13th Protein Expression in Animal Cells

Las Arenas Hotel
Valencia
Spain
September 24-28, 2017

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MESSAGE FROM THE CHAIR – PEACE

Dear Participants,

It is with great pleasure that the organizing committee welcome you to the 13th Protein Expression in Animal Cells (PEACe) Conference. The 13th conference consists of 7 scientific sessions and an industrial workshop covering cutting edge new science in animal cell expression and related areas. The extensive program has been designed by the committee to give as many early career scientists the opportunity to give oral presentations alongside the high profile and keynote speakers that we have attracted to the meeting. We thank all the speakers for agreeing to participate and those who submitted abstracts. Although there was not sufficient space in the program to give oral presentations to all those who wished speak, the extensive array of posters give multiple opportunities for authors to present their work and we encourage everyone to visit the posters and discuss the work with the authors.

The PEACe conference series started in 1992 and since then meetings have been held every two years. Over the past 25 years, the conference focus has adapted to address evolving developments in cell culture and recombinant protein expression technologies. The organizing committee and recognized scientific leaders in the field will serve as Session Co-Chairs at this meeting. Our Co-Chairs have worked hard to help identify speakers, review submitted abstracts, and encourage participation at the conference. This is a vital contribution to the success of the program that takes time outside of everyone's 'day job' and is very much appreciated. The PEACe conference operates as a not-for-profit organization and any funds remaining from this meeting will be used to support the next meeting in 2019. More details around this will be discussed at the General Assembly meeting during the conference.

The organizing committee would especially like to thank our sponsors. Their generosity enables us to hold this conference. Please take every opportunity to visit their booths and displays. Some of our funding initiatives have provided student bursaries for a number of graduate students to attend and present their research at the conference. The committee would also like to thank Bruno Begin, who has been the conference manager since 2003, for expert guidance and organization to keep the committee on track. I would also like to thank the rest of the organizing committee for undertaking all their assigned tasks in a timely manner and working together so well.

Finally, the location, the scientific program, and the social events have been designed to encourage networking and enhance scientific discussion amongst participants. It is anticipated that the oral and poster presentations in this inspiring environment will generate new ideas and the initiation of fruitful novel research projects and collaborations. Participants and their engagement make a scientific meeting and it is up to us all to ensure this is a successful conference that we all reflect upon favorably. We thank you for participating in the conference and wish you a successful and productive PEACe meeting in Valencia.

Mark Smales
Chair, 13th PEACe Meeting

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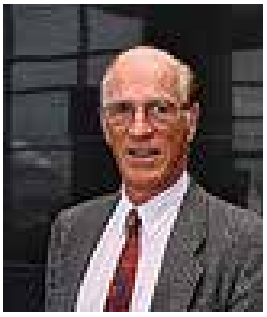

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CONFERENCE SCHEDULE

PROGRAM 13TH PEACE – SUNDAY, SEPTEMBER 24, 2017

17:00-18:00	Registration		*Foyer of Neptuno*
18:00-20:00	Welcome reception		
20:00-20:30	Tom Kost	GlaxoSmithKline	United States
	TIPS for Effective Presentations		
20:30-21:30	Jose Ferri	Valencia Guias	Spain
	A presentation of Valencia		

PROGRAM 13TH PEACE – MONDAY, SEPTEMBER 25, 2017

08:30-10:25 **CELL ENGINEERING 1 PRESENTED BY MIRUS BIO** *Sofia Auditorium*

KIM STUTZMAN-ENGWALL, CONSULTANT, USA
LINDA LUA, UNIVERSITY OF QUEENSLAND, AUSTRALIA

08:45	Yuansheng Yang	Bioprocessing Technology Institute	Singapore
	1,1 - CHO Targeted Integration (TI) cell line development and cell engineering for rapid, high level production of mAbs with controlled glycosylation		
09:15	Alan Dickson	University of Manchester	United Kingdom
	1,2 - Use of a Protein Engineering Strategy to Overcome Limitations in the Production of 'Difficult to Express' Recombinant Proteins		
09:45	Jennitte Stevens	Amgen	Canada
	1,3 - Mammalian Cell Line Development, Miniaturization, Automation, and Analysis using the Berkeley Lights Platform		
10:05	Nicolas Marx	University of Natural Resources and Life Sciences Vienna	Austria
	1,4 - CRISPR-based targeted epigenetic editing in Chinese Hamster Ovary cells		
10:25-10:55	Coffee Break		Foyer

10:55-11:35 **CELL ENGINEERING 2 PRESENTED BY MIRUS BIO** *Sofia Auditorium*

KIM STUTZMAN-ENGWALL, CONSULTANT, USA
LINDA LUA, UNIVERSITY OF QUEENSLAND, AUSTRALIA

10:55	James Budge	University of Kent	United Kingdom
	2,5 - Engineering Cellular Lipid Biosynthesis to Enhance Cell Growth and Difficult to Express Recombinant Protein Expression and Quality in CHO cells		
11:15	Hooman Hefzi	University of California San Diego	United States

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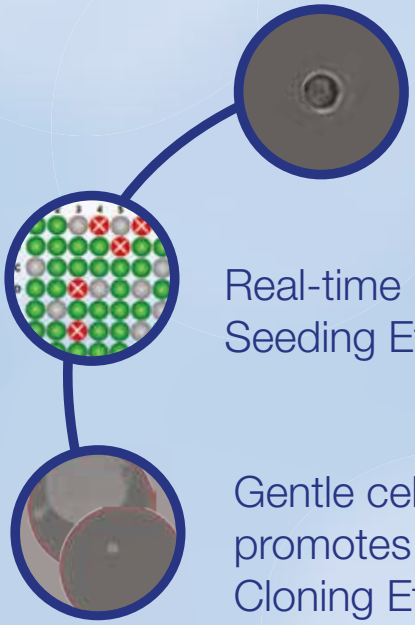

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2,6 - Elimination of lactate production in Chinese hamster ovary (CHO) cells via genetic engineering

11:35 - 12:35 **PEACE KEYNOTE LECTURE** *Sofia Auditorium*

11:35 Scott Estes Codiak Biosciences

The Potential of Exosome Based Therapeutics and the Challenges of Making Them a Reality

12:35-14:00 Lunch

14:00-15:20 **PROTEIN ENGINEERING 1** *Sofia Auditorium*

KIM STUTZMAN-ENGWALL, CONSULTANT , USA
RENÉ HUBERT, AGENSYS, USA

14:00 Umesh S. Muchhal Xencor Inc. United States

3,1 - Concept to Clinic : Development of Fc-containing XmAb® Bispecific Antibodies for Immunotherapy

14:30 Karl Griswould Dartmouth United States

3,2 - Engineering, Design, and Production of Antibacterial Enzymes to Treat Human Disease

15:00 Jennifer Keen Medimmune United Kingdom

3,3 - Antibody expression engineering through modelled rational design and mutagenesis

15:20-15:50 Coffee Break

15:50-16:50 **PROTEIN ENGINEERING 2** *Sofia Auditorium*

KIM STUTZMAN-ENGWALL, CONSULTANT , USA
RENÉ HUBERT, AGENSYS, USA

15:50 Amine Kamen McGill University Canada

4,4 - Accelerated Generation of Candidate Influenza Viral Vaccine To Respond to Pandemic Situations

16:10 Davide Vito University of Kent United Kingdom

4,5 - Translational Engineering Through The Non-Coding Genome in CHO: A transfer RNA and Long Non-Coding RNA Perspective

16:30 Catherine Huntington Medimmune United Kingdom

4,6 - Drug targets protein sequence variability : which one to select in drug discovery programs ? Using Haplosaurus, a public access Bioinformatics tool for rapid analysis of drug target haplotypes distribution.

17:00-19:00 Cocktail & Posters

20:00-21:00 Dinner

PROGRAM 13TH PEACE – TUESDAY, SEPTEMBER 26, 2017

08:30-09:50 **EXPRESSION SYSTEMS 1 PRESENTED BY MERCK** *Sofia Auditorium*

ALAN DICKSON, UNIVERSITY OF MANCHESTER, UK

08:30	Silvia Zucchelli	Università del Piemonte Orientale	Italy
	5,1 - SINEUPs: a new class of antisense long non-coding RNAs that specifically activate translation of targeted proteins		
09:00	Gary Pettman	Medimmune	United Kingdom
	5, 2 - Advances in CHO transient expression new areas of scale, productivity and novel applications		
09:30	Tanya Knight	University of Kent	United Kingdom
	5,3 - Inhibiting Protein Degradation Processes for Improved Recombinant Protein Production from CHO Cells		

09:50-10:10 Coffee Break

10:10-11:10 **EXPRESSION SYSTEMS 2 PRESENTED BY MERCK** *Sofia Auditorium*

ALAN DICKSON, UNIVERSITY OF MANCHESTER, UK

10:10	Meg Tung	Genentech	United States
	6,4 - Seed train culture conditions can affect production culture performance: A case study for a CHO cell culture process		
10:30	Margitta Schürig	Helmholtz Centre for Infection Research	Germany
	6,5 - Expression of Viral Surface Proteins for Functional and Structural Analysis		
10:50	Paul Kelly	Dublin City University	Ireland
	6,6 - Process-relevant concentrations of the leachable bDtBPP impact negatively on CHO cell production characteristics		

11:10-11:20 Short Break

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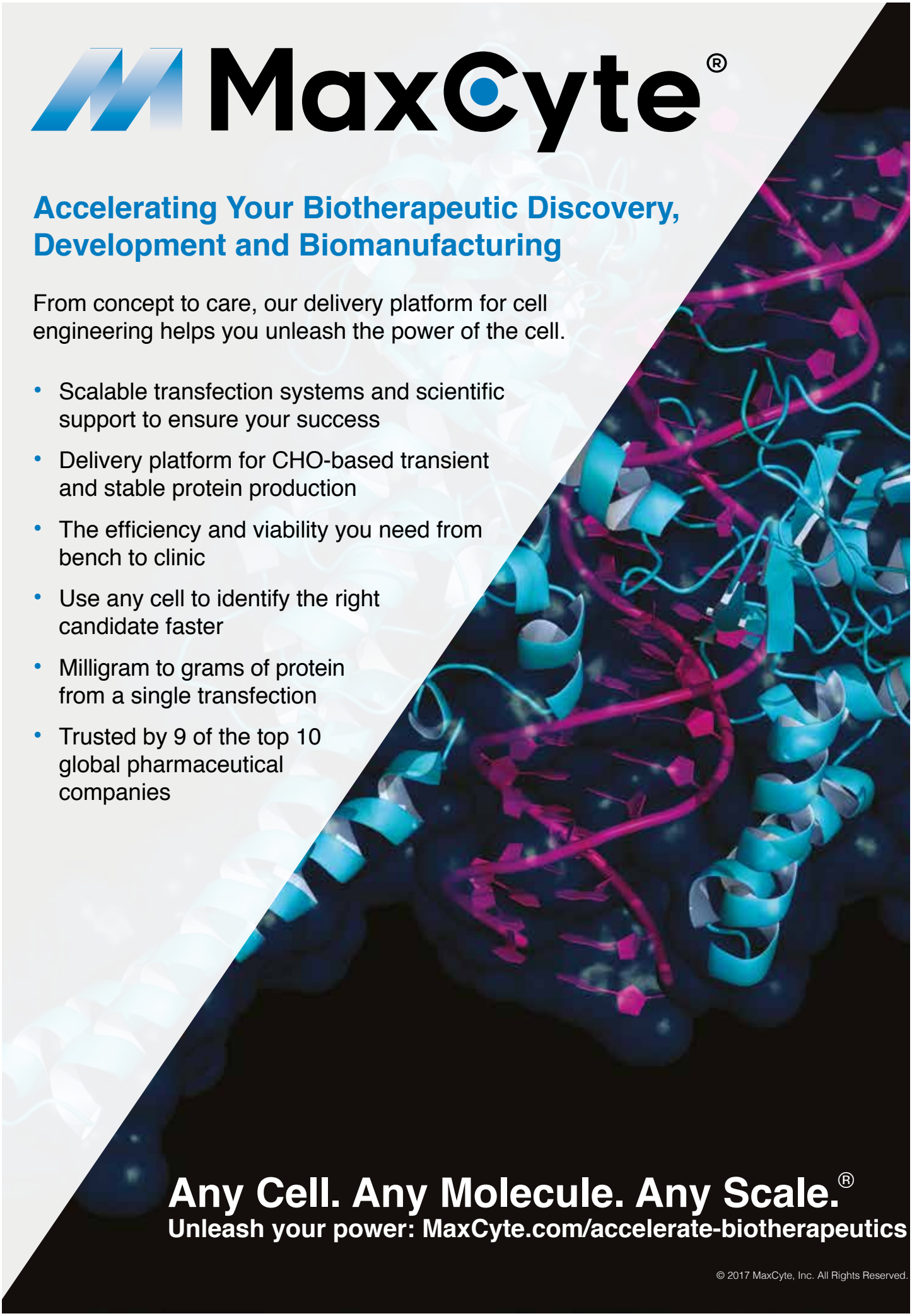

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11:20-13:05 **INDUSTRIAL WORKSHOPS** *Sofia Auditorium*

TOM KOST, CONSULTANT, USA

11:25	Jonathan Zmuda	Thermo Fisher Scientific	United States

7,1 - Latest Developments in Scalable, High-Titer Transient Protein Expression in the ExpiCHO Expression System

11:45	Ian Taylor	Solentim	United Kingdom

7,2 - A complete suite of instruments and software for clone generation, assurance and documentation in cell line development

12:05	Joseph Abad	Maxcyte	United States

7,3 Accelerating Biotherapeutic Development through Simultaneous High-Titer, CHO Transient Expression & Generation of High-Yield Stable Cell Lines Using Scalable Transfection

12:25	Ferenc Boldog	ATUM (formerly DNA2.0)	United States

7,4 - Novel Transposase Tools for Cell-Line Engineering

12:45	Tibor Anderlei	Adolf Kühner AG	Switzerland

7,5 - Orbital Shaken Bioreactors: The Scale Up Fast Track (from mL to 2500L)

13:00-14:00	Lunch
14:30-18:00	Valencia Tour
20:00-22:00	Dinner in restaurants on the beach

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PROGRAM 13TH PEACE – WEDNESDAY, SEPTEMBER 27, 2017

08:30-10:10 **DIFFICULT-TO-EXPRESS PROTEINS 1** *Sofia Auditorium*

GEORG SCHMID, FORMERLY AT F. HOFFMANN-LA ROCHE , SWITZERLAND
BERNIE SWEENEY , UCB , UK

08:30 Nicola Burgess-Brown — University of Oxford — United Kingdom
8,1 - Expression Screening and Production of Human Proteins for Structural and Functional Studies

09:00 Claudio Stustmann — Roche Pharma Research — Germany
8,2 - Expression and Characterization of Bispecific Antibodies @ Roche

09:30 Nikola Strempel — CEVEC Pharmaceuticals GmbH — Germany
8,3 - CAP-Go.2 cell line: Improved O-glycosylation for significantly prolonged plasma half-life of human recombinant C1 Inhibitor

09:50 Jill Cai — WuXi Biologicsw — China
8,4 - Strategies to Develop Difficult to Express Protein Therapeutics in CHO Cells

10:10-10:40 Coffee Break

10:40-11:20 **DIFFICULT-TO-EXPRESS PROTEINS 2** *Sofia Auditorium*

GEORG SCHMID, FORMERLY AT F. HOFFMANN-LA ROCHE , SWITZERLAND
BERNIE SWEENEY, UCB , UK

10:40 Valentina Ciccarone — MacroGenics, Inc. — United States
9,5 - Cell Line Development for Expression of Bispecific DART® and Trispecific TRIDENTTM Molecules

11:00 Sandra Markovic-Müller — leadXpro AG — Switzerland
9,6 - Production of crystal grade membrane proteins for biophysical methods to facilitate structure based drug discovery

11:20-12:20 **PEACE KEYNOTE LECTURE**

11:20 Nicole Borth — BOKU University of Natural Resources and Life Sciences — Austria
CHO in times of 'omics or 'There is much pleasure to be gained from useless knowledge' (Bertrand Russel, philosopher)

12:20-13:50 Lunch

13:50-15:10 **PROTEIN QUALITY CONTROL 1** *Sofia Auditorium*

GEORG SCHMID, FORMERLY AT F. HOFFMANN-LA ROCHE , SWITZERLAND
REINGARD GRABHERR, UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES, AUSTRIA

13:50	Yves Durocher	Mammalian Cell Expression, National Research Council Canada	Canada

10,1- Fc receptors biotinylation for oriented immobilization on surface plasmon resonance biosensors

14:20	Nico Callewaert	Center for Medical Biotechnology, VIB and Gent University	Belgium

10,2 - Glycosylation customizaton of eukaryotic biopharmaceutical expression hosts

14:50	Moritz Wolf	ETH Zürich	Switzerland

10,3 - Small-scale development and optimization of product quality control in mammalian cell perfusion cultures

15:10-15:40 Coffee Break

15:40-16:40 **PROTEIN QUALITY CONTROL 2** *Sofia Auditorium*

GEORG SCHMID, FORMERLY AT F. HOFFMANN-LA ROCHE , SWITZERLAND
REINGARD GRABHERR, BOKU UNIVERSITY OF NATURAL RESOURCES AND LIFE SCIENCES, AUSTRIA

15:40	Bernhard Sissolak	University of Natural Resources and Life Sciences	Austria

11,4 - Determination of monoclonal antibody charge heterogeneity directly from cell culture supernatants for quality by control realization

16:00	Kahina Mellahi	Ecole Polytechnique Montréal	Canada

11,5 - Process Development for an Inducible CHO Cell Line

16:20	Nicola MacKinnon	Merck	Switzerland

11,6 - Modulation of glycosylation by media design

16:45-17:30 General Assembly PEIS

17:30-19:00 Cocktail & Posters

19:30-24:00 Gala Dinner

PROGRAM 13TH PEACE – THURSDAY, SEPTEMBER 28, 2017

09:00-10:10 **INNOVATION VIA PREDICTION 1** *Sofia Auditorium*

HELEN FAUSTRUP KILDEGAARD, TECHNICAL UNIVERSITY OF DENMARK , DENMARK
JONATHON SOQUET, MERCK SERONO, SWITZERLAND

09:00	Nathan Lewis	University of California	United States
	12,1 - Engineering cell metabolism to enhance protein production		
09:30	Massimo Morbidelli	ETH Zürich	Switzerland
	12,2 - Perfusion reactors in the context of integrated manufacturing of therapeutic proteins		
09:50	Benjamin Bayer	University of Natural Resources and Life Sciences	Austria
	12,3 - Improved on-line monitoring of mammalian bioprocesses via fluorescence-based softsensors		

10:10-10:40 Coffee Break

10:40-11:40 **INNOVATION VIA PREDICTION 2** *Sofia Auditorium*

HELEN FAUSTRUP KILDEGAARD, TECHNICAL UNIVERSITY OF DENMARK , DANEMARK
JONATHON SOQUET, MERCK SERONO, SWITZERLAND

10:40	Michael Butler	National Institute for Bioprocessing Research and Training	Ireland
	13,4 - Dielectric monitoring of mammalian cells in a bioreactor		
11:00	Eduard Puente-Massague	Universitat Autònoma de Barcelona	Spain
	13,5 - Nanoscale characterization coupled to multi-parametric modelling of High Five cells transient transfection		
11:20	Kulwant Kandra	University of Natural Resources and Life Sciences	Austria
	13,6 - Application of Hybrid Modeling for Process Prediction of Mammalian Cell Culture Bioprocesses		

