelly, Lisa Strasser, Lin Zhang, Martin Clynes, Barry L. Karger, Niall Barron, Jonathan Bones, Colin Clarke

10:00 - 10:20 A RECONSTRUCTION OF THE MAMMALIAN SECRETORY PATHWAY IDENTIFIES MECHANISMS REGULATING ANTIBODY PRODUCTION

Jasmine Tat, University of California, San Diego; Amgen Inc, United States of America

Jasmine Tat, Helen Masson, Pablo Di Giusto, Nathan Lewis

10:20 - 10:40 PROTEIN LANGUAGE MODELS: IS SCALING NECESSARY?

Sarath Chandar, Mila - Quebec AI Institute, Canada

Quentin Fournier, Robert Vernon, Almer van der Sloot, Benjamin Schulz, Sarath Chandar, Christopher Langmead

10:40 - 10:50 COMPARATIVE ANALYSIS OF HEK293 CELLS: CHARACTERIZATION OF GENOMIC VARIABILITY

Georg Smesnik, BOKU University, Austria

Georg Smesnik, Nikolaus Virgolini, Astrid Dürauer, Nicole Borth

10:50 - 11:00 MULTIVARIATE DATA ANALYSIS AIDS SELECTION OF CHO CELLS CLONES EXPRESSING A MONOCLONAL ANTIBODY

Jimmy Gaudreault, Univeristé Laval, Canada

Jimmy Gaudreault, Petko Komsalov, Jason Kuipers, Lucas Lemire, Brian Cass, Linda Lamoureux, Christopher Corbeil, Traian Sulea, Robert Voyer, Simon Joubert, Yves Durocher, Olivier Henry, Phuong Lan Pham

OMICS, AI TOOLS AND INTENSIFICATION 2

ROOM: MONTREAL 1/3 (CONFERENCE LEVEL)

Chair: Olivier Henry

Chris Corbeil

11:30 - 11:50 ENABLING CONTINUOUS BIOMANUFACTURING FOR VIRUS-BASED EXPRESSION SYSTEMS VIA MULTI-STAGE BIOREACTORS

António Roldão, iBET, Instituto de Biologia Experimental e Tecnológica, Portugal

Ricardo Correia, Taja Zotler, Birhanu Hurisa, Gorben Pijlman, Paula Marques Alves, António Roldão

11:50 - 12:10 TARGETED MASS SPECTROMETRY-BASED PROTEOMICS FOR IDENTIFYING ER-RELATED STRESS IN RECOMBINANT CHINESE HAMSTER OVARY PRODUCTION CULTURES

Paula Meleady, Dublin City University, Ireland

Paula Meleady

12:10 - 12:20 QUANTITATIVE ANALYSIS OF PROTEOMIC DIFFERENCES IN CLONAL SUSPENSION MDCK CELL LINES INFECTED WITH HUMAN INFLUENZA A VIRUS

Jan Küchler, MPI Magdeburg, Germany

Jan Küchler, Tilia Zinnecker, Maximilian Wolf, Patrick Hellwig, Dirk Benndorf, Yvonne Genzel, Udo Reichl

12:20 - 12:30 ENHANCING CONTROL OF MAB PRODUCTION IN PERFUSION BIOREACTORS USING CONTINUOUS MONITORING

David Latulippe, McMaster University, Canada

Adrian Foell, Joel Baarbé, Claire Velikonja, Cindy Shu, Druty Savjani, Landon Steenbakkers, Nardine Abd Elmaseh, Nathan Mullins, William Pihainen-Bleeker, Mahshad Valipour, Chris McCready, Brandon Corbett, Prashant Mhaskar, David Latulippe

POSTER PRESENTATIONS

Monday, September 15th

FROM 18:00 TO 19:30

ROOM : ST LAURENT 1/2

POSTER SESSION 1 & COCKTAIL

POSTER # 2

Room: St Laurent 1/2 (Conference Level), Challenging Cell Death: Novel Anti-Apoptotic Targets for Extended Fedbatch Biomanufacturing, Javier Bravo-Venegas

Pontificia Universidad CatÛlica de ValparaÌso

POSTER # 4

Room: St Laurent 1/2 (Conference Level), Paving the Way for a Minimal CHO Genome: Investigating the Molecular Mechanism Driving Large-Scale DNA Deletions, Emely Walker

University of Applied Sciences Biberach

POSTER # 6

Room: St Laurent 1/2 (Conference Level), Redefining CHO-Chassis: Strategic Genome Reduction in CHO Cells Through Cas9-Mediated Megabase Deletions, Stefan Schneider

University of Applied Sciences Biberach

POSTER # 8

Room: St Laurent 1/2 (Conference Level), REMBAC - a Rapid Efficient Manifold Baculovirus Transduction Platform for Stable Cell Line Development, Manuel Reithofer

Institute of Molecular Biotechnology, BOKU University

POSTER # 10

Room: St Laurent 1/2 (Conference Level), Surrogate-Surface Marker Based Enrichment of CHO Stable Pools for Rapid Protein Production, Rohan Mahimkar

National Research Council Canada

POSTER # 12

Room: St Laurent 1/2 (Conference Level), Utilizing Whole-Genome CRISPR Screening to Develop Alternative Methods of Metabolic Selection, Corey Kretzmer

MilliporeSigma

POSTER # 14

Room: St Laurent 1/2 (Conference Level), CHOZNÆ GS-/- Cell Line Produces High Titer and High-Quality Bispecific Antibodies, Gabrielle Dowell

MilliporeSigma

POSTER # 16

Room: St Laurent 1/2 (Conference Level), Effect of individual Expression of AAV and AdV Elements on the Transcriptome and Proteome of CHO Cells, Jes˙s Lavado GarcÌa

Technical University of Denmark (DTU)

POSTER # 18

Room: St Laurent 1/2 (Conference Level), Enhancing Bispecific Antibody Production in CHO Cells Through Chain-Specific Signal Peptide Engineering, HaeWon Chung

Asimov

POSTER # 20

Room: St Laurent 1/2 (Conference Level), How we use Diverse Expression Platforms to Solve Problems for "Difficult-to-Express" Targets, Mark Elvin

Sygnature Discovery Limited

POSTER # 22

Room: St Laurent 1/2 (Conference Level), Optimizing IgM Production: an Integrated Platform Combining Phage Display Technology and CHO Cell Line Development, Fiona Zucchetti

Diasorin Italia SpA

POSTER # 24

Room: St Laurent 1/2 (Conference Level), Process for Generation of High-Producing CHO Cell Lines for Biologics Manufacturing, Simon Joubert

National Research Council of Canada

POSTER # 26

Room: St Laurent 1/2 (Conference Level), Purification and Characterization of Hetero-Oligomeric Variants of Proapoptotic Htra2 Using a Modified Tandem Affinity Purification Approach, Kakoli Bose

ACTREC, Tata Memorial Centre

POSTER # 28

Room: St Laurent 1/2 (Conference Level), Rapid Production of Bispecific Antibodies Using a Production-Phase Promoter, Michael Anbar

Lonza Integrated Biologics

POSTER # 30

Room: St Laurent 1/2 (Conference Level), Sfc1b5 a New Rhabdovirus-Free insect Cell Line That Improves the Yield of Secreted Proteins Expressed Using the Baculovirus System, Linda King

Oxford Expression Technologies Ltd

POSTER # 32

Room: St Laurent 1/2 (Conference Level), The Challenges in Bringing Recombinant Polyclonal Antibodies to the Clinic, Rena Mizrahi

Grifols

POSTER # 34

Room: St Laurent 1/2 (Conference Level), Towards Accessible and Sustainable Recombinant AAV Vector Supply via Optimization of Production Platforms, Yingchao Nie

Sanofi

POSTER # 36

Room: St Laurent 1/2 (Conference Level), Development of an Avian H5N1 Flu Vaccine Based on Recombinant H5 Antigen Expressed in CHO Cells, Olatz San Miguel

NRC - University of Montreal

POSTER # 38

Room: St Laurent 1/2 (Conference Level), AntibodyGPSÆ - Multiparametric Optimization of Antibodies Using Precise Characterization and Machine Learning, Claes Gustafsson

ATUM

POSTER # 42

Room: St Laurent 1/2 (Conference Level), Engineering a Genetically Encoded Fluorescent Sensor for D-Serine, Rochelin Dalangin

CERVO Brain Research Centre

POSTER # 46

Room: St Laurent 1/2 (Conference Level), Thomson 7L Flasks Replacing Bioreactor and Cell Bags Between 5L to 6L/Flask. Ability to Grow Up to 35L/Shaker., Alberto Estevez

Thomson Instrument Company

POSTER # 48

Room: St Laurent 1/2 (Conference Level), Characterization of Cellular Oxidative Stress in a CHO Transient Gene Expression Production Process Producing SARS-CoV-2 Spike Protein as a Subunit Vaccine Antigen, Annoj Thavalingam

National Research Council Canada

POSTER # 50

Room: St Laurent 1/2 (Conference Level), Enhancing Control of Mab Production in Perfusion Bioreactors Using Continuous Monitoring, Adrian Foell

McMaster University

POSTER # 52

Room: St Laurent 1/2 (Conference Level), High-Throughput Biomarker Discovery for Antibody Production Using Tag-Based Single-Cell RNA-Seq, Pavle Vrljicak

Lonza

POSTER # 54

Room: St Laurent 1/2 (Conference Level), Multivariate Data Analysis Aids Selection of CHO Cells Clones Expressing a Monoclonal Antibody, Jimmy Gaudreault

UniveristÈ Laval

POSTER # 56

Room: St Laurent 1/2 (Conference Level), Quantitative Analysis of Proteomic Differences in Clonal Suspension MDCK Cell Lines Infected with Human Influenza A Virus, Jan K¸chler

MPI Magdeburg

POSTER # 58

Room: St Laurent 1/2 (Conference Level), A Novel Strategy to Reduce Polysorbate Degradation? Influence of Cultivation Media on CHO Hydrolase Expression and Activity, Linus Weifl

University of Applied Sciences Biberach

POSTER # 60

Room: St Laurent 1/2 (Conference Level), CHO Cell Growth in 3-D Using Semi-Solid Alginate Enables 384 Well Plate Cutlures Without Shaking, Yimu Zhao

University of Toronto

POSTER # 62

Room: St Laurent 1/2 (Conference Level), Effects of Enzymatically-Synthesized Sialylated Oligosaccharides on the N- and O-Glycosylation of Etanercept in Recombinant CHO Cell Culture, Tae Ho Kim

Korea Research Institute of Bioscience & Biotechnology

POSTER # 64

Room: St Laurent 1/2 (Conference Level), Bio-Imaging Strategy to Follow Adeno-Associated Viruses Inside Cells, Laurence Paquet

Cervo Brain Research Centre; UniversitÈ Laval

POSTER # 66

Room: St Laurent 1/2 (Conference Level), Exploration of High Cell Density Intensification for the Production of Influenza A Virus, Tilia Zinnecker

Max Planck Institute for Dynamics of Complex Technical Systems

POSTER # 68

Room: St Laurent 1/2 (Conference Level), Novel Production Process for Herpes Simplex Virus-1 Utilizing a Suspension HEK293 Cell Line, David Ermert

Bioinvent International AB

Wednesday, September 17th

FROM 18:00 TO 19:30

ROOM : ST LAURENT 1/2

POSTER SESSION 2

POSTER # 1

Room: St Laurent 1/2 (Conference Level), A Novel Engineered CHO Host Cell Line for High-Titer Production of Biopharmaceuticals, Kristin Thiele

Sartorius Stedim Cellca GmbH

POSTER # 3

Room: St Laurent 1/2 (Conference Level), Advancing Single Cell Cloning Operations on the Beacon Optofluidic System for Development of High Quality Manufacturing Cell Lines, Ewelina Zasadzinska

Amgen Inc.

POSTER # 5

Room: St Laurent 1/2 (Conference Level), Fueling the Future: Engineering CHO Cells for Independent Essential Amino Acids Production, Alena Adler

Technical University of Denmark

POSTER # 7

Room: St Laurent 1/2 (Conference Level), SialMAX: a Streamlined Glycoengineering Workflow for Enhanced a-2,6-Sialylation in CHO Cells, Cristina Abascal Ruiz

University College Dublin

POSTER # 9

Room: St Laurent 1/2 (Conference Level), Targeted Gene Integration for Robust Performance of Inducible Transcriptional Circuits in CHO Cells, Sheryl Lim

University College Dublin

POSTER # 11

Room: St Laurent 1/2 (Conference Level), Towards a Minimal Genome: Exploring the Limits of CRISPR/Cas9-Mediated Large-Scale Genomic Deletions in CHO Cells, Melina Braeuer

Institute of Applied Biotechnology, Biberach University of Applied Sciences

POSTER # 13

Room: St Laurent 1/2 (Conference Level), A Hybrid Modeling Approach for Cell Culture Media Optimization to Enhance Monoclonal Antibody Production, Zahra Negahban

University of Waterloo

POSTER # 15

Room: St Laurent 1/2 (Conference Level), Glycosylation Profile of SARS-CoV-2 Spike-Based Subunit Vaccines Impacts Focusing of the Humoral Immune Response, Matthew Stuible

National Research Council Canada

POSTER # 17

Room: St Laurent 1/2 (Conference Level), H5N1 Pandemic Preparedness: Building a Comprehensive Immunological Toolbox, Nourelhouda Hammami

IQVIA Laboratories

POSTER # 19

Room: St Laurent 1/2 (Conference Level), Minimal Clonal Variation Eliminates the Need for Clone Selection and Recurring Bio-Process Optimization, Lasse Pedersen

The Technical University of Denmark

POSTER # 21

Room: St Laurent 1/2 (Conference Level), New Mid-Scale Workflows for 20ml - 50ml in Automation Friendly Format, Sam Ellis

Thomson Instrument Company

POSTER # 23

Room: St Laurent 1/2 (Conference Level), Optimizing Antigen-Expressing Stable Cell Line Development Using Transposases, Anett Ritter

Novartis

POSTER # 25

Room: St Laurent 1/2 (Conference Level), Power Meets Precision | HTP Antibody Production with ExpiTM Protein Expression Systems & GeneArt HTP Antibody Production, Matt McKenna

Thermo Fisher Scientific

POSTER # 27

Room: St Laurent 1/2 (Conference Level), Streamline Recovery of Immunoreactive Arboviral Antigens from E. Coli Inclusion Bodies for Diagnostic Use, Elisa Russo

University of S„o Paulo

POSTER # 29

Room: St Laurent 1/2 (Conference Level), Transposase Platform: a Comprehensive Approach to Cell Line Development Success, Alexandra MartinÈ

KBI Biopharma

POSTER # 31

Room: St Laurent 1/2 (Conference Level), A Human Lectibody Platform for Glycan Targeting and Exploration of Anti-Infective Potential, Michael Lehky

Helmholtz Centre for Infection Research

POSTER # 33

Room: St Laurent 1/2 (Conference Level), Developability Assessment of a CD28 x Nectin-4 Co-Stimulatory Bispecific for the Treatment of Bladder Cancer, Katja R¸ger

Rondo Therapeutics

POSTER # 35

Room: St Laurent 1/2 (Conference Level), Development of Multispecific VHHs for the Neutralization of SARS-CoV-2 and H5N1, Camila Brisighello

UniversitÈ de MontrÈal / NRC

POSTER # 39

Room: St Laurent 1/2 (Conference Level), A Surface Plasmon Resonance-Based Integrated Assay for Quantification and Glycosylation Characterization of Monoclonal Antibodies in Crude Heterogeneous Samples, Ilona Metayer

Polytechnique MontrÈal / CNRC

POSTER # 41

Room: St Laurent 1/2 (Conference Level), Challenges and Opportunities in Discovery of Large Molecule Therapeutics Designed with Generative Artificial Intelligence (AI) Technology, Jeffrey Mitchell

Generate Biomedicines

POSTER # 43

Room: St Laurent 1/2 (Conference Level), CHO Transcriptomics and Proteomics Using Quantitative Immunostaining Directed Laser Lysis of Single Cells, Jeremy Lant

University of Toronto

POSTER # 45

Room: St Laurent 1/2 (Conference Level), Comparative Analysis of HEK293 Cells: Characterization of Genomic Variability, Georg Smesnik

BOKU University

POSTER # 47

Room: St Laurent 1/2 (Conference Level), Glycomics and Glycoproteomics Methods for Applications in Biotherapeutic Products and Endogenous Biomarkers, Parastoo Azadi

University of Georgia

POSTER # 53

Room: St Laurent 1/2 (Conference Level), Implementation of Quality by Design Principles for Influenza A Virus Production, Tilia Zinnecker

Max Planck Institute for Dynamics of Complex Technical Systems

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POSTER # 55

Room: St Laurent 1/2 (Conference Level), Streamlining Bioprocesses by Converting a Dual-Feed into a Single-Feed System with Peptides, Zach Demorest

Evonik

POSTER # 57

Room: St Laurent 1/2 (Conference Level), Supplementation of Chemical Additives as a Strategy to Improve Novel Anti-sST2 IgG in CHO Cells System, Francisca Torres-GarcÌa

Pontificia Universidad CatÛlica de Valparaiso

POSTER # 59

Room: St Laurent 1/2 (Conference Level), Characterizing the In Vitro Antiviral Activity of Influenza A Virus Defective Interfering Particles Using a Systems Biology Approach, Patricia Opitz

Otto von Guericke University

POSTER # 61

Room: St Laurent 1/2 (Conference Level), CHO Cell Production of a Single Enveloped VLP Vaccine Targeting SARS-CoV-2, Influenza A and RSV, Zalma Vanesa Sanchez Martinez

National Research Council Canada (NRC) / University of Montreal (UdeM)

POSTER # 63

Room: St Laurent 1/2 (Conference Level), From CHO Mastery to Intensified HEK293 Innovation: Elevating Viral Vector Production, Anica Schmidt

Sartorius Xell GmbH

POSTER # 65

Room: St Laurent 1/2 (Conference Level), Mixture Design as a Tool for Improving Full-to-Empty Particle Ratios Across Various GOIs in rAAV, Jes˙s Lavado GarcÌa

Technical University of Denmark

POSTER # 67

Room: St Laurent 1/2 (Conference Level), Production of Virus-Like Particles for Antibody Development Using Baculovirus-Free Insect Cell Expression System, Seyhan Demiral

Technical University of Braunschweig

POSTER # 69

Room: St Laurent 1/2 (Conference Level), Reconstruction of the AAV Biosynthetic Pathway Enables Systems-Level Analysis of Viral Production Mechanisms in Mammalian Cells, Eunil Im

University of California San Diego


17th PEACe
Protein Expression
in Animal Cells Conference
September 14-18, 2025
Bonaventure Hotel • Montreal, Canada

ABSTRACTS

Advancing New Biomanufacturing with Alternative Hosts

J. Christopher Love[1,2]

[1] Department of Chemical Engineering, Koch Institute at MIT, U.S.A.,
[2]Alternative Host Research Consortium at MIT, U.S.A

Evolutionary development of eukaryotic microorganisms—and yeast in particular—has provided an ideal chassis for fast and predictable process development and realizing next-generation intensified manufacturing processes of recombinant proteins. This talk will explore the features of alternative hosts that make them well-suited for addressing both speed and costs, while retaining quality, in manufacturing recombinant proteins.

In an era of genomic sequencing and gene editing technologies, simple eukaryotic microorganisms like yeast have become 'software'-like and can be reprogrammed to make a range of recombinant proteins from cytokines to vaccines to nanobodies to monoclonal antibodies (mAbs). Alternative hosts offer great potential for predictable and fast cycles of development and process intensification and some have proven use in manufacturing FDA-approved products. There remains, however, conceptual and practical barriers to widespread adoption, including considerations of potential quality variations and sufficient productivity for commercial use in some applications, as well as limited shared experience with alternative hosts more broadly in the industry.

The AltHost Consortium at MIT has created a venue for sharing the risk of developing new host biology in an innovation-friendly model based on open-source principles akin to software. This talk will highlight examples of this approach to progress a model host, *Komagataella phaffii* ("Pichia"), to improve volumetric productivity of mAbs, create molecular tools for engineering, and remodel pathways for glycosylation. Some topics of discussion will include genome-scale screening for improved production, refinements of sequences for quality of produced mAbs, and ML-guided process-based improvements for improved production of proteins. Implications for these advancing capabilities for a next-generation platform for biomanufacturing will be presented.

SfC1B5 a new rhabdovirus-free insect cell line that improves the yield of secreted proteins expressed using the baculovirus system

Linda King, Adam Chambers, Mine Aksular, Mina Emamian, Ana Paula Pessoa Vilela, Robert Possee

Oxford Expression Technologies Ltd, Oxford UK

The baculovirus expression system is recognised as one of the main platform technologies for the production of proteins in insect cells. For many years, the Sf9 cell line has been popular for both the amplification of recombinant viruses and the production of proteins. It was produced by clonal selection from Sf21 cells, which were originally derived from the pupal ovarian cells of *Spodoptera frugiperda* (Fall armyworm). In this study, we aimed to produce a rhabdovirus-free insect cell line for production of proteins for use as human vaccines. We used a frozen vial of early passage Sf21 cells (Oxford, 1981) as our starting material. A number of cell lines were derived by rounds of single-cell cloning and testing for the presence or absence of the insect rhabdovirus by RT-PCR. Putative rhabdovirus-free cell lines were expanded in ES-AF (animal-free) medium and were also tested for the ability to support baculovirus amplification and protein production. One cell line, SfC1B5, was selected for banking and further study. The SfC1B5 cell line has since been adapted to grow in chemically-defined medium and extensive testing has demonstrated the cell line supports recombinant baculovirus production, amplification and protein yields similar to that obtained with Sf9 cells. We report use of the cell line to make Gc and Gn surface glycoproteins of Crimean Congo Hemorrhagic Fever virus as a candidate vaccine. However, for a range of secreted proteins, including viral antigens, higher yields were obtained in the SfC1B5 cell line. Where the secreted protein is to be used as a vaccine for human or animal health, the increase in yield significantly reduces the cost of vaccine production per unit dose.

How we use diverse expression platforms to solve problems for “difficult-to-express" targets

Mark Elvin

Sygnature Discovery Limited, Department of Protein Science and Structural Biology, Birchwood House, Larkwood Way, Macclesfield, Cheshire, SK10 2XR, United Kingdom

The utilization of diverse cell expression platforms, including insect cells, HEK (Human Embryonic Kidney) cells, CHO (Chinese Hamster Ovary) cells, and *Escherichia coli* (*E. coli*), has become a pivotal strategy in addressing challenges associated with “difficult-to-express" biological targets. Each cell expression platform offers unique advantages tailored to the specific requirements of the target proteins.

E. coli systems are favoured for their rapid growth and high yield, though they may struggle with complex post-translational modifications (PTMs). Insect cell systems, utilizing baculovirus vectors, excel in producing proteins with intricate PTMs, crucial for functional studies. HEK cells are widely used for their human-like PTMs and ease of genetic manipulation, making them ideal for producing proteins in their native conformation. CHO cells are indispensable for producing therapeutic proteins with human-like modifications and are extensively used in biopharmaceutical production. HEK and CHO cells also offer the advantage of secreting proteins into the surrounding medium making downstream processing much easier and cost effective.

As the Department of Protein Science and Structural Biology within Sygnature Discovery, we are a contract research organization (CRO) that provides custom made, soluble and membrane proteins for use in drug discovery, research applications as well as X-ray crystallography, NMR and Cryo-EM structures of both novel and precedented proteins. Here we show real examples of how we’ve utilized each of our different expression platforms (Insect, HEK, CHO, *E. coli*) to successfully express membrane proteins (for structural work), intrinsically disordered transcription factors, large multi subunit protein complexes (the largest being a 12 subunit protein complex) and labelled proteins for NMR studies. This was achieved by leveraging the strengths of each individual expression platform to overcome the limitations posed by “difficult-to-express" targets; thereby, facilitating advancements in drug discovery, structural biology and therapeutic development.

Non-Clonal CHO Cell Derived Material for Preclinical Studies of Complex Molecules

Kitty Agarwal

Process Cell Sciences, Biologics Process Research & Development, Merck & Co., Inc., Rahway, NJ, USA

The use of non-clonal CHO cell derived materials for preclinical studies has been found to be a valuable approach to accelerate the development of monoclonal antibodies (mAbs) for first-in-human (FIH) studies. In a comprehensive study, we assessed the culture performance, productivity, and product quality of non-clonal cell lines compared with clonal cell lines expressing various biologic modalities to determine if this approach can be applied to complex molecules. We evaluated a multi-specific antibody, a cytokine-Fc fusion protein, and a mAb produced using the stable pool, the pool of top clones, and the lead clone utilizing transposase-mediated integration. The results indicated that the attributes were comparable regardless of the source of cells. Building upon these findings, the study progressed to the preclinical manufacturing of two multi-specific antibodies using both the pool of top clones and the lead clone. Subsequently, clinical manufacturing of these multi-specific antibodies was performed using the lead clone. The batches produced with the pool of clones and the lead clone demonstrated a high level of comparability in culture performance, productivity, and product quality.

In conclusion, non-clonal CHO cell derived materials can be effectively utilized for preclinical studies of complex molecules without compromising their quality, allowing for accelerated development for FIH studies.

The Challenges in Bringing Recombinant Polyclonal Antibodies to the Clinic

Rena Mizrahi[1]

[1]GigaGen, a Grifols Company, U.S.A

Hyperimmune globulin drugs manufactured from pooled immunoglobulins from vaccinated or convalescent donors have been used effectively in treating infections where no treatment is available. This is especially important where multi-epitope neutralization is required to prevent the development of immune-evading viral mutants that can emerge upon treatment with monoclonal antibodies. Using microfluidics, flow sorting, and a targeted integration cell line, GigaGen established a platform for development and manufacturing of recombinant polyclonal antibodies (pAbs), which comprise a mixture of >1,000 individual antibodies produced en masse. Two drugs of this class have now entered clinical development: GIGA-2050, for treatment of SARS-CoV-2, and GIGA-2339, for treatment of chronic Hepatitis B virus. To achieve these milestones GigaGen overcame several key challenges, including development of a single site targeted integration cell line, optimization of the upstream process using that cell line, as well as development of novel methods to monitor the upstream and downstream processes to ensure lot to lot consistency.

Engineering of CHO genome using multiple orthogonal transposase/transposon pairs to increase yield and control output.

Claes Gustafsson, Mario Pereira, Ferenc Boldog, Jeremy Minshull

ATUM, 37950 Central Ct., Newark, CA 94560

Introducing therapeutic protein genes into mammalian cells can be done in several ways. Transposon systems consisting of a transposon encoding the genetic cargo and a transposase enzyme releasing the cargo from the transposon and inserting it into the genome have become a leading method for developing stable cell lines, effectively addressing issues with older random integration techniques.

We used orthologous transposase/transposon pairs to insert genes for increased protein production while reducing the activity of other genes. This allowed us to change cell behavior and improve the quality of the produced proteins. We sequentially introduced three orthogonal transposons into CHO cells using three corresponding transposases, creating a new CHO-K1 cell line. We then used this line to generate high-producing cell clones with desirable protein characteristics.

The first transposon introduced a selection marker. The second inserted a gene for an IgG therapeutic antibody, resulting in stable, high-producing clones yielding over 5 g/L. The third transposon limited the formation of glycans on the IgG, significantly reducing fucose levels to below 10%.

Throughout this process, we used advanced genetic analysis (TLA and NGS) to confirm accurate transposon integration, enzyme specificity, and the inserted genes' stability over time. Glycan modification did not affect cell growth or protein production, and we observed no loss or rearrangement of the transposons.

The produced IgG was functional and showed improved immune cell-killing activity in lab tests. The protein production and quality of the final engineered clone remained consistent for more than 60 cell generations.

Benchmarking Nicotinamide Phosphoribosyltransferase (NAMPT), a Novel CHO Metabolic Selection Marker, Against Glutamine Synthetase Selection for Production of Recombinant Proteins in Chinese Hamster Ovary Cells

Matthew Reaney[1], Zeynep Betts[1], Jon Dempsey[2] and Alan Dickson[1]

[1] Manchester Institute of Biotechnology, University of Manchester, UK
[2] Pathway Biopharma, Edinburgh, UK

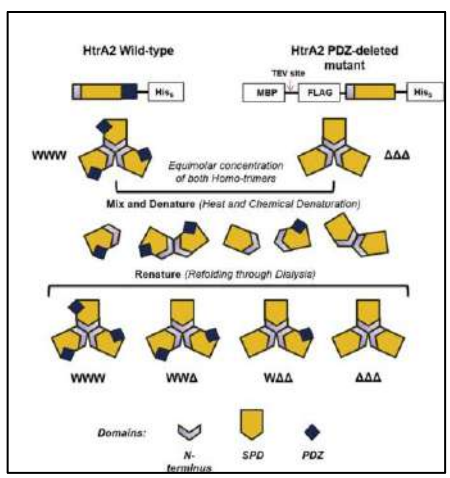
Metabolic selection systems, glutamine synthetase (GS) and dihydrofolate reductase (DHFR), have played a key role in establishing CHO cell processes capable of routinely generating high monoclonal antibody yields. In this study, we introduce nicotinamide phosphoribosyltransferase (NAMPT), an enzyme involved in cellular NAD^+ biosynthesis, as a novel metabolic selection marker for CHO cells and compare it with industry standard GS selection. NAMPT and GS were used to drive the expression of green fluorescence protein (GFP) and a trastuzumab biosimilar in NAMPT-knockout and GS-knockout host cells, respectively. Compared to the GS system, NAMPT selection demonstrated stronger selection stringency for GFP. Gene amplification, using small molecule inhibitors further enhanced the expression in both systems. In the production of trastuzumab, an industrially relevant recombinant protein, NAMPT-selected pools achieved comparable yields to those generated using the GS system. These data demonstrate the significant potential of NAMPT as a selection marker, offering a promising alternative to the industry-standard GS selection system for generating CHO cell populations for biopharmaceutical manufacturing.

Purification and Characterization of Hetero-Oligomeric Variants of Proapoptotic HtrA2 Using a Modified Tandem Affinity Purification Approach

Kakoli Bose

Integrated Biophysics and Structural Biology Lab, ACTREC, Tata Memorial Centre, HBNI, Navi Mumbai 410210, Maharashtra, India

Generating hetero-oligomers from a mixture of recombinantly expressed and purified proteins has always been arduous. It becomes even more challenging for large multimeric proteins. Using a series of ingenious modifications to existing techniques and tools, we have artificially generated variants of a trimeric proapoptotic serine protease, HtrA2, that is associated with several diseases, including neurodegeneration and cancer. With a sequential chemical denaturation/renaturation strategy followed by the introduction of variable and additional tags at the N- and C-termini of the macromolecules, we successfully purified these proteins using a modified affinity chromatography technique. This protocol generated hetero-oligomeric HtrA2 variants in a bacterial system by engineering and reorganizing the protomers obtained from the mixture of purified homo-oligomers differing in the number of a C-terminal protein-protein interaction domain - PDZ or active-site mutations (replacing the active serine with alanine). This effort was taken to explicate the contributions of each monomer and/or their domains in modulating the activity of the large multimeric HtrA2 ensemble. This will not only facilitate obtaining a homogenous population of difficult-to-purify hetero-oligomers with nominal differences in their physico-chemical properties from a set of recombinant proteins, but also help understand the contribution of each protomer through further biochemical and/or biophysical characterizations. This study, therefore, paves the way toward understanding the structural and functional intricacies of various proteins with biomedical and biotechnological importance.



Reference:

Parui A, Mishra V, Dutta S, Bhaumik P and Bose K. Inter-subunit Crosstalk via PDZ Synergistically Governs Allosteric Activation of Proapoptotic HtrA2. Structure-Cell Press. 2022 Jun 17; S0969- 2126(22)00232-5. doi: 10.1016/j.str.2022.06.001

Rapid production of bispecific antibodies using a production-phase promoter

Michael Anbar and Yusuf Johari

Lonza Integrated Biologics R&D, Cambridge, UK

Efficient expression of complex biopharmaceutical antibodies requires robust, high-titre cell lines and rapid manufacturing processes. Many conventional cell line development workflows involve generating mini-pools to enhance productivity and maintain stable expression over extended periods. Here, we present a novel synthetic production-phase promoter that enables high-level expression of complex and difficult-to-express molecules in bulk-pools, while significantly reducing recovery times. This promoter enhances the scalability of cell line development, improves consistency across production batches, and accelerates timelines, offering a promising strategy to meet the demands of rapid and cost-effective biopharmaceutical production.

Discovery and development of CRISPR-associated transposases for RNA-guided gene insertion

Samuel H. Sternberg

Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA

Howard Hughes Medical Institute, Columbia University, New York, NY, USA

In recent years, genome editing technologies have advanced from nuclease-based methods that generate DNA double-strand breaks (DSBs), which can cause undesired byproducts, to next-generation CRISPR approaches that perform controlled chemistry using DSB-independent strategies. Base editing and prime editing are ideally suited for small-scale modifications, but methods to achieve large-payload gene insertion have been lacking. To address this gap, I will present work from my lab describing a new family of CRISPR-associated transposases (CAST) that perform highly accurate, targeted integration of DNA payloads via RNA-guided transposition. Unlike conventional CRISPR systems that combine targeting and cleavage, CAST systems exploit nuclease-deficient CRISPR systems for RNA-guided DNA targeting, leading to the site-specific recruitment of transposase enzymes for DNA insertion. We have resolved molecular details of this pathway using a combination of high-throughput sequencing, biochemistry, genetics, and cryo-electron microscopy, revealing a hierarchical assembly pathway and a structural roadmap to guide engineering efforts. More recently, we leveraged phage-assisted continuous evolution (PACE) to identify transposase variants with ~200-fold improved activity in mammalian cells, yielding 10–25% integration efficiencies of kilobase-size DNA cargos across several human genomic sites. Our long-term goal is to harness CAST as a versatile platform technology for integrating large DNA payloads into the genome for basic research and to treat human disease.

Title: Consistency in Biomanufacturing from 4mL-50L with Thomson Tools for Plasmid, CHO, and HEK Manufacturing. Speeding up time to Clinic and Market

Author: Sam Ellis

Company: Thomson Instrument Company

Email: Sam@htslabs.com

Summary: Thomson solutions offer Optimum Growth line of flasks as scalable suspension growth platform for mammalian and insect cell lines. The system has shown consistent, reproducible results that scale from 24 and 6 well block formats, used in high throughput screening, to Thomson's 7L spouted flask for efficiently handling larger volumes. Automation friendly well based formats increase efficiency at the small and mid-scale formats. Availability of sizes 125ml, 250ml, 500ml, 1.6L, 2.8L, 5L, and 7L provide options seed trains and expression volumes that can greatly impact the speed of discovery.

Manipulating branched chain amino acid catabolism to improve CHO antibody titers: A combined cell line engineering and medium optimization approach

Camil A. C. Diaz[1], Cynthia Lam[1], Alyssa (Sargon) Getty[2], Zijuan Lai[1], Dewakar Sangaraju[1], Inn Yuk[1], Gavin Barnard[1], Shahram Misaghi[3]

[1]Genentech, U.S.A., [2]Formerly at Genentech, now at Flour & Fleur Cookies, U.S.A., [3]Formerly at Genentech, now at Prolific Machines, U.S.A.

Advancements in gene editing technology have enabled engineering Chinese hamster ovary (CHO) hosts with improved growth, viability, or productivity for recombinant monoclonal antibody (mAb) expression. One approach involved a knockout (KO) of the *BCAT1* gene, which encodes the first enzyme in the branched chain amino acid (BCAA) catabolism pathway. The *BCAT1* KO accumulated less BCAA byproducts including growth-inhibitory short chain fatty acids (SCFAs), ultimately improving growth and titer. However, because SCFA accumulation induces a metabolic shift to higher cell-specific productivity, SCFA supplementation during production aided in further translating the higher growth into higher titers.

Here we describe knocking out *BCKDHA* and *BCKDHB* genes, which act downstream of *BCAT1*, to reduce SCFA accumulation. We found that a partial KO of *BCKDHA* and *BCKDHB* introduced in the background of an apoptosis-resistant *BAX-BAK* host can achieve higher viabilities and titers. This was evident when SCFAs were added to boost productivity, as such additives negatively impacted culture viability in the wildtype but not *BAX-BAK* KO cells during batch production. Altogether, our findings suggest that SCFA addbacks can significantly increase productivity and mAb titers in the context of apoptosis-attenuated CHO cells with partial KO of BCAA genes. Such engineered CHO hosts can offer productivity advantages for expressing biotherapeutics in an industrial setting.

Reimagining CHO cell metabolism

Hooman Hefzi[1]

[1]Department of Biotechnology and Biomedicine, Technical University of Denmark, Denmark

Chinese hamster ovary (CHO) cells have been the primary workhorse for biotherapeutic protein production for nearly 40 years. Despite advances in process intensity and efficiency, universal mammalian cell phenotypes such as lactate and ammonia production, as well as the obligate requirement to supply essential amino acids, have led to challenges in process optimization without a one-size-fits-all solution. Over the last 9 years, we have developed genetic engineering strategies fundamentally reimagining these ubiquitous mammalian cell phenotypes and will present case studies around each in turn:

Lactate | Multiplex knockout of lactate dehydrogenase(s) and pyruvate dehydrogenase kinase(s) is sufficient to eliminate lactate production without impacting growth, protein production, or product quality. Intriguingly, this genetic engineering strategy appears to be generalizable to other mammalian cell lines such as HEK293.

Ammonia | Simultaneous knockout of asparaginase and glutaminases—responsible for the first step of catabolism for the respective amino acids—entirely eliminates ammonia production while cells are growing and reduces total ammonia levels over the full culture. This strategy can be combined with the lactate strategy described above, leading to cell lines with no lactate production and decreased ammonia production. Again, this strategy did not impact growth or product quality, while product titer actually improved using these cell lines as host cells for standard CLD workflows.

Essential amino acids | We have been able to restore biosynthesis for 2 essential amino acids (including 1 never before shown in a mammalian cell line) by introducing heterologous genes from a multiple microbes. While the resulting heterogenous pools show minor decreases in growth (15-25%), we are exploring if subcloning and/or additional optimization around pathway gene expression is sufficient to restore normal growth. We will also share the results of efforts to introduce these strategies into the engineered lactate/ammonia cell lines and an initial assessment of suitability for biotherapeutic production.

Collectively, these engineered cells have the potential to serve as next-generation manufacturing hosts, bypassing many of the existing limitations of mammalian cell lines.

Designing the Next Generation of CHO Cells Through Engineering Biology

Leon P. Pybus[1], Devika Kalsi[1], Alan J. Dickson[2], Dani Ungar[3], Susan Rosser[4]

[1]FUJIFILM Diosynth Biotechnologies, UK; [2]University of Manchester, UK; [3]University of York, UK; [4]University of Edinburgh, UK

Engineering biology can transform bioproduction by enabling precise, programmable control of cellular systems. This interdisciplinary approach combines synthetic biology, systems biology and -omics analytics to drive improvements in bioprocess phenotypes such as yield, cell line stability and product quality.

We have utilized synthetic biology strategies to enhance gene expression stability and productivity. Using a CRISPR-dCas9 system to deliver the catalytic domain of a histone acetyltransferase to the CMV promoter boosted mAb titres and cell line stability [1]. Complimentary to this, tRNA gene barriers have proven more effective than traditional UCOE elements in maintaining stable and high-level transgene expression [2,3].

Systems biology has provided deeper insight into CHO cell metabolism revealing ATF4 as a key regulator of lactate shifts induced by glutamine depletion and manipulating its expression improves cell performance [4]. Flux sampling of genome-scale metabolic models, constrained by transcriptomic data, has further identified metabolic signatures unique to high-producing CHO clones, suggesting nutrient supplementation strategies to enhance productivity [5]. Cell death pathway dissection shows non-apoptotic cell death dominate during fed-batch bioreactor conditions [6].

These findings demonstrate how engineering biology enables rationale CHO design. As the toolkit expands, future CHO expression systems will be increasingly modular, predictable and adaptable – designed from the genome up for next-generation biotherapeutics.

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2. Sizer RE et al. (2024) *Biotechnol J*, 19: e2400196.
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Engineering of endogenous retrovirus-like particle (RVLP)-deficient CHO cells by CRISPR or shRNA is facilitated by enrichment methods based on cell-surface expression of retroviral envelope protein

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ABSTRACT

Despite evidence that they are not functional or infective, retrovirus-like particles (RVLPs), originating from endogenous proviral sequences in Chinese hamster ovary (CHO) cells, present a safety risk for biotherapeutics manufactured using this cell line due to their resemblance to other mammalian leukemia viruses. The presence of RVLPs contributes significantly to the complexity and cost of therapeutic protein manufacturing using CHO cells: dedicated chromatography, low-pH or detergent-based viral inactivation and viral filtration steps are often necessary during DSP primarily for the purpose of reducing RVLP levels. Furthermore, these viral clearance/inactivation steps may be incompatible with some proteins and more complex biologics such as enveloped VLPs. Here, we demonstrate that CRISPR- and shRNA-based cell engineering strategies can be used to disrupt RVLP production by targeting the RVLP nucleotide sequences. Additionally, specific antibodies were generated to monitor RVLP protein expression, including RVLP envelope (Env) protein localized on the surface of CHO cells, greatly facilitating selection of RVLP-deficient clones. These modified CHO cells showed reduced RVLP production while maintaining or enhancing the ability to produce recombinant virus-like particles (VLPs), highlighting their potential application in biomanufacturing, especially for complex biologics that are incompatible with standard RVLP mitigation procedures, namely viral inactivation and nanofiltration.

Constitutive and Inducible UGCG Overexpression: Impacts on Viral Vector Production in a Targeted Integration Platform

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Viral vectors such as AAVs or VLPs are still produced via transient gene expression (TGE) due to the toxicity of certain elements, which makes constitutive expression difficult for host cells. However, scaling up TGE-based bioprocess is limited by the cell density effect (CDE), which reduces cell-specific productivity as cell concentration increases. Changes in the physiological state of cells at high density are believed to be one of the explanations for CDE. Previous research has revealed significant alterations in molecular components and metabolic pathways following transfection. Among these, the downregulation of glycosphingolipid biosynthesis has been identified as a key factor impacting cell viability and homeostasis during TGE[1]. The overexpression of UDP-glucose ceramide glucosyltransferase (UGCG), a key enzyme in glycosphingolipid biosynthesis, has demonstrated improvements in both transfection efficiency and VLP production[2]. However, since UGCG was only transiently expressed in these studies, its full impact was limited by CDE constraints. In this work, we evaluated the effect of constitutive and inducible UGCG overexpression in HEK293SF-3F6 cell lines using a targeted integration platform to assess its influence on transfection efficiency and VLP production. Our findings indicate that constitutive UGCG overexpression leads to significant metabolic and signaling pathway reprogramming, resulting in reduced transfection efficiency and lower VLP yields at low cell densities. Proteomic analysis identified key pathways responsible for these metabolic alterations. Conversely, inducible UGCG overexpression demonstrated improved transfection efficiency and enhanced VLP production at high cell densities upon induction. These insights highlight the importance of controlled UGCG expression to optimize recombinant VLP production strategies at high cell densities.

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Combining computationally guided protein engineering with multi-objective codon optimization (MOCO) for a novel and highly efficient transposase for cell line development

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Cell line development is a critical step in the production of biologics and plays a decisive role in determining the commercial viability of the final process. The method of transgene integration, in particular, significantly influences the efficiency of generating highly productive clones, while also impacting screening efforts and timelines. In recent years, several biopharmaceutical companies have transitioned from randomly integrating their gene of interest into host cells to using transposase-mediated transgene integration.

Transposases, which are typically co-transfected as DNA or mRNA along with the transgene flanked by the requisite transposon recognition sites, operate via a cut-and-paste mechanism, efficiently integrating the entire transgene expression cassette into the host cell genome at multiple locations. As most transposases were silenced by their originating host during evolution, it is difficult to identify a highly active variant suitable for the generation of industrially relevant cell lines.

In this study, we computationally mined and screened for previously unknown transposases and successfully identified two novel variants with a surprisingly high baseline activity. After highlighting the capability of these novel transposases by benchmarking them against internal and external controls, we applied computationally guided protein engineering to generate hyperactive variants. Additionally, we combined improvements on protein level with multi-objective codon optimization (MOCO) to synergistically enhance the efficiency for transgene integration of our novel transposase. In conclusion, this study highlights the deployment of protein and RNA optimization for maximum transposase efficiency leading to highly productive, recombinant protein expressing CHO cells.

Accelerating Drug Development:
Linking CHO Cell Genomics to Rapid Growth

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[1]Department of Gene Therapy, University of Ulm, Germany, [2]Corporate Research, Sartorius Stedim Cellca GmbH, Germany,

Commercial production cell lines must express biopharmaceutical proteins at high yields with suitable product quality in minimal time. To address the bottleneck of cell growth in cell line development and biopharmaceutical manufacturing, we employed an optimized lentiviral whole-genome knockout (KO) screen targeting 17,761 expressed genes with six gRNAs each to investigate the genetic mechanisms driving a fast-growth phenotype in CHO cells. This screen integrates refined multi-dimensional hit prioritization from multiple sampling time points and a high-throughput system for functional validation of growth-enhancing genes. The library was cultivated under shake flask conditions and in a 30-day, 3 L perfusion bioprocess, representing typical upstream biopharmaceutical processes. The screen achieved 70–80% single-copy integrations and >5,000x library coverage, significantly improving data quality compared to typical lentiviral genome-wide KO screens. We identified 198 growth-enhancing genes clustering into 69 functional gene sets with common pathways and functions. We use the established high-throughput validation system combining arrayed CRISPR KO libraries from the 198 gene hits with robot-assisted cultivation, measuring growth rates and viability. These findings provide insights into the genetic basis of fast-growth phenotypes, enabling targeted engineering of CHO cell lines to enhance efficiency in biopharmaceutical manufacturing. With this study, we established a comprehensive framework for genetic screening in CHO cells, addressing growth bottlenecks and enabling the rapid development of robust, high-yield production platforms for biopharmaceuticals.

Challenging cell death: novel anti-apoptotic targets for extended fedbatch biomanufacturing

Javier Bravo-Venegas[1], Camila Orellana[2,3], Mauro Torres[4,5], Mauricio Vergara[1], Alan Dickson[4,5], Marcela A. Hermoso[6,7], Julio Berríos[1], Claudia Altamirano[1,8,9]

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Chinese hamster ovary (CHO) cells are the preferred host for biopharmaceutical production. However, a major limitation is that they undergo apoptosis (programmed cell death) triggered by various stress factors restrigting culture lifespan and final titre. Though genetic engineering has been used to curb apoptosis, further improvements are needed. In this study, two novel anti-apoptotic genes (AAg) with broad protective functions were identified and overexpressed independently in an IgG-producing CHO cell line, as well as the reference anti-apoptotic gene bcl (BCL2) for comparison. Each modified line was compared to the parental IgG CHO cell line (control). After 48 h apoptosis-induction with camptothecin, the AAg2 cell line had 57% and 75% less cells in early and late apoptosis stages respectively, than the control. In batch cultures, both novel AAg cell lines achieved a peak viable cell density (VCD) similar to the control, while the Bcl-2 cell line reached 40% lower VCD. No extension in culture duration was observed in the AAg cell lines. However, the AAg2 cell line showed 31% higher IgG production and a 65% increase in cell-specific productivity compared to the control. In fed-batch culture, with sodium butyrate treatment, the AAg2 cell line extended culture duration by at least four days and increased IgG titer by 68% (Fig. 1). These findings demonstrate that the AAg2-modified line offers enhanced resistance to apoptosis and highlights their potential to enhance CHO cell viability and productivity.

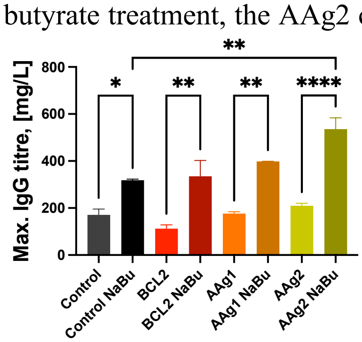

Figure 1. IgG production of control, Bcl-2 and AAg cell lines on Fed-batch cultures-

Paving the Way for a Minimal CHO Genome: Investigating the Molecular Mechanism Driving Large-Scale DNA Deletions

Emely Walker[1], Melina Bräuer[1], Stefan Schneider[1], Simon Fischer[2], Kerstin Otte[1]

[1]Department of Applied Biotechnology, University of Applied Sciences Biberach, Germany, [2] Cell Line Development, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

Chinese hamster ovary (CHO) cell lines are widely used in biopharmaceutical production and are continuously optimized for efficiency. Under bioreactor conditions, many endogenous cellular functions become redundant, imposing unnecessary transcriptional and translational burden to the cells. To address this, genome reduction via precise genome editing offers a powerful solution by removing non-essential DNA and reducing host cell protein levels to simplify downstream purification. Previously, large-scale genomic deletions of up to 1 megabase pair (Mbp) were achieved, laying the foundation for a minimal CHO genome. This study builds on this achievement by investigating the molecular mechanisms driving large-scale deletions in CHO cells.

To facilitate the generation of large-scale genomic deletions, various transfection methods of CRISPR/Cas9/sgRNA ribonucleoprotein (RNP) complexes were tested. Using the most effective method, large-scale model deletions of several Mbps in size were generated, and the cellular repair dynamics under physiological conditions were monitored over time via a deletion-specific quantitative PCR (qPCR) assay. The results indicated a very fast onset of large scale deletion repair mechanisms and that time-dependent formation of deletions is influenced by their size. To elucidate the repair mechanisms enabling these large-scale genome deletions, specific DNA double-strand break (DSB) repair pathways were selectively modulated using small molecule inhibitors. Establishing non-toxic modulator concentrations for CHO cells allowed for the application of a qPCR-based deletion assay to monitor early double-strand break repair events, revealing the involved key molecular mechanisms. Furthermore, the combined use of small molecules targeting several different DNA repair pathways led to a significant increase in deletion efficiency at later time points following RNP delivery, facilitating an efficient clone selection. These findings provide insights into the currently unexplored repair mechanisms and dynamics of large-scale genomic deletions. In addition, the finding that small molecule treatments improve CRISPR/Cas9-mediated deletion efficiency may has the potential to advance CHO cell engineering for biopharmaceutical applications in the future.