11:40-13:00 Lunch

13:00-15:10 **VACCINES AND VACCINE PRODUCTION** *Sofia Auditorium*

KENNETH LUNDSTROM, PAN THERAPEUTICS, SWITZERLAND
LAURA A. PALOMARES, UNIVERSIDAD NATIONAL AUTONOMA DE MEXICO, MEXICO

13:00	Leda Castilho	COPPE Universidade Federal do Rio de Janeiro	Brazil

14,1 - Development of technologies for the production of zika and yellow fever vaccine candidates

13:30	Dieter Palmberger	ACIB - Austrian Center of Industrial Biotechnology	Austria

14,2 - Process development for a flexible vaccine vector platform based on measles virus

13:50	Lovrecz George	CSIRO, BioManufacturing	Australia

14,3 - Platform technologies to assist the development of novel vaccines against emerging epidemic diseases

14:10	Irene González	Universitat Autònoma de Barcelona (UAB)	Spain

14,4 - Characterization of virus-like particle (VLP) production platform towards transient gene expression strategy

14:30	Joseph Shiloach	NIDDK/NIH	United States

14,5 - Improved protein expression from mammalian cells by targeting genes identified through genome scale sirna screening

14:50	Alexander Nikolay	Max Planck Institute for Dynamics of Complex Technical Systems	Germany

14,6 - Process intensification: Yellow fever virus and zika virus production in suspension cells

15:10-15:30 Awards and close

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ORAL PRESENTATIONS

1,1

CHO Targeted Integration (TI) cell line development and cell engineering for rapid, high level production of mAbs with controlled glycosylation

Yuansheng Yang
Animal Cell Technology, Bioprocessing Technology Institute, Singapore

Chinese hamster ovary (CHO) cell lines used for manufacturing monoclonal antibodies (mAbs) are traditionally generated by the random integration of the plasmid vectors into genome. The varied activity of each integration site results in significant clonal variation and thousands of clones need to be laboriously screened to identify suitable production clones with high titer, stable productivity and desirable product quality. To further improve the performance of established cell lines, cell engineering can be performed by knockout, knockdown and overexpression of one or more genes. The effectiveness of cell engineering could be impeded by inability to control the modified genes at optimal expression levels. At Bioprocessing Technology Institute, we have developed a FLP/FRT recombinase mediated cassette exchange (RMCE)-based targeted integration platform to avoid random integration for cell line development and to accurately control gene expression level for cell engineering. Accurate control of gene expression for cell engineering is achieved by combining our targeted integration technology with DNA regulatory elements of different strengths that we have also developed. We are able to generate high mAb producing CHO cell lines in eight weeks by isolating only tens of clones using this platform. We have also used these tools to engineer the glycosylation pathway in CHO cells for three applications: (1) Precise control of GnTIII expression in CHO cells for producing mAbs with low fucose for enhanced ADCC, (2) Production of mAbs with more homogeneous glycan structures, and (3) Developing biosimilar antibody producing cell lines with high similarity to innovator drugs.

Biography

Dr. Yuansheng Yang received his doctorate degree from Vanderbilt University's Department of Chemical Engineering in 2005. Upon graduation, he joined the Animal Cell Technology group, Bioprocessing Technology Institute (BTI) in Singapore as a research scientist. He had been an adjunct assistant professor of Nanyang Technological University from 2012 to 2015. He currently leads a group working on vector design, cell line development, cell engineering, and bioprocess optimization to enhance the yield and quality of biopharmaceutical drugs produced using CHO cells. He has published over 40 papers and filed several patents related to developing cell lines for high level expression of monoclonal antibodies and other recombinant proteins.

1,2

Use of a Protein Engineering Strategy to Overcome Limitations in the Production of 'Difficult to Express' Recombinant Proteins

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In recent years there has been an increased drive towards the production of recombinant proteins in large amounts using rapid cell culture processes. However, certain recombinant targets can be "difficult to express" and require extensive process optimisation. In this study, using model proteins from the Tissue Inhibitors of Metalloproteinase (TIMP) family, we present a systematic screen of the protein expression pathway to characterise the molecular mechanisms that limit production of recombinant proteins with high sequence homology. TIMP-2, -3 and -4, share significant sequence/structural homology, but show differences when produced in a transient Chinese Hamster Ovary (CHO) expression system. The data showed all three TIMPs were detectable at the mRNA and protein level within the cell but only TIMP-2 was secreted in appreciable amounts into the culture medium. Analysis of the intracellular protein suggested the post-translational processing of poorly expressed TIMPs was limiting. A protein engineering approach was employed to overcome challenges in the production of these 'difficult to express' TIMP proteins. The addition of a furin-cleavable pro-sequence from a secretory growth factor to TIMP-3 (non-secreted) and TIMP-4 (poorly secreted), resulted in their successful secretion and presents a novel strategy to increase the production of 'difficult-to-express' recombinant targets.

1,3

Mammalian Cell Line Development, Miniaturization, Automation, and Analysis using the Berkeley Lights Platform

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The biologic manufacturing process begins with establishing a clonally derived, stable production cell line. Generating a highly productive cell line is resource intensive and typically involves long timelines due to screening of large numbers of candidates and time required to adapt cell lines to suboptimal conditions. Often, miniaturization and automation strategies are employed to allow for reductions in resources and higher throughputs. However, we are beginning to reach the physical limitations of this approach. New nanofludic technologies offer solutions to move past these limits. One integrated platform is now being offered from Berkeley Lights Inc. The approach miniaturizes cell culture volumes (105 times smaller) through growing cells on custom nanofluidic chips. Cells are manipulated on a single cell level though use of OptoElectronic Positioning (OEP) technology, which utilizes projected light patterns to activate photoconductors that gently, repels cells. Common cell culture tasks can be programmed though software, and allow for thousands of cell lines to maintained at once. Finally, cultures can be interrogated for productivity and growth characteristics while on the chip. In this communication, we attempted to assess whether it was feasible to perform key cell line development work on this platform. We demonstrate that commercial production CHO cell lines can cultured on this environment. We next show that sub clone isolation, recovery, and selection can be achieved with very high efficiency. Finally, we demonstrate the ability to load transfected populations into the instrument and extract out viable clonally-derived production lines. Overall, this technology has potential to dramatically alter current cell line development workflows through the replacement of laborious manual processes with nanofludics, software and automation. Potential future applications can be focused toward increasing capacity, decreasing resource requirements, improving cell line quality, and decreasing cycle times

1,4

CRISPR-based targeted epigenetic editing in Chinese Hamster Ovary cells

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Extensive knowledge has been gathered by applying and generating –omics techniques and data towards a holistic understanding of the Chinese Hamster Ovary (CHO) cell's regulatory network. However, these data are far from universally explanatory. The epigenome, i.e. the genetic signature that contributes to modulation of gene expression, has not yet been fully explored. Interestingly, DNA methylation at the CMV promoter has been reported to result in a loss of productivity in CHO cells [1]. Additionally, a genome-wide study showed that the effect of DNA methylation is site-specific and dependent on the gene element [2]. Traditionally, epigenetic control in cell cultures has been executed by exposure to small inhibitor or enhancer molecules, which is inappropriate for controlling the expression of specific genes. A new and more elegant way to study epigenetic effects on CHO performance parameters would be the methylation or demethylation of specific promoters at their endogenous loci. Towards this aim, we have constructed CRISPR-based epigenetic editing tools that induce site-specific DNA methylation or demethylation rather than double strand breaks.

For proof of concept of transgene expression modulation, the current design targets two glycosylation enzymes aiming to turn off expression of α-(1,6)-fucosyltransferase and to induce expression of the silenced α (2,6)-sialyltransferase. This epigenetic tool set would not only allow to build a new layer of cell control that complements existing techniques (e.g. genome engineering), but also to enable a more sensitive investigation of gene function by induction and repression of genes without altering the DNA sequence. Finally, other than gene knockout or overexpression studies, the modulation would be readily reversible.

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[2] Feichtinger, J. et al. Biotechnology and Bioengineering (2016). doi:10.1002/bit.25990

2,5

Engineering Cellular Lipid Biosynthesis to Enhance Cell Growth and Difficult to Express Recombinant Protein Expression and Quality in CHO cells

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Chinese hamster ovary (CHO) cells are the leading mammalian cell host for the production of recombinant biotherapeutics, as they are robust and capable of human-like post-translational modifications. Whilst CHO hosts and bioprocesses are well established for the production of monoclonal antibodies, specifically IgGs, the emergence of new-format, non-native therapeutic protein molecules such as fusion proteins and multispecific antibodies as well as development of multimeric glycoprotein therapies, present new challenges to these established expression systems and are often difficult to express (DTE) in CHO or any other expression system. These next-generation biologics (NGBs), are frequently being developed for diseases with no current treatment. Inefficient manufacturing processes equate to: an inability to evaluate the potential of these molecules as drugs; a high failure rate; and a high cost-of-goods. We therefore embarked upon an investigation of an approach to reprogramming the CHO cells' ability to 'cope' with DTE proteins by globally enhancing the secretory pathway capacity and quality-control attributes. We have engineered lipid biosynthesis components in the CHO cells. Upon modification of lipid metabolism in CHO cells, we have observed improved yield and quality of a number of different DTE molecules. Furthermore, this approach has been applied to successfully enhance production of existing recombinant CHO cell lines.

2,6

Elimination of lactate production in Chinese hamster ovary (CHO) cells via genetic engineering

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Background and novelty

Toxic bioproducts such as lactate and ammonia have posed considerable challenges in bioprocessing since they limit cell growth and impact critical quality attributes by altering the regulation of biosynthetic enzymes. To mitigate the negative effects of lactic acid accumulation and control the culture pH, base is added to the media during the course of a bioprocess. However, the base addition increases osmolarity over time, which also negatively impacts the bioprocess by inhibiting growth and shortening the length of time in which the cells can produce the recombinant protein. Thus, it is of great interest to reduce or eliminate lactate production. We report the first complete elimination of lactate production in CHO cells via genetic engineering.

Experimental approach

Knockout of necessary genes was accomplished via CRISPR/Cas9 and verified at multiple levels. The knockouts were introduced into both nonproducing and producing CHO cell lines.

Batch and fedbatch were carried out for both producing and nonproducing cell lines to characterize the phenotypic impact of the knockouts on important cellular attributes.

Results and discussion

In fedbatch, nonproducing cells had a prolonged growth period to higher VCDs than the wildtype CHO-S cells, likely due to lower osmolarity as base addition to maintain optimal pH was unnecessary. We additionally observed higher productivity and titer in producing cells. Deeper characterization of the cells in both batch and fedbatch is currently underway. We will present results on the effect of the knockout on growth, protein production, product quality and nutrient utilization for all experiments.

Keynote presentation

The Potential of Exosome Based Therapeutics and the Challenges of Making Them a Reality

Scott Estes

Head of Upstream Process Development & Manufacturing, Codiak Biosciences., MA USA

Exosomes are small vesicular bodies of roughly 50-150nm that arise from the endosomal compartment. They are released from virtually all cells and are thought to have evolved early in the tree of life. Although initially discovered in the early 80s, exosomes remained relatively obscure for many years. This began to change with the seminal discoveries that exosomes played a role in antigen presentation and contained functional nucleic acid that could alter gene expression in recipient cells. With this realization came a significant upsurge in exosome research and within the last few years, burgeoning translational efforts to harness their capabilities for diagnostic and therapeutic applications. Regarding the latter, these efforts have typically taken two paths; leveraging the potential disease modifying properties of the endogenous cargo or alternatively harnessing the cellular delivery capabilities of exosomes to deliver an exogenous therapeutic payload. This talk is intended to explore the potential applications of exosomes in medicine and the steps underway to build the robust bioprocessing platforms and analytical capabilities necessary to produce safe, efficacious and scalable exosome based products capable of meeting unmet medical needs.

3,1

Concept to Clinic : Development of Fc-containing XmAb® Bispecific Antibodies for Immunotherapy

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Bispecific antibodies offer unique advantage of eliciting biological effects that require simultaneous binding to two targets. Efforts in bispecific antibody design have historically been frustrated by poor molecular stability, difficulties in production and short in vivo half-life. We have developed a modular bispecific antibody platform employing a heterodimeric Fc region that allows for high stability, easy manufacturability and favorable antibody-like pharmacokinetics. This optimized bispecific format resembles a standard monoclonal antibody, with one of the Fab arms replaced by a stability-optimized scFv. The engineered Fc region enables a molecular distribution heavily skewed toward the desired heterodimer, and allows for easy purification and characterization of the heterodimeric bispecific from minor homodimer side products.

Utility of this plug-and-play format was first explored with T-cell targeting bi-specifics containing an anti-CD3 scFv, a Fab region targeting various tumor antigens, and an Fc region with abolished FcγR binding. These bi-specifics potently target and kill tumor cells via redirected T cell-cytotoxicity while avoiding off-target FcγR-mediated crosslinking and activation of T cells. The cross-reactivity of the anti-CD3 variable region enabled evaluation of pharmacokinetics, pharmacodynamics, and toxicity profiles in cynomolgus monkeys - clearly demonstrating their potent cytotoxic activity and antibody-like serum half-life. Our XmAb format also allows us to tune the potency of T-cell killing potentially improving the tolerability of tumor immunotherapy.

Stable CHO cell lines expressing these bi-specifics were generated for our lead molecules using optimized transfection ratios of the three chains, and produce virtually pure heterodimers at high yields. Downstream purification used standard 3 step process routinely used for monoclonal antibodies, with high process yield and purity. This platform approach has allowed us to take a parallel early development path for multiple programs. We have completed GMP manufacturing for three candidates, and two are undergoing clinical testing. The plug-and-play nature of each domain has enabled the rapid discovery and development of a portfolio of differentiated drug candidates with a diversity of mechanisms, targets and potential disease indications.

3,2

Engineering, Design, and Production of Antibacterial Enzymes to Treat Human Disease

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Drug-resistant bacteria represent an urgent threat to human health, and these dangerous microbial pathogens are a leading cause of death and excess economic burden world-wide. Bacteriolytic enzymes have the potential to treat such drug-resistant infections, but as exogenous proteinaceous molecules, they present the pharma industry with unique design challenges and developmental risks. First, due to their innate function, lytic enzymes can be difficult to produce in standard microbial hosts, yet they likewise have proven poorly suited to production in eukaryotic systems. Second, most lytic enzymes are derived from non-human sources, and they are subject to immune surveillance in the human body. Antidrug immune reactions can alter pharmacokinetics, neutralize therapeutic activity, and can cause more serious adverse events including a range of allergic type reactions and deposition of toxic immune complexes in tissues and vasculature.

One prototypical case study is the bacteriocin lysostaphin, a selective lytic enzyme with potent activity towards the dangerous and wide-spread pathogen Staphylococcus aureus. For more than five decades lysostaphin has undergone extensive drug development efforts, but commercialization has yet to be realized in part due to the enzyme's immunogenicity in diverse animal models and even human subjects. Mitigating immunogenicity risk is therefore central to enabling successful clinical deployment of lysostaphin, and development of a commercially scalable production platform will also be necessary.

We have engineered the lysostaphin gene and protein sequences towards addressing the above limitations to clinical translation and practical application. In this talk we will describe genetic engineering approaches that enable high level expression of fully functional lysostaphin in eukaryotic systems, including the yeast Pichia pastoris, for the purposes of commercial scale production. We will further detail genetic engineering of lysostaphin so as to silence immunogenic T cell epitopes across genetically diverse patient populations. Highlights of the presentation will include preclinical immunogenicity testing in humanized mice, parallel ex vivo cellular immunoassays using human peripheral blood mononuclear cells from diverse human subjects, and in vivo infection models in which we demonstrate superior efficacy relative to wild type lysostaphin.

3,3

Antibody expression engineering through modelled rational design and mutagenesis

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The developability and manufacture of successful therapeutic antibodies frequently depends on high levels of protein expression. Two related antibodies Ab001 and Ab008 were found to have noticeable differences in titre when expressed in a Chinese hamster ovary (CHO) cell line. To investigate the reduced titre of Ab001 compared to Ab008, single-chain gene expression vectors were used in co-transfections together with structural analysis to locate the problem to the light chain variable region (VL). Two methods were explored to improve the titre of Ab001. One was the mutagenesis of 9 single amino acid residues in the VL to revert them back to the equivalent residues in Ab008. Only two of the mutants showed a statistically significant increased titre but they did not restore full activity as compared to Ab008. Therefore a second in silico structural approach was then taken to try to understand the differences between Ab001 and Ab008 protein structure. A structural model was generated and five more VL variants were designed and tested. In stable cell lines the most improved mutation, D53N, produced expression levels ~25 fold higher than Ab001. None of the structurally-designed VL mutants affected potency. This strategy, using a structural-guided design could be used in future to make informed changes to amino acids in specific positions to increase the expression of problematic proteins.

4,4

Accelerated Generation of Candidate Influenza Viral Vaccine To Respond to Pandemic Situations

Ernest Milián , Johnny Montes, Emma Petiot, Sven Ansorge and Amine Kamen

Over the last decade, public health authorities invested massively to support the development of rapid responses to influenza pandemic threats. As an alternative to egg-based manufactured influenza vaccines, two cell culture-based novel influenza vaccines (FluBlok, Protein Science Corp. and Flucelvax from Novartis) were approved. Other seasonal and pandemic influenza candidate vaccines are in late phases clinical evaluations. The most advanced approaches rely on Viral-like particle (VLP) strategies as proposed with VLPs produced in insect cell cultures (Novavax) or plant cells (Medicago). Whereas the quest for a universal influenza vaccine is still a dominant line of research in many laboratories.

Despite major advances in developing capacities and alternative technologies to egg-based production of influenza vaccines, responsiveness to an influenza pandemic threat is limited by the time it takes to generate a Candidate Viral Vaccine (CVV) as reported by the 2015 WHO Informal Consultation report titled “Influenza Vaccine Response during the Start of a Pandemic”.

In previous work, we have shown that HEK-293 cell culture in suspension and serum free medium is an efficient production platform for cell culture manufacturing of influenza candidate vaccines. This report, took advantage of, recombinant DNA technology using Reverse Genetics of influenza strains, and advances in the large-scale transfection of suspension cultured HEK-293 cells. We demonstrate the efficient generation of H1N1 with the PR8 backbone reassortant under controlled bioreactor conditions in two sequential steps (transfection/rescue and infection/production). This approach could deliver a CVV for influenza vaccine manufacturing within two-weeks, starting from HA and NA pandemic sequences. Furthermore, the scalability of the transfection technology combined with the HEK-293 platform has been extensively demonstrated at > 100L scale for several biologics, including recombinant viruses.

Thus, this innovative approach is better suited to rationally design and mass produce the CVV within timelines dictated by pandemic situations and produce effective responsiveness than previous methodology

4,5

Translational Engineering Through The Non-Coding Genome in CHO: A transfer RNA and Long Non-Coding RNA Perspective

Davide Vito, Mark Smales

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Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides without an annotated coding potential and have emerged in the last few years as key regulators in many biological processes, ranging from epigenetic variations to splicing and selective microRNA regulation of translation. The aim of our work is to assess the role of lncRNAs in mammalian cell factories, where the investigation of these molecules is in its infancy. We initiated our study by comparing the coding and non-coding transcriptome of a suspension Chinese hamster ovary (CHO) cell line grown in suspension cultured in batch and fed-batch mode. We determined cell growth and viability as our key phenotypic parameters and harvested samples at two different time points throughout the culture for transcriptome analysis. The results revealed up- and down-regulated coding and non-coding transcripts, providing evidence for the first time of a significant role for lncRNAs in CHO cell biology. From this data we have identified a panel of lncRNAs targets for manipulation to improve the growth and productive characteristics of CHO cell lines. This talk will describe the results of these studies.

Alongside these studies, we are determining the impact of transfer RNA (tRNA) variations in terms of abundance and chemical modifications to nucleotides on the production of recombinant proteins and on the cell as a whole. The reliable sequencing and measurement of the abundance of tRNAs have always been impaired as a result of technical limitations, despite being central for basic applications in protein production such as codon optimization and specific enhancement of translation and folding. As new techniques are being developed to overcome these limitations, studies are emerging that indicate new mechanisms of action by which tRNAs regulate translation and potentially change drastically the quantity and quality of recombinant proteins of interest. Here we have adapted a dedicated RNA-seq protocol which allows the analysis of tRNA abundance in industrially relevant cell lines under different conditions, including cold shock and batch and fed-batch cultures. Our analysis has allowed the identification of tRNA variations that will facilitate the rational manipulation of tRNA genes and the optimization of codon sequences based on their presence and availability. Here we discuss the application of this work to optimization of CHO cell factories.

4,6

Drug targets protein sequence variability : which one to select in drug discovery programs? Using Haplosaurus, a public access Bioinformatics tool for rapid analysis of drug target haplotypes distribution.

Catherine Huntington

MedImmune Ltd, Antibody Development & Protein Engineering Dept. Granta Park, Cambridge,

Appreciation of the effect of human genetic variation on pharmacology is growing as drug development becomes increasingly influenced by precision medicine. Natural protein diversity is driven by combinations of co-inherited protein altering genetic variants (protein haplotypes). Drug Discovery programs aim at developing drug molecules to be effective for the widest possible spectrum of protein target haplotypes found in a patient population to bring therapeutic benefit to as many patients as possible. Biotherapeutic targets with a high number of significant protein haplotypes pose the greatest risk with regard to population efficacy coverage. The complexity of inferring protein haplotypes from available databases meant this has up to now been largely challenging and overlooked. We describe here Haplosaurus a novel public resource developed from an industry/academic collaboration. Haplosaurus enables the rapid exploration of cDNA/protein haplotype sequences from the entire 1000 Genomes dataset and subpopulations. We discuss the importance and impact of haplotype analysis in two real-world biologics drug discovery projects. We demonstrate the advantages of using the new resource and discuss its impact for increasing the efficiency of development of Biologics Drugs. We envisage this open resource to be of use to many applications across the biological sciences, its potential application to a whole druggable protein landscape or extension to animal populations for toxicology studies.

5,1

SINEUPs: a new class of antisense long non-coding RNAs that specifically activate translation of targeted proteins

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Despite recent efforts in discovering novel long non-coding RNAs (lncRNAs) and unveiling their functions in a wide range of biological processes their applications as biotechnological or therapeutic tools are still at their infancy. Recently we have discovered a new functional class of natural and synthetic antisense lncRNAs that stimulate translation of sense mRNAs. These molecules have been named SINEUPs since their function requires the activity of an embedded inverted SINEB2 sequence to UP-regulate translation in a gene-specific manner. The overlapping region at the 5' end is indicated as the Binding Doman (BD) and dictates SINEUP specificity; the embedded inverted SINEB2 element is the Effector Domain (ED) and controls enhancement of mRNA translation. By swapping BD, synthetic SINEUPs can be designed to the mRNA of choice representing the first scalable tool to increase protein synthesis of potentially any gene of interest. SINEUPs function in an array of cell lines and can be efficiently directed toward N-terminally tagged proteins, secreted proteins as well as endogenous proteins. Their biological activity is retained in a miniaturized version within the range of small RNAs length. In summary, SINEUPs represent the first scalable tool to increase synthesis of proteins of interest. We propose SINEUPs as reagents for molecular biology experiments, in protein manufacturing as well as in therapy of haploinsufficiencies.

5,2

Advances in CHO transient expression – new areas of scale, productivity and novel applications

Gary Pettman

Medimmune, UK

MedImmune has developed a proprietary high yielding, scalable and easy to use CHO cell based transient expression system. The system is used routinely and very successfully for early stage material supply for projects within the R&D organisation at both AstraZeneca and MedImmune.

To further build on the success achieved to date with the system, there is a continued desire to optimise the process even further in a number of areas, including; increasing expression levels and increasing scalability. As part of the continued development, work has been performed to develop a process for use in a 500L Single Use Bioreactor (SUB) and also to implement a more rapid (7 day) but equally productive, transient process. Data will be presented on the scalability of the CHO transient process, including successful scale up to 500L, which, to our knowledge, is an industry first. The new processes show comparable product quality profiles for material generated from stirred SUB and rocking bioreactor cultures at different scales, suggesting that this transient process can be used to rapidly generate 100's of grams of recombinant protein and potentially accelerate drug development timelines.

In addition, data will be presented demonstrating the use and versatility of transient expression for some novel applications which are being utilised to generate proteins with specific molecular attributes and modifications.

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5,3

Inhibiting Protein Degradation Processes for Improved Recombinant Protein Production from CHO Cells

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During the synthesis of secreted recombinant proteins from eukaryotic cell expression systems, such as when using Chinese hamster ovary (CHO) expression systems, the polypeptide is co-translationally fed into the ER where it is folded, assembled if required as in the case of antibodies, and post-translation modifications can be undertaken (e.g. disulphide bond formation, addition of N-linked glycans). Overload of the ER can result in the induction of the unfolded protein response (UPR), alongside the activation of ER-associated degradation (ERAD), key processes initiated to deal with misfolded or unassembled proteins during protein synthesis and assembly in the ER. It is thought that 30% of newly translated polypeptides are targeted for degradation, potentially due to misfolding. This degradation plays a key role in alleviating stress on the ER and incidentally provides a source of 'recycled' amino acids to the cell. Here we have investigated the impact of manipulating protein degradation and turnover via ERAD in recombinant-producing CHO cell lines and then used this information to develop new recombinant cell pools with enhanced product yields and quality. The impact of inhibiting degradation on the survival and overall recombinant productivity of model recombinant CHO cell lines was determined using chemical inhibitors of both the translocation of the polypeptide during ERAD, and the proteasome, which is typically responsible for the final degradation of the polypeptide. A correlation between productivity and susceptibility to inhibition was observed, with a decreased sensitivity observed in higher-producing cell lines. We then used proteasome inhibition as a selection pressure during a cell-pool construction process. Rounds of cell-pool construction were performed with the additional proteasome selection pressure and improved recombinant protein production was observed in those pools with chemical selection compared to control pools with standard selection only.

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6,4

Seed train culture conditions can affect production culture performance: A case study for a CHO cell culture process

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A pattern of slow growth behavior was observed in inoculum train passaging and subsequent production cultures of a CHO cell line expressing a recombinant mAb. The affected runs had progressive slowdown in growth from inoculum train through production, smaller cell sizes, low viability in production culture, and low titer (60-85% decrease from normal). Such a significant shift in phenotypic behavior had not been previously observed in our CHO cell culture development pipeline. Cultures exhibiting this slow-growth phenotype can rapidly "switch" from slow growth to normal growth behavior within a single seed train passage of several days. When investigating the potential root cause it was determined that seed train culture conditions, in particular culture pH, correlated with the observed slow growth phenotype in the subsequent inoculum train and production culture. Numerous seed train and production culture experiments were conducted in controlled bioreactors to identify which culture parameter(s) could affect inoculum train and production culture performance. In addition, different cell biology assays, including cell cycle, apoptosis and ER size analysis by FACS, mRNA transcript level determination by PCR and, Western blotting for analysis of proteins involved in protein secretion/folding, were applied in an effort to better understand the biology of this slow-growth phenotype. Bioreactor studies demonstrated that a slightly lower seed train culture pH could trigger the observed slow growth phenotype in the subsequent inoculum train and production cultures. Of the cell biology assays evaluated, Western blot analysis of binding immunoglobulin protein (BiP), an Endoplasmic Reticulum (ER) chaperone and part of the Unfolded Protein Response (UPR) pathway, was found to be a reliable biomarker for the slow-growth phenotype. Higher intracellular BiP levels in seed train cultures correlate with subsequent poor performance in the inoculum train and the production culture, leading to the hypothesis that lower pH in seed train cell cultures increases the level of ER stress. Study and optimization of seed train pH resulted in mitigation of this phenotypic behavior.

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Expression of Viral Surface Proteins for Functional and Structural Analysis

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For many aspects of infection research viral surface proteins are required. However, these proteins often need post-translational modifications and are therefore difficult to express. This project deals with the expression of two different viral targets for vaccination and crystallization: Hemagglutinin (HA) of the influenza A virus and the envelope glycoprotein E2 of HCV.

HA is a surface glycoprotein of the influenza A virus playing a critical role in infection. It is produced as an inactive precursor that needs to be activated via proteolytic cleavage through serine proteases. This cleavage leads to conformational reorganization of the HA homotrimer resulting in fusion with the host cell membrane initiating the infection. Because of that HA is of special interest for novel approaches to affect the influenza virus infection process. It was shown in cell culture that HA proteins of diverse human influenza viruses can be activated by the serine protease TMPRSS2 through monobasic cleavage. Therefore, co-crystallization of different HA subtypes with TMPRSS2 should reveal important structural information about proteolytic activation of the influenza virus. Furthermore efficient large scale expression for vaccination is intended. The Baculovirus Expression Vector System (BEVS) was used for recombinant HA expression of the subtypes H1, H3 and H7. HA could be expressed in Hi5 insect cells and unprocessed protein was successfully purified and crystallized for the subtypes H1 and H3.

The envelope glycoproteins E1 and E2 of HCV are critical to mediate virus receptor interactions and to permit virus uptake into human liver cells. Besides they are the target of neutralizing antibodies, which inactivate circulating viruses in vivo. However, HCV has evolved strategies that ensure virus persistence in the presence of large amounts of glycoprotein-specific antibodies. Hence different soluble variants of E2 (sE2) were generated with the aim to re-direct antibody responses towards more conserved viral epitopes. Those sE2 variants could be expressed in Hi5 insect cells. After successful purification the different variants will be used by our collaboration partners at the TWINCORE to immunize mice.

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6,6

Process-relevant concentrations of the leachable bDtBPP impact negatively on CHO cell production characteristics

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The Chinese hamster ovary (CHO) cell has been the bedrock of the biopharmaceutical industry for the past 3 decades for the production of high quality recombinant therapeutic proteins. Despite vast advancements in bioprocess design and cell line development leading to g/L titres, the cost of drug production is at an all-time high thus impacting on the cost of these life-saving therapies. Minimizing the initial capital investment associated with hard-piped stainless steel facilities is currently being explored through the implementation of single-use disposable systems such as wave bag bioreactors. However, the introduction of new materials into the bioprocessing pipeline comes with it a cohort of unknown, potentially toxic compounds, the majority of which remain unidentified.

We have demonstrated that a leachate from Low-density polyethylene (LDPE) bioreactor bags, bis(2,4-di-tert-butylphenyl)phosphate or bDtBPP poses a risk to the maximum efficiency of a batch bioprocess by reducing Chinese hamster ovary (CHO) cell growth at concentrations found to accumulate in culture media in real-time (0.035-0.1 mg/L). By applying a comprehensive discovery phase including GC-MS, UHPLC, LC-MS and ICP-MS, sampled from 37 single-use disposable plastic bioreactor bags supplied by industrial partners covering a range of vendors and manufacturing dates, a comprehensive list of leachable compounds have been identified.

Cytotoxic evaluation of these leachates has revealed that never seen before compounds in this context are toxic to CHO cell growth at concentrations comparable to bDtBPP. This further raises the concern that the cocktail of leachates accumulated over the course of a bioprocess will have a synergistic negative effect on the maximum production potential. Similar to bDtBPP originating from the anti-oxidant Irgafos-168 required for plastic manufacturing, these novel leachates also originate from materials selected to “enhance” the performance of these single-use disposables as bioreactors. Finally, incomplete removal of these contaminants during downstream processing poses a serious risk to the patient to the extent of being endocrine disruptors.

7,1

Latest Developments in Scalable, High-Titer Transient Protein Expression in the ExpiCHO Expression System

Jonathan Zmuda, Chao Yan Liu, Jian Liu, Wanhua Yan, Kyle Williston, Henry Chiou1,

Thermo Fisher Scientific, Frederick, MD, U.S.A
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The ExpiCHO™ transient expression system offers an all-in-one solution for generating high-titer recombinant proteins for therapeutic drug development, reagent generation, and for proteins that express poorly in 293-based transient systems. The ExpiCHO system has become an integral part of the drug development workflow for many biopharmaceutical companies, helping to bridge the gap between transient and stable protein generation by minimizing changes in protein quality that are inherent when moving from 293-based transient systems to stable CHO expression. In the present study we provide the latest data for optimal expression and purification of various classes of proteins in the ExpiCHO system (i.e.antibodies, Fabs, membrane proteins, etc) for volumes ranging from less than 1mL in 96 well plates to liter-scale volumes in standard shake flasks, and all scales in between. Additionally, we provide strategies for automation of the ExpiCHO expression system, including a case study on the ambr®15 micro bioreactor system. Lastly, we present data on scale up of the ExpiCHO system to 10L expression volumes using the WAVE ReadyToProcess 25™ bioreactor system. Together, these protocols allow for the high titer transient expression of a broad range of proteins from milliliter to multi-liter scales to address unmet needs in protein expression workflows.

7,2

A complete suite of instruments and software for clone generation, assurance and documentation in cell line development

Dr Ian Taylor

Solentim

Objectives

The cell line development workflow has evolved over the past 5 years or so with the acceptance by the regulator of one round of cloning combined with whole well imaging.

Traditionally, many CLD labs have used FACS or the limiting dilution method for single cell deposition, each of which have their own merits and drawbacks. More recently, cell printer devices have also appeared on the market.

Solentim introduce and explain a new single cell seeding approach called Verified In-Situ Plate Seeding (or VIPS™). We will also present the case for a more complete probability calculation for clonality.

Methods

We will explain how the VIPS system works using a proprietary process of SMART LD™ (Limiting Dilutions); a combination workflow will be presented for high efficiency seeding of 96 well plates and the subsequent assurance of clonality using a whole well imager.

Results

Example images from the VIPS will be shown of single cells seeded as droplets

Practical examples will also be given for the key factors impacting the probability calculation.

Discussion

Overall workflow efficiency and data integrity improvements will be discussed using the VIPS seeder in combination with the Cell Metric CLD imager for daily clone screening and the Clonality Report for full documentation of chosen clones submitted to the regulator or client.

7,3.

Accelerating Biotherapeutic Development through Simultaneous High-Titer, CHO Transient Expression & Generation of High-Yield Stable Cell Lines Using Scalable Transfection

Joseph Abad, Field Applications Scientist

Transitioning early stage discovery efforts with later stage development activities is critical for moving biological therapies into the clinic in an efficient and timely manner. MaxCyte's Flow Electroporation™ Technology allows gram-scale transient expression of antibodies and other proteins in cell types that are relevant to biomanufacturing. At the same time, the high levels of efficiency and viability provided by Flow Electroporation Technology, can shorten timelines and reduce labor involved in generating clonal cell lines. We will present data demonstrating gram-scale production of therapeutic antibodies using multiple strains of CHO cells, the streamlined generation of high-yield stable cell lines within 6-8 weeks of transfection and the production of other therapeutic moieties, such as Fc-fusion proteins.

7,4

Novel Transposase Tools for Cell-Line Engineering

Ferenc Boldog, Kate Caves, Mark Welch and Jeremy Minshull

ATUM (formerly DNA2.0), U.S.A

ATUM has discovered, characterized, engineered and patented new transposases that work comparable to, or better than previously commercialized transposases. The technology is highly valuable for protein expression and genome engineering applications. It enables a specified sequence to behave as a transposon, a mobile genetic element, which can efficiently transpose between vectors and chromosomes via a "cut & paste" mechanism. The Leap-In Transposase® catalyzes the integration of a transposon containing your gene into TTAT sites in the target genome. During transposition, the Leap-In Transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and moves the contents from the original sites and efficiently integrates them into TTAT chromosomal sites. Similar technologies report integration of up to 20 copies of the transposon into unique locations in the genome 72 hours post transfection leading to very high expression levels of payload gene. Furthermore, transposase technologies are highly valuable because of their ability to integrate large payloads. This technology significantly accelerates stable pool and cell-line generation and can be used in conjunction with metabolic selections such as dihydrofolate reductase (DHFR) and glutamine synthetase (GS) or more generic drug selections such as puromycin and neomycin. We have designed transposon based multi-ORF vectors that allow expression of target proteins at controlled ratios. These vectors combined with two orthogonal sets of engineered hyperactive transposases allows multiple levels of genome engineering. The valuable features of the system and performance characterization will be discussed with cell line development and cell engineering case studies.

7,5

Orbital Shaken Bioreactors: The Scale Up Fast Track (from mL to 2500L)

Tibor Anderlei[1], Tim Bürgin [1]

[1]Kühner AG, Birsfelden, Switzerland

Since more than 60 years the shake flask is the standard shaken bioreactor in biotechnology. Regarding cell cultivation the shake flask has been used intensively since more than 15 years. The presentation gives an overview of the variety of shaken bioreactors from µL to 2500L scale (microtiter plate, TubeSpin, Erlenmeyer flask, ORBShaker, …) focusing on the engineering parameters. Furthermore, application and scale up results of antibody and vaccine productions using shaken bioreactors will be shown. Since more than 63 years Kuhner AG, Switzerland is building shakers for the biotechnology field. Kuhner works strongly together with the EPFL, Switzerland and the Technical University of Aachen, Germany.

8,1

Expression Screening and Production of Human Proteins for Structural and Functional Studies

Nicola Burgess-Brown, Ph.D., Principal Investigator Biotechnology

SGC, University of Oxford

The SGC (Structural Genomics Consortium) is a not-for-profit, public-private partnership that promotes research advancement through our open access policy, providing knowledge and tools freely available and in the absence of IP. Globally, the SGC has solved more than 1700 human soluble protein structures in addition to 8 novel integral membrane proteins (IMPs). Respectively, this corresponds to 20% and 10% of proteins solved worldwide. Although we have made a significant contribution to structural biology and protein production for functional studies, there are many highly desired targets, particularly large proteins, protein complexes and known drug targets which remain a challenge to obtain. Our well established expression systems using E. coli and baculovirus/insect cells will be presented in addition to new strategies applied to our processes to aid with production of these difficult proteins, including BacMam expression, alternative tags for purification and mutagenesis.