REMBAC - A Rapid Efficient Manifold Baculovirus Transduction Platform for stable cell line development

Manuel Reithofer[1], Sophie Huber[1], Sandra Díaz Sánchez[1], Miriam Klausberger[1] and Reingard Grabherr[1]

[1]Institute of Molecular Biotechnology, Boku University, Austria

Efficient recombinant protein production often depends on stable cell lines, especially for multisubunit complexes like virus-like particles (VLPs) and adeno-associated viruses (AAVs) used in vaccines and gene therapy. Nevertheless, generating stable cell lines is time-consuming and challenging, especially for products requiring multiple and large transgenes. Current biopharmaceutical production processes are based on stably transfected Chinese hamster ovary (CHO) cells, however for various products, human-like post-translational modifications are required. Thus, there is a need for a versatile, cell type-independent platform for fast stable cell line development.

Our system addresses this need by using baculoviral transduction of mammalian cells (BacMam), which is cost-effective, scalable, and efficient. BacMam has several key advantages: (i) it doesn't require high-biosafety laboratories, (ii) it efficiently transduces various cell types, and (iii) it is suitable to deliver large DNA fragments into the cellular nuclei. Hence, we developed the REMBAC platform (Rapid Efficient Manifold Baculovirus Transduction), enabling site-specific genome integration of large transgenes with customizable expression levels based on BacMam. Our expression cassettes have been optimized by including several beneficial elements such as insulators to protect against host-cell silencing. Our system ensures efficient gene delivery across different cell types and is designed to integrate the transgenes without leaving viral footprints. By combining BacMam's versatility with homologous recombination for site-specific integration, and using a homing endonuclease for precise transgene excision, REMBAC allows the co-expression of multiple transgenes at controlled levels. Thus, REMBAC facilitates stable cell line development for a wide range of biopharmaceutical applications, including biologics like monoclonal antibodies or bionanoparticles such as VLP vaccines or AAV gene therapy vectors.

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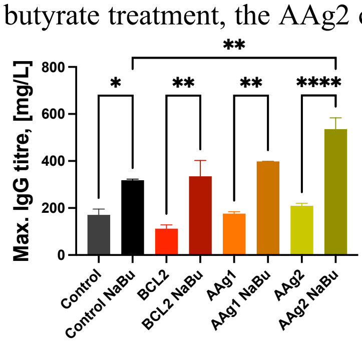

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Paving the Way for a Minimal CHO Genome: Investigating the Molecular Mechanism Driving Large-Scale DNA Deletions

Emely Walker[1], Melina Bräuer[1], Stefan Schneider[1], Simon Fischer[2], Kerstin Otte[1]

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To facilitate the generation of large-scale genomic deletions, various transfection methods of CRISPR/Cas9/sgRNA ribonucleoprotein (RNP) complexes were tested. Using the most effective method, large-scale model deletions of several Mbps in size were generated, and the cellular repair dynamics under physiological conditions were monitored over time via a deletion-specific quantitative PCR (qPCR) assay. The results indicated a very fast onset of large scale deletion repair mechanisms and that time-dependent formation of deletions is influenced by their size. To elucidate the repair mechanisms enabling these large-scale genome deletions, specific DNA double-strand break (DSB) repair pathways were selectively modulated using small molecule inhibitors. Establishing non-toxic modulator concentrations for CHO cells allowed for the application of a qPCR-based deletion assay to monitor early double-strand break repair events, revealing the involved key molecular mechanisms. Furthermore, the combined use of small molecules targeting several different DNA repair pathways led to a significant increase in deletion efficiency at later time points following RNP delivery, facilitating an efficient clone selection. These findings provide insights into the currently unexplored repair mechanisms and dynamics of large-scale genomic deletions. In addition, the finding that small molecule treatments improve CRISPR/Cas9-mediated deletion efficiency may has the potential to advance CHO cell engineering for biopharmaceutical applications in the future.

Redefining CHO-Chassis: Strategic Genome Reduction in CHO Cells Through Cas9-Mediated Megabase Deletions

Stefan Schneider[1], Melina Bräuer[1], Emely Walker[1], Simon Fischer[2], Kerstin Otte[1]

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[2] Cell Line Development, Boehringer Ingelheim Pharma GmbH & Co KG

Chinese hamster ovary (CHO) cells are the predominant mammalian production host for complex therapeutic glycoproteins, yet they retain numerous functions that are superfluous in industrial bioprocesses. Given the intrinsic limitations in cellular resources such as ATP and ribosomal capacity, we hypothesized that targeted elimination of non-essential genomic regions could liberate these resources for bioproduction, ultimately enhancing cellular performance. Building on our previous demonstration of large-scale deletions up to 800 kilobase pairs, we expanded our approach to achieve megabase-scale deletions via a Cas9-mediated strategy. Non-essential genomic regions were identified by mapping all publicly available essential genes onto the CHO genome, while additional boundaries were defined by predicted essential metabolic genes derived from metabolic models tailored to growth and recombinant protein production data. Bioenergetic costs for replication, transcription, and translation of genes within these regions were calculated using cell-line-specific expression data, allowing us to prioritize targets with high cumulative costs. The deletion strategy involved an initial transfection with sgRNAs targeting selected boundaries, yielding monoallelic deletion clones, followed by a second transfection to obtain biallelic deletions in this diploid system. Tiling PCR and whole-genome nanopore sequencing confirmed precise excision, with observed reintegration events—ranging from complete reintegration to reassembled large fragments (up to multi million base pairs)—that did not result in detectable gene expression. Fed-batch cultivations of both the host and recombinant antibody-producing clones demonstrated that even megabase-scale deletions encompassing regions with highly expressed genes did not compromise key bioprocess parameters such as viable cell density, productivity, or cell viability compared to the parental cell line. These findings provide a promising blueprint for developing a streamlined, genome-reduced CHO cell chassis that reallocates cellular resources from energetically expensive, non-essential processes to enhanced bioproduction, setting the stage for the next generation of optimized CHO cell factories.

REMBAC - A Rapid Efficient Manifold Baculovirus Transduction Platform for stable cell line development

Manuel Reithofer[1], Sophie Huber[1], Sandra Díaz Sánchez[1], Miriam Klausberger[1] and Reingard Grabherr[1]

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Our system addresses this need by using baculoviral transduction of mammalian cells (BacMam), which is cost-effective, scalable, and efficient. BacMam has several key advantages: (i) it doesn't require high-biosafety laboratories, (ii) it efficiently transduces various cell types, and (iii) it is suitable to deliver large DNA fragments into the cellular nuclei. Hence, we developed the REMBAC platform (Rapid Efficient Manifold Baculovirus Transduction), enabling site-specific genome integration of large transgenes with customizable expression levels based on BacMam. Our expression cassettes have been optimized by including several beneficial elements such as insulators to protect against host-cell silencing. Our system ensures efficient gene delivery across different cell types and is designed to integrate the transgenes without leaving viral footprints. By combining BacMam's versatility with homologous recombination for site-specific integration, and using a homing endonuclease for precise transgene excision, REMBAC allows the co-expression of multiple transgenes at controlled levels. Thus, REMBAC facilitates stable cell line development for a wide range of biopharmaceutical applications, including biologics like monoclonal antibodies or bionanoparticles such as VLP vaccines or AAV gene therapy vectors.

Surrogate-surface marker based enrichment of CHO stable pools for rapid protein production

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Rapid and scalable production of biotherapeutics, including vaccine antigens, is critical for responding to public health emergencies. Typical workflows for developing production-ready clonal Chinese Hamster Ovary (CHO) cell lines are lengthy, requiring 8–10 months. To expedite early-stage clinical material generation, non-clonal CHO stable pools offer a promising alternative, reducing timeline from several months to a few weeks. However, the productivity of random-integration stable pools is lower than that of clones due to heterogeneous expression profiles, with some cells being high producers, some moderate and others non-producing. Identifying and fishing out high producers from a heterogenous pool would thus improve its overall productivity. Because assessing expression levels of cells in a stable pool is challenging as most of the recombinant protein product is secreted in the culture medium, we describe a surrogate surface marker-based approach to identify and enrich for high-producers from a heterogenous stable pool expressing a CD200-Fc fusion protein. We developed a bipartite vector system wherein the strength of the promoter driving the surface marker cassette can be modulated, such that its expression has minimal impact on target protein production. We tested two classes of surface markers-glycosylphosphatidylinositol (GPI)-anchored (GPI-mRFP) and transmembrane domain (TMD)-based (PD-L1, CD4) to compare their effectiveness in labeling and selectively enriching for high CD200-Fc-expressing cells. Furthermore, we characterized and compared this approach with an established methodology known as cold capture, wherein under cold conditions, the secreted product is transiently retained at the CHO cell surface. Our results show that cell surface expression of both GPI-anchored and TMD based surrogate marker correlates with target protein expression. This enabled us to identify and sort for high-expressing subpopulations, which showed higher transgene copy numbers, mRNA expression levels, and cell specific productivity, leading to a 2-fold increase in the volumetric titers. Overall, this strategy represents a significant advancement to shorten biomanufacturing timelines and support global health initiatives.

Utilizing whole-genome CRISPR screening to develop alternative methods of metabolic selection

Corey Kretzmer[1] and David Razafsky[1]

[1]Process Solutions Upstream Research and Development, MilliporeSigma, Saint Louis, Missouri, USA

Chinese hamster ovary (CHO) cells have been the industry standard for biotherapeutics production for several decades. During this time, great efforts have been made to improve supporting infrastructure and environmental controls, while other advances came in the form of bespoke medias for optimized clone bioproduction. However, the CHO genome as an engineerable means of improving manufacturing outcomes has remained elusive, largely due to difficulties in target identification. Previously, our group developed and validated a first-in-class CHO CRISPR guide RNA library, built upon the de novo assembly and annotation of the CHO-K1 based CHOZN® $GS^{-/-}$ genome. As large-scale genomics studies become more financially accessible through cheaper sequencing costs, implementation of these unbiased, genome-scale screening tools becomes more practical for the CHO bioprocessing community. Here, we demonstrate the utility of our CHOZN®$GS^{-/-}$ CRISPR library in identifying new metabolic selection mechanisms via a genetic depletion screen and confirm the utility of these target genes via the development and testing of isogenic knockout clones which could ease the cell line development process for bispecific antibodies.

CHOZN® GS-/- Produces High Titer and High-Quality Bispecific Antibodies

Gabrielle Dowell[1], Jim Mahon[1], Kelsey Reger[1], Jayson Stoner[2], Sarah Brown[2], Channing McLaurin[3], Felicia Riordan[4], Carolyn Cash[4], Sophia Melzer[4], Amber Henry[4], Pei Liu[4], Brandon Medeiros[2], Jana Mahadevan[3], Benjamin Cutak[4], Kevin Ray[4], Amber Petersen[1] , and Wilson Fok[1]

[1]Biopharma Expression Team, Upstream R&D, MilliporeSigma, U.S.A, [2]Upstream Applications, Single Use and Integrated Systems, MilliporeSigma, U.S.A [3]CHO Media Innovation, Upstream R&D, MilliporeSigma, U.S.A [4]Analytical R&D, MilliporeSigma, U.S.A

The CHOZN® GS-/- CHO cell line is a robust production platform for monoclonal antibodies and other recombinant protein therapeutics. With the increasing use for bispecific antibodies in antibody therapies, we aim to demonstrate the platform's performance in bispecific cell line development and manufacturing. Utilizing various 3-chain bispecific designs such as SEEDbodies and either common light chains or scFv; our resulting bispecific clones exhibit high productivity, stability, and product quality following our standard cell line development workflows and templated fed-batch and perfusion bioreactor processes.

We employed a two-plasmid system, with each vector housing a unique heavy chain and its corresponding light chain. Two selection strategies were tested: either glutamine selection for both plasmids, or sequential puromycin and glutamine selection. Both selection strategies yielded productive, stable, and high quality clones.

Our top clones maintain a minimum of 80% heterodimer; and have similar glycosylation and charge variant profiles across clones and assays. These clone attributes extend from spin tube studies to 3L Mobius® single use fed batch bioreactors and Mobius® Breez 2mL dynamic perfusion microbioreactors. Spin tube fed batch titers reach 2-3.5 g/L, while 3L Mobius® fed batch exceeds 3.5 g/L, and Mobius® Breez perfusion systems reach 3.5 g/L/day in volumetric productivity. Our performance metrics highlight the CHOZN® GS-/- CHO platform's potential in bispecific cell line development and manufacturing.

Effect of individual expression of AAV and AdV elements on the transcriptome and proteome of CHO cells

Konstantina Tzimou[1], Lars Keld Nielsen[1,2] and Jesús Lavado García [1]

[1]Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denamrk [2] University of Queensland, Australia

Recombinant adeno-associated virus (rAAV) vectors have emerged as the leading platform in gene therapy due to their favourable safety profile and high transduction efficiency. However, biomanufacturing constraints, particularly low production yields in HEK293-based systems, limit their scalability and drive the search for alternative hosts. Chinese Hamster Ovary (CHO) cells, the gold standard for biopharmaceutical production, present a promising alternative, yet no CHO line has been established for efficient rAAV production, and the cellular response to AAV and adenoviral (AdV) gene expression remains largely unexplored.

This study represents the first comprehensive analysis of CHO cell responses to individual AAV and AdV proteins at both the transcriptomic and proteomic levels. We engineered expression constructs for isolated AAV and AdV gene products and systematically evaluated their impact on CHO cellular pathways. Surprisingly, functionally redundant AAV proteins elicited distinct cellular responses, with limited overlap in differentially expressed transcripts. Both transcriptomic and proteomic data suggest novel roles for AAV capsid and accessory proteins beyond their canonical functions observed in HEK293 systems. Additionally, AdV proteins displayed unexpected regulatory activities. Notably, expression of viral proteins resulted in the downregulation of immune and energy metabolism pathways in CHO cells.

Our findings uncovered key host-virus interactions leading to potential candidate targets for host cell engineering. This work contributes to a better understanding of the bottlenecks associated with rAAV production in CHO cells and highlights potential directions for future optimization strategies.

Enhancing Bispecific Antibody Production in CHO Cells Through Chain-Specific Signal Peptide Engineering

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Chinese Hamster Ovary (CHO) cells remain the dominant host system for the production of therapeutic monoclonal antibodies (mAbs), routinely achieving titers exceeding 10 g/L. However, the growing demand for more complex biologics, such as bispecific antibodies (bsAbs) capable of engaging multiple antigens simultaneously, presents significant challenges in cell line development. These engineered, multi-chain formats often suffer from imbalanced chain expression, inefficient assembly, and substantially reduced productivity—posing a major bottleneck to their commercialization.

To address this, we developed a novel signal peptide (SP) cleavage prediction algorithm that surpasses current state-of-the-art models in both precision and recall. Applying this predictor to the CHO-K1 proteome, we identified over 800 previously uncharacterized CHO-derived signal peptides. These were combined with existing human SP sequences to construct a diverse library of approximately 4,000 signal peptides.

We systematically applied this SP library to the heavy chains of various complex bsAb formats—including scFv-Fc and CrossMab—enabling chain-specific fine-tuning of molecule expression. This approach led to as much as a threefold increase in antibody titers, demonstrating the critical role of signal peptide selection in optimizing the expression of structurally complex antibodies.

In conclusion, our work highlights the utility of chain-specific signal peptide engineering—particularly leveraging CHO-derived sequences—as a powerful and generalizable strategy to enhance bispecific antibody production in CHO cells.

How we use diverse expression platforms to solve problems for "difficult-to-express" targets

Mark Elvin

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The utilization of diverse cell expression platforms, including insect cells, HEK (Human Embryonic Kidney) cells, CHO (Chinese Hamster Ovary) cells, and *Escherichia coli* (*E. coli*), has become a pivotal strategy in addressing challenges associated with "difficult-to-express" biological targets. Each cell expression platform offers unique advantages tailored to the specific requirements of the target proteins.

E. coli systems are favoured for their rapid growth and high yield, though they may struggle with complex post-translational modifications (PTMs). Insect cell systems, utilizing baculovirus vectors, excel in producing proteins with intricate PTMs, crucial for functional studies. HEK cells are widely used for their human-like PTMs and ease of genetic manipulation, making them ideal for producing proteins in their native conformation. CHO cells are indispensable for producing therapeutic proteins with human-like modifications and are extensively used in biopharmaceutical production. HEK and CHO cells also offer the advantage of secreting proteins into the surrounding medium making downstream processing much easier and cost effective.

As the Department of Protein Science and Structural Biology within Sygnature Discovery, we are a contract research organization (CRO) that provides custom made, soluble and membrane proteins for use in drug discovery, research applications as well as X-ray crystallography, NMR and Cryo-EM structures of both novel and precedented proteins. Here we show real examples of how we've utilized each of our different expression platforms (Insect, HEK, CHO, *E. coli*) to successfully express membrane proteins (for structural work), intrinsically disordered transcription factors, large multi subunit protein complexes (the largest being a 12 subunit protein complex) and labelled proteins for NMR studies. This was achieved by leveraging the strengths of each individual expression platform to overcome the limitations posed by "difficult-to-express" targets; thereby, facilitating advancements in drug discovery, structural biology and therapeutic development.

Optimizing IgM Production: An Integrated Platform Combining Phage Display Technology and CHO Cell Line Development

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Recent advancements in biotechnology, coupled with the increasing recognition of the unique properties of recombinant immunoglobulin M (IgM), have generated growing interest in these antibodies for therapeutic and diagnostic applications. A major driver of this progress is the widespread adoption of phage display technology, a valuable tool in antibody discovery and engineering that allows the complete synthetic generation of antibodies. The combination of phage display methodology and CHO expression system provides a powerful platform to produce recombinant IgMs addressing several challenges arising from the complex nature of these antibodies. The recombinant IgMs offer a promising alternative to traditional reagents in immunodiagnostic kits, with many advantages including the reduced reliance on human sera. In this work, we employed phage display technology to identify single-chain variable fragment (scFv) sequences with high affinity for the Rubella viral particle, a key reagent in the Diasorin IgM Rubella LIAISON® assay. This strategy allowed the isolation of scFv in only 18 days. Six pairs of sequences were then assembled on IgM scaffolds and produced as full-length antibodies in transient expression. The six antibodies were characterized in term of productivity and immunochemical performance, leading to the selection of the best IgM to proceed with stable expression. Finally, a hyper-producing IgM anti-Rubella stable clone was generated by our improved CHO cell line development process. Fed-batch production yielded 1.2 g/L of recombinant IgM, while preserving its multimeric structure. The recombinant IgM excellently replaces human positive sera for IgM anti Rubella, currently used in Diasorin IgM Rubella LIAISON® assay as calibrators and positive control. Signal emission and consistency were the parameters used to evaluate the comparison. Additionally, the long-term stability of the recombinant IgM was assessed. Along with optimal reactivity performance, its high production titer enables the expansion of the kit calibration range. This significant achievement highlights the potential of this integrated platform and marks a significant step toward leveraging recombinant IgM in diagnostic and therapeutic applications.

Process for generation of high-producing CHO cell lines for biologics manufacturing

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Chinese hamster ovary (CHO) cells are the most widely used mammalian host for industrial-scale production of mAbs and other protein biologics. Selection of high-producing cell lines is a key step in the process of manufacturing a novel biologic and requires an extensive and lengthy screening campaign of several hundreds of clonally-derived cell lines. We have previously reported the development of an efficient cumate-inducible expression system. Here, we present a new GMP-banked parental cell line, CHO^{2353}, amenable to both constitutive or cumate-inducible expression. We first present our process for selecting CHO pools and then cell lines using a semi-automated approach, where imaging analysis provides >99% probability that selected cell lines are single-cell derived. Following stable pool selection with MSX, fluorescently labeled cells are deposited at one cell per well in 384-well plates using FACS and pictures of each well are taken to assess monoclonality. Hundreds of cell lines are then screened in 96-well plate format to identify top producers, which then a subset of clones enter expression stability study in a 6-deepwell plate format (~20 mL). High-producing, stable cell lines are then tested in 1-5 L bioreactors. We found that ~75% of selected cell lines show stable expression after > 60 generations in culture. We also present how we have engineered our platform for the production of antibodies with reduced fucosylation, and recent development of a minipools selection approach allowing to select more productive CHO clones. Antibody productivity for pools reached 2-4 g/L while that of clones reached 6 g/L. Finally, we present recent data with a newly engineered CHO cell line (CHO^{NRC4}), where expression from CHO pools reached titers of 4.0 g/L and 6.5 g/L for two model proteins.

Purification and Characterization of Hetero-Oligomeric Variants of Proapoptotic HtrA2 Using a Modified Tandem Affinity Purification Approach

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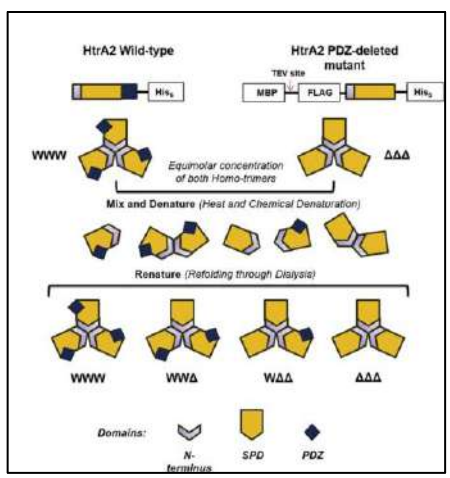
Generating hetero-oligomers from a mixture of recombinantly expressed and purified proteins has always been arduous. It becomes even more challenging for large multimeric proteins. Using a series of ingenious modifications to existing techniques and tools, we have artificially generated variants of a trimeric proapoptotic serine protease, HtrA2, that is associated with several diseases, including neurodegeneration and cancer. With a sequential chemical denaturation/renaturation strategy followed by the introduction of variable and additional tags at the N- and C-termini of the macromolecules, we successfully purified these proteins using a modified affinity chromatography technique. This protocol generated hetero-oligomeric HtrA2 variants in a bacterial system by engineering and reorganizing the protomers obtained from the mixture of purified homo-oligomers differing in the number of a C-terminal protein-protein interaction domain - PDZ or active-site mutations (replacing the active serine with alanine). This effort was taken to explicate the contributions of each monomer and/or their domains in modulating the activity of the large multimeric HtrA2 ensemble. This will not only facilitate obtaining a homogenous population of difficult-to-purify hetero-oligomers with nominal differences in their physico-chemical properties from a set of recombinant proteins, but also help understand the contribution of each protomer through further biochemical and/or biophysical characterizations. This study, therefore, paves the way toward understanding the structural and functional intricacies of various proteins with biomedical and biotechnological importance.



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Rapid production of bispecific antibodies using a production-phase promoter

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Efficient expression of complex biopharmaceutical antibodies requires robust, high-titre cell lines and rapid manufacturing processes. Many conventional cell line development workflows involve generating mini-pools to enhance productivity and maintain stable expression over extended periods. Here, we present a novel synthetic production-phase promoter that enables high-level expression of complex and difficult-to-express molecules in bulk-pools, while significantly reducing recovery times. This promoter enhances the scalability of cell line development, improves consistency across production batches, and accelerates timelines, offering a promising strategy to meet the demands of rapid and cost-effective biopharmaceutical production.

SfC1B5 a new rhabdovirus-free insect cell line that improves the yield of secreted proteins expressed using the baculovirus system

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Oxford Expression Technologies Ltd, Oxford UK

The baculovirus expression system is recognised as one of the main platform technologies for the production of proteins in insect cells. For many years, the Sf9 cell line has been popular for both the amplification of recombinant viruses and the production of proteins. It was produced by clonal selection from Sf21 cells, which were originally derived from the pupal ovarian cells of *Spodoptera frugiperda* (Fall armyworm). In this study, we aimed to produce a rhabdovirus-free insect cell line for production of proteins for use as human vaccines. We used a frozen vial of early passage Sf21 cells (Oxford, 1981) as our starting material. A number of cell lines were derived by rounds of single-cell cloning and testing for the presence or absence of the insect rhabdovirus by RT-PCR. Putative rhabdovirus-free cell lines were expanded in ES-AF (animal-free) medium and were also tested for the ability to support baculovirus amplification and protein production. One cell line, SfC1B5, was selected for banking and further study. The SfC1B5 cell line has since been adapted to grow in chemically-defined medium and extensive testing has demonstrated the cell line supports recombinant baculovirus production, amplification and protein yields similar to that obtained with Sf9 cells. We report use of the cell line to make Gc and Gn surface glycoproteins of Crimean Congo Hemorrhagic Fever virus as a candidate vaccine. However, for a range of secreted proteins, including viral antigens, higher yields were obtained in the SfC1B5 cell line. Where the secreted protein is to be used as a vaccine for human or animal health, the increase in yield significantly reduces the cost of vaccine production per unit dose.