Biography

Nicola Burgess-Brown is the Principal Investigator of the Biotechnology Group at the SGC, responsible for managing all biotech research for the Oxford site. The group collaborates and interacts closely with the other SGC teams, to develop methods for increasing protein expression, parallel processing and increasing throughput for soluble and membrane proteins involved in human disease. Prior to her role as PI, Nicola has been responsible for optimising the high-throughput screening processes from cloning to expression and purification of human proteins for structural and functional studies. Since June 2009, she developed a similar pipeline for production of human integral membrane proteins (IMPs). Nicola obtained a First Class degree in Applied Biochemical Sciences from the University of Ulster in 1997 and spent the following year working as a molecular biologist for SmithKline Beecham. She received her Ph.D. in Molecular Microbiology at the University of Nottingham in 2001 and then moved back to industry to work on high-throughput cloning and validation of therapeutic cancer antigens for Oxford Glycosciences and subsequently Celltech R&D.

8,2

Expression and Characterization of Bispecific Antibodies @ Roche

Claudio Sustmann

Head Molecular Design & Engineering, Large Molecule Research, Roche Pharma Research and Early Development, Roche Innovation Center Munich, Germany

Bispecific engineered antibodies are an important drug class. They offer enhanced functionality in comparison to standard antibodies. The Roche Crossmab technology enables convenient assembly of bispecific molecules in an IgG-like scaffold making use of all features of the constant domain as well. More than four different CrossMabs targeting different diseases are already in clinical evaluation. The talk will focus on design principles, expressability and analytical aspects of CrossMabs also in comparison to standard antibodies.

Bio Sketch

Claudio Sustmann studied chemistry in Marburg and Munich and obtained his Ph.D. from the University of Freiburg working at the MPI of Immunobiology and Epigenetics, Freiburg, Germany. Since 2008 Claudio works for Roche in different positions with a particular focus on bi- and multispecific therapeutic antibodies and antibody conjugates in the field of oncology. Since 2015 Claudio is head of a department focusing on molecular design and engineering of therapeutic antibodies. LinkedIn profile: https://de.linkedin.com/in/claudio-sustmann-51292029

8,3

CAP-Go.2 cell line: Improved O-glycosylation for significantly prolonged plasma half-life of human recombinant C1 Inhibitor

N Strempel, S Wissing, J Woelfel, H Kewes, C Niehus, C Bialek, S Hertel, N Faust

CEVEC Pharmaceuticals,, Germany,

The development of therapeutic proteins has accelerated immensely over the past years. However, the recombinant expression of highly glycosylated therapeutic proteins such as blood coagulation factors or serum proteins, has remained a challenging task.

C1-Inhibitor (C1-Inh) is glycosylated with 7 N-glycans and 8 O-linked glycans. Plasma derived C1-Inh (Berinert, Cinryze) as well as recombinant C1-Inh from transgenic rabbits (Ruconest) are approved for the treatment of acute attacks in patients with hereditary angioedema. However, the recombinant product shows dramatically reduced plasma half-life in comparison to the plasma-derived versions.

We have developed the CAP-Go protein expression platform, an expression system based on human cells, to confer optimal glycosylation to complex glycoproteins such as C1-Inh. The CAP-Go.1 cell line has been modified to facilitate expression of proteins with fully sialylated N-glycans. Recombinant proteins like human alpha-antitrypsin or human placental alkaline phosphatase produced with CAP-Go.1 show a significantly prolonged serum half-life in rats. However, expression of rhC1-Inh in CAP-Go.1 cells had no positive impact on the pharmacokinetic profile.

Expression of rhC1-Inh in CAP-Go.2 cells, which in addition addresses the O-linked glycosylation patterns, results in a significantly increased serum half-life which is actually indistinguishable from the plasma-purified protein. O-glycan analysis shows that rhC1-Inh expressed by CAP-Go.2 cells contains only highly sialylated core1 O-glycan structures, highly comparable to plasma-derived Berinert. Our results demonstrate that in addition to N-glycosylation, the structure of O-linked glycans plays a crucial role in bioavailability and pharmacokinetic properties of glycoproteins.

In conclusion, rhC1-Inh expressed from CAP-Go.2 cells, matches serum-derived C1-Inh in all analyzed aspects - specific activity, serum half-life, and glycosylation pattern - and offers the advantage of being producible at large scale on a safe platform

8,4

Strategies to Develop Difficult to Express Protein Therapeutics in CHO Cells

Jill Cai and Weichang Zhou

Biologics Development, WuXi Biologics, Shanghai, China

A versatile and efficient protein expression platform using CHO cells has been established for production of various protein types including monoclonal antibodies, bispecific antibodies, recombinant and fusion proteins. This platform has been used for rapid production of micrograms to multiple grams during different stages of discovery and development. Several case study examples will be given to highlight our approach to developing different formats of bispecific antibodies; how a highly hydrophobic protein therapeutics is expressed and purified; and finally we will present an assay to prove monoclonality in addition to using imaging system for manufacturing cell lines.

9,5

Cell Line Development for Expression of Bispecific DART® and Trispecific TRIDENTTM Molecules

Valentina C. Ciccarone, Ph.D.

Principal Scientist, Cell Line Development, Antibody Engineering, MacroGenics, Inc. Rockville, MD, USA

In order to achieve enhanced biological activity, we have developed multispecific DART In order to achieve enhanced biological activity, we have developed multispecific DART antibody-like characteristics. Bispecific DART molecules are designed to target two independent antigens on the same or separate cells, while TRIDENT molecules can target up to three independent antigens. By varying the specificity as well as the valency of the individual arms, DART and TRIDENT proteins can be designed to utilize multiple mechanisms of action for specific clinical indications, including re-directing cytolytic effector cells to tumor cells, targeting two signaling molecules on the same cell, the simultaneous blockade of immune checkpoint targets, multiple co-inhibitory receptors, or the neutralization of multiple viral epitopes. These molecules are being evaluated as clinical candidates in several indications including oncology and autoimmune disorders. DART and TRIDENT molecules are composed of two to four different peptide chains that need to assemble correctly for functional activity. The appropriate expression of each component by the production cell line is important in achieving high expression levels and product quality that meet criteria for the successful manufacture of the clinical product. By incorporating molecule engineering strategies, proper vector selection and optimization together with conventional cell line screening, we have been able to obtain correctly assembled molecules at high expression levels that retain the biological activity of these complex, multi-chain molecules.

9,6

Production of crystal grade membrane proteins for biophysical methods to facilitate structure based drug discovery

Sandra Markovic-Müller, Robert Cheng, Mathieu Botte, Nicolas Bocquet, Sophie Huber, Michael Brauchle and Michael Hennig

leadXpro AG, PARK INNOVAARE, Switzerland

LeadXpro is an emerging structure based lead discovery company focusing on membrane protein drug targets, including G-protein coupled receptors (GPCRs), ion channels and transporters. The company is co-located with the Paul Scherrer Institute and leverages state of the art facilities for structure determination like the synchrotron SLS and the X-ray Free Electron Laser SwissFEL. In collaboration with the University of Basel leadXpro has access to single particle cryo-electron microscopy.

In projects dealing with structure of integral membrane proteins the quality of the In projects dealing with structure of integral membrane proteins the quality of the In projects dealing with structure of integral membrane proteins the quality of the conditions, to ultimately obtain high quality sample enabling structure determination conditions, to ultimately obtain high quality sample enabling structure determination our initial screening assays are designed for transient expression in mammalian cells and contain C-terminal GFP to allow for early assessment of expression level and monodispersity using fluorescence size exclusion chromatography. We regularly vary many parameters during our construct design to influence expression level, biochemical properties, functionality, stability and crystallizability of a membrane protein of interest. Most common modifications include: N- and C-terminal truncations, testing of different signal sequences, introduction of different fusion proteins as well as introduction of signal sequences, introduction of different fusion proteins as well as introduction of expression in insect cells. In most of the cases we observe good correlation in expression expression in insect cells. In most of the cases we observe good correlation in expression expression in insect cells. In most of the cases we observe good correlation in expression choice. Finally, throughout all expression and purification steps we apply various analytical methods to test for functionality of produced membrane proteins.

Keynote presentation

CHO in times of –omics or "There is much pleasure to be gained from useless knowledge" (Bertrand Russel, philosopher)

Nicole Borth and Team[1,2]

[1] *BOKU University Vienna, Austria*
[2] *acib - Austrian Center of Industrial Biotechnology*

During the last six years, more than a dozen different CHO cell lines have been sequenced as well as the Chinese hamster genome. With a greatly improved reference genome coming out, it is time to review how this sequence information has actually changed our understanding of how CHO cells operate and function and what new possibilities are now open to improve the platform technology for therapeutic protein production. Have we found explanations for CHO´s adaptability and its striking efficiency to produce large amounts of proteins that provide no benefit to the cell itself? Are the limits of productivity and titers already reached or can we expect another surge now that we can define correlations between gene expression patterns and cellular phenotypes? How do the abilities to calculate the entire metabolism of cells or to fine-edit the genome by deleting, replacing or altering defined genes translate into improved processes that are faster, cheaper and more efficient? Or is it really epigenetic control which will provide a more stringent control of phenotypes? Many questions, indeed, but by now, despite only six years since genome availability, several studies indicate that the systematic use of the rather elaborate and expensive multi-omics approaches on an industrial scale may be entirely justified. More importantly, and here I refer to the citation above: we are having a lot of fun

10,1

Fc receptors biotinylation for oriented immobilization on surface plasmon resonance biosensors.

Yves Durocher

Mammalian Cell Expression, National Research Council Canada

The use of surface plasmon resonance (SPR) biosensors represents a relevant tool to evaluate the kinetic constants of IgG/FcγR interactions. A variety of experimental protocols is currently being used (choice of the partner to be immobilized or captured, ‘ow rates and densities) that yield to inconsistencies among reported kinetic values. In many cases, kinetic pro’les deviate from a simple Langmuirian model and this can be attributed to the intrinsic complexity of the biological interactions under study or suboptimal experimental procedures. We present our strategy to reduce inconsistencies and increase robustness of the IgG/Fcγ SPR assay.

10,2

Glycosylation customizaton of eukaryotic biopharmaceutical expression hosts

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Glycan structures characterize the molecular environment immediately outside of all cell types and hence have critical functions in interactions of any cell with its environment (cell-cell, cell-pathogen, cell-molecule). The field of glycobiotechnology is concerned with understanding and re-engineering of these glycosylation-dominated interactions. In particular, the understanding of the synthetic pathways and functions for eukaryotic N- and O-glycosylation, gained over the past few decades, has enabled the rewiring of these pathways for the benefit of pharmaceutical applications. Based on the conservation of the core pathways between eukaryotes, it has been possible to transfer the efficient synthesis of particular human-specific glycan structures to other eukaryotes such as yeasts and plants. This is enabling the cost-effective production of biopharmaceutical proteins with glycosylation patterns customized to particular therapeutic functionality (e.g. targeting to particular glycan receptors, or customized for particular pharmacokinetic behaviour). I will illustrate our work with regard to the production of human IgG-like glycosylation patterns in yeast1, and the production of mannose-6-phosphate modified lysosomal enzymes for the treatment of human inherited lysosomal storage diseases2. Whereas these earlier synthetic biology endeavours were geared towards copying the synthesis of complex mammalian glycan structures in other eukaryotes, more recently we have generated mammalian cells, plants and yeast in which glycosylation complexity has been reduced to the bare minimum, while still being compatible with eukaryotic cell life and protein productivity. This 'GlycoDelete' technology3,4 opens up many new structural biology and biopharmaceutical applications that are currently being explored in our laboratory.

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2. Tiels, P. et al. A bacterial glycosidase enables mannose-6-phosphate modification and improved cellular uptake of yeast-produced recombinant human lysosomal enzymes. Nat. Biotechnol. 30, 1225–1231 (2012).

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10,3

Small-scale development and optimization of product quality control in mammalian cell perfusion cultures

Moritz Wolf, Daniel Karst and Massimo Morbidelli

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The continuous production of biopharmaceuticals in perfusion bioreactors combines several advantages: the use of smaller equipment, faster and more efficient processing, higher volumetric productivities, as well as a more uniform product quality. However, the knowledge on time and cost effective development and scale-up procedures to achieve a reliable reactor operation and desired product characteristics is still limited. Therefore, this study aims at the definition of a comprehensive optimization framework for mammalian cell cultures utilizing a combination of small-scale spin tube and bench top bioreactor experiments.

First, suitable operating conditions have been rapidly evaluated in small scale simulated perfusion cultures. This allowed the identification of key process parameters to facilitate steady state operation and process control. Their choice was further refined in a stirred tank perfusion bioreactor setup by sequential screening of steady states targeting improved product yields and desired product quality characteristics. The measurements of extra- and intracellular metabolites, product concentration and quality attributes were used to optimize the reactor performance. Comparable growth behavior and metabolite consumption/production rates were observed between experimental scales.

The variation of key cell culture parameters led to a superior performance of the perfusion culture. The decrease of the cell specific perfusion rate prevented excessive cellular growth and significantly reduced the loss of product in the bleed stream. Constant patterns of product quality attributes such as N-linked glycosylation and charge isoforms were observed within each steady state but slightly varied between different operating points. Overall, this study underlines the high potential of perfusion cultures to simultaneously achieve high productivities while tuning towards desired characteristics of consistently expressed therapeutic proteins.

11,4

Determination of monoclonal antibody charge heterogeneity directly from cell culture supernatants for quality by control realization

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he Quality by Design (QbD) concept implies that the process defines quality. However, this approach does not implicitly focus on the controlling perspective. The concept of Quality by Control (QbC) goes one step further and aims on a highly dynamic model predictive process. The implementation of such requires an enhanced analytical platform which helps to elucidate the critical process parameters (CPP) affecting the product's quality attributes and developing robust process models. One important critical quality attribute (CQA) is the charge heterogeneity of a monoclonal antibody (mAb). The commonly used analysis is ion exchange chromatography (IEX) of pre-purified product utilizing a salt gradient. However, this approach is time consuming and predominantly suitable for harvest- and pre-purified samples only. Yet, to establish mathematical models, predicting this CQA within the bioprocess, a method that allows reliable measurement without pre-processing is desired.

Here, we present an IEX method which was successfully adapted to determine charge heterogeneity of mAbs directly out of the supernatant. We show that no major impurities were altering the results and that matrix effects were negligible. The established approach was successfully used to characterize the CQA variation during processes performed in a two dimensional full factorial design of experiment setup. Differences in the charge heterogeneity over time as well as dramatic impacts of temperature and glucose concentration in the feed media were determined. The variation of temperature enhanced the formation of basic-, whereas the variation of glucose concentration influenced the formation of acidic variants. Indeed, the achieved data sets provide a valuable insight into the mechanisms of product formation and are going to be incorporated into the development of a robust process model. In general, this method is one important step towards QbC implementation to mammalian bioprocesses.

11,5

Process Development for an Inducible CHO Cell Line

Kahina Mellahi[1], Michel Perrier[1], Sylvie Perret[2], Yves Durocher[2] and Olivier Henry[1]

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Mammalian inducible expression systems are gaining increasing interest for recombinant protein production. By allowing to decouple the growth and the production, they offer the unique ability to independently optimize each phase. Despite the growing availability of efficient inducible expression systems, very little work has been devoted to the process optimization of biphasic cultures. In particular, the timing of induction is one of the most critical parameter determining the performance of a culture, as it will ultimately impact cell growth, culture duration and protein productivity.

In this work, we have specifically investigated how the cell culture state at the time of induction influences the cumate-inducible expression of recombinant rituximab by a GS-CHO cell line (Glutamine synthetase Chinese Hamster Ovary cells). To this end, cells grown in batch and fed-batch cultures were induced at increasing cell densities (1 to 10e6 cells/mL) and the resulting kinetics of growth and production were analyzed. A temperature shift to mild hypothermia conditions was performed at the time of induction in all the cultures.

Compared to control cultures in batch, a dynamic feeding strategy employing a concentrated nutrient solution and applied prior and post induction allowed to significantly increase the integral of viable cells (IVC) and led to a 4-fold increase in the volumetric productivity (monoclonal antibody titers up to 1.4 g/L). Interestingly, the temperature shift did not significantly impact the cell specific growth rate, as similar maximum cell concentrations were achieved in both high and low cell density inductions. While high product yields were consistently achieved under most conditions, cultures induced during the late stationary phase, after the cells transitioned from lactate production to consumption, were associated with a shortened production phase and a concomitant decrease in volumetric productivity. Taken together, these results indicate that the cell culture state at the time of induction is a key determinant of culture performance and the design of a fed-batch strategy able to maintain the cells in a favorable metabolic state is essential for productive high cell density induction.

11,6

Modulation of glycosylation by media design

Nicola Mac Kinnon

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N-glycosylation is a complex post-translational modification, which impacts the structure of the Fc part of the antibody and thereby its effector functions and pharmacokinetics. The modulation of N-glycosylation is an important aspect of antibody development to ensure the required quality and safety of a therapeutic drug. While tight fine-tuning modulation is required in the frame of product characterization, wider ranges of glycosylation may be explored during early process development or to investigate the limits of bioactivity and product safety. The screening of media components, starting at the early stage of cell line development and throughout the entire development process does not only allow for improved product understanding but does also provide important levers for the management of process changes. High throughput tools are excellent means to screen for an important number of media components. In this presentation it is shown how microscale bioreactors are used to evaluate media components at an early stage of development, with robust upscaling to lab-scale bioreactors. Moreover a novel approach to examine time-profiles of intracellular nucleotides and sugars of CHO-S fedbatch cultures by MALDI-TOF MS contributes highly to a more optimized strategy for media modulation through the direct targeting of these glycosylation intermediates with specific media components. Multivariate analysis by PCA was used to provide statistical relevant correlations between intracellular component, media supplements and glycan profiles.

12,1

Engineering cell metabolism to enhance protein production

Nathan E. Lewis

University of California, San Diego

Over the past 3 decades, mammalian cells have become the predominant production hosts for biotherapeutics, and now produce 6 of the top 10 grossing pharmaceuticals. However, the complexity of the protein-based drugs and the host cells pose major challenges that must be controlled to improve the safety, efficacy, and affordability of these pharmaceuticals. The connection of metabolism to these attributes has been long appreciated, but a comprehensive view of CHO metabolism has been lacking. To address this need, we have identified >1700 metabolic genes in the Chinese hamster genome and mapped out thousands of active metabolic reactions in CHO cells. In this talk I will demonstrate how these models provide insights into the protein-production capacity of CHO cells, and how it influences the metabolic needs differ across products. We further explore how these resources allow us to control the production of toxic by-products, such as lactic acid and thereby improve bioprocess phenotypes.

12,2

Perfusion Reactors in the context of integrated manufacturing of therapeutic proteins

Moritz Wolf, Nicole Ulmer, Fabian Steinebach and Massimo Morbidelli

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Continuous integrated manufacturing of therapeutic proteins is a novel paradigm in the production of biopharmaceuticals. The decrease of capital and operating costs on the one hand, but especially higher volumetric productivity as well as more homogeneous product quality are considered to alleviate regulatory issues. These advantages have increased interest in continuous unit operations and their integration. Herein, we evaluate the performance of mammalian cell perfusion bioreactors as part of an end-to-end integrated production stream.

The following unit operations were connected in an integrated manner: A perfusion bioreactor equipped with an external hollow fiber device, a continuous two column capture process, a virus inactivation step, a semi-continuous polishing step (twin-column MCSGP) and a batch-wise flow-through polishing step. In each unit, internal recycle loops allowed to improve the performance: Increased volumetric productivity and capacity utilization are obtained in the bioreactor and capture step, respectively; in addition, the purity-yield trade-off typically encountered in batch-wise bind-elute polishing steps could be overcome.

This study focuses on the influence of operating conditions (media composition, viable cell density) on product quality and the overall performance of the end-to-end integrated process. The end-to-end integration was carried out for 17 cycles yielding to more uniform product quality compared to batch-wise operations. Especially, the perfusion bioreactor operation facilitated the downstream purification of the protein compared to traditional fed-batch processes. The steady-state operation was fully characterized in terms of product titer and quality considering both product (aggregates, fragments) and process (HCP, DNA, leached Protein A) related impurities.

12,3

Improved on-line monitoring of mammalian bioprocesses via fluorescence-based softsensors

Bayer, B.[1], Kandra, K.[1], Sissolak, B.[1], Melcher, M.[2], Sommeregger, W.[3], Striedner, G.[1]

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Since mammalian bioprocesses are expensive and time consuming, batch rejection may result in a high loss of time and money. Therefore, the development of strategies for direct monitoring and control of product quality is of high priority. The main challenge of real-time monitoring and control is that many relevant molecules or product properties are not directly measurable. In this project quality linked process variables of a monoclonal antibody produced in Chinese hamster ovary (CHO) fed-batch processes represent the targets to be predicted and controlled in real-time. For the implementation of this Quality by Control (QbC) concept, much attention was given to the development of meaningful on- and off-line process analyses, rational design of experiments (DoE) to investigate the process under varying conditions (temperature and glucose concentration in the feed media), process characterization and the identification of product relevant process inputs (critical process parameters). Here we want to focus on the on-line application of a non-invasive multichannel fluorescence detection system, which automatically scans the 2D emission-excitation fluorescence spectrum of the cell suspension from 270 nm to 590 nm. The obtained fluorescence data from several DoE settings was used to find correlations to off-line measurements via chemometric modelling, including multivariate partial least squares regression (nPLS), Pearson correlation coefficient, Parallel Factor Analysis (PARAFAC) and different regression models. Thereby, various novel and promising soft-sensors for several relevant process variables including amino acid, glucose and biomass concentrations were established. These results can now be used for real-time on-line monitoring of the process and in combination with a hybrid process model for dynamic process control.

13,4

Dielectric monitoring of mammalian cells in a bioreactor

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Dielectric spectroscopy is an analytical technology with the potential to revolutionize the control of biopharmaceutical manufacturing. The frequency-dependent profile of ionic polarization of cells in response to an alternating electromagnetic field changes with respect to cell type, metabolism and media conductivity. The method has been adopted to measure the growth of cells through in situ capacitance measurements at a single frequency. However the power of such measurements can be enhanced through the use of a dielectrophoretic flow cytometer (DEP) that can measure the changing trajectory of single cells passing through a microbore tube subjected to an electromagnetic field controlled by a bank of electrodes. We have used this principle to analyze cells in a bioprocess to identify changing sub-populations of cells during apoptosis. This has led to the early detection of changes that lead to the eventual loss of productivity and viability. The presentation will include comparative data from five alternative measurements of cell growth and viability. Each method provides a different profile which can be used to decipher changes in viability and metabolism of the cells during the production process. The value of these methods will be discussed in relation to the production of monoclonal antibodies from Chinese hamster ovary (CHO) cells. Our data shows that dielectric cell monitoring provides unique information that can be related to more conventional methods of biochemical monitoring by fluorescent agents.

13,5

Nanoscale characterization coupled to multi-parametric modelling of High Five cells transient transfection

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High Five cells are one of the preferred platforms towards the production of recombinant proteins with the Baculovirus Expression Vector System (BEVS). Although it is a powerful system, the use of BEVS entails several limitations inherently associated to the viral nature of the infection. Growing interest has emerged on exploiting the potency of this host devoid of BEVS usage. Despite of numerous efforts involving the use of polyethylenimine (PEI)-based reagents or expensive transfection carriers, there is not an efficient and scalable protocol for these cells yet. Here, a PEI-based transient transfection method has been developed for suspension adapted High Five cells in Erlenmeyer flasks. Method optimization was performed with the model proteins intracellular enhanced Green Fluorescent Protein (eGFP) and Secreted Alkalyne Phosphatase (SEAP).

Nanoscale characterization was performed to select NaCl 150mM as the DNA:PEI complexation solution. Cryo-Electron Microscopy (Cryo-EM) and Dynamic Light Scattering (DLS) techniques were used to monitor the dynamics of complex formation and particle size. These analysis revealed complexation solution and incubation time of DNA:PEI as relevant factors influencing transient transfection.

DoE-based Response Surface Methodologies were used to find the optimum condition of cell density at the time of transfection (1.5•106 cells/mL), DNA (2.1 µg/mL) and PEI concentration (9.3 µg/mL). Of note, this optimum condition was found according to a maximization desirability criteria simultaneously applied to different response variables, namely cell viability, % of transfection and specific production. This methodology implies to give different weights to each of the three response variables regarding their importance. Under these conditions, a ~60% transfection and 198 ± 17 relative fluorescence units (r.f.u) were achieved. In addition, characterization of the optimum DNA:PEI concentrations showed that small complexes (< 1 µm) were more efficient for the transfection of High Five cells.

13,6

Application of Hybrid Modeling for Process Prediction of Mammalian Cell Culture Bioprocesses

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Mammalian bioprocesses are complex, since they are subjected to many influences (e.g. biology of the cell, operational input and raw materials). To gain overall process understanding and ensure product quality, it is of high importance to understand how critical process parameters affect the critical quality attributes as highlighted in the Process Analytical Technology initiative. During the step of bioprocess development often design of experiment settings with response surface type models are used, which generally provide a static model. Whereas the concept of hybrid modeling allows to precisely predict process states, because existing process knowledge is additionally considered and the structure of the experimental data is inherent dynamic. Hybrid models can not only increase process knowledge, but also help integrating and structuring information obtained from different sources. Therefore, we applied this approach aiming for advanced process control and monitoring. In this project a Chinese hamster ovary cell line, producing a monoclonal antibody was chosen as model for fed-batch cultivations at different scales (shake-flask: 0.3L and bioreactor: 15 L, 100 L) in an intensified design of experiment setting. Enhanced process monitoring was achieved by applying a broad on- and off-line analytical platform. A relatively small number of shake-flask experiments were sufficient to establish a hybrid model, where a good prediction performance for estimating specific process variables could be demonstrated (such as viability, total cell concentration, several amino acids and metabolites). The model based on small scale experiments in combination with data from few bioreactor runs could furthermore be transferred to describe the process performance in the bioreactor. Concluding, the generated models enable process prediction as well as simulation, provide enhanced process knowledge and are the basis for advanced bioprocess control.

14,1

Development of technologies for the production of zika and yellow fever vaccine candidates

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The years 2016 and 2017 have shown the health threats posed by zika (ZIKV) and yellow fever (YFV) viruses, both mosquito-borne viruses belonging to the family Flaviviridae, genus Flavivirus. ZIKV quickly spread to approximately 60 countries around the globe, and its association with serious congenital malformations was confirmed, making clear the need for a ZIKV vaccine. Concurrently, major YFV outbreaks in Angola (2016) and Brazil (2017) demanded dozens of millions of doses of the existing egg-derived vaccine, causing depletion of stockpiles and leading to the emergency use of a fractional (1/5) vaccine dose. This made clear the urgent need for developing a new, non-egg derived YFV vaccine.

In this talk, we will show efforts towards developing new vaccine candidates for zika and yellow fever viruses. In a first approach, for the case of YFV, the work focused on the development of a purified inactivated vaccine candidate produced from the attenuated YFV 17DD strain in Vero cells. High virus titers (~1E8 pfu/mL) were achieved under serum-free conditions, using microcarriers in stirred-tank bioreactors. Based on the isoelectric point of the envelope protein of YFV, membrane-based anion-exchange chromatography was used as a first purification step and allowed for a high degree of concentration and removal of host-cell DNA. The subsequent step using a multimodal resin in flow-through mode allowed for separation of host-cell proteins.

In a second approach, virus-like particles (VLPs) were explored as potential vaccine candidates. The production of ZIKV and YFV VLPs was first investigated in suspension-adapted mammalian cell lines (HEK293, CHO.K1, BHK-21 and MDCK) by transient lipofection. HEK293 and CHO.K1 cells showed to be the most favorable host cell lines among those evaluated, so stable cell lines derived from these cells were generated. The supernatants were purified by chromatographic techniques, also including ion-exchange and multimodal chromatography. Purified VLPs analyzed by electron microscopy were 30-50 nm in size, as expected for flaviviruses, and antigenicity was confirmed by immunoassays.

14,2

Process development for a flexible vaccine vector platform based on measles virus

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Vaccines are one of the most important, safe and efficient interventions to protect people from illness, disability and death. In recent years several new viral outbreaks where no vaccines are currently available were reported worldwide. Therefore, the development of flexible processes for the production of vaccines is urgently needed. This project aims at developing a platform process for the production of different viral vaccines. The core technology is based on the fact that large recombinant genes coding for selected, foreign antigens can be inserted into the genome of a well-established measles virus vaccination vector. The vaccine delivers the selected antigens directly to macrophages and dendritic cells, the most potent and effective antigen-presenting cells, thereby triggering a specific immune response to the selected antigens. As a replicating vector, the vaccine continuously expresses antigens even after immunization. This setup results in a powerful, antigen-focused immune response, which is expected to confer long-term immunity as shown for the measles vaccine.

The challenges in production process design for such a vaccine are the establishment of a robust cell expansion and infection strategy as well the development of efficient downstream processing methods including flow through chromatography, ultra-diafiltration and employment of bio recognition principles. The implementation of a meaningful real-time process monitoring/characterization concept furthermore serves as a basis for reliable in-process control strategies (e.g. the prediction of the optimal infection/harvesting time point).

14,3

Platform technologies to assist the development of novel vaccines against emerging epidemic diseases

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A range of emerging epidemic infectious diseases was identified by the World Health Organization (WHO: Blueprint for Action to Prevent Epidemics, 2016). Viruses such as Ebola, MERS-CoV, Nipah, Zika, Lassa and Chikungunya were listed to have a potential to pose a significant threat to societies and individuals. Unfortunately, there are many cases where market incentives alone do not foster vaccine development against these diseases. The Coalition for Epidemic Preparedness Innovations (CEPI) aims to facilitate and finance the creation of new vaccines including their clinical testing and stockpiling. There is a plan to institute a vaccine development network in Australia to establish platform technologies and manufacturing capabilities that can be deployed rapidly against outbreaks of various pathogens.

This paper summarizes our current experience with recombinant glycoprotein-based vaccine candidates (Hendra and Ebola viruses) and suggest novel vaccine development ideas and capabilities needed to create a successful vaccine development pipeline. Focussing on our experience, we will discuss genetically engineered virus-like particles (VLPs) to increase immunogenicity and the 'molecular clamp' technology which is a propriety stabilization domain offering significant advantages over traditional vaccines in terms of safety, efficacy, and thermal stability.

14,4

Characterization of virus-like particle (VLP) production platform towards transient gene expression strategy.

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Virus-like particles (VLPs) have become a promising platform for vaccine production and antigen presentation. VLPs are formed by structural viral proteins that inherently self-assemble when expressed in a host cell. They represent a highly immunogenic and safe system, due to the absence of the viral genome and its high protein density. In this work, a platform for the obtention of complex enveloped VLPs in the human cell line HEK 293 is presented by means of transient gene expression strategies (TGE). The DNA/PEI complex ability to mediate TGE processes has been widely studied in the last twenty years. However the relationship between DNA/PEI complex physicochemical properties and its biological activity has not been yet mastered.

The aim of this study is to characterize and better understand the role of the different parameters that critically affect the platform for its further bioprocess improvement. The obtention of HIV-1 GagGFP VLP was used as a scaffold model.

Characterization of DNA/PEI complexes has been successfully performed by means of particle tracking techniques. Complexes are characterized as compacted particles of 250 nm that suffer an aggregation process during its incubation. Moreover, X-ray spectroscopy coupled to electron microscopy analysis was used to analyze DNA/PEI complexes morphology and composition. The effect of the aggregation status in DNA/PEI complexes was tested in TGE in the presence of specific endocytosis inhibitors. Those experiments revealed that despite DNA/PEI complexes may enter throughout all the different endocytosis pathways, micropinocytosis and hence aggregates of bigger size are the major contribution specimen to TGE efficiency.

Secondly the behavior of DNA/PEI complexes at each intracellular step was characterized and the main bottlenecks were identified. HIV-1 GagGFP VLP production was recorded by fluorescence-based techniques, confocal microscopy and molecular biology methodologies. Colocalization of DNA-Cy5 and PEI-Cy3 analysis during TGE showed how DNA and PEI are tightly interacting just after its addition to the cell culture and the de-complexation process is produced meanwhile complexes travel into the cells. pGagFP mRNA levels appears just 30 minutes after transfection and increase in two orders of magnitude until 10 hours post transfection (hpt) were a plateau is achieved. Intracellular GagGFP expression start at 4 hpt and is increased until 48 hpt and the final HIV-1 GagGFP VLPs increment during all the production phase.

Finally, the characterization of the obtained VLPs, and the discrimination between VLPs and other vesicular bodies, has been performed using electron microscopy techniques such as CryoTEM, but more remarkably the nanostructure of the VLPs could also be characterized applying novel techniques based on Atomic Force Microscopy (AFM). The application of advanced multifrequency AFM unveils new opportunities for studying the capsid protein composition and nanomechanical qualities and its further use in the development of proper and efficient strategies in the obtention of high quality VLP candidates for vaccination purposes.

14,5

IMPROVED PROTEIN EXPRESSION FROM MAMMALIAN CELLS BY TARGETING GENES IDENTIFIED THROUGH GENOME SCALE SIRNA SCREENING

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High-throughput whole-genome RNA interference screen was conducted in HEK 293 cells expressing firefly luciferase. 21,585 human genes were silenced with three different siRNAs for each gene. From the 56 genes, whose silencing caused the greatest improvement in the luciferase expression, 10 top genes that significantly enhanced the protein expression, were validated by their effect on the expression of four different recombinant proteins. Among the validated genes, the gene encoding the ornithine decarboxylase antizyme1 was selected for further investigation, since its silencing improved the reporter protein production without affecting cell viability. It was found that silencing this gene caused an increase in the ornithine decarboxylase enzyme and the cellular levels of putrescine and spermidine, a hint that increased cellular polyamines enhanced the expression of the recombinant protein. This study suggests that this gene can be a novel target for improving recombinant proteins expression. Subsequent deletion of this gene by molecular biology tools confirmed this observation: enhanced expression was observed from cells missing this gene without affecting their growth properties. The screening process, the gene identification and the cell line creation will be described as well as the effect of direct involvement of noncoding RNAs. In summary, the genome-scale screening described can be used for targeted design of an efficient mammalian cell platform for different biotechnological applications.

14,6

Process intensification: Yellow fever virus and zika virus production in suspension cells

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The viral emergence and rapid spread of flavivirus-related diseases, like yellow fever and zika, are a global health concern. Recent outbreaks exposed the urgent need for a reliable supply of sufficient yellow fever virus (YFV) vaccines, but also depict the lack of preventive countermeasures against zika virus (ZIKV). If suspension cells are available as suitable flavivirus cell substrate, process intensification may impact the supply of these low-yield viruses.

Baby hamster kidney (BHK-21) cells were identified to be a suitable producer cell line compared to Vero cells. Adaptation to growth in suspension and serum-free conditions enabled a scale-up into perfused stirred tank bioreactors with tangential flow filtration (TFF). We achieved high BHK-21SUS cell concentrations of 2.8 x 107 cells/mL and YFV-17D titers of up to 1.6 x 108 PFU/mL (infectious virions per mL). This setup can generate virus material for more than 1 Mio live-attenuated vaccine doses in a 1 L working-scale and 10 days operation time. To meet the urgent demand of Brazilian ZIKV material for virological studies, we transferred the YFV process in BHK-21SUS cells to ZIKV. Different ZIKV isolates were screened and a high producer seed virus was identified. Observed low cell-specific yields might be related to intracellular accumulation of viral copy numbers and poor virus release. Overcoming this limitation, the production process was likewise intensified by a perfused bioreactor with alternating tangential flow filtration (ATF), and BHK-21SUS cells were now adapted to chemically-defined medium. This led to cell concentrations of 1.1 x 107 cells/mL and reasonable ZIKV titers of 3.6 x 107 PFU/mL. This setup allowed to provide large amounts of ZIKV material for research and is a first step towards process development for manufacturing inactivated or live-attenuated ZIKV vaccines [1]. As demonstrated, BHK-21 cells are a promising cell substrate and its approval for human vaccine production processes may be reconsidered.

[1] Nikolay at al. Propagation of Brazilian Zika virus strains in static and suspension cultures using Vero and BHK cells. Vaccine (2017), 10.1016/j.vaccine.2017.03.018

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P-1

Functional Analysis of Phage N15 Protelomerase

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Bacteriophage N15 has a unique life-cycle that enable its prophage to replicate in linear form using its protelomerase (TelN), which function specifically on telomere occupancy sites (tos). TelN is a prokaryotic protein that cut at circular tos-containing double-stranded DNA, rejoins the ends to form hairpins, and replicates the prophage in linear form. Among a group of telomere resolvases, TelN is the only enzyme demonstrated its functional activities in vitro and in vivo of bacterial host. Our group showed TelN expression in Escherichia coli has its cleaving-rejoining function retained in bacterial host for the development of linear species, paving the way for solution associated with the vector integration problem in genome. More significantly, TelN also function in maintaining existence of linear species in bacterial host, potentially creating long-term expression of heterologous gene. Previous achievements of TelN-tos in bacterial cells suggest its functional activities may be retained when it is demonstrated in different hosts, due to its occurrence happens independent of host-encoded factor. Our works further explore the mechanism of TelN and study the functionality of its natural activities in terms of versatility, feasibility and stability. Efforts to improve the understanding of TelN-tos functionality system will be potentially useful in the study of long-term gene expression and providing preliminary solution to the vector integration issue. (213 words)

P-2

Leap-In transposases for the generation of recombinant mammalian cell lines for research purposes

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In the early phases of Biologics development projects in the pharmaceutical industry various protein and cellular reagents need to be prepared. For example, recombinant cell lines presenting the target protein on their cell surface are used as cellular antigens for the generation of therapeutic antibody candidates by phage display or hybridoma approaches. Also, recombinant cell lines producing secreted proteins like antigens, antibodies or various tool proteins are applied for the generation soluble protein reagents. In this context the objective of this study was to compare the commercially available Leap-In transposase system for the generation of stable recombinant mammalian cell lines to established in-house processes for stable pool generation and transient transfection in terms of quality, productivity, stability, timelines and ease-of-use.

Stable pools were generated for three different model proteins (GFP, human IgG1, and a pharmaceutically relevant type I single-pass transmembrane protein) and in three different mammalian host cell lines (HKB11, CHO and 300.19 mouse preB cells).

Generation of stable pools was achievable within 2-4 weeks and especially for HKB11 cells was significantly faster than with the reference process. High-producing pools could be generated for host cell lines and proteins where established processes only resulted in low-producing pools. The pools generated with the help of transposases appeared to be more stable than pools generated without transposases.