The Challenges in Bringing Recombinant Polyclonal Antibodies to the Clinic

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Hyperimmune globulin drugs manufactured from pooled immunoglobulins from vaccinated or convalescent donors have been used effectively in treating infections where no treatment is available. This is especially important where multi-epitope neutralization is required to prevent the development of immune-evading viral mutants that can emerge upon treatment with monoclonal antibodies. Using microfluidics, flow sorting, and a targeted integration cell line, GigaGen established a platform for development and manufacturing of recombinant polyclonal antibodies (pAbs), which comprise a mixture of >1,000 individual antibodies produced en masse. Two drugs of this class have now entered clinical development: GIGA-2050, for treatment of SARS-CoV-2, and GIGA-2339, for treatment of chronic Hepatitis B virus. To achieve these milestones GigaGen overcame several key challenges, including development of a single site targeted integration cell line, optimization of the upstream process using that cell line, as well as development of novel methods to monitor the upstream and downstream processes to ensure lot to lot consistency.

Towards Accessible and Sustainable Recombinant AAV Vector Supply via Optimization of Production Platforms

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Abstract

Recombinant adeno-associated viral vectors (rAAV) have emerged as a transformative platform in treating rare genetic diseases, evidenced by seven recent FDA approvals. These vectors are distinguished by their long-term expression capabilities, favorable safety profiles, and diverse capsid types that enable targeting of different tissues. While initially successful in treating rare monogenic disorders, rAAV applications have expanded to include more common conditions in ophthalmology and CNS disorders. Beyond gene replacement and regulation, rAAV vectors are now being explored as delivery vehicles for antibodies, cytokines in immunotherapy, and vaccine development.

Despite their versatile potential, recombinant Adeno-Associated Virus (rAAV) treatments have experienced suboptimal adoption rates. A key challenge lies in the cost-effective manufacturing of high-quality AAV vectors at scale compared to traditional biologic products. Streamlining manufacturing processes and reducing costs while maintaining vector quality could help overcome the barriers to wider adoption. Currently, transient transfection-based production in HEK293 cells represents the most widely used platform, while stable producer cell lines emerge as the preferred future production system. Although recent years have seen significant improvements through process understanding and optimization, comprehensive knowledge of these production systems remains limited. This study presents an overview, progress, and outlook of stable producer cell line-based AAV production systems, focusing on helper virus mechanisms, host cell pathway analysis, and digital tools advancing AAV production excellence.

Development of an avian H5N1 flu vaccine based on recombinant H5 antigen expressed in CHO cells

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H5N1 is a highly pathogenic avian influenza virus that is responsible of the highly infectious respiratory illness called “bird flu”. Since 1996, the virus has expanded across the globe spilling into different animal groups such as poultry, wild and domestic mammals, as well as over 900 humans including one case in BC, Canada (November 2024). Since 2022 an increase of the outbreak has been reported, causing the death of millions of birds and leading to the death of approximately 20 million hens as of January 2025, and one human death in the USA

The virus is constantly evolving, and it has diversified and accumulated mutations throughout the years. Despite no transmission between humans having been reported yet, there are concerns that the virus is only a few mutations away from being able to transmit between humans, leading to a new potential pandemic.

Most of the current available H5N1 vaccines are manufactured through a lengthy egg-based production process and the protection they provide is often based on outdated H5N1 strains. An alternative option for flu vaccines is the use of recombinantly produced antigens, such as hemagglutinin. Since CHO is the industry’s most used cell factory to produce r-proteins with a glycosylation similar to humans, their use is more and more considered for vaccine manufacturing. In my presentation, I will detail our strategy to produce a H5N1 recombinant hemagglutinin (H5) antigen in CHO cells that is able to generate immunogenicity in vivo, as well as improving its manufacturability.

Thomson 7L Flasks replacing Bioreactor and Cell Bags between 5L to 6L/Flask. Ability to Grow up to 35L/Shaker.

Sam Ellis and Alberto Estevez

Thomson Instrument Company, Carlsbad, CA, 92008

This work introduces a spouted variant of the Thomson 7L Optimum Growth flask, currently the largest working volume flask available in the market. The design features a basal spout with integrated tubing, engineered as a closed system for cellular transfer operations. The system's low retention volume architecture optimizes culture transfer efficiency for seeding several liters or serving as an inoculation vessel for large-scale bioreactors. This configuration reduces both culture exposure risks and the total number of vessels required for large-volume cell culture operations.

Characterization of cellular oxidative stress in a CHO transient gene expression production process producing SARS-CoV-2 spike protein as a subunit vaccine antigen

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Chinese Hamster Ovary (CHO) cells are widely used for the manufacturing of antibodies and, more recently, subunit vaccines. To this end, transient transfection is a rapid and cost-effective means of expressing lead candidates in CHO cells and is particularly useful when needing to produce novel subunit vaccine variants within a short time frame. In a conventional process, the timing of transfection is generally decided by a predetermined viable cell density (VCD) trigger. However, this strategy may not translate well when culturing CHO cells using continuous cultures or N-1 perfusion, due to the cultures not following conventional growth curves. To determine an ideal time to carry out transient transfection in such cultures, an oxidation-reduction potential (ORP) probe may aid in this decision-making process. ORP is an indirect measure of the presence of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide, which in higher concentrations can negatively impact cellular productivity and quality attributes of the products such as post-translational modifications.

To determine whether ORP levels at the time of CHO cell transfection have any impact upon production yield of the therapeutic marker, namely SARS-CoV-2 spike protein, transient transfection was performed in 1 L bioreactors while monitoring readings from an online ORP probe. The discovery of an ideal ORP range and VCD combination for transient transfection may provide a useful parameter for CHO cell readiness for transfection. Furthermore, monitoring of the ORP signal post-transfection can serve as an indicator of the general health and productivity of the culture. Changes in redox potential captured with the ORP probe may be correlated with production rate that generally decline after a few days post-transfection. This would in turn help us further improve cellular productivity by adding antioxidants, for instance, at the appropriate time to reduce cellular oxidative stress.

Enhancing Control of mAb Production in Perfusion Bioreactors using Continuous Monitoring

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Perfusion bioreactors are emerging as a powerful platform for continuous monoclonal antibody (mAb) production in Chinese Hamster Ovary (CHO) cells with advantages in productivity, product quality, and operational efficiency. However, conventional perfusion processes typically rely on offline sampling and delayed analytics to guide process decisions. This piecewise approach often leads to reactive rather than proactive control, where flow rates, feed concentration and downstream adjustments are only made after metabolite imbalances or performance issues arise. In this study, we present the design of an advanced perfusion bioreactor platform built around the Sartorius Biostat® B-DCU 2L bioreactor system coupled with a Repligen ATF (alternating tangential flow) controller. Enhanced with integrated online sensors, the system enables real-time process awareness and more responsive control. Alongside standard sensors (pH, dissolved oxygen, temperature) the system incorporates a multi-spectrum UV sensor and a capacitance probe to provide continuous data on metabolic state, nutrient consumption, cell density, and product yield. The central aim of this work is to demonstrate how a real-time sensing strategy can overcome the limitations of traditional offline workflows. Leveraging the Sartorius Cellca 2 CHO strain for maximum protein production, the system uses continuous monitoring to enable early detection of metabolic shifts to support dynamic control of perfusion rates, nutrient supplementation, and process parameter shifts including temperature and dissolved oxygen. This level of process visibility has the potential to boost productivity over extended culture periods, improve overall titre, and maximize space-time yield while simultaneously informing downstream operations. By transitioning from manual adjustments to adaptive control, this platform reflects a broader push toward integrated and data-driven biomanufacturing. Future work will focus on leveraging these sensor outputs to develop predictive control algorithms and fully closed-loop systems for robust, scalable mAb production.

High-throughput Biomarker Discovery for Antibody Production Using Tag-Based Single-Cell RNA-Seq

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Chinese Hamster Ovary (CHO) cells are the industry gold standard for expressing therapeutic monoclonal antibodies (mAbs), and selecting high-producing clones early in the production pipeline is essential for efficient patient delivery. This can be achieved by identifying biomarkers that accurately predict cell productivity. However, current approaches for biomarker discovery rely heavily on low-throughput, labour-intensive methods that limit scalability and slow development timelines. These limitations hinder comprehensive assessment of large clone panels and obscure the full extent of transcriptional heterogeneity at early stages. To address these challenges, we present a novel high-throughput, multiplexed approach for early clonal characterization using single-cell RNA sequencing (scRNA-seq). By co-culturing and barcoding multiple CHO clones in a pooled format, we enable efficient, parallelized transcriptomic profiling of thousands of individual cells across multiple clones in a single experiment. This strategy reduces batch effects and accelerates screening, providing a more holistic view of clonal behaviour at the single-cell level. Our analysis revealed consistent transcriptomic signatures associated with high-productivity clones and identified productive subpopulations within clones. Beyond biomarker discovery, this platform offers a scalable, data-rich framework for characterizing transcriptional heterogeneity and performance across clones — delivering valuable insights for early decision-making in cell line development.

Multivariate Data Analysis Aids Selection of CHO Cells Clones Expressing a Monoclonal Antibody

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The development of cell lines that reliably express large titers of biologics such as monoclonal antibodies (MAbs) is an important step of bioprocess development. After transfection of CHO cells, stable pools are obtained, and clones are isolated. As each clone can behave distinctly when cultivated, clone selection is crucial. This study proposes an adaptable multifactorial data analysis method for clone selection that takes multiple process parameters into account, in addition to the traditionally considered titer and cell growth. We applied our innovative approach on existing in-house data corresponding to the generation of CHO clones producing Omalizumab, an IgG1 monoclonal antibody. Twenty-four clones were chosen for expression stability screening tests conducted in extra deep well plates (18 mL). Among them, eight stable clones were selected for scalability evaluation performed in benchtop stirred-tank bioreactors (0.75-1 L). Considering parameters related to productivity, cell growth and expression stability led to the selection of different clones for bioreactor scalability assessment experiments compared to those originally selected based on a conventional method. As efficient CO_2 stripping is challenging in large-scale bioreactors, experiments were conducted with and without addition of air in the bioreactor headspace to modulate the concentration of CO_2 in the media. We found that cultures without overlay air addition reached a pCO_2 of up to 190 mmHg. Of note, we showed the increased concentration of CO_2 to be beneficial. Indeed, on average, we measured 1.31-fold higher final titer, 1.14-fold higher cell specific productivity, and 1.13-fold greater peak viable cell density for cultures exposed to a greater pCO_2. In addition, these cultures benefitted from 3 to 5 more days above 80% viability, and their titer kept increasing until the end of the culture (17-21 days). We integrated statistical tools to reliably analyze datasets relating to productivity, cell growth, and key cellular metabolites. This new approach helped selection of a robust clone which performed similarly for low and high pCO_2, offering a better potential for subsequent scale-up. These findings underscore the great potential of MVDA to improve bioprocess development.

Quantitative analysis of proteomic differences in clonal suspension MDCK cell lines infected with human influenza A virus

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Suspension MDCK cells are a highly relevant cell substrate for scalable and efficient production of influenza A virus (IAV). Considering the high heterogeneity within conventional cell populations, the development of clonal cell lines has resulted in candidates with superior growth characteristics and high IAV yields (Zinnecker et al., 2024). However, proteomic analysis could help to further understand specific properties of such clonal cell lines and help to identify best producers for vaccine manufacturing.

In the present study, we compare proteome alterations between two IAV-infected suspension MDCK cell clones (C59 & C113, Sartorius, Germany) to elucidate differences in cell growth, size, metabolism and IAV productivity. Using advanced mass spectrometry, a total of 5177 host cell proteins were detected in both cell clones. Protein network analysis of the differentially expressed proteins with respect to cell growth revealed that fatty acid oxidation and branched-chain amino acid degradation were upregulated in the highly productive cells, whereas steroid biosynthesis and DNA replication were more active in the faster growing cells. After infection, 122 proteins were significantly upregulated ($\log_2$ fold change ≥ 1) in the high-producing cell line, including proteins associated with membrane trafficking. In addition, proteins that have cross-links to the IAV-NS1 protein and proteins that support virus production were identified. In addition, 98 proteins associated with antiviral signaling pathways such as Met and TNF signaling were downregulated ($\log_2$ fold change ≤ 1). In the less producing cell line, 77 proteins were downregulated and 57 upregulated after infection. Here, RNA metabolism seemed to be downregulated, whereas the TCA cycle and stress response were upregulated.

Overall, we were able to identify important differences between a fast-growing and a high-producing clonal MDCK cell line, revealing potential bottlenecks and providing further insights into the efficient production of IAV in cell cultures.

A Novel Strategy to Reduce Polysorbate Degradation? Influence of Cultivation Media on CHO Hydrolase Expression and Activity

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As a crucial surfactant, polysorbate (PS) is deployed in the final formulation buffer to stabilize biologics – often monoclonal antibodies – and thus ensuring the safety and efficacy of the drug product. However, in recent years it was shown that Chinese hamster ovary (CHO) host cell proteins (HCPs) possessing hydrolytic activity could persist the purification process of the biotherapeutic, and were found at trace levels in the final drug product. Over time, even trace amounts of hydrolases can possibly cleave PS ultimately compromising stability of the drug product and might even lead to the occurrence of (sub)-visible particles. Tackling strategies encompass the entire bioprocess, including cell line engineering, downstream optimization, or alternative formulation approaches. However, mitigation strategies during upstream processing are scarce and usually only focus on the harvest procedure to avoid leakage of intracellular HCPs. Here, we present a novel strategy to reduce PS degradation via choice of cultivation media.

During head-to-head cultivation of CHO cell lines in various cultivation media, we revealed strongly differing PS degradation properties when assaying the harvested cell culture supernatant. This phenomenon seemed to be lipoprotein lipase (LPL) related, since PS degradation was substantially reduced in LPL knockout cells even in media that showed pronounced PS degradation. Consequently, PS degradation clearly correlated with LPL protein abundance in the supernatant. In order to elucidate the connection between media and varying LPL levels, we analyzed media dependent cellular LPL expression and could rule out differences in transcription as the root cause. Consequently, we focused on media dependent effects on LPL protein expression, secretion and stability. In addition, media components were correlated to LPL expression to identify possible stabilizing or destabilizing agents in the media composition.

The understanding of the influence of cell culture media components on hydrolytic HCP expression and hence varying PS degradation offers a novel solution to mitigate PS degradation in CHO cell bioprocesses. Our results suggest that by rational media design we might be able to reduce the activity of critical PS-degrading hydrolases in the future.

CHO Cell Growth in 3-D Using Semi-Solid Alginate Enables 384 Well Plate Cutlures Without Shaking

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Antibody-producing cells like CHO are typically cultivated in shake flasks, limiting miniaturization and throughput due to volume and high-velocity shaking requirements. We propose a high-throughput microfluidic bioprocess in a 384-well plate format, where CHO cells are encapsulated in a hydrogel and receive nutrients and oxygen via rocking induced media flow through hydrogel channels, enabling efficient mass transfer with minimal infrastructure and energy needs. We identified alginate as the optimal hydrogel for CHO cell encapsulation, preserving their rounded morphology. Alginate enabled easy cell retrieval via EDTA-mediated Ca^{2+} chelation, achieving a 97 ± 16% yield, facilitating downstream analysis. We established CHO cell cultivation in alginate within a perfusable 384-well plate, using a microfabricated platform (OrganoBiotech) where encapsulated cells occupy the middle well, and gravity-driven flow is maintained by differential media levels and rocking-induced perfusion. To enhance mass transfer, 20 μm-scale granules of gelatin were incorporated at the cell encapsulation stage. Gelatin was removed upon alginate crosslinking and temperature elevation, leaving behind a porous path. To fabricate the slab, 25 μL of the alginate solution with CHO cells ($1x10^{6}$ mL) were deposited into the middle well, allowing the solution to evenly spread across the well, followed by a rapid crosslinking using a $CaCl_2$ solution yielding an even distribution of live cells in 3-dimensions. After 10 days, CHO cells cultivated in alginate with rocking induced flow, exhibited higher density and viability compared to those cultivated in a static alginate slab, or a standard 384-well plate with cells growing in 2-dimensions at the bottom of the wells . Quantification of the IgG concentration using the Octet platform further highlighted the superiority of the microfluidic platform, with significantly higher total mAb amount under flow compared to a static cultivation in alginate or a standard 384-well plate (**Fig. 1**).

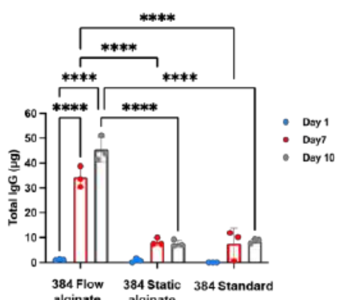

Fig. 1 mAb production in microfluidic 384-wells

Acknowledgements: The authors thank Amgen for funding, Rene Hubert, Tracy Lee, Brett Eyford and Eric Gislason for helpful discussion.

Effects of enzymatically-synthesized sialylated oligosaccharides on the *N*- and *O*-glycosylation of etanercept in recombinant CHO cell culture

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In mammalian cell-based bioprocesses, glycosylation of recombinant therapeutic proteins requires stringent control and monitoring as it may affect therapeutic efficacy and safety. The sialylation of glycosylated moieties plays an important role in determining the in vivo clearance of therapeutic proteins by extending their serum half-life. In this study, six enzymatically synthesized oligosaccharides (3′-sialyllactose, 6′-sialyllactose, sialyllacto-*N*-tetraose a, disialyllacto-N-neotetraose, lacto-*N*-tetraose, and lacto-*N*-neotetraose) and lactose were evaluated for their effectiveness on the *N*- and *O*-glycan profiles of etanercept, a therapeutic Fc-fusion glycoprotein consisting of 3 *N*- and 13 *O*-glycosylation sites, produced in recombinant Chinese hamster ovary (rCHO) cells. Of these seven compounds, only sialylated oligosaccharide supplementation increased the proportion of the acidic isoforms, di-sialylated *N*-glycan and di-sialylated *O*-glycan, as well as the total sialic acid content of etanercept, although the degree of the effect varied. Increased sialylation resulted from increased concentrations of intracellular CMP-sialic acid, with the highest increase observed for disialyllacto-*N*-neotetraose, which contains the most di-sialic acid residues. In contrast, four sialylated oligosaccharides did not enhance the sialylation of etanercept produced by human embryonic kidney 293 (HEK293) cells. Taken together, enzymatically synthesized sialylated oligosaccharides represent novel supplements to enhance the sialylation of therapeutic Fc-fusion glycoproteins in rCHO cell culture.

Bio-imaging strategy to follow adeno-associated viruses inside cells

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Adeno-associated viruses (AAVs) are indispensable viral vectors for fundamental and clinical research. In fact, AAVs are composed of a single-stranded DNA contained in a 25 nm capsid. These two components can be modified to create recombinant AAVs (rAAVs), which can then be designed for a specific use. For example, some rAAVs allow the delivery of optogenetic tools and render the study of *in vivo* mechanisms possible. Other rAAVs can cross the blood brain barrier and represent an interesting avenue for central nervous system gene therapy. However, while rAAVs enable a panoply of applications, the transduction of the viral particle itself remains very intricate and underknown. There is a critical need to further our understanding of this tool to optimize its use and therefore lead to the development of highly efficient viral vectors. Thus, the goal of this project is to develop a bio-imaging and analysis method to follow the journey of rAAVs throughout cells in real time. First, AAV capsids are labelled using dibenzocyclooctyne (DBCO) molecule paired with a fluorophore via click chemistry. This unique AAV labelling strategy was optimized to minimize the impacts on the viral capsid integrity and its transduction properties. Second, subcellular regions like the cell membrane and the nuclear envelope are tagged to create checkpoints during the rAAV transduction. Neurons will be the main cells of interests considering AAVs' central nervous system gene therapy perspective. Third, the monitoring of the fluorescently labelled rAAVs infection process is made using real-time videorate fluorescence microscopy. Fourth, analysis and quantification of the AAV transduction will then be realized to obtain unprecedented data on this pathway. Furthermore, a wide variety of cellular models and AAV serotypes are compatible with the developed labelling methods, which will open the door to further studies on this viral vector and contribute to its widespread and optimized use.

Exploration of high cell density intensification for the production of influenza A virus

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Influenza vaccine production in cell culture offers a scalable and efficient alternative to egg-based manufacturing. Process intensification strategies can be used to further increase yields, reduce facility footprint, and shorten production timelines. This study evaluates different approches for intensified influenza A virus (IAV) production using two clonally-derived suspension MDCK cell lines, C59 and C113 (Sartorius, Germany), with distinct characteristics [1].

To drive cells to high cell density (HCD), we implemented a semi-perfusion strategy in shake flasks, achieving maximum densities of 42×10^6 C59 cells/mL and 17×10^6 C113 cells/mL. Upon infection, high hemagglutinin titers of 3.5 and 3.6 $\log_{10}$(HAU/100 µL) were reached for C59 and C113, respectively. Based on the small-scale results, the process was transferred to a 3 L bioreactor coupled to a KrosFlo tangential flow depth filtration (TFDF) perfusion lab scale system (Repligen, USA) with perfusion rates tailored to the specific needs of each cell line. The filter allowed for cell retention with continuous virus transmission enabling continuous clarification and final harvest, resulting in a 10-fold increase in space-time yield for C59 and a 4-fold increase for C113 over batch operation.

In addition, we evaluated seed train intensification strategies, including N-1 perfusion and HCD cryopreservation for direct inoculation of the production bioreactor. Cells from N-1 perfusion showed reduced doubling times, but virus titers were unaffected compared to control infections. Direct inoculation from HCD cryovials enabled accelerated process initiation without additional precultures, demonstrating its applicability for streamlining manufacturing workflows.

Overall, our study provides important insights into process intensification strategies for improved IAV production. We demonstrate scalable, high-yield solutions that increase productivity, reduce production times, and support the transition from batch to intensified cell culture-based influenza vaccine manufacturing.

[1] Zinnecker et al., 2024, *Eng. Life Sci.*, https://doi.org/10.1002/elsc.202300245

Novel production process for Herpes Simplex Virus-1 utilizing a suspension HEK293 cell line

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Traditionally, Herpes Simplex Virus-1 is produced in adherent cells, typically Vero. The use of adherent cells, despite the availability of roller bottle systems, cell factories, or fixed bed/microcarrier bioreactors, poses significant challenges for handling, upscaling, and production. We, therefore, aimed to set up a suspension-cell process that would enable the production of high titers of HSV-1. Moreover, the process should allow for certain flexibility in production, suitable for both research-scale production and supply for potential clinical studies.

First, we aimed to identify a suitable suspension cell line to propagate HSV-1. Different cell lines commonly used for virus production were evaluated. To our surprise, HEK293 cells proved to be the most promising for HSV-1 production. Following the identification of HEK293 as the most productive cell line, three different HEK-derived cell lines were tested in combination with various media. The best producing cell line, HEK-LTV cultured in Dynamis medium (Thermo Fisher Scientific), produced approximately 2×10^8 TCID50/ml of HSV-1. To further optimize the batch process, the influence of temperature during the infection and production phases was analyzed. Lowering the temperature to 35°C or 33°C did not improve viral production compared to culture at 37°C. Furthermore, cell density effects in both batch and semi-perfusion settings were studied. Finally, the batch process was transferred to a stirred tank bioreactor, which resulted in a comparable HSV-1 infectious titer of 1.3×10^8 $TCID_{50}$/ml. In this setting, different agitation speeds were tested to assess the impact of shear stress on HSV-1 production.

Current commercial HSV-1 production utilizes adherent cells, highlighting the need for the development of a suspension cell process that yields high viral titers. Here, we demonstrate a scalable HSV-1 production process in suspension HEK293 cells, yielding high viral titers.

Transforming Therapeutic Protein Engineering

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To accelerate the design and success of therapeutic proteins, we have evolved from a traditional rational design and measurement approach to a predictive, AI/ML-driven platform. This next-generation platform combines predictive screening with generative biology to rapidly engineer proteins optimized for manufacturability, efficacy, and safety. Our AI/ML tools are tailored to protein therapeutic challenges—guiding engineering decisions to improve properties such as low viscosity for high-dose administration and eliminating chemical liabilities like oxidation or isomerization. Critically, our predictive platform is coupled with our Next Generation CHO expression system that mirrors our manufacturing process, using the same host cell and vector system. This system has demonstrated predictive production yields and product quality across a range of therapeutic modalities ensuring design decisions made early in discovery are highly translatable to scale-up and GMP production.

Trans-splicing mediated recombination to generate multi-specific antibodies

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Multi-specific antibodies resemble an extreme diversity of formats and designs that facilitate a wide range of functionalities and modes of action. The search for suitable candidates with the desired properties is quite tedious and limits a timely identification of the best lead molecules. Already the rather simple combination of different binding arms in bispecific antibodies representing a variety of affinities, epitopes, distances and flexibility can exhaust the capacity of conventional methods quite quickly.

Therefore, we established a platform based on trans-splicing that allows the seamless *in vitro* reconstitution of many individual antibody fragments into fully functional multi-specific antibodies, thus enabling a fast turnover of well-defined building blocks amenable to high throughput and automation. Furthermore, this universal platform process avoids the generation of product related impurities, is agnostic to a wide variety of binders and enables fast reshuffling into the final candidate.

This approach resolves the bottleneck of molecule prototyping through combinatorial screening. The achieved quantity and purity is sufficient for a multitude of functional assays, speeding up the discovery process for multi-specific antibodies which are in high demand for modern therapies.