In conclusion, the application of Leap-In transposases proved to be an attractive and effective method for the generation of stable recombinant mammalian cell lines for various research purposes in the context of antibody and therapeutic protein discovery projects.

P-3

Lentivirus transgenesis for high yield production of human alpha-Galactosidase A in suspension CHO-K1 cells

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Fabry disease is an X-linked recessive disorder caused by a deficiency in lysosomal α-Galactosidase A. Currently, two enzyme replacement therapies (ERT) are available. However, access to orphan drugs continues to be limited by their high cost. Selection of adequate high-expression systems still constitutes a challenge for alleviating the cost of treatments. Several strategies have been implemented, with varying success, trying to optimize the production process of recombinant human α-Galactosidase-A (rhαGAL) in CHO cells. Herein, we have replaced the methotrexate amplification strategy (through which the highest rhαGAL producer clone described until this moment was developed) by a strategy based on third-generation lentiviral particles (LP) transduction of suspension CHO-K1 cells. LP transduction improved the global production process, as clones with productivities higher that the ones previously reported (3.5 to 59.4 pg.cell-1.d-1) with high specific enzyme activities were obtained. After two purification steps, the active enzyme was recovered (2.4 x 106 U.mg-1) with 100% purity and 60% overall yield. Michaelis-Menten analysis demonstrated that rhαGAL was capable of hydrolyzing the synthetic substrate 4MU-α-Gal at a comparable rate to Fabrazyme®, the current CHO-derived ERT available for Fabry disease (Vmax 60.1 ± 12.4 nM.min-1 and 63.2 ± 25.3 nM.min-1; Km 1.6 ± 0.6 mM and 1.2 ± 0.2 mM; Kcat 20.04 ± 4.14 and 21.1 ± 8.4 s-1 for rhαGAL and Fabrazyme®, respectively). Although glycosylation pattern and in vitro stability in plasma of both molecules were similar, rhαGAL contained 40% higher level of sialic acid. In summary, our process achieves the highest rhαGAL productivity reported to date (8-fold higher), maintaining the biochemical properties of the commercial product. Even more, considering the glycosylation characteristics of rhαGAL, which might provide advantages regarding pharmacokinetics, our enzyme could be postulated as a promising alternative for therapeutic use in Fabry disease.

P-4

Identification of novel promoters and genetic control elements derived from Chinese hamster ovary cells

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Typically, for recombinant protein production in mammalian cell lines strong viral promoters, like the Cytomegalovirus (CMV) promoter, are used. As these promoters evolved to ensure high transcription rates of viral RNA irrespective of the state of the infected cells, they consequently circumvent many of the cellular feedback controls in place to integrate gene expression with cellular state and environment. Due to their constitutive overexpression, viral promoters are subjected to a higher rate of silencing than endogenous promoters. Endogenous promoters would also have the advantage of better response to cellular signaling networks and thus a lower level of stress caused by uncontrolled overexpression of the transgene. Here we describe the identification of endogenous promoters and their regulatory elements from Chinese hamster ovary (CHO) cells. A promoter candidate list with the top 100 expressed genes was created based on RNA-seq expression data. For cloning of candidates, the CHO-Epigenome database (http://cho-epigenome.boku.ac.at/) was used to detect precise transcription start sites and promoter location. For measuring the promoter activity, a test system was established for normalized analysis of promoter strength in comparison to the Simian virus 40 (SV40) promoter without enhancer (set to 100% promoter activity). Different endogenous promoters were found showing between 2 and 21 fold higher expression values than the SV40 promoter, and up to 1.7 fold higher expression than the CMV promoter without enhancer. Computational analysis of tested promoters was performed using several free online prediction tools to identify transcription factor binding sites and regulatory elements.

P-5

Engineering CHO Cells

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Over the past two decades, the Chinese Hamster Ovary (CHO) Cell has become increasingly used as the expression host of choice in the industrial production of therapeutic proteins. Despite this fact, the CHO cell as production host is poorly understood and most of the development within this area has been done to optimise the expression of a specific protein product as the end goal. As the three main drivers that have been driving the cell factory development in the microbial field, (1) whole genome sequences and analytical ”omics”, (2) efficient genetic modification tools and (3) genome-scale in silico models, have now become available for the CHO cells, it is now possible to adress the CHO cells as cell factories for the production of therapeutic proteins in a completely novel and more general way.

The NNF-Center for Biosustainability (CFB) is engaged in an 8-year project, based on genome scale science to develop CHO cell lines optimised for the industrial production of therapeutic proteins. Using full scale metabolic models, cutting edge genetic engineering tools and high throughput technologies, we have been systematically engineering CHO cell lines to improve expression, secretion, growth, glycosylation, metabolism, etc… to obtain a panel of optimised CHO cell lines specialised for high productivity of therapeutic proteins with custom-designed homogenous glycosylation, made for optimal performance under large scale bioprocessing conditions.

Using the approach mentioned above, we are generating a growing panel of engineered CHO cells, with tailored glycanprofiles and improved bioprocess features, that will make the production of therapeutic proteins easier, cheaper and more efficient, and that also opens up the possibility to produce recombinant proteins in CHO cells, that can not be produced from CHO cells today. Among the cell lines made are glycosylation engineered CHO cells that only make biantennary glycans, CHO Cell lines that can not produce lactate and CHO cell lines resistant to some virus strains.

P-6

IMPROVING PRODUCTION OF RETROVIRAL VECTOR FROM PG13 CELLS FOR T CELL THERAPY

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Adoptive T-Cell therapy is a growing field for cancer treatment using the patient's immune system to battle the cancer cells. Tumor specific T cells are either isolated from a tumor or created by modifying the T cells and after expansion are administered to the patient. The modifications include adding specific T cell receptors (TCR) or chimeric antigen receptors (CAR) by way of retroviral vector, lentiviral vector, or other method. One method is to use PG13 cells, which are derivatives of NIH3T3 mouse fibroblasts, to stably produce a retroviral vector that is used to transduce the T cell. PG13 cells are anchorage dependent cells that grow in roller bottles or cell factories to produce the viral vector and recently in a fixed bed bioreactor. To improve the production of the viral vector we explore the possibility of its production using PG13 cells grown on microcarriers in a bioreactor. Microcarriers are small, approximately 100-300 μm, charged beads that support the attachment of the cells and are suspended in the growth media in the bioreactor that provide controlled growth conditions. In this way parameters, such as oxygen concentration, pH, and nutrient are monitored and controlled. The result is higher cell concentration and consequently virus titer. There was no effect on the specific virus titer or the efficacy of the vector in transducing t cells indicating that using microcarriers in a bioreactor is a good method for scaling up stable production of gamma retroviral vector in PG13 cells.

P-7

Cell cycle and transcriptome analysis of CHO-K1 cell culture bioprocessing

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Chinese Hamster Ovary (CHO) cells are the preferred host for the production of a wide range of biopharmaceuticals. Knowing how CHO cells behave during bioprocessing has until now relied heavily on empirical studies with a limited knowledge of the intracellular dynamics. The recent availability of CHO genome reference and several -omics data has led to new areas of research for better understanding of CHO cells metabolic behavior. The purpose of this work was to study the distribution of cell cycle phases and to perform transcriptome analysis, using RNA-Seq, during the culture stages of a typical industrial bioprocess of suspension-adapted CHO-K1 cells.

During 20 days, three replicates of CHO-K1 cells were cultured in 1 L bioreactors. Equal operating conditions were used and the temperature was shifted gradually from 37°C to 31°C. For cell cycle study, a daily sample was taken and DNA content was determined by propidium iodide staining followed by flow cytometry analysis and cell cycle modeling. Besides, RNA samples were collected on day 1, and during the exponential (days 7 and 8) and the stationary (days 14 and 15) growth phases for strand-specific RNA-Sequencing analysis. Differential gene expression (DE) between culture phases, Gene Ontology enrichment and pathways analysis were performed. Due to temperature shift, an S phase cell population reduction was observed and G0/G1 arrest over culture time, particularly during stationary phase nearly 80% of the cells analyzed were in G0/G1. Clustering analysis reveals that days 7 and 8 clustered together with day 1, and days 14 and 15 comprised another group, indicating that the major expression changes occur during stationary phase with respect to the beginning of the culture. A number of key regulatory genes and pathways involved in modulating the response to cold, metabolic and growth were identified. The DE analysis showed that at 31°C 443 genes were up-regulated and 182 genes were down-regulated. These genes can be utilized as targets for cellular and metabolic engineering to improve CHO cell bioprocessing.

P-8

Understanding the sources of variation in protein N-glycosylation patterns of recombinant proteins generated in Chinese Hamster Ovary cells

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Increasing the performance of Chinese Hamster Ovary (CHO) cells for the production of complex therapeutic proteins has been an ongoing process ever since the cell line was established for many blockbuster products. Among other factors the ability to generate proteins with human-like N-glycosylation has assured their position as major production system. Nevertheless, the resulting glycan patterns are often heterogeneous, their processing is considerably affected by bioprocess conditions and clonal variation and is difficult to control. One main objective in cell line optimization is to engineer CHO cells which stably produce defined N-glycan profiles to assess the influence of different N-glycan patterns on the pharmacokinetics of individual protein therapeutics. The regulation of N-glycan maturation is multilayered and there is only limited insight into the underlying processes. To some extent the variety of resulting N-glycan structures is determined by differences in the occurrences of glycosyltranferases resident in the Golgi. Transcriptomic data sets have revealed the theoretical capacity for N-glycan maturation in CHO cells, but the roles of the individual isoforms of Golgi glycosyltransferases have not been fully elucidated. Previous work has aimed to identify the key-players in the N-glycosylation cascade, but the results obtained are not fully consistent and the collected information is restricted to few model proteins.

The challenge to render the process of N-Glycosylation more stable and to reduce heterogeneity without lowering the degree of complexity of the mature N-glycan structures remains. In this project work is focused on understanding the contributions of the individual isoforms by generating a panel of multiple knock-out cell lines. The resulting changes are assessed with several, transiently produced model proteins, in order to additionally investigate the impact of glycoprotein structure and amino acid sequence on N-glycosylation. Based on these results, engineering strategies for further improvement with the aim to push desired reactions, potentially combined with a reduction of Golgi enzymes, will be developed.

P-9

Incorporation of a Super Vector and Transcriptome-guided Design for Enhancing CHO Cell Productivity

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Efficient production and secretion of recombinant protein in mammalian cells depends on combination of genetic metabolic and culture condition optimization. A major aim of our study is to improve the yields of therapeutic antibodies by utilizing optimized vector and engineered host. Rational vector design includes two genetic components (promoter and signal peptide). We've development a super vector by means of combination of selected promoters and signal peptide named J1.0. The expression titer of the stable pool J/DXB11 can be up to 300 mg/L (6 day batch culture) by simply increasing selection stringency, and it has twice the productivity when compared with pCHO1.0/CHO-S system. In order to identify genes that contribute to high IgG productivity in CHO cells, the CHO-specific microarray transcriptomics technology was carried out on high and low IgG producer clones with 7 fold difference in productivities. Hence, we generated four knockdown pools which targeting gene H, gene D, gene C and gene B respectively. Transfection of vector J 1.0 into these pools results in 1.65 to 2.4 fold increase in the expression. In conclusion, combining engineered CHO with optimized vector provides an attractive system for generating mAb in CHO cells with high productivity.

P-10

Investigation of Relationship between EBNA-1 Expression Level and Specific Foreign Protein Productivity in Transient Gene Expression of HEK293 Cells

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In an attempt to determine the relationship between the Epstein–Barr virus nuclear antigen-1 (EBNA-1) expression level and specific foreign protein productivity (qp), EBNA-1-amplifed HEK293 cells, which achieved a higher EBNA-1 expression level than that achieved by HEK293E cells, were established using dihydrofolate reductase (dhfr)-mediated gene amplification. Compared with a control culture in a null pool, Fc- fusion protein production by transient transfection in the EBNA-1-amplified pool showed a significant improvement. qp was linearly correlated with the EBNA-1 expression level in the transient transfection of EBNA-1-amplified clones, as indicated by the correlation coefficient (R2 = 0.7407). The Fc-fusion protein production and qp in a transient gene expression-based culture with EBNA-1-amplified HEK293 cells, E-amp-68, were approximately 2.0 and 3.2 times, respectively, higher than those in a culture with HEK293E cells. The increase in qp by EBNA-1 amplification mainly resulted from an enhancement in the amount of replicated DNA and level of mRNA expression but not an improved transfection efficiency. Taken together, it was found that EBNA-1 amplification could improve the therapeutic protein production in an HEK293 cell-based transient gene expression system.

P-11

Reprogramming Amino Acid Catabolism in CHO Cells With CRISPR-Cas9 Genome Editing Improves Cell Growth and Reduces By-Product Secretion

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Chinese hamster ovary (CHO) cells are the preferred host for production of biopharmaceuticals. Amino acids are biologically important precursors for CHO metabolism; they serve as building blocks for proteogenesis and are utilized for growth and cellular maintenance. In this work, we studied the physiological impact of disrupting amino acid catabolic pathways in CHO cells. We aimed to reduce secretion of a wide range of growth inhibiting metabolic by-products derived from amino acid catabolism, including lactate and ammonium. To achieve this, we engineered nine genes in seven different amino acid catabolic pathways using the CRISPR-Cas9 genome editing system. For identification of target genes, we used a metabolic network reconstruction of amino acid catabolism to follow transcriptional changes in response to antibody production, which revealed candidate genes for disruption. We found that disruption of single amino acid catabolic genes reduced specific lactate and ammonium secretion while specific growth rate and integral of viable cell density were increased in many cases. Disruption of multiple amino acid catabolic genes further reduced secretion of lactate and ammonium, but did not increase growth. Taken together, this study demonstrates the potential in engineering the amino acid catabolism in CHO cells to achieve an improved phenotype for bioprocessing.

P-12

High-throughput knockout of difficult-to-remove and troublesome CHO host cell proteins to create a clean CHO cell

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The Chinese hamster ovary (CHO) Cell Line Engineering department is addressing the need to obtain high yields and quality of protein biopharmaceuticals produced in optimized CHO cells through genome-scale-based methodologies. Host cell proteins (HCPs) are one of many process-related impurities generated during the production of biotherapeutic protein products. Although many HCPs are effectively removed in downstream purification processes, a small population of HCPs are particularly challenging. Here, we present our ongoing effort to create a clean CHO cell by CRISPR/Cas9-mediated disruption of multiple genes. Targets were selected based on three criteria: (i) abundance in the CHO secretome as analyzed by mass spectrometry, (ii) co-purification with mAbs, and (iii) negative impact on product quality. MiSeq analysis and targeted proteomics were used to check for indels and confirm the absence of our proteins of interest, respectively. We analyzed total HCP content of a 6-fold, 11-fold, and 14-fold knockout and characterized their growth in shake flasks and bioreactors. We observed a strong reduction (~70-65%) in total HCP content and slightly improved growth. Protein A and ion exchange purification of a monoclonal antibody (mAb) produced in these cell lines also led to a strong reduction in HCP content when measured at different steps during the purification process. A collaboration with KAIST has recently been started to address the effect of deleting the targets that have been reported to have a negative impact on mAb quality.

P-13

Engineering of the CHO Secretory Pathway for Enhanced Secretory Recombinant Protein Production

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The secretory pathway is comprised of a series of membrane enclosed organelles that provide the required environments for the correct folding, assembly, post- translational modification, quality control and targeting of proteins in eukaryotic cells. Recombinant biotherapeutic protein production in such eukaryotic cells typically targets polypeptides to the secretory pathway to undertake these processes and deliver the target protein into the external environment. The overall goal of this project is to improve the efficiency of the translocation of material from one compartment to the next and the ability of individual organelles to process recombinant material in a quantitative and qualitative way in Chinese hamster ovary (CHO) cells, the workhorse of the biopharmaceutical industry.

The particular focus of this work is on the manipulation of a specific superfamily of proteins involved in the vesicle traffic and sorting aspects of the secretory pathway, the SNARE (Soluble N-ethylmaleimide-sensitive factor Attachment protein Receptor) family. Through manipulation of proteins involved in this aspect, the aim is to improve the secretion of proteins of interest in a quantitative manner but also in a qualitative manner by improving the secretion of difficult-to-express proteins. To this end, the project is based on overexpressing target proteins transiently or stably, alongside products of interest to determine the impact on the cells, specifically regarding cell growth and cell productivity. To date we have cloned and stably expressed 5 proteins implicated in control of secretory transport and related processes and expressed these transiently and stably in CHO-S cells grown in CD-CHO media. Each protein has been tagged with a fluorescent reporter to allow tracking of expression and localisation in the cell. Stable expression resulted in a pool of cells, as shown by flow cytometry, microscopy and western blot, expressing each target to different extents. Limited dilution cloning was therefore undertaken and the resulting engineered cell lines assessed to identify cell lines with a range of expressions for each target. Characterisation of these cell lines exhibited a range of cell growth phenotypes. Preliminary transient expression of two model recombinant proteins in the engineered cells, an FC fusion protein (Etanercept) and a monoclonal antibody (Adalimumab), have shown that engineering of some specific secretory pathway targets and levels enhances secreted recombinant protein amounts whilst other targets and levels actually reduced secreted methods. In conclusion, we present evidence that novel secretory pathway engineering can be used to enhance secreted recombinant protein amounts from CHO cells whilst maintaining appropriate growth characteristics.

P-14

Untangling the Mechanism of 3-Methyl Adenine (MA) in Enhancing Specific Productivity: Transcriptomic Survey into Recombinant CHO Cells Treated with 3-MA

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3-Methyladenine (3-MA) is a chemical additive that enhances specific productivity (qp) in recombinant Chinese hamster ovary (rCHO) cell lines. Different from its widely known function of inhibiting autophagy, 3-MA has shown to rather increase autophagic flux in various rCHO cell lines. Thus, the mechanism of 3-MA behind enhancing the qp needs to be elucidated. In order to evaluate the effect of 3-MA on transcriptome dynamics in rCHO cells, RNA-seq using next-generation sequencing was performed with Fc-fusion protein-producing rCHO cells treated with 3-MA. By analyzing genes that were differentially expressed by the addition of 3-MA during rCHO cell cultures, the role of 3-MA in biological processes of rCHO cells was identified. One of the pathways that were heavily influenced by the addition of 3-MA was unfolded protein response (UPR). Having a close correlation with autophagy, UPR is a process that reestablishes protein folding homeostasis under ER stress. Numerous studies have demonstrated that the bottleneck of increasing the qp lies within the secretory pathway in ER and promoted UPR in order to increase the ER capacity and consequently the production of recombinant proteins. The addition of 3-MA has shown to increase the expression of key regulators of UPR, such as Atf4, Ddit3, and Creb3l3, which further supports the idea that the enhancement in ER capacity acts as a key in increasing the qp. Consequently, the downstream effectors of UPR, which includes autophagy promoting genes, were also upregulated as well. Hence, the role of 3-MA in increasing UPR pathway could have taken a salient contribution to the increased autophagic flux in rCHO cells. Taken together, the transcriptome analysis allowed a better understanding of 3-MA's role in gene expression dynamics in rCHO cells and its mechanism in enhancing the q_p.