SPASE: A Web-Based Tool for Streamlining Protein Engineering

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Advances in protein engineering are driving transformative applications in biocatalysis, therapeutics, enzyme design, and synthetic biology. A major challenge in experimental protein optimization lies in efficiently designing stable, soluble, and expressible variants prior to synthesis and characterization. To address this, we have developed SPASE (Soluble Protein Analog Selection Engine), a web-based tool that leverages deep learning and computational filtering to generate high-quality protein analogs, with favorable solubility, stability, and reduced aggregation propensity. SPASE takes an input protein structure (PDB format, monomeric) and generates 10,000 synthetic sequence variants using ProteinMPNN, retaining the overall 3D fold of the original template while enhancing key properties. The generated sequences are then filtered through a automated multi-step computational pipeline to ensure solubility and reduced aggregation propensity: 1) Protein-Sol assesses solubility, 2) ESMFold predicts the 3D folds of the top 100 candidates, 3) Aggrescan3D identifies and removes sequences with high aggregation propensity to further refine the selection. Finally, a selection of sequences with the lowest aggregation propensity is obtained, prioritizing candidates with the highest confidence folding scores. The final output is a curated set of ~25-35 high-quality protein analogs optimized for experimental expression and characterization. Importantly, SPASE allows users to preserve functionally critical residues—such as catalytic or binding sites—ensuring that designed proteins remain biologically relevant. By integrating these computational tools into an easy-to-use web interface, SPASE lowers the barrier for experimentalists to incorporate rational and combinatorial *in silico* library design into their workflows, accelerating the development of improved biocatalysts, therapeutics, enzymes, and other functional proteins. We hope that SPASE will help facilitate the transition from computational predictions to experimental validation, bridging the gap between *in silico* design and real-world applications. Our aim is to demonstrate how computational advancements can streamline protein engineering, making it more accessible and effective for experimentalists while democratizing *in silico* protein design across diverse fields, from green chemistry to industrial biotechnology and pharmacology.

Flow cytometric approaches to controlling product quality during cell line generation

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A long-standing challenge in the biopharmaceutical industry has been the need for a rapid Cell Line Development (CLD) process capable of screening a significant number of clones for the ability to produce high amounts of drug substance with the desired product of interest. To achieve high expression of monoclonal antibodies (mAbs), we have employed a flow-based cytometric reporter system to screen for productivity. FLARE (FLow-cytometric Attenuated Reporter Expression) uses an alternate start cell surface reporter which serves as a specific productivity surrogate. FLARE can be used analytically to identify productivity differences in both pools and clones. It can also be manipulated via sorting to isolate individual cells (clone generation) and pooled populations (bulk sorting) to yield either pools or clones with enriched productivities. It has proven to be a powerful tool for both enriching populations and yielding high producing clonal mAb cell lines.

In recent years, multispecific antibodies have emerged as promising candidates for new therapeutics. Although these are mAb-like, they typically are of greater complexity, in part due to being comprised of > 2 peptide chains. These molecules can pose a challenge for FLARE, as enrichment of just one peptide chain coding transcript could lead to a transgene expression imbalance. To this end, we developed a second reporter system (FLARE 2.0), that allows for the ability to simultaneously enrich for cells expressing two different transgenes, at various levels. This presentation will highlight the utility of the FLARE 2.0 system in the establishment of cell lines expressing a 3-chain multispecific molecule with optimal product quality.

Enhancing the ApolloX Process Development Toolbox: Strategies for navigating Complex Modalities

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With the biotherapeutic market gearing towards the production of a new era of modalities, novel challenges await Chinese hamster ovary (CHO)-based process development. Multi-specific antibodies present complications due to the requirement of efficient multi-chain expression. This poses challenges for the expression system, cell line selection strategy, analytical characterisation and downstream purification. High levels of productivity are often driven by optimised vector designs that achieve delicately balanced chain expression. Additionally, consideration of product purity earlier in the upstream process is paramount since product related impurities are common with these complex proteins.

In this study, we investigated a holistic approach to bespoke process development for bispecific antibodies (BsAbs). Early in the ApolloX™ cell line development process, we evaluated the use of a vector and transfection design toolbox to rapidly evaluate various multi-gene vector constructs and identify optimal DNA amounts/selection pressure stringency for balanced chain expression. Selected pools were taken forward into single cell clone generation based on their growth and heterodimer production performance. Screening stages in cell line development were complemented by advanced analytics such as high-throughput product purity analysis and mass spectrometric evaluation of product related impurities. Targeted downstream purification development approaches based on molecule bioinformatics were also adopted.

Application of this toolbox-style approach boosted target BsAb titres for a 4-chain BsAb by 3-fold in transfectant pools. Additionally, average heterodimer purity of over 80% was achieved across 3 different model BsAb formats. By adopting a testing toolbox strategy catered to the variability of multi-specific antibody structures, we were able to successfully navigate the complexity of BsAb production.

Developability assessment of a CD28 x Nectin-4 co-stimulatory bispecific for the treatment of bladder cancer

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Effective T-cell activation is driven by three signals: Signal 1 delivered through antigen recognition via the T-cell receptor, signal 2 through co-stimulatory receptors, and signal 3 mediated by cytokines. CD3-targeting T-cell engagers provide signal 1 to T cells and have shown significant clinical benefit in hematological malignancies but have faced challenges in solid tumors due to on target off tumor toxicities. Emerging clinical data supports the hypothesis that for efficient and sustained activity in the presence of signal 1, engagement of co-stimulatory molecules like CD28 could be important for effective anti-tumor activity in solid tumors.

We present the development of a panel of bispecific antibodies (bsAbs), targeting the costimulatory molecule CD28 and the tumor associated antigen (TAA) Nectin-4, a cell adhesion molecule overexpressed in bladder cancer and other malignancies. To select our development candidate RNDO-564, we screened a panel of bsAbs with varying CD28-potencies for both functional activity and biophysical stability to assess and reduce manufacturability risks.

Functional characterization of our bsAbs showed robust tumor cytotoxicity and IL-2 secretion while posing a lower safety risk providing that the activity is dependent on presence of signal 1 and expression of the TAA. Additionally, we demonstrated that the panel of CD28 binders can be paired with various TAA-binding arms, enabling the design of new bispecific antibodies for different indications.

In addition to the desired biological activity, the CD28 x Nectin-4 bsAbs also exhibited favorable developability profiles as demonstrated by subjecting the panel to accelerated stress conditions like thermal stress at elevated temperatures for extended periods of time or low pH hold.

This extensive functional and biophysical characterization enabled the selection and successful manufacture of our clinical candidate RNDO-564.

Protein structure, a genetic encoding for glycosylation

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DNA, RNA, and proteins are synthesized using template molecules, but glycosylation is not believed to be constrained by a template. However, if cellular environment is the only determinant of glycosylation, all sites should receive the same glycans on average. This template-free assertion is inconsistent with observations of microheterogeneity—wherein each site receives distinct and reproducible glycan structures. Here, we test the assumption of template-free glycan biosynthesis. Through structural analysis of site-specific glycosylation data, we find protein-sequence and structural features that predict specific glycan features. To quantify these relationships, we present a new amino acid substitution matrix that describes "glycoimpact" -- how glycosylation varies with protein structure. High-glycoimpact amino acids co-evolve with glycosites, and glycoimpact is high when estimates of amino acid conservation and variant pathogenicity diverge. We report hundreds of disease variants near glycosites with high-glycoimpact, including several with known links to aberrant glycosylation (e.g., Oculocutaneous Albinism, Jakob-Creutzfeldt disease, Gerstmann-Straussler-Scheinker, and Gaucher's Disease). Finally, we validate glycoimpact quantification by studying oligomannose-complex glycan ratios on HIV ENV, differential sialylation on IgG3 Fc, differential glycosylation on SARS-CoV-2 Spike, and fucose-modulated function of a tuberculosis monoclonal antibody. In all, we show glycan biosynthesis is accurately guided by specific, genetically-encoded rules, and this presents a plausible refutation to the assumption of template-free glycosylation.

Enhancing our understanding of viroceuticals using an optimized mass spectrometry-based lipidomics approach

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Viroceuticals are emerging tools for vaccination, genetic therapies, and novel cancer treatments. While effective, many have large costs associated with their manufacturing and delivery. We have conducted novel studies on viroceutical products and production via mass spectrometry-based lipidomics. Lentivirus (LV) technology descends from human immunodeficiency virus (HIV), whose lipid envelope has been previously measured and shown to have a direct impact on its transduction efficiency. Madin-Darby Canine Kidney (MDCK) cells are a widely used host cell line for influenza A virus (IAV) production as an alternative to egg-based production and exhibit a pronounced heterogeneity that can significantly affect viral yields. We have developed a rapid, robust, and sensitive untargeted lipidomics pipeline to elucidate novel biomolecular signatures that affect both LV and IAV biotherapeutic production pipelines. The impact of 48 hours of LV production on the lipidome of HEK 293T cells was measured and 151 lipids were identified, 84 of which had fold changes with FDR-corrected $P<0.05$ compared to HEK 293T treated with media. LV contained 102 lipids, half of which were determined to be unique LV virion lipids after subtracting the media lipidome. We also investigated two MDCK clones (C59 and C113) that differ in biochemical properties and viral production attributes and examined their lipid dynamics upon influenza A virus (IAV) infection at 24, 48, and 72 hours. C113 had elevated levels of lipid species across all major lipid classes with the exception of ether lipids compared to C59. IAV infection in C59 led to lipid droplet (LD) accumulation, elevated levels of ceramides and diacylglycerols as well as lysophospholipid and phospholipid depletion. IAV infection in C113 led to a decrease in LDs and lysophospholipids. Lipidomic analysis of the purified progeny virions from C59 and C113 yielded only subtle differences with an overall strong positive correlation in lipid profile (R^2 = 0.77), suggesting similar lipid raft domain composition between clones.

Multi-objective optimization of monoclonal antibody titer and product quality using data-driven and hybrid models

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Asimov, Inc

The production of monoclonal antibodies in CHO cells requires both high titer and favorable product quality. Understanding and controlling the complex, high-dimensional dependencies of quality attributes on genetic and bioprocess design is challenging—particularly when data and experimental capacity are limited, which is typical for bioprocess development. These multivariate relationships vary across cell lines, antibodies, pools, and clones, and we often lack prior knowledge of how a specific antibody's quality profile will respond to different design decisions. Further, improving product quality often comes at the expense of reduced titer, making this a challenging multi-objective optimization problem.

The ideal approach to model-based product quality optimization depends on the type and quantity of available data and the constraints on experimental scope. We employed distinct modeling strategies for three specific cases: when initial data was sparse and multiple sequential experiments were feasible, when initial data was plentiful but few validation experiments were feasible, and when initial data was plentiful and inference alone was required with experimental validation deferred.

All strategies predicted quality attributes and titer of different mAbs from process conditions and plasmid design features as inputs. First, we used Bayesian optimization in the context of sparse data and iterative experiments to optimize charge variant profiles by changing media and feed types and quantities. For four combinations of antibody and cell line, we achieved a 22–25% reduction in acidic species while maintaining ≥3 g/L pool titer. Second, we applied a hybrid model of metabolism and post-translational modifications to optimize media choices, feeding strategies, and temperature and pH setpoints in the context of plentiful data and a single validation experiment. This yielded a 25% reduction in acidic species with no detrimental effect on titer. Third, when preparing to extend process optimization from charge variants to glycan distributions with plentiful initial data to inform future experimental plans, we used multivariate analysis via PLS regression to identify process variables and clonal phenotypes associated with favorable charge variant and glycan distributions. These results demonstrate that high quality, high titer processes may be best achieved through application of modeling strategies appropriately selected based on data availability and experimental scope constraints.

Glycosylation site Asn168 is important for slow *in vivo* clearance of recombinant human diamine oxidase heparin-binding motif mutants

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Elevated plasma and tissues histamine concentrations can cause severe symptoms in mast cell activation syndrome, mastocytosis or anaphylaxis. Endogenous and recombinant human diamine oxidase (rhDAO) can rapidly and completely degrade histamine, and administration of rhDAO represents a promising new treatment approach for diseases with excess histamine release from activated mast cells.

We recently generated heparin-binding motif mutants of rhDAO with considerably increased *in vivo* half-lives in rodents compared to the rapidly cleared wildtype protein. Herein we characterize the role of an evolutionary recently added glycosylation site asparagine 168 in the *in vivo* clearance and the influence of an unusually solvent accessible free cysteine 123 on the oligomerization of DAO. Mutation of the unpaired cysteine 123 strongly reduced oligomerization without influence on enzymatic DAO activity and *in vivo* clearance. Recombinant hDAO produced in ExpiCHO-S™ cells showed a 15-fold reduction in the percentage of glycans with terminal sialic acid at Asn168 compared with CHO-K1 cells. Capping with sialic acid was also strongly reduced at the other glycosylation sites. The high abundance of terminal mannose and N-acetylglucosamine residues in the four glycans expressed in ExpiCHO-S™ cells compared to CHO-K1 cells resulted in rapid *in vivo* clearance. Mutation of Asn168 or sialidase treatment also significantly increased clearance. Intact N-glycans at Asn168 seem to protect DAO from rapid clearance in rodents. Full processing of all glycoforms is critical for preserving the improved *in vivo* half-life characteristics of the rhDAO heparin-binding motif mutants.

Without a Trace: Identification, Characterization and 9x Knockout of CHO Hydrolases to Tackle the Polysorbate Degradation Challenge

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Enzymatic degradation of polysorbates (PS) in biologics formulations are often caused by hydrolytic host cell proteins (HCPs) and can lead to particle formation and reduced shelf-life. Because PS-degrading HCPs are critical even in miniscule amounts, their identification, characterization and removal pose great challenges to the biopharmaceutical industry. To this day, the occurrence of difficult-to-remove HCPs in the final drug product is mainly tackled by molecule-tailored optimization of downstream purification steps.

Here, we present an alternative: the genomic knockout (KO) of 9 critical HCPs within a CHO host cell line. In the first part of this work potentially critical HCPs were identified in industrially relevant drug formulations, followed by artificial overexpression, purification and detailed characterization. Hydrolytic HCPs were ranked based on expression level, occurrence rate in monoclonal antibody (mAb) products, PS degradation activity and cellular localization providing a broad dataset for further risk management. In part two, we sequentially knocked out these PS-degrading HCPs giving rise to a novel CHO host cell line variant with substantially reduced hydrolytic activity. By means of precise genome editing tools, we knocked out 9 critical HCPs including large carboxylesterase gene clusters of >1 megabase in size. Surprisingly, all 9 HCP KOs could be combined in a clonal CHO cell line without significantly compromising cell culture performance. This paves the way for a potentially novel platform host cell line with improved properties for future biologics production. Additional KOs of the pro-apoptotic genes *Bax* and *Bak1* further improved cell growth and viability of the multi-KO cell line. Finally, the suitability as host cell line was verified in two cell line development campaigns, where our multi-KO host cell line was able to deliver high mAb titers while maintaining high product quality. Most importantly, hydrolytic activity and thus PS degradation could be dramatically reduced. The presented multi-hydrolase KO strategy delivers a universal solution for the elimination of PS-degrading HCPs in a CHO-based bioprocess. Currently, we are further investigating the impact of repeated hydrolase gene editing on the geno- and phenotype of the multi-KO cell line, generating valuable insights for future multi-KO endeavors of other detrimental host genes and potential bioprocess optimizations.

Glycomics and glycoproteomics methods for applications in biotherapeutic products and endogenous biomarkers

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The structural characterization of glycoproteins both as biotherapeutic products and as endogenous biomarkers in plasma and tissues remains one of the most analytically demanding tasks in modern bioanalysis. Glycosylation is a highly complex, heterogeneous, and context-dependent post-translational modification that influences protein folding, function, pharmacokinetics, and immunogenicity. High-throughput (HTP) glycomics and glycoproteomics methods have recently been developed as essential tools to dissect this complexity, and their implementation into routine workflows for industry has been tested.

Accurate glycoprotein analysis requires resolving site-specific glycoform variations across batches and navigating complex plasma and tissue samples with high dynamic range and structural isomers. Tissue analysis adds challenges like limited material and extracellular matrix interference. Accurate interpretation depends on advanced bioinformatics tools and machine learning algorithms, which must navigate an enormous search space of potential glycan structures and account for variable ionization efficiencies, incomplete fragmentation, and co-eluting species. Despite these hurdles, precise glycoprotein characterization is essential for therapeutic safety and for leveraging glycans as biomarkers in diseases such as cancer and neurodegeneration. Ongoing innovation is key to advancing high-throughput glycoanalysis in clinical and biopharmaceutical settings.

We have developed HTP glycomics and glycoproteomics mass spectrometry-based methods to address these challenges. We have now optimized technologies ensuring the retention of labile residues such as sialic acids and fucose, and non-carbohydrate substituents like acetylation and sulfation. We have worked out advanced workflows that combine enrichment techniques, specialized digestion and derivatization protocols, and orthogonal mass spectrometry platforms (such as LC-MS/MS with EThcD, stepped HCD and UVPD) to structurally elucidate and preserve native glycosylation patterns. These developments are a continuation of our ongoing efforts of using state-of-the-art MS instrumentation to address newly arising difficulties in glycoprotein characterization and applying these tools to assist the industry in characterizing new biologics products.

Implementation of Quality by Design Principles
for Influenza A Virus Production

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Ensuring consistent product quality in cell culture-based vaccine manufacturing requires a thorough understanding of the process parameters that affect titers, yields, and impurity levels. This study applies Quality by Design (QbD) principles to an influenza A virus (IAV) production process operated in batch mode using two monoclonal suspension MDCK cell lines, C59 and C113 (Sartorius, Germany), with distinct charcteristics, focusing on process robustness and optimization.

Based on knowledge from previous process development [1], a quantitative risk assessment including biological and technical parameters was performed to identify Critical Process Parameters (CPPs). Using a Design of Experiments (DoE) approach in an Ambr® 15 scale-down system, four key CPPs (pH value, dissolved oxygen concentration, viable cell concentration at time of infection, and multiplicity of infection) were investigated at three levels. After data analysis and modeling, we obtained dedicated design spaces for each cell clone characterized by high process robustness with a less than 1% risk of failure and even some indications for virus titer and yield improvement, while keeping process-related impurities such as DNA and total protein concentration low. Scale-up experiments in a 2 L single-use stirred tank bioreactor confirmed the validity of these conditions. Total virus titers of 2.95±0.06 $\log_{10}$(HAU/100 µL) and 3.13±0.12 $\log_{10}$(HAU/100 µL) were obtained for C59 and C113 cells, respectively [2].

By applying QbD principles, this study not only improves IAV production but also demonstrates a framework applicable to manufacturing of other cell culture-based vaccines. The results provide valuable insights for optimizing manufacturing processes, reducing batch failure risks, and supporting regulatory approval through data-driven process characterization.

[1] Zinnecker et al., 2024, *Eng. Life Sci.*, https://doi.org/10.1002/elsc.202300245
[2] Zinnecker et al., 2025, *Eng. Life Sci.*, https://doi.org/10.1002/elsc.70027

A Surface Plasmon Resonance-Based Integrated Assay for Quantification and Glycosylation Characterization of Monoclonal Antibodies in Crude Heterogeneous Samples

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The rise in cancer, autoimmune, inflammatory, and infectious diseases in recent decades has led to a surge in the development of monoclonal antibodies (mAbs) therapies, now the most widely used family of biologics. To meet the growing global demand, biopharmaceutical industries are intensifying their production processes. One approach to achieve more efficient production of effective mAbs is to develop tools for real-time quality monitoring. Specifically, the glycosylation profile of mAbs must be closely monitored, since it greatly impacts their therapeutic efficacy and innocuity, making it a critical quality attribute. In this study, we developed a surface plasmon resonance-based integrated assay allowing for the simultaneous quantification and glycosylation characterization of mAbs in crude samples, hence permitting the at-line analysis of bioreactor cell cultures. Thanks to the high specificity of the interaction between biosensor surface-bound protein A and the Fc region of mAbs, we quantified crude IgG samples under mass transport limitations. Next, by flowing running buffer on the surface, impurities contained in the mAbs samples were washed away from the biosensor surface, allowing subsequent recording of the kinetics between the captured mAbs and injected FcγRII receptors. Of interest, with this strategy, we were able to quantify terminal galactosylation and core fucosylation of IgG lots, two important glycan modifications for mAb efficacy.

PEACe 2025 Abstract Template

Suplementation of chemical aditives as an strategy to improve novel anti-sST2 IgG in CHO cells system

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A novel monoclonal antibody (mAb) (anti-sST2) (U. Chile) with therapeutic potential for autoimmune disease Ulcerative colitis and other similar diseases with high levels of sST2 soluble protein was designed. For the production of this mAb, a possible secretory bottle neck is hyphotesized, observed at a previous analysis. Unfolded/misfolded proteins could cause reticulum stress, activating the unfolded protein response and possible apoptosis; also an increase on mAb demand could raise reactive oxygen species (ROS) leading to an accumulation of protein in the endoplasmic reticulum. To improve this scenario, process engineering approaches were studied, supplementing the culture medium with three different molecules: a novel and promissory antioxidant, a recognized antioxidant and a chemical additive, to decrease folding and aggregation problems, and with it is expected to enhance production of the novel mAb. Independent supplementation improved specific production (qP), with a value of 47%, 43.5% and 46.8% more than control for novel antioxidant, recognized antioxidant and chemical additive, respectively. Also, CHO cells were able to grow at optimal conditions on a studied range of the novel antioxidant, maintaining cell viability over 85% in culture, and improving the specific cell growth rate (μ) (0.55 1/d), a 30% higher than control, which also improved qP. As expected, supplementation of both a known antioxidant and a novel antioxidant were able to reduce intracellular ROS.

Targeted Gene Integration for Robust Performance of Inducible Transcriptional Circuits in CHO Cells

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Inducible transcriptional circuits are useful tools to fine-tune gene expression and enable real-time control over desirable cellular phenotypes. In contrast to conventional inducible systems that function in an on/off manner, Linearizer circuits enable gradual gene expression in response to increasing inducer molecule concentrations, somewhat akin to dimmer light switches. Linearizer circuits have extensive potential applications across biopharmaceutical cell culture processes, for example: (i) controlled and coordinated expression of peptide chains that form multimeric therapeutic proteins to enhance the yield of bi- and multi-specifics, (ii) inducible and tuneable expression of adeno-associated viral vector (AAV) components to maximise gene therapy vector yields, and (iii) real-time glycosylation control to enable robust quality assurance of therapeutic glycoproteins.

A key challenge in deploying Linearizer circuits is that the stoichiometry of their components (repressor operon sites, repressor protein, and gene of interest) must be ensured for optimal performance (minimal basal GOI expression, fold induction, and linear dose response to inducer concentrations). This study evaluates the performance of two negatively autoregulated Linearizer circuits (TetR_Lin and PhlF_Lin) in Chinese hamster ovary (CHO) cells using two different genomic integration strategies, targeted integration (TI) and random integration (RI). Each circuit consists of a fluorescent protein reporter (eGFP or mCherry) and a repressor gene (TetR or PhlF). For RI, Linearizers were deployed using transfection and antibiotic resistance selection. For TI, orthogonal landing pads with the Cre/lox and Bxb1/att recombinase mediated cassette exchange systems were used.

Our results show that RI results in high basal expression, low fold induction, and a nonlinear response across inducer concentrations. In contrast, TI achieves lower basal expression, a strong linear response to inducer molecule concentration, and a broader induction range. Our results suggest that the suboptimal performance observed with RI is likely due to stoichiometric imbalances of Linearizer circuit components, and that the issues are resolved with targeted integration. By optimising Linearizer circuit performance in CHO cells, this project paves the way towards real-time control and optimisation – at the cellular level – of biopharmaceutical cell culture processes.

Thermo Fisher / Abstract

Expi293™ PRO Expression System: Achieving Higher Titers and Higher Throughput to Support the Ever-Expanding Needs of Protein Expression Scientists

Producing increasingly complex proteins with higher yields and higher throughput is critical to accelerating breakthroughs in protein research and therapeutic drug development. Here, we introduce the Expi293 PRO Expression System, a next-generation transient expression system developed to address the unmet needs of protein expression scientists. The Expi293 PRO Expression System comprises: 1) a new, clonally-derived HEK293 cell line capable of producing difficult to express proteins and supporting milliliter to multi-liter scale protein production, 2) optimized culture medium and transfection reagent to enable high density cell transfection, 3) components and protocols designed for easy incorporation into automated workflows, and 4) a streamlined protocol with the fewest steps possible to minimize hands-on time and maximize the number of transfections that can be performed in a workweek. Together, these optimized components enable the Expi293 PRO Expression System to produce multi-fold improvements in protein yields across a broad variety of proteins compared to existing 293 expression systems with protein titers up to 4 grams per liter, making the Expi293 PRO Expression System an ideal next-generation offering to meet the ever-expanding needs of protein expression scientists.