P-15

Comparison of Distinct Promoters and their Impact on Antibody Production in a CHO DG44 Expression System

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CHO cells are widely used for recombinant protein production. Sartorius Stedim Cellca GmbH continuously enhances the performance of their cell line development platform, which profits from the coordinated interplay of the CHO DG44 host cell line, the expression vector, media system and upstream process design. The present study utilizes vector engineering in order to optimize the promoter controlling the gene of interest (GOI) in Cellca's standard expression vector. Using a 25 mL scale batch cultivation screening experiment with stably transfected cell pools, seven alternative promoters were tested and compared to the standard simian virus 40 (SV40) promoter. Lead parameter was the titer of an IgG model Ab (antibody). Citrate synthase promoter (CS) and human antigen R promoter (HuR) showed titers similar to the control, whereas extremely low titers were observed for all other tested promoters (UbC (Ubiquitin C), hCMV (human Cytomegalovirus), GRP78 (glucose regulated protein 78), EF1-α (elongation factor 1-α), FAS (fatty acid synthase)), although their benefit was highlighted in literature (Damdindorj et al. 2014; West 2014; RunningDeer & Allison 2004; Qin et al. 2010; der & Lars Kober 2013). CS and HuR promoter as well as the mouse cytomegalovirus promoter (mCMV), which has been identified as a promising candidate in previous studies, were investigated in a 25 mL scale fed-batch experiment with stable pools. Based on final titers, promoter strength can be ranked as follows: CS<HuR<SV40<mCMV. Using mCMV, the mean final titer was highly improved compared to SV40 (1038 µg/mL vs. 332 µg/mL). Interestingly, the data show, that the increased titers are achieved by an elevated viable cell integral (567 cells*d/mL vs. 240 cells*d/mL) rather than by increased productivities (18.7 pg/cell/d vs. 16 pg/cell/d). In order to confirm the superior performance of the mCMV promoter, it was tested with three additional model Abs. The results showed that mean final product concentrations in a 25 mL scale fed-batch experiment were increased by 46 % to 176 % with mCMV compared to SV40. This was mainly due to a higher viable cell integral for two out of three model Abs and due to increased cell specific productivities for the third Ab. Taken together, we could demonstrate that the mCMV promoter clearly outperformed the current standard. SV40 promoter, resulting in enhanced expression levels in a product independent manner.

P-16

Metabolic Evolution of CHO Cells Varying in Synthetic Biomass Capacity

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What is the optimal CHO cell factory metabolic phenotype and how do we obtain host cells exhibiting this phenotype? Fundamentally, a successful host cell has to achieve two related, yet distinct objectives during a fed-batch production process, (i) replicate engineered genomes (i.e. make many recombinant gene copies) and (ii) synthesize recombinant product (make biomass). With respect to the latter, we assume that ability to make product biomass is generally proportional a cell's ability to make its own biomass – it is synthetic capacity. How do we obtain a metabolic phenotype that efficiently achieves both objectives? To address this question we have analysed cellular metabolic diversity deriving from multiparallel evolution of discrete CHO clonal isolates. Through detailed mechanistic dissection of mitochondrial and glycolytic functions, we have investigated how CHO cells vary in their ability to create and maintain cellular biosynthetic capacity during fed-batch culture to achieve an optimal combination of rapid exponential proliferation and extended maintenance of high cell biomass concentration. Our data reveal that for the vast majority of isolated subclonal populations, metabolic phenotype and culture performance drift over extended subculture, with only a small proportion of subclones exhibiting functional stability. Taken together, we show that (i) exploitation of functional diversity in metabolic programs arising through evolutionary subculture is a logical route to obtain parental CHO hosts with significantly improved ability to synthesize biomass and (ii) cell engineering strategies to holistically improve CHO cell culture performance should target control of mitochondrial biogenesis and oxidative metabolism.

P-17

miRNA engineering of CHO cells to develop impactful productive phenotypes

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Introduction

MicroRNAs have gained considerable attention as a breakthrough in cell and molecular biology as a potent cell engineering tools to improve process performance of CHO cell line. They are engineering alternatives to gene-based methods because they are not translated into proteins, avoiding a translational burden to the production cell line, and moreover, their ability to simultaneous regulate the expression of multiple genes.

Objectives

The role of miRNAs in several cellular pathways and uncertainty in their target recognition still remains a challenge. For this reason, our objective is to demonstrate their role as potential engineering targets to genetically enhance relevant phenotypes in CHO cells, widely used in the biopharmaceutical industry production. We aim to better understand the molecular mechanisms of promising miRNAs candidates to achieve an extended culture lifespan and higher product titer.

Methods

Cell culture and transient transfection

CHO DP12 were seeded at 2x105 in CHO-S-SFM-II media, supplemented with 2% polyvinyl alcohol, cultured at 170 rpm and 5% CO2 in an orbital shaker at 37°C. Cells were transient transfected with miRNA mimics at a final concentration of 25nM using INTERFERin siRNA transfection reagent.

Cell density and viability

Viable cell number/mL was measured using ViaCount assay on a Guava EasyCyte benchtop flow cytometer

Titer of expressed IgG

Productivity of CHO DP12 was analysed from supernatants using enzyme linked immunosorbent assay (ELISA).

Results

Our miRNA engineering approach has generated a significant amount of novel information on the role of microRNAs in both pathways, cell death and cell cycle. Our data suggests a new knowledge on how the expression of specific miRNAs such as miR-31*, miR-125b or miR-326, could exert a beneficial impact on bioprocess-relevant phenotypes.

The upregulation of miR-125b and miR-326 generate an increase in the viability in the last days of batch culture without a noticeable difference in the product titer. Meanwhile, the upregulation of miR-31* generates an arrest in the proliferation, an increase in the viability and a higher product titer.

Conclusion

This data provide significant evidence that host cell engineering using fine-tune miRNAs technology represent an attractive tool to improve the most impactful phenotypes, making the industry more sustainable and driving down the cost of and access to medicines to patients.

P-18

Pooled siRNA screening to identify genes that impact recombinant protein yield and cell growth in suspension CHO cells

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The industry workhorse of mammalian therapeutic protein production is the Chinese hamster ovary (CHO) cell system. Due to the continued growing demand for biopharmaceuticals, and despite enhancements in the ability of the CHO platform to express recombinant proteins, studies continue to pursue the improvement in recombinant protein production in order to achieve higher product titers while maintaining optimal product quality. Traditional strategies of cell engineering commonly rely on low- throughput overexpression or introduction of single genes which are beneficial for cell performance. Recently, CHO cell research has experienced a paradigm shift due to the availability of a variety of omics data. The Chinese hamster, CHO K1 and other CHO cell line genomes have been sequenced and published along with a library of epigenetic, proteomic, and transcriptomic data. This presents the community with new tools and datasets that can be used to rationally investigate and engineer the underlying biochemical process of industrially relevant CHO cell lines.

Here we present a process for identifying genes that influence growth rate and qP using a meta-analysis of CHO transcriptomics literature based on their frequency and pathway enrichment analysis. These targets have then been used to conduct a 1000 gene pooled RNA interference (RNAi) screen on a CHO GFP secreting suspension cell line. We present the results of this RNAi screen and the identification of genes whose down- regulation positively impacts upon cell growth and/or qP. We also present the design and characterization of a range of CHO cell lines that produce erythropoietin and Adalimumab with reporters for rapid screening that can be used as models for further high throughput screening experiments.

P-19

Knock-Down of RAD21 Leads to Productivity Enhancement in Recombinant CHO Cells

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Chinese Hamster Ovary cells are the working horse of pharmaceutical production and higher producing CHO cells are an important contribution. Despite its importance for industry, currently no siRNA library for CHO exists. A recent high throughput screen of a mouse siRNA library in a suspension CHO cell line yielded two possible targets for productivity enhancement, CHD4 and RAD21. CHD4 plays an important role in epigenetic repression of genes, whereas RAD21 is involved in the repair of DNA double strand breaks.

Various siRNAs were tested against each gene and the best applied together to reduce possible off target effects. Cell viability, cell density and productivity were monitored. Knockdown of RAD21 was able to enhance specific protein productivity (qP) to 56% higher compared to the mock sample in a Herceptin producing CHO-S cell line, while CHD4 did not show any effect. These effects were confirmed in other Herceptin producing CHO lineages. However slower growth was observed in these cells, which was not seen in CHO-S.

The results partly confirm the screening results, but also demonstrate that an emerging effect of the knock down on growth seems to be cell line specific. Thus engineering strategies may have to be designed specifically for each cell line and CHO lineage.

P-20

A new cell line development platform for high efficiency single cell deposition with in-situ image verification

Ian Taylor, Andrea Gough and Sky Jiang

Objectives

Deposition of single cells and associated seeding efficiency is currently regarded as a discrete step in the sequential cell line development workflow. After seeding, the cells then need to be independently verified in the wells of a microplate using an imager to confirm single cell origin.

Many labs currently use FACS or the limiting dilution method for single cell deposition, each of which have their own drawbacks. For FACS, it is possible to get very high seeding efficiency, however, many of the cells fail to grow following the harsh treatment resulting in low cloning efficiency. For limiting dilution, the reverse is the case; good cell survival but low seeding efficiency.

Novel cell printing approaches new to market are claiming efficiency improvements, however, these approaches still have their drawbacks:

- They still require separate whole well image verification
- They are difficult to validate
- Their use requires the replacement of expensive consumables after every few plates

In this poster, we introduce a new approach called Verified In-Situ Plate Seeding (or VIPSTM), which combines single cell deposition with concurrent in-situ image verification of the single cell in a well.

Methods

We will demonstrate the performance of the new VIPS technology using CHO suspension cells and assessing seeding efficiencies. Outgrowth or cloning efficiencies will be assessed using the Cell Metric whole well imager.

Results

VIPS images will be shown along with experimental data for the seeding and cloning efficiencies.

Discussion

Workflow benefits of verified in-situ of seeding will be discussed including:

- Elimination of ghost wells
- Validation of the seeding system
- Real-time image verification of a single cell in the well of a plate
- Improved seeding and cloning efficiencies
- Potential for high throughput application
- No expensive or proprietary consumables

P-21

Unravelling and Engineering the Role of Trace Metals on Recombinant Therapeutic Protein Synthesis and Heterogeneity from Chinese Hamster Ovary Cells

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In the bioprocessing industry, mammalian cell expression systems, such as Chinese hamster ovary (CHO) cells, are used to produce high value biotherapeutic recombinant proteins, such as monoclonal antibodies (mAbs). Recent improvements in yields have been driven by the selection/development of improved host cell lines, expression vector advancements, enhanced screening techniques, the optimization of medium and feeding formulations and feeding strategies. One area that has not been widely investigated is the role of trace metals, found in the medium and feeds, on cell attributes (e.g. growth) and recombinant protein production and quality. This is somewhat surprising as around a third of all proteins and half of all enzymes require metal cofactors, whilst trace metal amounts influence a range of cellular properties that impact upon cell growth and survival. At a recombinant protein level, trace metals also impact upon post-translational modifications (e.g. glycosylation), enzymes and proteins involved in recombinant protein folding, assembly and secretion, and on critical quality attributes (e.g. cleavage of monoclonal antibodies). Here we report on the examination of the intra- and extracellular flux of metal ions using ICP-MS and characterize the relationship of trace metals and their availability to model recombinant mAb production, quality and cell line characteristics. We show that manipulation of particular metals in the system evaluated can impact upon the flux of other trace metals and ultimately on product yields and cell growth. In addition, we have manipulated the amounts of specific metal transporters expressed in CHO cells, both mammalian and microbial transporters that have been introduced into CHO cells, and show how this can impact upon the flux of specific metals into CHO cells, cell growth and recombinant protein product yields and quality. Finally, we discuss how manipulation of such transporters could be utilized to enhance recombinant protein production and quality.

P-22

Generation of Cell Lines following Combinatorial Genome Editing and Evaluation in High Throughput Bioreactor System and Next-Generation Sequencer

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Gene editing technology such as CRISPR/Cas9 is a valuable tool for determining the effect of genetic elements on commercially relevant cell lines. The aim of this project is to generate CHO cell variants with multiple genome edits that may enhance CHO host characteristics and performance. Target parameters include: increased cell density and culture viability, favourable metabolic profile and improving the yields of difficult-to-express proteins. A workflow for editing individual genes was developed and genes of interest were knocked out both individually and in combination. Clones of these edited host cells were established and the absence of targeted protein demonstrated by Western blot. The parental clone was screened in the Ambr250 bioreactor system to determine the optimal cultivation conditions. Cells were taken during the run and transcriptome analysis performed on an Ion Torrent 5S instrument. Differences in the expression of genes could be seen depending on whether the cells were slow or fast growing and higher or lower producers. Genes involved in ER homeostasis, protein translation and protein folding were upregulated in higher producing cultures. Transfer RNA genes were upregulated in faster growing cultures. These results provide information on genes that may be relevant targets for the desirable host cell properties.

P-23

A CHO Media Platform to Facilitate Cell Line Development

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Chinese hamster ovary (CHO) cells continue to be the workhorse host cell line for manufacturing protein therapeutics. The development of a top performing clone entails multiple activities which include transfection, clone selection, and productivity assessment. Irvine Scientific has developed the chemically-defined, animal component- free BalanCD® CHO media platform to allow media consistency from development to manufacturing. Implementing this media platform throughout cell line development can help simplify workflow and shorten development time by reducing the need to optimize the media at each step. In this work, we demonstrate how we used rational design to develop 1) the BalanCD Transfectory CHO medium that allows for high transfection efficiency of CHO cells ensuring productive yields of recombinant protein, 2) the CloneMedia CHO Growth A semi-solid media to promote rapid and robust growth of single cell colonies and 3) the BalanCD CHO Feed media with enriched formulation to support high performing CHO growth and productivity during fed-batch production. Overall, the compatibility of Irvine Scientific's BalanCD CHO media platform provides a seamless, time-saving solution from gene to lead clones within 6-8 months as demonstrated through case studies.

P-24

Discovery of novel expression enhancers for transient transfection by transcriptomics-based pathway analysis

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Expression of proteins including antibodies by transient transfection (TT) represents a widely used technology in basic research and drug discovery. Small molecule enhancers of expression are applied in order to maximize protein titers. Short chain fatty acids and derivatives such as valproic acid and butyrate have been described as potent expression enhancers. Inhibition of histone deacetylases inhibition (HDACi) has been proposed as the major mode of action of the short chain fatty acids to increase productivity in TT. However, this class of molecules induces inhibition of cell growth and cell death thereby limiting its application as expression enhancers. By using comparative whole-genome transcriptomics in conjuction with bioinformatics pathway analysis including upstream regulator analysis, a variety of target molecules and pathways were identified as potential novel expression enhancers in a HEK293-based transfection system. In follow-up experiments, nuclear factor kappa B (NFkappaB) was validated as key pathway to increase productivity. By interfering in the NFkappB pathway by activation using e.g. flagellin, interleukin-1, tumor necrosis factor and inhibition by BAY 11-7082, an irreversible inhibitor of IkappaB-alpha phosphorylation, protein expression was increased and decreased, respectively. In addition, activation of NFkappaB during HDACi-induced gene expression was shown. Besides NFkappaB, other inflammation-related pathways were identified as source of potential novel expression enhancers. Moreover, specific HDACs of class I were shown to be critical for productivity increase in TT by this method resulting in identification of potent HDACi as expression enhancers.

In summary, novel expression enhancers were identified for increasing productivity in transient transfection systems for small-scale and large-scale applications by a transcriptomics-bioinformatics approach. This strategy may also be relevant for development and optimization of bioprocesses in fields different from TT.

P-25

Expression of full-length HER2 protein on the surface of budded virus-like particles

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Biomarkers of cancer are often glycosylated membrane receptor proteins present on the cellular surface. In order to develop new antibodies for cancer diagnostics or treatment, it is a main pre-requisite that the target proteins are available in a native conformation. However, membrane receptor proteins are notoriously difficult to produce due to their hydrophobic nature and complex structures. Here, we used the baculovirus-insect cell expression system to produce budded virus like particles (VLPs) as the scaffold for the presentation of complex membrane proteins. Since the epidermal growth factor receptor 2 (HER2) is known to be overexpressed in a number of cancers it was chosen as the model for a tumor antigen. VLPs displaying full-length HER2 on the surface were produced in Sf9 insect cells and were purified by sucrose gradient ultracentrifugation. The number of secreted particles was quantified by nanoparticle tracking analysis. To confirm the presence of full-length HER2 on the surface, VLPs were labeled with gold-conjugated antibodies and were analyzed by transmission electron microscopy. To evaluate functionality of displayed HER2, VLPs were used in an ELISA and a newly-established biolayer interferometry based method. Detection was accomplished using Herceptin and phages displaying a single-chain variable fragment (scFv) of an anti-HER2 antibody. Significant stronger binding of Herceptin and anti-HER2 phages to HER2-displaying VLPs compared to control VLPs demonstrate that Sf9 insect cells are highly feasible to produce various budded VLPs that serve as an ideal platform for full-length membrane receptor presentation. Using our strategy, many other membrane proteins including cancer antigens, immune cell markers and immune receptors could be expressed. Therefore, VLP surface display ma contribute markedly to antibody selection and engineering as well as cancer vaccine design and diagnostics.

P-26

HIV-1 VLP Production Enhancement by the Use of iRNA and Chemical Additives

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Gag polyprotein from HIV-1 is able to generate Virus-Like Particles (VLPs) when recombinantly expressed in animal cell platforms. HIV-1 VLP production in HEK293 cells can be improved by the use of different strategies for increasing product titters. One of them is the so-called Extended Gene Expression (EGE), based on repeated medium exchanges and retransfections of the cell culture to prolong the production phase. Another approach is the media supplementation with gene expression enhancers such as valproic acid and caffeine, despite their detrimental effect on cell viability. Valproic acid is a histone deacetylase inhibitor while caffeine has a phosphodiesterase inhibition effect.

This work has two main objectives. First, the combination of the EGE protocol with valproic acid and caffeine supplementation to maximize VLP production; and second, the replacement of these chemical additives by iRNA for obtaining the same inhibition action.

The combination of the EGE protocol with caffeine and valproic acid supplementation resulted in a 1.5-fold improvement in HIV-1 VLP production compared with the EGE protocol alone, which is an 18-fold improvement over a conventional batch cultivation. shRNAs encoded in the expression vector were tested to substitute valproic acid and caffeine. This novel strategy enhanced VLP production by 2.5 fold without any detrimental effect on cell viability, which results in obtaining higher quality VLPs. Finally, the combination of shRNA with EGE resulted in more than 14 fold improvement compared with the batch standard protocol traditionally used. This protocol enables the production of high-quality HIV-1 VLPs avoiding toxic effects of the additives but maintaining high product titters.