Funcionalized Viral Protein Assemblies as Scaffolds for Neural Tissue Engineering

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Viral protein assemblies provide a unique scaffold for cell growth. They are biocompatible and provide a structured surface ideal for tissue cell growth. This study presents the construction, characterization, and brain implantation of a nano-biorganometallic material called µChipVP6-Au, comprised of viral protein nanotubes (rotavirus nVP6) functionalized with gold nanoparticles (nVP6-Au) printed on a poly(vinyl alcohol co-vinyl acetate) chip as electric in series or in parallel nVP6-Au circuits. Coating of PAcVA with nVP6-Au increased its conductivity by 169-fold. Atomic force microscopy (AFM) analysis revealed accumulation zones of nVP6-Au on PAcVA with an electrical conductivity of -60 pA. Additionally, nVP6-Au increased the roughness of PAcVA, creating depressions and crests measuring -6 µm and 3.9 µm, respectively. A549 and mHypoE-N1 cell lines were cultured on nVP6, nVP6-Au, and µChipVP6-Au, exhibiting a similar cell growth than on commercial culture plates. No cytotoxicity or oxidative stress was observed, demonstrating the innocuousness of nVP6-Au for mammalian cell cultures. Furthermore, as a proof of concept, µChipVP6-Au loaded with L-DOPA was intracranially implanted in the brain of a mouse model of Parkinson's disease and in control mice. Motor coordination was restored at 8 days post-implantation, and it was comparable with the control group. µChipVP6-Au is a potential versatile scaffold for cell culture, enabling cell replacement therapies, in situ drug release, and electrical stimulation. This study presents the first functional nano-bioorganometallic material based on viral protein assemblies for brain implantation.

Engineering Ferritin Nanoparticles For Precise Antigen Display

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Ferritin (Ft) nanoparticles provide a stable, self-assembling scaffold suitable for antigen display. Two complementary strategies have been established to enable precise and functional antigen display for vaccine development, with broader applicability to other biotechnology fields.

Tyrosinase-mediated catalysis was applied to *Pyrococcus furiosus* ferritin (PfFt), a cysteine-free archaeal nanoparticle. Through targeted mutagenesis, single cysteine residues were introduced to enable site-specific, irreversible antigen conjugation under physiological conditions. This method preserved nanoparticle structure and allowed repetitive antigen distribution, as confirmed by native PAGE, DLS, HPLC, and mass photometry. Biolayer interferometry demonstrated nanomolar affinities to neutralizing antibodies, indicating maintenance of conformational epitopes. This platform is compatible with microbial expression systems and broadly applicable to areas requiring precise, site-specific protein–protein conjugation.

In parallel, a genetic fusion approach was used to express antigen-Ft constructs in animal cells. Flexible glycine-serine linkers promoted structural separation, contributing to uniform antigen display, proper protein folding, and nanoparticle assembly, validated by cryo-EM analysis. An *in vitro* immunological evaluation pipeline was established using THP-1-derived dendritic cells. Nanoparticle uptake was visualized by confocal microscopy, and immune activation was evaluated through flow cytometry of surface markers, cytokine profiling using Olink® multiplex assays, and transcriptional analysis by real-time qPCR.

Together, these strategies offer modular and versatile Ft-based platforms for precise antigen display and immune characterization. Their compatibility with various expression systems and analytical pipelines underscores their relevance to vaccine development, immunotherapy, and targeted bioconjugation applications.

SYSTEMSBIOLOGY, CELL LINE AND PROTEIN ENGINEERING FOR INCREASED AAV PRODUCTION

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AAV based gene therapy has revolutionized medicine and provided curative treatments for several indications. The lack of adequate platforms and the complex nature of the drug has affected COG significantly owing to low yield, poor quality of viral vectors and high required dosages to reach therapeutic effects. This results in low accessibility and hinders targeting of larger indications through novel AAV gene therapies. In the multidisciplinary GeneNova innovation milieu, we innovate the manufacturing of AAV by simultaneous targeting of several limitations in the AAV development pipeline.

Here we report on improvements of AAV2 manufacturing using rational domain swopping of AAV2 and AA8 as well library approaches allowing for selection of improved variants with >10x improved secreted titers at the expense of lower infectivity. We further exemplify the ability to restore infectivity and steer tropism using modular grafting of 58 aa Affibody scaffolds onto the surface (VR4 or VR10) of the AAV. This platform allowed for selective targeting of cell types of interest including EGFR, HER2, HER3, IGF1R, PDGFRb or VEGFR2 positive cells lines, while reducing off targeting to unwanted tissues such fibroblasts. We show that this is of particular utility for applications delivering toxic cargo such as in the case of AAVs packed with herpes simplex virus thymidine kinase used in conjunction with ganciclovir as a cytotoxic drug for oncolytic therapies. To increase upstream yield, we performed a systems biology approach comparing AAV production in more efficient adherent 293T cells with less productive suspension HEK293F cells. Transient validation studies aimed at up/down regulating genes of key pathways correlating with improved AAV productivity, e.g. relating to viral response and cell homeostasis, was performed followed by development of stable cell lines and balancing of such genes in HEK293. Productivity fold-improvements and packaged yield for engineered HEK293 will be presented. To assure correct packaging of desired target ssDNA, adequate sequencing methods fit for AAV are needed. To avoid introduction of bias present in typical NGS methods we designed a fragmentation and amplification free sample preparation and data analysis pipeline for quality control of encapsidated viral genomes using Oxford Nanopore sequencing. We show the ability capture full length sequences of encpasidated DNA of both transgene and undesired DNA sequence originating from the bioprocess.

Process intensification for high cell density oncolytic Newcastle disease virus production

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Interest in therapeutic viruses, such as oncolytic viruses, is growing with the first oncolytic therapeutics on the market and several candidate viruses in clinical trials. Currently doses of up to 10^{12} infectious viruses/dose are envisioned for use of these viruses and remain a challenge for the upstream processing. Oncolytic viruses are naturally occurring, or genetically engineered viruses that selectively infect and kill cancer cells. To tackle the coming demand, intensified production processes need to be developed. Here, we present development of a high-yield perfusion process for the production of oncolytic Newcastle disease virus (NDV) in suspension EB66 cells.

EB66 duck cells (Valneva SE) were cultivated in shake flasks and in a DasGip 3 L reactor (Eppendorf) using CDM4 Avian medium at 150 rpm, 37°C and a pH of 7.2. Infections were performed at a MOI of 10^{-4} with the NDV-GFP LaSota strain. Small-scale batch and semi-perfusion experiments were used for process characterization and optimization. In perfusion mode, cells were fed at a perfusion rate of 36 pL/(cell x day) and a TrypLE concentration of 2.5 U/mL, to cleave and activate the NDV fusion protein. Tangential flow depth filtration (TFDF, Repligen) was used for cell retention. Infectious virus titers were determined by $TCID_{50}$ assay with adherent Vero cells, total virus titers by HA assay. With optimized batch conditions, maximum titers of up to 4.2 x 10^8 $TCID_{50}$/mL were achieved. Infection of EB66 cells at 20.1 x 10^6 cells/mL resulted in a 3-fold higher maximum titer of 1.3 x 10^9 $TCID_{50}$/mL with a 1.5-fold higher volumetric virus productivity and increased cell-specific virus yield, compared to batch processes. Moreover, TFDF allowed direct clarification and harvesting of virus through the depth filter, enabling upstream and downstream process integration with a reduction of one unit operation. This resulted in continuous virus harvest with 99.4% cell retention and 90% turbidity reduction. In a next step, we aim to produce NDV at very high cell densities >100 x 10^6 cells/mL to challenge the EB66 cell specific productivity and the TFDF system.

In conclusion, we present a complete process development and intensification of oncolytic NDV production starting from batch experiments in shake flasks to a perfusion process yielding infectious virus titers above 10^9 $TCID_{50}$/mL.

Recombinant AAV production: Insights from Stable Cell Lines and Adenovirus Infection

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Adeno-associated viruses (AAVs) naturally integrate into the AAVSI locus of the host genome. With a helper virus like Adenovirus (AdV), their genome is amplified, producing AAV progeny. Recombinant AAVs (rAAVs), which carry a gene of interest (GOI) instead of AAV genes, are widely used in gene therapy due to their safety and production scalability. However, current triple transfection process is expensive and leads to batch variability. Unlike wild-type AAVs with 90–100% full capsids, rAAVs show only 10–30% full-to-empty ratios, and the cause of this low packaging efficiency remains unclear.
To explore key factors in rAAV production and assess the benefit of stable GOI integration, we engineered a HEK293 cell line with a single-copy "CMV-eGFP-WPRE" construct integrated at the AAVSI locus via RMCE. rAAVs were produced using this stable line with co-transfection of two plasmids (*rep*, *cap*, and AdV helper genes) and compared to the standard triple transfection in parental HEK293 cells. To study the low full-to-empty capsid ratio, both lines were also infected with replication-competent AdV. As high eGFP expression may reduce AAV yield in the stable line, the effect of siRNA-mediated eGFP repression was tested. rAAVs were analyzed by ELISA, ddPCR and mass photometry for a more accurate full to empty ratio.
Our experiments showed that stable cell lines produce lower amounts of filled rAAVs as compared to the standard triple transfection. However, this effect can be alleviated by AdV infection and siRNA co-transfection.
Wild type AAVs are being produced in viral replication complex (VRC) in the nucleus which are formed by the helper virus. Recombinant AAV production was more efficient with the infection of adenovirus as compared to the helper plasmid which contained the DNA binding protein, E4 & VA RNA from adenovirus. This indicates that vital genes are missing in the helper plasmid. We have taken electron microscopy images of the VRC to investigate the differences between adenovirus infection and helper plasmid transfection.
This investigation of rAAV production from a stabe cell line with a genome integrated GOI offers a lot of possibilities to explore process optimization from a molecular biology aspect. This highlights that AAV production is still not fully understood and that the helper functions play a vital role in AAV productivity.

Functionalized HIV-1 Gag VLPs for targeted delivery to CXCR4-positive cancer cells

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The use of virus-like particles (VLPs) as nanocarriers for biomolecules is a promising approach in drug delivery research. CXCR4 is a cell surface receptor associated with several types of cancer. The T22 peptide is a known antagonist of this receptor that efficiently binds to and penetrates CXCR4-positive cells. In this work, we produced Gag-GFP VLPs in HEK293 cells via transient transfection. We then optimized a four-step downstream process for these VLPs, including clarification via depth and standard filtration, intermediate purification using tangential flow filtration or multimodal chromatography, capture through ion exchange, hydrophobic interaction, and heparin affinity chromatography, and final polishing with size exclusion chromatography resulting in an overall recovery of 38% and a purity of 64%, with host cell DNA and protein levels complying with regulatory standards. These VLPs were then functionalized with the T22 peptide via click chemistry. In vitro studies revealed the ability of Gag-GFP VLPs-T22 to penetrate CXCR4-positive cells in a dose- and time-dependent manner. CXCR4-positive cells exposed to 8.0×10^9 functionalized Gag-GFP VLPs/mL exhibited 30-fold higher fluorescence than those exposed to non-functionalized Gag-GFP VLPs after 24 hours of incubation, showing the internalization of functionalized VLPs. Also, the absence of Gag-GFP VLPs-T22 internalization in CXCR4-negative cells confirmed specificity via the CXCR4 receptor. The functionalization of VLPs with the T22 ligand enables selective targeting of CXCR4-positive cells, which is significant due to the established role of CXCR4 in promoting cancer progression and metastasis. By specifically targeting CXCR4, these VLPs can selectively deliver therapeutic biomolecules to cancer cells, potentially enhancing treatment efficacy while minimizing off-target effects and reducing systemic toxicity. The presented results show that this targeted nanoplatform holds great potential for the development of safer and more effective therapies against CXCR4-overexpressing tumors, paving the way for precision medicine approaches in oncology.

CHO cell production of a single enveloped VLP vaccine targeting SARS-CoV-2, Influenza A and RSV

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The overlapping circulation of SARS-CoV-2, Influenza A virus (IAV), and Respiratory Syncytial Virus (RSV) continues to strain global healthcare systems, particularly among vulnerable populations. A single vaccine targeting all three pathogens could streamline immunization efforts and enhance protection, while reducing manufacturing costs. We previously showed that CHO cell–derived enveloped virus-like particles (eVLPs) formed through expression of full-length SARS-CoV-2 spike (S) protein not only exhibit high S density and strong immunogenicity, but also serve as a platform to co-display heterologous antigens such as IAV hemagglutinin (H1) and neuraminidase (N1). Here, we extend this approach by producing a trivalent eVLP candidate that simultaneously displays SARS-CoV-2 S, IAV H1, and the RSV pre-fusogenic fusion (F) protein. These S/H1/F eVLPs were successfully produced using both transient and stable gene expression in CHO cells and were purified via affinity chromatography. The presence of all three antigens on the same particles was confirmed by Western blot and immuno-electron microscopy. Their immunogenicity is currently being evaluated *in vivo* to assess their potential as a single vaccine against SARS-CoV-2, IAV, and RSV.

Mixture Design as a tool for improving full-to-empty particle ratios across various GOIs in rAAV production

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Optimization of rAAV production is essential for effective gene therapy applications. However, multiple factors affect the rAAV productivity in mammalian cells, and often they interact with each other, making the optimization process highly challenging.

In this study, we optimized the triple transfection method for rAAV production using a design of experiments (DoE) approach. Building on our prior work identifying mixture design (MD) with face-centered composite design (FCCD) as optimal for enhancing yield and cell viability, we now evaluate two more therapeutic genes, msh2 and bdnf, along with the percentage of full-to-empty capsids as a quality metric. Samples were harvested at 72 hours post-transfection (hpt), capsid quantification was performed using ELISA, and viral titers measured with qPCR. Statistical analysis was conducted using JMP 16 Pro.

Our results show that rAAV optimization is highly gene-dependent, with each gene-of-interest (GOI) requiring specific plasmid ratios and conditions. Optimal settings for one response differed from those for others, emphasizing the need for tailored strategies. While volumetric productivity was relatively consistent across GOIs, the full-to-empty capsid ratio varied significantly, likely due to GOI-specific biological properties. Interestingly, GC content, ΔG, and construct length seem to have minimal impact on packaging, while genome secondary structure correlated with full-to-empty capsid ratio in each case. Overall, we show how MD coupled with FCCD is a robust method that can be used to improve different responses in rAAV production with different GOIs, achieving an improvement of almost 100-fold in Log(Vp) in the case of egfp-expressing rAAV, and a 12-fold increase in bdnf-expressing full rAAV capsids. These were followed by validation runs, supporting the accuracy of the models' predictions.

Leap-In Transposase and discoCHO; Tools for Rapid, Stable, Scalable Biologics Down Selecting and Manufacturing

Claes Gustafsson

ATUM, 37950 Central Ct, Newark, CA 94560

As the demand for high-quality biologics accelerates, biopharmaceutical developers face increasing pressure to optimize cell line development for speed, quality, stability, and scalability. ATUM's Leap-In Transposase technology transforms the protein expression landscape, enabling rapid, predictable, and high-yielding stable cell lines. Unlike traditional random integration methods, the Leap-In Transposase system facilitates highly efficient transgene integration at transcriptionally active loci, leading to enhanced expression, improved genetic stability, and accelerated development timelines. This presentation will showcase how Leap-In Transposase technology empowers biopharma teams to achieve faster workflows while eliminating the need for labor-intensive single-cell cloning. We will present case studies demonstrating its impact across monoclonal antibodies, multispecifics, and novel therapeutic proteins, highlighting significant improvements in titers, scalability, and manufacturability. Furthermore, we will discuss its seamless integration with CHO and HEK cell systems, making it a solution for early-stage discovery and commercial-scale production.

Abstract – Sartorius Workshop PEACe 2025

The Power of Data - Combining Analytics and Model-Driven Approaches to Optimize Cell Lines, Media and Processes

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Key Words: AI & ML (Artificial Intelligence & Machine learning), Cell line development (CLD), Multivariate Data Analysis (MVDA), Cell Culture Media, Fed-Batch Cell Culture, Bioprocessing, Gene and Cell Therapy, Adeno-Associated Virus (AAV)

In the rapidly evolving landscape of cell line development and bioprocessing, powerful analytics with the integration of data models, artificial intelligence (AI) and machine learning (ML) as predictive tools offers transformative potential. To keep up with this expanding field, we introduce novel approaches for the development of cells and production processes, combining advanced analytical techniques and multivariate data analysis (MVDA). Applications range from manufacturing of classical protein-based therapeutics in CHO cells to gene and cell therapies, which represent promising avenues for treating a variety of genetic disorders and diseases. Implementation of these new tools for data analysis and modelling will further increase our process understanding and accelerate the pipeline to manufacturing.

The workshop will interactively assess the audience's use of analytics, data and modeling tools and then discuss use cases of powerful analytics and data-modeling for bioprocess optimization.
Three case studies from our teams will show the potential of data- and model-based approaches:

1) Clone selection in CHO cell line development by combining MVDA with machine learning algorithms → Identification of clones with 40-60% higher titer in fed-batch
2) Predicting cell culture media quality and performance through untargeted mass spectrometry and MVDA → Fingerprinting approach that allows identification of bad media lots without in-process testing
3) Unraveling HEK-based AAV production through combination of UHPLC-Orbitrap-MS for the analysis of metabolome and secretome with targeted methods (e.g. trace element analysis and titer determination) → Generates models with >1000 metabolites to find markers for high AAV production

A Novel Engineered CHO Host Cell Line for High-Titer Production of Biopharmaceuticals

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CHO cell lines are the most important mammalian expression system for producing protein-based biopharmaceuticals. Over the last decades, continuous advancements in cell line development strategies—focusing on expression system optimization, clone selection, and media and process innovations—have significantly improved product yield and quality. To keep pace with the rapid development of new and more complex therapeutics, genetic engineering of cell lines to enhance growth, productivity and optimize product quality attributes is a promising approach.
In this study, we developed a genetically engineered CHO cell line as a new innovative host, aiming to create stable cell lines with further enhanced titer and productivity for biopharmaceutical manufacturing.
The newly engineered host was employed alongside the wild-type (WT) cell line in various cell line development campaigns to generate production clones expressing DTE, IgG, and Fc-fusion antibodies. Sartorius' CHO CLD technology, optimized for accelerated generation and effective identification of high-producing clones, was utilized. Additional evaluations under fed-batch conditions in different Ambr® system scales using 4Cell® SmartCHO media and feeds demonstrated significantly enhanced productivity across all tested molecules, marking this new engineered cell line as a promising new host for Sartorius' cell line development platform.

Advancing Single Cell Cloning operations on the Beacon Optofluidic system for development of high quality manufacturing cell lines

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The growing number of candidate therapeutic biologics currently under development has increased the demand for innovative solutions from Process Development operations to deliver these medicines. A substantial number of biologics are manufactured using live cell systems, where mammalian Chinese Hamster Ovary (CHO) cells are predominantly used for production of recombinant protein therapeutics. To meet regulatory requirements and ensure a safe and robust process, the manufacturing cell lines must be derived from a single cell origin. Typically, Cell Line Development single cell cloning and screening campaigns are considerably challenging and resource intensive as hundreds to thousands of clones need to be isolated and evaluated to identify high quality candidates suitable for clinical and commercial manufacturing. At Amgen, we implemented the Beacon Optofluidic system for single cell cloning, which is a fully integrated nanofluidic cell culture platform that allows isolation of up to 1758 clonal cell lines on a single nanofluidic chip. Commercially available Spotlight® assay reagents, implemented in Beacon CLD workflows, rely on fluorescently conjugated anti-fragment crystallizable (Fc) or anti-Light chain (LC) probes to quantify the steady-state secreted protein level for each individual clone. However, there is a substantial amount of recombinant protein therapeutics that may not be compatible with commercially available Spotlight® reagents due to an incompatible molecule type or protein sequence engineering. We introduced differentiating clone selection strategies, including the development of novel assays to quantify protein secretion for engineered therapeutic modalities. Furthermore, we optimized the cell export workflow through media additives and export process parameters to eliminate cell adhesion of difficult to manipulate clones and achieve 99% clonality assurance with sticky cell lines. These advancements enhance confidence in single cell derivation and clone selection process at the point of Single Cell Cloning to ensure high quality of manufacturing cell lines.

Fueling the Future: Engineering CHO Cells for Independent Essential Amino Acids Production

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The production of life-saving biotherapeutics depends heavily on Chinese hamster ovary (CHO) cell culture systems, which require precise control of essential amino acids (EAAs) supplementation. Improper EAA levels—whether in excess or deficiency—can lead to cellular stress and death, complicating manufacturing processes. Our research addresses this fundamental limitation by engineering CHO cells capable of synthesizing their own essential amino acids through the integration of heterologous synthetic biosynthetic pathways.

We have successfully achieved random genomic integration of two essential amino acid biosynthetic pathways, followed by rigorous selection in amino acid dropout media. Expression of the transfected genes has been confirmed through qPCR, with pathways integrated at ~1 copy per cell. Gene expression varied due to different promoter/polyA combinations, ranging between 0.2 – 10 % of Gapdh expression. These engineered cells demonstrate the ability to grow in media lacking the matching essential amino acid, validating functional restoration of biosynthetic capacity. We will also present preliminary assessment of the transcriptional response to restoring these evolutionarily ancient, foreign pathways, providing insights into the regulatory networks activated during this metabolic reprogramming.

Our approach represents a significant advancement toward creating more sustainable and robust bioproduction systems. By reducing dependency on precise external amino acid supplementation, these engineered CHO cell lines have the potential to enhance manufacturing flexibility and process control. Ongoing work focuses on pathway optimization, characterization of cellular adaptations through multi-omics approaches, and evaluation of these pathways as novel selection systems. Additionally, we will assess how these prototrophic hosts impact product titer using standard manufacturing workflows and evaluate product quality attributes such as glycosylation.

SialMAX: A Streamlined Glycoengineering Workflow for Enhanced α-2,6-Sialylation in CHO Cells

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The global biopharmaceutical market, projected to surpass $550 billion by 2028, is increasingly dependent on monoclonal antibodies (mAbs). While α-2,6-sialylation can improve the therapeutic efficacy of mAbs by enhancing serum half-life, mAbs produced in wild-type CHO cells typically exhibit low levels of sialylation, predominantly in the form of α-2,3 linkages. To overcome this limitation, we introduce SialMAX, a glycoengineering platform designed to promote α-2,6-sialylation in CHO cells, supported by a robust and flexible cell line development workflow.

Our strategy began with the targeted knockout of ST3GAL4, a key gene responsible for α-2,3-sialylation, using CRISPR/Cas9 and USER cloning for plasmid construction. Post-transfection, clones were selected via single-cell sorting. To support this workflow, we developed a novel lectin-assisted confocal laser scanning microscopy technique that enables direct visualization of cell surface glycosylation—providing a rapid, non-invasive, early-stage phenotypic screening tool. Rather than replacing traditional selection methods, this microscopy approach adds a novel, flexible characterization step post-single-cell sorting, enabling real-time assessment of cell surface glycan profiles before product extraction. It functions as a final check prior to product purification or as a mini-pool strategy when single-cell sorting is not feasible, aiding efficient clone triage and prioritization. To validate this workflow beyond SialMAX, we applied it to additional cell lines within our group engineered for distinct glycan motifs. In all cases, the approach enabled efficient characterisation of Glyco-phenotypes.

As we move towards final product purification and mass spectrometry-based glycan profiling, this integrated approach stands to significantly accelerate cell line development, ensuring more consistent and therapeutically effective mAbs. SialMAX exemplifies how precision glycoengineering and real-time phenotyping can shape the future of biopharmaceutical manufacturing.

Targeted Gene Integration for Robust Performance of Inducible Transcriptional Circuits in CHO Cells

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Inducible transcriptional circuits are useful tools to fine-tune gene expression and enable real-time control over desirable cellular phenotypes. In contrast to conventional inducible systems that function in an on/off manner, Linearizer circuits enable gradual gene expression in response to increasing inducer molecule concentrations, somewhat akin to dimmer light switches. Linearizer circuits have extensive potential applications across biopharmaceutical cell culture processes, for example: (i) controlled and coordinated expression of peptide chains that form multimeric therapeutic proteins to enhance the yield of bi- and multi-specifics, (ii) inducible and tuneable expression of adeno-associated viral vector (AAV) components to maximise gene therapy vector yields, and (iii) real-time glycosylation control to enable robust quality assurance of therapeutic glycoproteins.

A key challenge in deploying Linearizer circuits is that the stoichiometry of their components (repressor operon sites, repressor protein, and gene of interest) must be ensured for optimal performance (minimal basal GOI expression, fold induction, and linear dose response to inducer concentrations). This study evaluates the performance of two negatively autoregulated Linearizer circuits (TetR_Lin and PhlF_Lin) in Chinese hamster ovary (CHO) cells using two different genomic integration strategies, targeted integration (TI) and random integration (RI). Each circuit consists of a fluorescent protein reporter (eGFP or mCherry) and a repressor gene (TetR or PhlF). For RI, Linearizers were deployed using transfection and antibiotic resistance selection. For TI, orthogonal landing pads with the Cre/lox and Bxb1/att recombinase mediated cassette exchange systems were used.

Our results show that RI results in high basal expression, low fold induction, and a nonlinear response across inducer concentrations. In contrast, TI achieves lower basal expression, a strong linear response to inducer molecule concentration, and a broader induction range. Our results suggest that the suboptimal performance observed with RI is likely due to stoichiometric imbalances of Linearizer circuit components, and that the issues are resolved with targeted integration. By optimising Linearizer circuit performance in CHO cells, this project paves the way towards real-time control and optimisation – at the cellular level – of biopharmaceutical cell culture processes.

Towards a Minimal Genome: Exploring the Limits of CRISPR/Cas9-Mediated Large-Scale Genomic Deletions in CHO Cells

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Chinese hamster ovary (CHO) cell lines are the predominant host for biopharmaceutical production and are continuously engineered to enhance productivity and product quality. A novel approach for eukaryotic systems, though previously applied in prokaryotes, is the minimal genome strategy, which seeks to reduce genome size by removing non-essential genomic regions. Our group previously demonstrated genomic deletions of up to ~1 megabase (Mb), but given the CHO genome size of 2.45 gigabases (Gb), larger deletions are needed to achieve substantial genome reduction. This study therefore aimed to explore the upper size limits of genomic deletions in CHO cells. CRISPR/Cas9-mediated deletions were performed by transfecting Cas9 protein and single guide RNAs (sgRNAs) flanking genomic target regions selected based on gene expression and essentiality. Genomic regions of up to 13 Mb with low gene expression were identified and deletions thereof performed with the aim of complete genomic excision. Even larger regions of up to 130 Mb including highly expressed and essential genes were addressed via monoallelic deletions to test the maximum feasible deletion size. Following single cell sorting, individual clones exhibiting deletions of the target sequences were identified and validated through PCR and sequencing. All deletions were verified, with confirmation at clonal level for up to 50 Mb and confirmation at cell pool level for up to 130 Mb deletions. Flow cytometry-based ploidy analysis revealed an increased DNA content in some clones, indicating polyploidy and emphasizing the need for comprehensive clone characterization post editing. Bioprocess compatibility was finally evaluated through batch cultivation and assessment of IgG productivity, cell growth, and viability. Interestingly, partially improved productivity and harvest viability was observed with no significant negative impacts attributed to genome reduction. This study demonstrates targeted CRISPR/Cas9-mediated genomic deletions of unprecedented size in CHO cells, thereby establishing a basis for creating a significantly genome-reduced CHO cell line with potentially enhanced recombinant protein production capabilities and reduced cellular complexity.

A Hybrid Modeling Approach for Cell Culture Media Optimization to Enhance Monoclonal Antibody Production

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Monoclonal antibodies (mAbs) represent the most commercially successful category of biopharmaceuticals. However, the high production costs associated with mAb synthesis in CHO cell cultures limit accessibility for many patients. Optimizing protein productivity in biopharmaceutical manufacturing is heavily dependent on advancements in cell culture media, which play a critical role in the production process. This study aims to model how variations in culture media influence protein production to facilitate medium optimization for improved biopharmaceutical yield.

Since most commercially available media consist of proprietary formulations with undisclosed compositions, industrial optimization practices typically involve blending different commercial media in varying proportions to enhance cell culture performance. While statistical methods can assist in identifying optimal conditions, they often necessitate extensive experimental trials. To reduce the cost and experimental burden of media optimization, this study proposes a novel hybrid modeling approach integrating dynamic flux balance analysis (dFBA) with partial least squares (PLS)-based empirical modeling. Using this hybrid framework, mAb production is optimized by determining the optimal proportions of commercial media in the final mixture. The resulting regression models are then incorporated as kinetic constraints within the dFBA framework with specific tolerances. The optimization problem is subsequently formulated to maximize mAb production, with decision variables representing media components and constraints defined by the PLS-dFBA hybrid model. Optimization results are experimentally validated, generating new data that iteratively refine the model, facilitating a continuous search for improved conditions. This systematic approach harnesses the complementary strengths of data-driven PLS modeling and dFBA metabolic pathway optimization, ultimately contributing to the development of enhanced cell culture media for optimized mAb production.

Glycosylation Profile of SARS-CoV-2 Spike-Based Subunit Vaccines Impacts Focusing of the Humoral Immune Response

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Protein subunit vaccines have a strong track record of efficacy and safety and have been widely applied for prevention of a variety of infectious diseases. The impacts of post-translational modifications of vaccine antigens are often overlooked, despite the fact that they can vary significantly depending on the expression hosts (e.g., bacteria, yeast, plant, insect or mammalian cells) and the culture conditions used for their manufacturing. Using SARS-CoV-2 spike trimers as model antigens, we sought to evaluate the immunological impact of modulating their state of glycosylation. Spike proteins rich in complex-type (CT), high-mannose (HM) or paucimannose (PM) N-linked glycans were produced using Chinese Hamster Ovary (CHO) cells (cultured with or without the mannosidase inhibitor kifunensine) or insect cells. We found that when these antigens are adjuvanted with liposomes composed of sulfated lactosyl archaeol (SLA), all glycoforms are highly immunogenic and induce abundant spike-specific serum IgG and IFN-γ producing T-cells in a mouse model. The spike antigen with CT glycans induces a significantly more potent neutralizing immune response, which directly correlates to more abundant receptor binding domain (RBD)-specific IgG when comparing to the antigen with HM glycans. This observation remains true whether the spike is resistin- or T4 foldon-trimerized, indicating that the glycosylation effect is not trimerization domain-specific. Spike with PM glycans induces remarkably low titers of neutralizing antibodies and RBD-specific IgG. Our results highlight the significant impacts of a vaccine antigen's glycosylation profile in directing the immune response, which should be an important consideration for design of protein-based vaccines.

H5N1 Pandemic Preparedness: Building a Comprehensive Immunological Toolbox

Martine Boyer, Patrick Rheault, Vitalie Samoil

Abstract:
H5N1, a highly pathogenic avian influenza subtype, has caused severe poultry outbreaks and sporadic human transmission with mortality rates over 50%. As the virus evolves, the need for effective preparedness becomes urgent. Immunological tools play a central role, offering insights into disease mechanisms, advancing vaccines, enhancing diagnostics, and guiding therapeutics. This poster highlights key innovations to build a comprehensive toolbox for H5N1 pandemic preparedness.

Highlight the key immunological tools that form a comprehensive toolbox for H5N1 pandemic preparedness. This poster emphasizes the role of these tools in advancing vaccine development, monitoring viral evolution, analyzing immune responses, and guiding therapeutic strategies to enhance global health security and pandemic resilience.

Minimal clonal variation eliminates the need for clone selection and recurring bio-process optimization

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When generating CHO based production clones, a significant amount of time is spent selecting clones and creating an optimal bioprocess. This is due to high clone to clone variation.

Our previous research has indicated that the clonal variation can be nearly entirely removed simply by employing a recombinase-based targeted gene integration strategy rather than using methods relying on double stranded breaks.

In this latest work, we have compared the results of a fed-batch bioprocess optimization conducted on several clones either created using RMCE or random integration.

The results show that the most important factors for RMCE clones are process parameters such as e.g. pH or choice of feed. The most important factor for clones generated with random insertion is which clone you selected.

In fact, the most optimal bioprocess for the RMCE clones was optimal for all clones, despite the clones producing different products, indicating that one bioprocess optimization is all you need.

New Mid-Scale Workflows for 20mL - 50mL in Automation Friendly Format

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The Thomson 6-well plate system provides a mid-scale culture format with individual well working volumes of 20-50mL, serving as an alternative to conical bioreactor tubes. The plate's integrated lid includes splash guards and helps reduce evaporation during culture periods. Each well's 50mL maximum working volume allows for larger culture volumes, which can be particularly useful when working with low-expressing proteins where additional culture volume is needed for protein accumulation. The multi-well design enables processing multiple samples within a single unit, increasing throughput compared to individual tubes.

Optimizing Antigen-Expressing Stable Cell Line Development Using Transposases

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Cell lines expressing specific membrane proteins are essential tools in research and drug development. Phage display, a key technology in this field, enables the identification of highly specific monoclonal antibodies. Whole-cell panning in phage display further advances this process by using membrane protein-expressing cell lines to present target antigens in their native context. These cell lines are also instrumental in screening hit compounds to identify antigen binders and performing bioassays to evaluate drug potency and efficacy.

Generating stable cell lines with diverse type I, II, and III membrane proteins across various cell types, from different organisms, and tissues often necessitates customized strategies. The key challenge lies in developing efficient and streamlined strategies for stable cell line generation. Here, we evaluated the DirectedLuck® transposase from ProBioGen AG for research cell line production. Our findings highlight its ability to accelerate the selection of expressing pools with high expression levels, and maintain stability of target genes, offering a promising platform for optimizing cell line development.

Title: Streamline Recovery of Immunoreactive Arboviral Antigens from *E. coli* Inclusion Bodies for Diagnostic Use

Authors: Pereira, .A.S., Russo, E.M.S.

Affiliation: Ribeirão Preto School of Pharmaceutical Sciences, University of São Paulo.

Abstract:

Escherichia coli is a widely used host for recombinant protein production due to its fast growth, well-characterized genetics and cost-effectiveness. However, heterologous expression often leads to inclusion body formation, requiring solubilization and protein-specific refolding protocols. Here, we describe a streamlined strategy for recovering diagnostically relevant arboviral antigens from inclusion bodies.

Three arboviral proteins were expressed in E. coli Rosetta(DE3): non-structural protein 1 from dengue virus (DENVNS1), envelope domain III from dengue virus (DENVE3), and non-structural protein 1 from Zika virus (ZIKVNS1). The recombinant proteins predominantly accumulated in inclusion bodies demanding a systematic evaluation of solubilization (urea 8M) and renaturation conditions to obtain correctly folded and immunoreactive proteins suitable for diagnostic applications. After expression and purification under denaturing conditions, proteins were refolded using a redox-buffer system (reduced/oxidized glutathione) and gradual urea removal via G-25 size-exclusion chromatography. This dilution-based approach provided a simplified and reproducible strategy for oxidative protein folding.

Refolded protein yields reached 8 mg/L (DENVNS1), 20 mg/L (DENVE3), and 5 mg/L (ZIKVNS1). Dynamic light scattering revealed that preparations contained both high-molecular-weight aggregates and a smaller population of lower-order species, suggesting partial success in recovering monomeric forms.

These findings support the use of this refolding protocol as a practical approach for producing immunoreactive arboviral antigens suitable for diagnostic assay development.

Transposase Platform: A Comprehensive Approach to Cell Line Development Success

Alexandra Martiné [1]

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The successful development of biotherapeutic molecules begins with the generation of a robust cell line. Choosing the right Cell Line Development (CLD) path is essential and should be focused on both comprehensive analysis of protein-to-be-expressed attributes and specific programs needs assessment. We have developed a robust transposase-based expression platform that overcomes these challenges and deliver consistent high-expressing cell lines up to 12g/L with sustained stability over 60 generations. To reach this goal, we have leveraged a proprietary CHO-M mammalian cell to design comprehensive CLD workflows without compromising the successful inherited assets from our former random expression platform. In this "trust our experts" approach, we offer flexible solutions and risk mitigation strategies to ensure the best cell line development path for any therapeutic protein format.

A Human Lectibody Platform for Glycan Targeting and Exploration of Anti-Infective Potential

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Lectins are carbohydrate-binding proteins involved in crucial biological processes like cell-cell recognition, immune responses, and host-pathogen interactions [1,2]. Their high specificity for sugar moieties enables them to recognize glycosylated proteins on host cells or pathogens, making them valuable tools for studying glycan-mediated interactions and developing targeted therapies [3]. Among the various lectin families, C-type lectins, are of particular interest due to their calcium-dependent binding mechanism and prominent roles in immune surveillance and pathogen recognition [4]. To enhance their utility, lectibodies, chimeric molecules combining lectin domains with antibody Fc regions, have been developed [5]. These fusions improve stability and avidity and convey effector functions, enabling targeted applications in glycan-rich structures such as tumors and pathogen surfaces [5,6]. Here, we describe the construction and characterization of an off-the-shelf human lectibody collection for subsequent assessment of glycan recognition and applicability against infectious agents. Over 100 annotated lectin domain sequences from the human genome were cloned in the lectibody format and are expressed via transient gene expression in mammalian cells. Proteins are purified using bio-affinity followed by size exclusion, yielding high-quality samples. Protein quality is judged based on purity and biophysical properties, while glycan-binding specificity is evaluated using a wide variety of carbohydrate structures. Finally, we outline future directions for functional assays, protein engineering, and systematic screening of lectibodies as potential anti-infective candidates.

1. Sharon N, Lis H., *Glycobiology*. 2004 Nov;14(11):53R–62R.
2. Varki A., *Glycobiology*. 2017 Jan;27(1):3–49.
3. Van Kooyk Y, Rabinovich GA., *Nat Immunol*. 2008 Jun;9(6):593–601.
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Developability assessment of a CD28 x Nectin-4 co-stimulatory bispecific for the treatment of bladder cancer

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Effective T-cell activation is driven by three signals: Signal 1 delivered through antigen recognition via the T-cell receptor, signal 2 through co-stimulatory receptors, and signal 3 mediated by cytokines. CD3-targeting T-cell engagers provide signal 1 to T cells and have shown significant clinical benefit in hematological malignancies but have faced challenges in solid tumors due to on target off tumor toxicities. Emerging clinical data supports the hypothesis that for efficient and sustained activity in the presence of signal 1, engagement of co-stimulatory molecules like CD28 could be important for effective anti-tumor activity in solid tumors.

We present the development of a panel of bispecific antibodies (bsAbs), targeting the costimulatory molecule CD28 and the tumor associated antigen (TAA) Nectin-4, a cell adhesion molecule overexpressed in bladder cancer and other malignancies. To select our development candidate RNDO-564, we screened a panel of bsAbs with varying CD28-potencies for both functional activity and biophysical stability to assess and reduce manufacturability risks.

Functional characterization of our bsAbs showed robust tumor cytotoxicity and IL-2 secretion while posing a lower safety risk providing that the activity is dependent on presence of signal 1 and expression of the TAA. Additionally, we demonstrated that the panel of CD28 binders can be paired with various TAA-binding arms, enabling the design of new bispecific antibodies for different indications.

In addition to the desired biological activity, the CD28 x Nectin-4 bsAbs also exhibited favorable developability profiles as demonstrated by subjecting the panel to accelerated stress conditions like thermal stress at elevated temperatures for extended periods of time or low pH hold.

This extensive functional and biophysical characterization enabled the selection and successful manufacture of our clinical candidate RNDO-564.

Development of multispecific VHHs for the neutralization of SARS-CoV-2 and H5N1

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The rise of H5N1, a highly pathogenic avian flu virus capable of infecting mammals, and the continuous dissemination of SARS-CoV-2 variants highlight the need for effective viral therapies. Current antiviral treatments and vaccines often fail due to viruses high mutation rate, leading to immune evasion. Camelidae produce functional immunoglobulin G molecules lacking a light chain and CH1 domain, with a variable region consisting of a single domain called VHH. Although they are much smaller than full-length antibodies (15 vs. 150 kDa), VHHs can be expressed as single domain antibodies while keeping similar binding specificity and affinity. Their small size allows them to access and recognize cryptic antigenic sites that are inaccessible to human antibodies, have high solubility and stability, and can be administered in aerosolized form. VHHs are also modular and can be fused together to generate bispecific or multispecific antibodies that typically exhibit increased avidity compared to individual monomers. The fusion of two different VHHs targeting non-overlapping epitopes on the same antigen results in biparatopic antibodies that may render them less sensitive to viral mutations. We produced libraries of VHHs against SARS-CoV-2 spike and avian flu H5 and N1 antigens. Various VHH designs against spike have been generated and produced in CHO cells including Fc fusions to enhance their *in vivo* half-life. These modalities will be tested in surrogate neuralization assays to identify the best candidates for further development as potential therapeutic or diagnostic tools.

A Human Lectibody Platform for Glycan Targeting and Exploration of Anti-Infective Potential

Michael Lehky[1], Jie Jiao[2], Stefan Mereiter[3,4], Kristin Metzdorf[1], Josef Penninger[1,2,3,4]

[1] Department of Innovative Organoid Research, Helmholtz Centre for Infection Research, Germany, [2] Department of Medical Genetics, Life Sciences Institute, University of British Columbia, Canada, [3] Department of Laboratory Medicine, Medical University of Vienna, Austria, [4] IMBA, Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Austria

Lectins are carbohydrate-binding proteins involved in crucial biological processes like cell-cell recognition, immune responses, and host-pathogen interactions [1,2]. Their high specificity for sugar moieties enables them to recognize glycosylated proteins on host cells or pathogens, making them valuable tools for studying glycan-mediated interactions and developing targeted therapies [3]. Among the various lectin families, C-type lectins, are of particular interest due to their calcium-dependent binding mechanism and prominent roles in immune surveillance and pathogen recognition [4]. To enhance their utility, lectibodies, chimeric molecules combining lectin domains with antibody Fc regions, have been developed [5]. These fusions improve stability and avidity and convey effector functions, enabling targeted applications in glycan-rich structures such as tumors and pathogen surfaces [5,6]. Here, we describe the construction and characterization of an off-the-shelf human lectibody collection for subsequent assessment of glycan recognition and applicability against infectious agents. Over 100 annotated lectin domain sequences from the human genome were cloned in the lectibody format and are expressed via transient gene expression in mammalian cells. Proteins are purified using bio-affinity followed by size exclusion, yielding high-quality samples. Protein quality is judged based on purity and biophysical properties, while glycan-binding specificity is evaluated using a wide variety of carbohydrate structures. Finally, we outline future directions for functional assays, protein engineering, and systematic screening of lectibodies as potential anti-infective candidates.

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Challenges and Opportunities in Discovery of Large Molecule Therapeutics Designed with Generative Artificial Intelligence (AI) Technology

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Generative artificial intelligence (AI) is transforming the design of large molecule therapeutics, significantly accelerating the pace and efficiency of drug development. Traditional discovery approaches rely on screening semi-random sequences, with optimization achieved through similarly stochastic diversification. This random exploration of sequence space yields unpredictable success rates and necessitates the evaluation of vast numbers of variants, making the process both time-consuming and costly. In contrast, AI-guided design offers a more informed, targeted strategy—marking a shift from a largely empirical discovery exercise to an engineering-driven discipline grounded in iterative design-build-measure-learn cycles. At Generate Biomedicines, this technology has been deployed to generate high-quality in silico variant sequences with exceptional speed and precision. However, while sequence design has advanced rapidly, the technologies supporting production and screening of these variants have not progressed at the same pace. As a result, wet lab processes—namely protein production, purification, and analytical chemistry—have become the new rate-limiting steps in the discovery pipeline. To overcome this bottleneck, the Protein Sciences Department at Generate has expanded its capabilities in protein expression, purification, and analytical chemistry through focused investment. The resulting infrastructure is notable for a company of our size and underscores our commitment to building a platform with lasting translational potential. This robust capability underpins our biotherapeutic discovery workflow, supporting all processes from high-throughput screening of thousands of variants to the delivery of comprehensive Lead Candidate Selection (LCS) and Development Candidate Nomination (DCN) packages to bolster our clinical pipeline.

CHO Transcriptomics and Proteomics Using Quantitative Immunostaining Directed Laser Lysis of Single Cells

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Early manufacturability prediction of antibody-producing cell lines can be a lengthy and costly process necessary in the transition of lead biotherapeutics to process development and manufacturing. Significant time is required to identify and expand single CHO cell clones to practical culture volumes for in-depth cell line and recombinant protein characterization. We present a novel approach which enables -omics level (transcriptomic and proteomic) analysis of single and up to 10 cell clusters directly from stable cell pools using microlitres of culture. The method combines two novel developments: (1) a recombinant antibody secretion-dependent quantitative immunostaining approach to identify single cells expressing at relative levels, and (2) a microfluidic and laser-cell-lysis-based approach to pool cell lysates for -omics analyses. Using a panel of stable antibody-producing CHO cell pools, we demonstrate that the immunostaining approach quickly ranks cultures with good correlation to titer measurements made from shake flasks. Indeed, the immunostaining approach required only one hour expression to detect expression from pools compared to seven days needed for standard shake culture assays. Our novel quantitative immunostaining approach gives a relative ranking of CHO cell antibody expression at single cell resolution within a pool thereby informing which individual cell should be lysed for transcriptomics and proteomics. Based on the immunostaining signal intensity with high, medium, and low expression, we lysed and collected 1-to-10-cell lysates in microdroplets for downstream -omics analysis from several stable antibody expressing CHO cell pools. Heterogeneity in transcriptomics and proteomics profiles from single CHO cells within and between stable antibody producing pools was investigated. The approach is mostly automated using digital microfluidics, with potential for a fully autonomous platform in future iterations. We envision that this method will enable rapid and powerful investigations into the manufacturability of lead biotherapeutics by elucidating proteomic and transcriptomic signatures of maximally productive cells in a shortened timeline.

Comparative analysis of HEK293 cells: Characterization of genomic variability

Georg Smesnik[1], Nikolaus Virgolini[1], Astrid Dürauer[1], Nicole Borth[1]

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Human embryonic kidney cells (HEK293) serve as cell factories in viral vector manufacturing, particularly for recombinant adeno-associated virus (rAAV) production. They find widespread utilization in industrial applications, but a comprehensive characterization of the HEK293 genome and epigenome stability is still missing. To address this knowledge gap, the study employs a systematic approach to examine the genetic landscapes of various HEK293 cell lines. The objective is to evaluate their responses to changing environmental conditions and improve the current understanding of how these molecular mechanisms might influence rAAV production processes. Therefore, adherent HEK293 cells were adapted to suspension growth using various commercially available serum-free media formulations. Following successful adaptation, whole-genome deep sequencing was performed on both adapted and parental cell lines. The sequenced reads were then aligned to the human reference genome, enabling the assessment of genome stability, by evaluation of identified structural variants. Comparative analysis of these cell lines along with publicly available genome sequences of different HEK293 derivatives revealed a characteristic genetic signature common to all HEK293 cells, independent of cultivation conditions, phenotypic divergence or phylogenetic distance. Alterations in the distribution of structural variants including insertions and deletions, and of single nucleotide polymorphisms, indicate a continuous accumulation of genetic changes over time in culture, rather than abrupt genomic shifts in response to altered cultivation conditions. In contrast, adenoviral genes integrated into HEK293 cells appear to be highly conserved, as indicated by their stable copy number and consistent integration site. Overall, this work offers novel insights into the cellular response of various HEK293 cells to different cultivation conditions. Furthermore, it lays the groundwork for more comprehensive omics characterization, to support the development of cell lines with higher production efficiency.

Glycomics and glycoproteomics methods for applications in biotherapeutic products and endogenous biomarkers

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The structural characterization of glycoproteins both as biotherapeutic products and as endogenous biomarkers in plasma and tissues remains one of the most analytically demanding tasks in modern bioanalysis. Glycosylation is a highly complex, heterogeneous, and context-dependent post-translational modification that influences protein folding, function, pharmacokinetics, and immunogenicity. High-throughput (HTP) glycomics and glycoproteomics methods have recently been developed as essential tools to dissect this complexity, and their implementation into routine workflows for industry has been tested.

Accurate glycoprotein analysis requires resolving site-specific glycoform variations across batches and navigating complex plasma and tissue samples with high dynamic range and structural isomers. Tissue analysis adds challenges like limited material and extracellular matrix interference. Accurate interpretation depends on advanced bioinformatics tools and machine learning algorithms, which must navigate an enormous search space of potential glycan structures and account for variable ionization efficiencies, incomplete fragmentation, and co-eluting species. Despite these hurdles, precise glycoprotein characterization is essential for therapeutic safety and for leveraging glycans as biomarkers in diseases such as cancer and neurodegeneration. Ongoing innovation is key to advancing high-throughput glycoanalysis in clinical and biopharmaceutical settings.

We have developed HTP glycomics and glycoproteomics mass spectrometry-based methods to address these challenges. We have now optimized technologies ensuring the retention of labile residues such as sialic acids and fucose, and non-carbohydrate substituents like acetylation and sulfation. We have worked out advanced workflows that combine enrichment techniques, specialized digestion and derivatization protocols, and orthogonal mass spectrometry platforms (such as LC-MS/MS with EThcD, stepped HCD and UVPD) to structurally elucidate and preserve native glycosylation patterns. These developments are a continuation of our ongoing efforts of using state-of-the-art MS instrumentation to address newly arising difficulties in glycoprotein characterization and applying these tools to assist the industry in characterizing new biologics products.

Implementation of Quality by Design Principles for Influenza A Virus Production

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Ensuring consistent product quality in cell culture-based vaccine manufacturing requires a thorough understanding of the process parameters that affect titers, yields, and impurity levels. This study applies Quality by Design (QbD) principles to an influenza A virus (IAV) production process operated in batch mode using two monoclonal suspension MDCK cell lines, C59 and C113 (Sartorius, Germany), with distinct charcteristics, focusing on process robustness and optimization.

Based on knowledge from previous process development [1], a quantitative risk assessment including biological and technical parameters was performed to identify Critical Process Parameters (CPPs). Using a Design of Experiments (DoE) approach in an Ambr® 15 scale-down system, four key CPPs (pH value, dissolved oxygen concentration, viable cell concentration at time of infection, and multiplicity of infection) were investigated at three levels. After data analysis and modeling, we obtained dedicated design spaces for each cell clone characterized by high process robustness with a less than 1% risk of failure and even some indications for virus titer and yield improvement, while keeping process-related impurities such as DNA and total protein concentration low. Scale-up experiments in a 2 L single-use stirred tank bioreactor confirmed the validity of these conditions. Total virus titers of 2.95±0.06 $\log_{10}$(HAU/100 µL) and 3.13±0.12 $\log_{10}$(HAU/100 µL) were obtained for C59 and C113 cells, respectively [2].

By applying QbD principles, this study not only improves IAV production but also demonstrates a framework applicable to manufacturing of other cell culture-based vaccines. The results provide valuable insights for optimizing manufacturing processes, reducing batch failure risks, and supporting regulatory approval through data-driven process characterization.

[1] Zinnecker et al., 2024, *Eng. Life Sci.*, https://doi.org/10.1002/elsc.202300245
[2] Zinnecker et al., 2025, *Eng. Life Sci.*, https://doi.org/10.1002/elsc.70027

PEACe 2025 Abstract Template

Suplementation of chemical aditives as an strategy to improve novel anti-sST2 IgG in CHO cells system

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A novel monoclonal antibody (mAb) (anti-sST2) (U. Chile) with therapeutic potential for autoimmune disease Ulcerative colitis and other similar diseases with high levels of sST2 soluble protein was designed. For the production of this mAb, a possible secretory bottle neck is hyphotesized, observed at a previous analysis. Unfolded/misfolded proteins could cause reticulum stress, activating the unfolded protein response and possible apoptosis; also an increase on mAb demand could raise reactive oxygen species (ROS) leading to an accumulation of protein in the endoplasmic reticulum. To improve this scenario, process engineering approaches were studied, supplementing the culture medium with three different molecules: a novel and promissory antioxidant, a recognized antioxidant and a chemical additive, to decrease folding and aggregation problems, and with it is expected to enhance production of the novel mAb. Independent supplementation improved specific production (qP), with a value of 47%, 43.5% and 46.8% more than control for novel antioxidant, recognized antioxidant and chemical additive, respectively. Also, CHO cells were able to grow at optimal conditions on a studied range of the novel antioxidant, maintaining cell viability over 85% in culture, and improving the specific cell growth rate (μ) (0.55 1/d), a 30% higher than control, which also improved qP. As expected, supplementation of both a known antioxidant and a novel antioxidant were able to reduce intracellular ROS.

Characterizing the in vitro antiviral activity of influenza A virus defective interfering particles using a systems biology approach

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Influenza A virus (IAV) defective interfering particles (DIPs) hold great promise for the prevention and treatment of IAV infections. DIPs inhibit IAV replication and spread and counteract unrelated viral infections by activating the innate immune system. To investigate their potential use as antivirals, we used a systems biology approach that integrates mathematical modelling with experimental data to uncover the mechanisms underlying the effects of IAV DIPs.

We focused on "OP7", an unconventional DIP characterised by multiple point mutations in its genome. Co-infection scenarios were tested using human Calu-3 cells infected with IAV at different multiplicities of infection (MOIs) and co-infected with different concentrations of OP7. To evaluate a prophylactic and therapeutic treatment window, cells were treated with OP7 and subsequently infected with PR8 at different time points. Total and infectious virus titres were quantified and vRNA segment numbers and gene expression were measured by quantitative real-time RT-qPCR.

The results showed that co-infection with OP7 reduced infectious virus titres, even at low doses. Remarkably, OP7 completely suppressed IAV replication in low MOI scenarios, highlighting its strong antiviral potential. Both co-infection and OP7 infection alone enhanced type I IFN responses. In addition, co-infection with OP7 reduced NS1 protein expression by more than 1.5 logs. As NS1 is a known IFN antagonist, its reduction is likely to account for the increased IFN response compared to PR8 infection alone.

Mathematical modelling calibrated with these data suggested a therapeutic window in which OP7 administration at 9 hours after PR8 infection could still significantly reduce infectious virus titres. Experimental validation confirmed that OP7 suppressed infectious titers by 4 logs in low MOI infections (MOI 10E-3) within a 12-hour time window. Future studies will evaluate OP7 in prophylactic applications and further refine the model to deepen the understanding of its antiviral mechanisms. Taken together, these findings highlight the potential of IAV DIPs as potent antiviral agents.

CHO cell production of a single enveloped VLP vaccine targeting SARS-CoV-2, Influenza A and RSV

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The overlapping circulation of SARS-CoV-2, Influenza A virus (IAV), and Respiratory Syncytial Virus (RSV) continues to strain global healthcare systems, particularly among vulnerable populations. A single vaccine targeting all three pathogens could streamline immunization efforts and enhance protection, while reducing manufacturing costs. We previously showed that CHO cell–derived enveloped virus-like particles (eVLPs) formed through expression of full-length SARS-CoV-2 spike (S) protein not only exhibit high S density and strong immunogenicity, but also serve as a platform to co-display heterologous antigens such as IAV hemagglutinin (H1) and neuraminidase (N1). Here, we extend this approach by producing a trivalent eVLP candidate that simultaneously displays SARS-CoV-2 S, IAV H1, and the RSV pre-fusogenic fusion (F) protein. These S/H1/F eVLPs were successfully produced using both transient and stable gene expression in CHO cells and were purified via affinity chromatography. The presence of all three antigens on the same particles was confirmed by Western blot and immuno-electron microscopy. Their immunogenicity is currently being evaluated *in vivo* to assess their potential as a single vaccine against SARS-CoV-2, IAV, and RSV.

From CHO Mastery to Intensified HEK293 Innovation: Elevating Viral Vector Production

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The emergence of cell and gene therapies in recent years, combined with an increasing number of approved therapies, has meant that more and more previously incurable diseases can now be treated. However, the availability and cost of these therapies remains a major challenge that needs to be addressed. One way to make life-saving therapies more accessible and affordable for patients worldwide is through process intensification (PI). PI can be achieved by optimizing various aspects of the cell culture process, such as cell density, media formulation, and process type, resulting in more robust and scalable processes. While PI is well established for CHO-based protein production, this field is still largely uncharted territory in HEK293-based gene and cell therapies.

Sartorius Xell leverages extensive expertise in developing advanced cell culture media, alongside a comprehensive portfolio of analytical techniques and equipment scaling options, to enhance cell culture processes. This toolbox has facilitated the creation of a specialized cell culture medium for HEK293 suspension cultures, capable of sustaining exceptionally high cell densities of 100 million cells per milliliter over extended durations. Building on established techniques from CHO-based therapeutic protein production, the HEK293 cell line, renowned for its applications in recombinant protein production and gene therapy, was integrated into a continuous perfusion process. This approach ensured optimal growth conditions and nutrient supply, allowing the cells to maintain their ability to be transiently transfected even at elevated densities. The study demonstrates the feasibility of gene transfer under intensified conditions, highlighting the potential of perfusion systems to revolutionize cell-based production processes. These advancements promise increased bioproduct yields while preserving the functional integrity of cells for transient transfection applications, paving the way for more efficient and scalable biomanufacturing strategies.

Mixture Design as a tool for improving full-to-empty particle ratios across various GOIs in rAAV production

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Optimization of rAAV production is essential for effective gene therapy applications. However, multiple factors affect the rAAV productivity in mammalian cells, and often they interact with each other, making the optimization process highly challenging.

In this study, we optimized the triple transfection method for rAAV production using a design of experiments (DoE) approach. Building on our prior work identifying mixture design (MD) with face-centered composite design (FCCD) as optimal for enhancing yield and cell viability, we now evaluate two more therapeutic genes, msh2 and bdnf, along with the percentage of full-to-empty capsids as a quality metric. Samples were harvested at 72 hours post-transfection (hpt), capsid quantification was performed using ELISA, and viral titers measured with qPCR. Statistical analysis was conducted using JMP 16 Pro.

Our results show that rAAV optimization is highly gene-dependent, with each gene-of-interest (GOI) requiring specific plasmid ratios and conditions. Optimal settings for one response differed from those for others, emphasizing the need for tailored strategies. While volumetric productivity was relatively consistent across GOIs, the full-to-empty capsid ratio varied significantly, likely due to GOI-specific biological properties. Interestingly, GC content, ΔG, and construct length seem to have minimal impact on packaging, while genome secondary structure correlated with full-to-empty capsid ratio in each case. Overall, we show how MD coupled with FCCD is a robust method that can be used to improve different responses in rAAV production with different GOIs, achieving an improvement of almost 100-fold in Log(Vp) in the case of egfp-expressing rAAV, and a 12-fold increase in bdnf-expressing full rAAV capsids. These were followed by validation runs, supporting the accuracy of the models' predictions.

Production of virus-like particles for antibody development using baculovirus-free insect cell expression system

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High expression levels of virus-like particles (VLPs) are essential for vaccination and diagnostic applications. Although the Baculovirus Expression Vector System (BEVS) in insect cells is widely employed for the production of VLPs due to its high yields, it faces significant bottlenecks. These challenges include optimization problems of the required ratios of structural proteins and the co-production of baculoviral proteins and particles, resulting in a cumbersome purification. Our plasmid-based High Five insect cell expression system overcomes these limitations. In a direct comparison of BEVS and the plasmid-based system regarding the yield and quality of Noro, Entero, and Rota VLPs, we were able to highlight the advantages of the plasmid-based system.

Furthermore, we produced SARS-CoV-2 VLPs for antibody development. The quality of these VLPs was validated by Nanotracking Analysis, ELISA, cytometry, and microscopy, confirming a diameter of ~145 nm, ACE2 binding and the typical "corona" aura. These fluorescent SARS-CoV-2 VLPs have been used in cell-based assays and enable high-throughput screening of anti-SARS-CoV-2 antibody candidates for their capability to inhibit binding to ACE2 positive cells through cytometry. We now transfer this system to other viruses like Hantavirus and West Nile virus.

In summary, we demonstrate the successful production of VLPs in our plasmid-based insect cell system, achieving both high quality and high yield, and their subsequent application in antibody development.

Reconstruction of the AAV biosynthetic pathway enables systems-level analysis of viral production mechanisms in mammalian cells

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Adeno-associated virus (AAV) is a leading viral vector for in vivo gene therapies. However, current AAV production titers and quality remain insufficient for large-scale applications. To improve AAV production, understanding the biomolecular mechanism of the AAV biosynthesis pathway is crucial. In the context of an AAV manufacturing platform that relies on infection of a stable producer cell line (PCL) with a wild-type adenovirus, a particular interest is how the host cell machinery is exploited by both the adenoviral helper virus and AAV. As AAV is replication-deficient on its own, it requires co-infection with a helper virus such as adenovirus, which reprograms the host environment to enable AAV genome replication and capsid assembly. However, the molecular steps underlying this coordinated process remain poorly understood, making it challenging to pinpoint where deficiencies arise in AAV-producing PCL clones. Here we present a genome-scale reconstruction of the AAV biosynthesis pathway, comprising 2,863 manually curated genes annotated into 125 process terms across 22 subsystems. This reconstruction also incorporates the induction of AAV production via wild-type adenovirus infection, providing a comprehensive resource for systems-level analysis of AAV biosynthesis. We overlaid transcriptomic data from AAV-producing clones onto the biosynthetic pathways to identify functional modules associated with variations in AAV productivity and quality. Comparative gene set enrichment analyses using the AAV-specific reconstruction versus conventional Gene Ontology (GO) annotations demonstrate improved contextualization of regulated pathways under different conditions. Network-based visualization and clustering approaches enable the mapping of gene expression dynamics onto the reconstructed network, revealing condition- and time-dependent changes in pathway activity. Identified pathways of interest may be further correlated with AAV titer and vector quality to investigate potential mechanisms driving productivity variance. Together, the reconstruction serves as a systems-level framework for interpreting gene regulation during AAV production. This resource supports transcriptomic profiling, facilitates bioprocess optimization, and lays the groundwork for future identification of key functional interplay modules between producer cell line, AAV and adenovirus during AAV biosynthesis.

Declaration of interest statement

This research is funded by Sanofi. Authors employed by Sanofi may hold shares and/or stock options in the company. N.E.L is a co-founder of Augment Biologics and NeuImmune. He is also a board member for CHO Plus and Neion Bio. The remaining authors declare no competing interests.

A reconstruction of the mammalian secretory pathway identifies mechanisms regulating antibody production

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The secretory pathway processes >30% of mammalian proteins, orchestrating their synthesis, modification, trafficking, and quality control. However, its complexity—spanning multiple organelles and dependent on coordinated protein interactions—limits our ability to decipher how protein secretion is controlled in biomedical and biotechnological applications. To advance such research, we present secRecon—a comprehensive reconstruction of the mammalian secretory pathway, comprising 1,127 manually curated genes organized within an ontology of 77 secretory process terms, annotated with functional roles, subcellular localization, protein interactions, and complex composition. Using secRecon to analyze multi-omics data, we identified distinct secretory topologies in antibody-producing plasma cells compared to CHO cells. Genes within proteostasis, translocation, and N-glycosylation are deficient in CHO cells, highlighting them as potential engineering targets to boost secretion capacity. Applying secRecon to single-cell transcriptomics and SEC-seq data, we uncovered secretory pathway signatures underlying secretion diversity among IgG-secreting plasma cells. Different transcriptomic clusters had unique secretory phenotypes characterized by variations in the unfolded protein response (UPR), endoplasmic reticulum-associated degradation (ERAD), and vesicle trafficking pathways. Additionally, we discovered specific secretory machinery genes as new markers for plasma cell differentiation. These findings demonstrate secRecon can identify mechanisms regulating protein secretion and guide diverse studies in biomedical research and biotechnology.

Protein Language Models: Is Scaling Necessary?

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Public protein sequence databases contain samples from the fitness landscape explored by nature. Protein language models (pLMs) pre-trained on these sequences aim to capture this landscape for tasks like property prediction and protein design. Following natural language processing, pLMs have been continuously scaled up. However, the assumption that scale leads to better performance assumes that databases accurately represent the fitness landscape, which is likely false. Assuming that most UniRef sequences are real and not pseudo-genes or the result of sequencing errors, then UniRef100 is a more representative sample of the fitness landscape. This is because redundancy is a sign of observation count, which we should treat as a measure of confidence, and clustering is in fact expected because of the nature of protein evolution - these clusters represent families of related proteins that are common for functional reasons. Removing sequences that show up in large clusters downweights confident sequences and ultimately upweights singleton sequences that are more likely to derive from sequencing errors and other sources of noise. Equipped with this understanding, and thanks to an efficient codebase and modern architecture, we introduce AMPLIFY, a best-in-class pLM that is orders of magnitude less expensive to train and deploy compared to previous models like ESM2 that are trained on clustered versions of UniRef. To support the scientific community and democratize the training of pLMs, we have open-sourced AMPLIFY's pre-training codebase, data, and model checkpoints.

Comparative analysis of HEK293 cells: Characterization of genomic variability

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Human embryonic kidney cells (HEK293) serve as cell factories in viral vector manufacturing, particularly for recombinant adeno-associated virus (rAAV) production. They find widespread utilization in industrial applications, but a comprehensive characterization of the HEK293 genome and epigenome stability is still missing. To address this knowledge gap, the study employs a systematic approach to examine the genetic landscapes of various HEK293 cell lines. The objective is to evaluate their responses to changing environmental conditions and improve the current understanding of how these molecular mechanisms might influence rAAV production processes. Therefore, adherent HEK293 cells were adapted to suspension growth using various commercially available serum-free media formulations. Following successful adaptation, whole-genome deep sequencing was performed on both adapted and parental cell lines. The sequenced reads were then aligned to the human reference genome, enabling the assessment of genome stability, by evaluation of identified structural variants. Comparative analysis of these cell lines along with publicly available genome sequences of different HEK293 derivatives revealed a characteristic genetic signature common to all HEK293 cells, independent of cultivation conditions, phenotypic divergence or phylogenetic distance. Alterations in the distribution of structural variants including insertions and deletions, and of single nucleotide polymorphisms, indicate a continuous accumulation of genetic changes over time in culture, rather than abrupt genomic shifts in response to altered cultivation conditions. In contrast, adenoviral genes integrated into HEK293 cells appear to be highly conserved, as indicated by their stable copy number and consistent integration site. Overall, this work offers novel insights into the cellular response of various HEK293 cells to different cultivation conditions. Furthermore, it lays the groundwork for more comprehensive omics characterization, to support the development of cell lines with higher production efficiency.

Multivariate Data Analysis Aids Selection of CHO Cells Clones Expressing a Monoclonal Antibody

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The development of cell lines that reliably express large titers of biologics such as monoclonal antibodies (MAbs) is an important step of bioprocess development. After transfection of CHO cells, stable pools are obtained, and clones are isolated. As each clone can behave distinctly when cultivated, clone selection is crucial. This study proposes an adaptable multifactorial data analysis method for clone selection that takes multiple process parameters into account, in addition to the traditionally considered titer and cell growth. We applied our innovative approach on existing in-house data corresponding to the generation of CHO clones producing Omalizumab, an IgG1 monoclonal antibody. Twenty-four clones were chosen for expression stability screening tests conducted in extra deep well plates (18 mL). Among them, eight stable clones were selected for scalability evaluation performed in benchtop stirred-tank bioreactors (0.75-1 L). Considering parameters related to productivity, cell growth and expression stability led to the selection of different clones for bioreactor scalability assessment experiments compared to those originally selected based on a conventional method. As efficient CO_2 stripping is challenging in large-scale bioreactors, experiments were conducted with and without addition of air in the bioreactor headspace to modulate the concentration of CO_2 in the media. We found that cultures without overlay air addition reached a pCO_2 of up to 190 mmHg. Of note, we showed the increased concentration of CO_2 to be beneficial. Indeed, on average, we measured 1.31-fold higher final titer, 1.14-fold higher cell specific productivity, and 1.13-fold greater peak viable cell density for cultures exposed to a greater pCO_2. In addition, these cultures benefitted from 3 to 5 more days above 80% viability, and their titer kept increasing until the end of the culture (17-21 days). We integrated statistical tools to reliably analyze datasets relating to productivity, cell growth, and key cellular metabolites. This new approach helped selection of a robust clone which performed similarly for low and high pCO_2, offering a better potential for subsequent scale-up. These findings underscore the great potential of MVDA to improve bioprocess development.

Enabling continuous biomanufacturing for virus-based expression systems via multi-stage bioreactors

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The lytic nature of virus-based expression systems, used for production of many vaccines and viral vectors, limits their transition from traditional batch-based to intensified continuous bioprocessing. In this work, we implemented a multi-stage bioreactor setting to leverage continuous operation using virus-based expression systems.

The multi-stage bioreactor set-up was used for continuous production of influenza virus-like particles (iVLPs) as vaccine candidate, using the insect cell-baculovirus expression vector system (IC-BEVS). Additionally, this system is being validated to produce another two biopharmaceutical products: (i) adenovirus-like particles (ADDomer) as a nanoparticle-based snake-bite therapy alternative, using IC-BEVS, and (ii) recombinant adeno-associated viruses (rAAV) as gene therapy vector, using the HeLaS3 packaging cell line (PCL) infected with wild-type adenovirus type 5 (wtAd5). Critical process parameters (CPP) being fine-tuned to maximize productivity include cell line, virus construct, cell concentration at infection (CCI), residence time (RT) in the production bioreactor, and perfusion rates (PR). For iVLPs, optimization of cell line, baculovirus construct, and RT, allowed for consistent iVLPs titer (34 ±14 HA titer/mL) throughout the period of continuous operation (20 days). For ADDomer, cell line and PR have been optimized to achieve 8-fold higher ADDomer expression via high cell density (HCD) culture (10-20 x10^6 cell/mL) using perfusion. This HCD approach will be employed into the continuous production using the multi-stage bioreactor set-up. A similar approach is being undertaken for continuous rAAV production, but using lower CCI (0.5-1 x10^6 cell/mL); RT will be further fine-tuned for optimal rAAV expression using the HeLaS3-PCL expression system.

This work showcases the potential of multi-stage bioreactors for continuous production of different types of biopharmaceutical using virus-based expression systems across multiple cell hosts. This approach allows seamless integration with continuous downstream processing, paving the way for fully integrated, end-to-end biomanufacturing.

Targeted Mass Spectrometry-Based Proteomics for Identifying ER-Related Stress in Recombinant Chinese Hamster Ovary Production Cultures

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Chinese hamster ovary (CHO) cells remain the most widely used host cell line for biotherapeutics production. Despite their widespread use, understanding endoplasmic reticulum (ER) stress conditions in recombinant protein production remains limited, often creating bottlenecks preventing improved production titers and product quality. Excessive protein production, nutrient deprivation, and other cell culture media conditions (e.g. waste build-up) in CHO cell cultures can lead to protein unfolding and misfolding, which in turn triggers ER stress. When ER stress occurs, cells activate the unfolded protein response (UPR) to restore protein homeostasis and maintain ER folding capacity. However, if ER stress persists and remains unresolved, the UPR can trigger apoptosis, leading to cell death. Monitoring UPR levels is therefore essential for maintaining high productivity and ensuring product quality.

In our study we have carried out a comprehensive whole cell proteomic and ubiquitinated proteomic investigation of CHO cells in a range of bioprocess conditions such as temperature shift, culture longevity, waste build-up, and high and low productivity, to identify potential protein biomarkers of ER-stress. Using open-source R packages, the proteomic data compiled from the various stress conditions was analysed using PCA and regression analysis; and randomForest and Support Vector Machine machine learning models. We identified a panel of ER-stress related biomarkers, including well known markers of ER-stress such as BiP and ATF6, and developed a targeted mass spectrometry based assay, i.e. parallel reaction monitoring (PRM), to monitor stress related biomarkers in CHO cell cultures. Using PRM, we were able to accurately quantify a well-defined set of peptides associated with a panel of protein biomarkers linked with ER stress.

Understanding UPR regulation under varying culture conditions could establish this assay as a screening tool for assessing media composition or process changes, and also during cell line development for the selection of high-performing clones.

Quantitative analysis of proteomic differences in clonal suspension MDCK cell lines infected with human influenza A virus

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Suspension MDCK cells are a highly relevant cell substrate for scalable and efficient production of influenza A virus (IAV). Considering the high heterogeneity within conventional cell populations, the development of clonal cell lines has resulted in candidates with superior growth characteristics and high IAV yields (Zinnecker et al., 2024). However, proteomic analysis could help to further understand specific properties of such clonal cell lines and help to identify best producers for vaccine manufacturing.

In the present study, we compare proteome alterations between two IAV-infected suspension MDCK cell clones (C59 & C113, Sartorius, Germany) to elucidate differences in cell growth, size, metabolism and IAV productivity. Using advanced mass spectrometry, a total of 5177 host cell proteins were detected in both cell clones. Protein network analysis of the differentially expressed proteins with respect to cell growth revealed that fatty acid oxidation and branched-chain amino acid degradation were upregulated in the highly productive cells, whereas steroid biosynthesis and DNA replication were more active in the faster growing cells. After infection, 122 proteins were significantly upregulated ($\log_2$ fold change ≥ 1) in the high-producing cell line, including proteins associated with membrane trafficking. In addition, proteins that have cross-links to the IAV-NS1 protein and proteins that support virus production were identified. In addition, 98 proteins associated with antiviral signaling pathways such as Met and TNF signaling were downregulated ($\log_2$ fold change ≤ 1). In the less producing cell line, 77 proteins were downregulated and 57 upregulated after infection. Here, RNA metabolism seemed to be downregulated, whereas the TCA cycle and stress response were upregulated.

Overall, we were able to identify important differences between a fast-growing and a high-producing clonal MDCK cell line, revealing potential bottlenecks and providing further insights into the efficient production of IAV in cell cultures.

Enhancing Control of mAb Production in Perfusion Bioreactors using Continuous Monitoring

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Perfusion bioreactors are emerging as a powerful platform for continuous monoclonal antibody (mAb) production in Chinese Hamster Ovary (CHO) cells with advantages in productivity, product quality, and operational efficiency. However, conventional perfusion processes typically rely on offline sampling and delayed analytics to guide process decisions. This piecewise approach often leads to reactive rather than proactive control, where flow rates, feed concentration and downstream adjustments are only made after metabolite imbalances or performance issues arise. In this study, we present the design of an advanced perfusion bioreactor platform built around the Sartorius Biostat® B-DCU 2L bioreactor system coupled with a Repligen ATF (alternating tangential flow) controller. Enhanced with integrated online sensors, the system enables real-time process awareness and more responsive control. Alongside standard sensors (pH, dissolved oxygen, temperature) the system incorporates a multi-spectrum UV sensor and a capacitance probe to provide continuous data on metabolic state, nutrient consumption, cell density, and product yield. The central aim of this work is to demonstrate how a real-time sensing strategy can overcome the limitations of traditional offline workflows. Leveraging the Sartorius Cellca 2 CHO strain for maximum protein production, the system uses continuous monitoring to enable early detection of metabolic shifts to support dynamic control of perfusion rates, nutrient supplementation, and process parameter shifts including temperature and dissolved oxygen. This level of process visibility has the potential to boost productivity over extended culture periods, improve overall titre, and maximize space-time yield while simultaneously informing downstream operations. By transitioning from manual adjustments to adaptive control, this platform reflects a broader push toward integrated and data-driven biomanufacturing. Future work will focus on leveraging these sensor outputs to develop predictive control algorithms and fully closed-loop systems for robust, scalable mAb production.