P-27

Improving the production of recombinant human bone morphogenetic protein-4 in CHO cell cultures through inhibition of its undesirable self-regulatory mechanism

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Recombinant human bone morphogenetic protein-4 (rhBMP-4), a potential therapeutic reagent for the treatment of bone and cartilage, is considered to be a "difficult to express" protein. BMP-4 is a multifunctional growth factor that participates in diverse biological processes beyond bone induction, and its expression is tightly regulated by numerous antagonists that act intra- or extracellularly. In this study, the distinct feature of rhBMP-4 as a signaling molecule was investigated to improve its production in CHO cells. Extracellular rhBMP-4 can be entrapped by three types of BMP-binding proteins: receptors on cell membranes; secreted BMP antagonists; and membrane-associated proteins, such as heparan sulfate proteoglycan (HSPG). This may contribute to the poor acquisition of secreted rhBMP-4 in CHO cells. When CHO DG44 cells were incubated in the medium containing 10 μg/mL of rhBMP-4, the rhBMP-4 concentration in the medium decreased to 5.9 ± 0.3μg/mL (n = 5) after 24 h of incubation. A significant portion of the rhBMP-4 molecules in the culture medium was associated with and/or internalized into the cells during the incubation. It was found that essential components of BMP-4 regulation, including BMP receptors and several extracellular antagonists, were conserved in the CHO cells. The addition of endocytosis inhibitors (chlorpromazine, monensin, and genistein) to the medium was unable to maintain the rhBMP-4 concentration during the 24 h incubation of the cells, and nor could downregulation of BMP receptor 2, which binds directly to rhBMP-4. However, the addition of dextran sulfates or heparin (competitive inhibitors of HSPG binding) to the medium achieved maintenance of the rhBMP-4 concentration during the indicated incubation period, suggesting that the rhBMP-4 in the medium is regulated by HSPG-mediated pathways. In fact, addition of the HSPG inhibitors to the cultures of rhBMP-4-producing CHO cells resulted in an over 1.4-fold increase of specific productivity and maximum concentration of rhBMP-4 without affecting cell growth. Taken together, these results indicate that the rhBMP-4 secreted in CHO cell cultures is regulated mainly by HSPG-mediated pathways, and the HSPG inhibitors are potent culture supplements for improving rhBMP-4 production in CHO cells.

P-28

IgA Difficult to Express Protein Expression in CHO Cells is Dependent Upon the Variable Heavy Chain Region: Bottlenecks in Heavy Chain Assembly and Secretion

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Production of IgG proteins used as biopharmaceuticals has revolutionized the treatment of many diseases with a number of blockbuster IgG molecules on the market. IgGs manufacture in cultured Chinese hamster ovary (CHO) cells has been enhanced dramatically over the last 2-3 decades such that yields in excess of 10 g/L have been reported. However, although IgA, the second most prevalent antibody class in humans, has recently been reported as a promising new immunotherapeutic that can prevent and treat infections at mucosal surfaces, there are few reports on the development and manufacturing of these molecules as they are difficult to express compared to IgGs. We have generated three cassette expression system to evaluate transient and stable expression in CHO-S cells CHO codon optimized IgAs using model systems of humanized IgA1 and IgA2 antibodies against Shiga toxin 2 (Stx-2) and IgA1 and IgA2 antibodies against Human epidermal growth factor receptor 2 (Her2). In our model system, both IgAs have the same light (LC) and heavy chain (HC) stable domain, but differences in the variable domains with homology between variable regions being 45.8% (light chain) and 57.5% (heavy chain). Along with the HC and LC of the IgAs we have also expressed a common J-chain (joining chain). Using these we have generated a panel of monoclonal cell lines cultivated in serum free conditions (CD-CHO medium) and stably expressing IgA1 or IgA2 antibodies (heavy chain, light chain and J-Chain). To understand differences and to explore potential expression and secretion limitations we have characterized growth in 37° and 32°C, intracellular expression and secretion of IgAs, potential UPR stress response and evaluated the distribution of light and heavy chain within the cell. We find that at reduced temperature both intracellular and secreted IgA amounts are enhanced. In particular, HC expression is enhanced at lower temperature, however at both temperatures HC tends to be retained within the cell limiting the amount of secreted IgA observed. Here we describe and discuss the limitations upon IgA expression in CHO cells and potential approaches to alleviate these.

P-29

Utilizing Stuffer DNA Strategies to Improve Stable Expression of Difficult-to-Express Proteins

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CHO stable expression platforms have been the workhorse in Amgen's Protein Technology group because of their high productivity and much improved timeline compared to clonal stable expression. With the increasing need of complex proteins such as bi-specific antibodies and membrane proteins we often face challenges including failed stable cell recovery or poor protein productivity/quality. Optimizing DNA dosing is a common strategy in transient mammalian expression in order to achieve high titers. In this report we show that the addition of empty vector DNA can rescue stable cell pool recovery for certain difficult-to-express proteins and for toxic membrane proteins. Interestingly, we also observe that less GOI (gene of interest) dosing improves titers and purified yield of some difficult-to-express proteins.

P-30

Improving the Utility of FACS when Cloning Cells

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Recent scrutiny of cloning methods has led to concerns over the reliability of monoclonality within the industry. Moreover, there has been a reliance on vendor data to support the FACS (fluorescent activated cell sorting) of single cells. Lonza have identified key set-up measures and controls that, when implemented, can result in cell lines with a high probability of being monoclonal (≥0.99).

Following comprehensive instrument set-up, a series of gates are applied to exclude cell debris, non-viable cells and cell aggregates. A representative sample of cells are fluorescently stained, single cell sorted onto a 96-well plate-lid and visually assessed using fluorescent microscopy for the presence of 0 cells, 1 cell or ≥ 2 cells per well position. These data are used to estimate the probability of monoclonality using a prior to posterior Bayesian analysis. Since use of the FACS assumes that each droplet contains a cell, statistical methods based upon random distribution of cells in the droplets are not appropriate.

FACS system performance is monitored throughout each cloning session and monoclonality at intervals immediately before and after each sorting session. Any changes in instrument performance are corrected following appropriate control strategies to return the FACS equipment to optimal performance for cloning.

The use of such control strategies increases the confidence that a well contains a single cell. With this increased control over single cell deposition, Lonza believe that FACS is a highly appropriate method for generating monoclonal cell lines for bioprocessing uses.

P-31

Rabies virus-like particles expressed in insect cells

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Rabies is a zoonotic disease responsible for more than 60.000 deaths each year. The rabies affects different species of mammals, the vaccine prevention is considered the main method to contain the disease, the rabies virus glycoprotein (RVGP) is the only protein exposed on the virus surface and it is able to induce neutralizing antibodies that make it interesting to be expressed in many systems. Different vaccine proposals have been studied in order to establish an immune response mediated by neutralizing antibodies more effective and improve the level of cellular immune response, among them, the use of virus-like particles (VLPs). It is assumed that the matrix protein (RVM) constitutes major structural components of the rabies virus, and it has an important role in virus assembly and budding. It is our interesting that recombinant baculovirus system be able to producing VLPs containing the RVGP of higher quality. Therefore, the production of VLPs containing the RVM and RVGP would be system safer and cheaper vaccine candidates. The focus of this paper is to produce recombinant baculovirus bearing RVM and RVGP proteins of rabies virus and to produce vlps that are able to inducing immune response. The gene encoding RVM protein was synthesized according to the procedure of gBlock Gene Fragments (IDT Technologies) with restriction sites. The RVM was amplified by PCR and cloned in pFastBac1 vector (Bac-to-Bac - Baculovirus Expression System, Thermofisher). The pFast-M product was transformed into DH5a, for further processing in DH10 Bac. The RVGP gene was amplified from pMTGPV-Higro vector with restriction sites. Subsequently, the RVGP gene was cloned in pFastBac1 vector and it was transformed into DH5a, for further processing in DH10 Bac. Sf9 cells were transfected with 1 μg recombinant bacmid. The baculovirus recombinant, the matrix gene (BVM) or the RVGP gene (BVG), were collected 96 h post transfection (Lot 1). The supernatant (L1) was used to infect Sf9 cells with different volume, 500 μL and 1 mL. After 96 h post infection the supernatant and cells were collected and analyzed by dot blot or western blotting. Sf9 cells infected with BVM or BVG showed typical cytopathic effects such as ceasing of cell growth and decreasing cell viability. Additionally, the matrix protein rabies virus was detected by western blotting assay in infected cells and the RVGP protein was detected by western blotting assay. Thus, the BVM and the BVG are able to express the proteins in their conformational structure. It is our expectancy that a co-infection of BVM and BVG produce rabies VLPs.

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P-32

Determination of CHO biomass composition

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Chinese hamster ovary (CHO) cells are the primary host organism for the production of protein biopharmaceuticals. Significant improvements in product yield and cell growth were achieved in the past years by bioprocess and media optimization, directed evolution and targeted genetic engineering. However, a deeper understanding of the underlying processes in the cells is still limited. Recently, for the first time, a CHO-specific genome scale metabolic model was created in a large community effort. This model is a comprehensive resource of CHO metabolism. Using the toolsets provided by metabolic modelling, we are now starting to get valuable insights into the cells' metabolism, their protein production capabilities and their possible limitations. One of the essential inputs for the model is biomass composition. It has been shown that using strain and condition specific biomass together with bioprocess data improves the predictions made by the model. Currently, however, the model uses estimates and literature values, since comprehensive data about CHO cell composition, specifically of individual cell lines or strains, are lacking. In this work, methods for the determination of CHO biomass composition (proteins and amino acids, lipids, DNA, RNA, lipids and cell dry weight) were established. These include chromatography and mass spectrometry determination of lipids/fatty acids and amino acids, fluorimetric and spectrophotometric quantification of nucleic acids and gravimetric quantification of cell dry weight. Biomass compositions of various CHO cell lines (CHO-K1, CHO-S, CHO-DHFR-, antibody- and EpoFc-producer cell lines) are being evaluated throughout batch cultures to answer the question whether a generic CHO composition is sufficient for modelling or whether it is necessary to use strain or condition specific biomass data.

P-33

Simple and fast Baculovirus-free expression system in High 5 cells results in high protein yields

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The Baculovirus Expression Vector System (BEVS) has been used very successfully for the production of recombinant target proteins over many years. However, BEVS has two crucial drawbacks: Firstly, it is time- and work-intensive to generate recombinant baculovirus. Secondly, the quality of the target protein might be diminished in the late stage of infection due to the viral remodeling of the host cells.

A way to overcome these limitations while maintaining the high level of protein expression is the optimization of baculovirus-free, plasmid-based protein production in insect cells. Hereto, Polyethylenimine (PEI) was applied for plasmid-transfection as it presents a very cheap alternative to other commercial transfection reagents. The PEI transfection protocol was optimized by adjusting the PEI:DNA ratio, the PEI concentration, cell densities and the PEI type. Furthermore, different available expression vectors for baculovirus-free protein production were evaluated and an optimized High 5 cell line was generated. Finally, the resulting optimal protocol was used to compare yields of different intracellular and secreted target proteins to expression in BEVS. Most secreted target proteins resulted in the higher yields in the plasmid-based approach than in the BEVS system. Intracellular target proteins obtained similar or less protein yields to BEVS. Interestingly, the protein yields of our plasmid-based system were also in the range of the commercially available HEK293-6E cell system.

In conclusion, the plasmid-based approach is a valuable alternative to BEVS while being at the same time simpler to handle and faster. Furthermore, it avoids difficult separation of baculoviral particles during the production of viral target particles. Additionally, it simplifies the production of protein complexes as plasmid ratios can be very simply adjusted to meet the required expression rates.

P-34

Improving Transient Gene Expression in Insect cells

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Plasmid based transient gene expression (TGE) in mammalian cell lines is an attractive method to screen for expression constructs and produce high quality recombinant mammalian proteins. However, this technology largely depends on licencing EBNA 1 expressing HEK293 or CHO cell lines and using expensive cultivation media.

For large scale use of the technology we established the plasmid based TGE method in Hi5 insect cells. Using an optimised vector with eGFP we were able to reach up to 50% of the expression level compared to the Baculoviral Expression System. Although, the plasmids are not replicated and retained by the cell, the expression of the protein lasts for 48 to 60 hours. In this presentation we will show which steps have been successful to get to this level of expression. Upon thorough selection of the optimal plasmid, promoter, transfection agent, cell line and cultivation media, an improved procedure was established. Additional miniaturization using an automated micro-fermentation system allowed us to implement a high throughput splitGFP screen for expressible constructs. We will show that the TGE method in the insect cell line Hi5 is a fast and cheap alternative for production of substantial amounts of challenging recombinant proteins for functional and structural analysis.

P-35

Assurance of Monoclonality of Recombinant CHO Cell Lines Using High-Resolution Imaging

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It is a regulatory requirement that a production cell line used for the manufacture of biotherapeutics should be derived from a single cell progenitor. It is therefore standard procedure to include at least one cloning step during the development of a recombinant cell line. Numerous techniques are available for single cell cloning, but regardless of the method used there should be appropriate evidence to support that the method is fit for purpose. FUJIFILM Diosynth Biotechnologies (FDB) employ a two-step cloning strategy which combines the ClonePix™ as a cloning and screening tool followed by a second cloning step using the industrially accepted method of limiting dilution cloning (LDC). This paper describes the establishment of an imaging method to strengthen the LDC stage of our current cloning approach by providing evidence of the single cell stage of cell line development (CLD).

In order to demonstrate that a colony has been derived from a single cell, it is essential that all cells seeded into a well have been imaged. This paper focuses on the main areas of investigation performed at FDB to integrate a high-resolution imaging system into our CLD workflow; including the assessment of the suitability of a range of 96 and 384 well microplates and animal component free (ACF) medium types. The work carried out here identifies an optimum plate type and ACF cloning medium for cell growth and optical clarity. An imaging time point after cell seeding on day 0 was identified after which all cells could be visualised on the well surface. Further data to support this was then obtained which demonstrates that all colony outgrowth can be traced back to a visible cell on day 0.

P-36

Comparison of three different mammalian expression systems for the production of mammalian ion channels

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The production of milligram amounts of complex membrane proteins for structural studies remains a biotechnological challenge. Membrane proteins fold and assemble in the endoplasmic reticulum by a poorly understood, inefficient process, which can lead to large amounts of misfolded membrane protein accumulating in intracellular membranes when they are expressed from strong promoters. Complex membrane proteins are expressed preferably in mammalian cells, because other expression systems such as recombinant baculovirus or yeast often result in poor expression of functional protein. Here we compare the expression of a human voltage-gated sodium channel fused to GFP in three different mammalian expression systems. A tetracycline-inducible HEK293 expression system (T-ReX, Invitrogen) was investigated by either transient transfection using polyethylenimine or by the construction of a monoclonal stable cell line. The mammalian baculovirus transduction system (BacMam) was also compared. Transient transfection was the fastest technique, but the efficiency of channel expression was limited due to the high heterogeneity in expression levels measured by flow cytometry. The establishment of inducible monoclonal cell lines gave more homogenous expression levels per cell, but required the screening of several cell lines and was time consuming. The BacMam system involved moderate time investment and allowed the comparison of different cell lines. For each expression system, expression levels were quantified through the detection of the GFP-fusion protein by flow cytometry. All three expression systems produced a small amount of functional channels in the plasma membrane as detected by electrophysiology. Confocal microscopy studies indicated that the majority of the expressed protein did not reach the plasma membrane. The presence of aggregated protein was also observed and was assessed using fluorescence-detection size exclusion chromatography (FSEC). Degradation of the channels was revealed by western blot analysis of the solubilized protein. Of the three expression systems tested, the BacMam system seemed to be the most attractive as it is efficient, easy to modulate and can be used to screen other cell lines. However, none of the expression systems produced the sodium channels efficiently, indicating that further work is required to understand channel biogenesis and to develop effective expression systems for their overexpression for structural studies.

P-37

Strategy for Automation Implementation in Cell Line Development

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Automation solutions in the biotechnology sector have become a fundamental driver of progress for manufacturers and service providers with a global laboratory automation market of USD 3.92 billion in 2016. Under the aspect of 'speed to clinic', clients attach great importance to both shorter time lines in the cell line development (CLD) process and consistently high quality recombinant products. Sartorius Stedim Cellca GmbH's platform approach to cell line development for high-titer recombinant protein expression profits from the harmonized interplay of CHO DG44 host cell line, expression vector, media system and upstream process design. For the automation of our CLD process, which addresses both easy- and difficult-to-express proteins in a very short time frame (4-5 months from DNA to research cell bank (RCB)), we developed a strategy that uses forward-looking technologies while considering the need to ensure maintained high product quality. In a stepped approach, we plan to automate significant portions of the process and to minimize the manual workload to few quality-relevant steps. Currently, already a number of fully- or semi-automated instruments are used (Cellavista (Synentec), Vi-Cell (Beckman), ambr 15 (Sartorius)). Further islands of automation are being implemented, utilizing flexible robotic microtiter plate handlers that can operate a variety of semi-automated instruments (Cellavista, Multidrop dispenser (Thermo), iQue Screener (Intellicyt)), freeing scientists from the need to perform repetitive tasks. Subsequently, a high degree of automation will be realized in a flexible system, fully enclosed in a class 2 type enclosure with connect ports designed to optimize access to the internal instruments without compromising the internal environment. Choosing a system design with instruments on removable carts enables continued manual instrument usage and ensures maximum return on the investment while also having the flexibility to adapt the process to latest developments. Combined with the benefits of a high-standard laboratory information management system (LIMS) and data bank system, automation will allow us to process larger numbers of samples while ensuring sample traceability throughout the entire process and produce datasets relevant for documentation and evaluation. This automation strategy for both customer business and research activities is designed to accelerate standardization as well as discovery, while eliminating human error and increasing productivity and work package delivery.

P-38

Rolling Cycle Translation of Circularized Infinite Open Reading Frames; Fooling the Ribosome

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Background and Novelty: Recent findings highlighting the abundance of endogenous circular RNA (circRNA) in human and mouse (Jeck et al, 2013) and functional validation of numerous circRNA species (Hansen et al, 2013, Memzcak et al, 2013) point towards new genetic engineering targets. Naturally occurring circRNA demonstrate greater stability over their linear mRNA counterparts, and artificial circularization of exonic sequence has been achieved (Hansen et al, 2013, Li et al, 2015). Other reports suggest a new function for these molecules, translation of circularized RNA open reading frames (Wang et al, 2015; Abe et al, 2015; Yang et al, 2017).

Small and pilot scale production of new biologics are routinely done with transient transfection of an expression vector. The loss of expression through plasmid dilution is always going to be an issue with the approach. However, by generating a circular RNA open reading frame that is highly stable and infinitely translatable, it may be possible to greatly reduce this effect of plasmid dilution. The plasmid will still be lost at the same rate, however, each transcript from the expression cassette is not prone to enzymatic degradation, making it more stable over linear mRNA. The greater stability means transcript will not be lost over the duration of the transient batch.

Objectives: To investigate the potential of circularized open reading frames, as a means of improving RNA output. To achieve this, it was first thought to circularize a single exon as a proof of principle, following this, a comparison of linear and circular RNA of the same ORF. The human glycoprotein Erythropoietin (EPO) was used as a model to study synthetic circRNA translation in Chinese Hamster Ovary (CHO) cells.

Experimental approach: Taking advantage of the complementary intron pairing pathway (Hansen et al, 2013), vectors were generated to express the linear or circular form of a human exonic sequence as a proof of concept. To demonstrate the potential of this technology, the same structured intron method was used to circularize the human EPO gene. Four circular EPO (circEPO) constructs were designed, each harboring unique elements. The production of EPO from both linear and circular RNA is compared by yield in transient transfection, monitored by an ELISA and Western Blot.

Results: Efficient circularization of the human ACTB exon 3 sequence has been achieved, with >90% of the RNA transcript circularizing. Using the same approach, circularization of the EPO ORF was achieved in CHO K1 cells. Circularization was monitored by PCR, and validation by RNase R treatment of total RNA. The protein expression from these constructs was monitored by ELISA and Western Blot of supernatant from transient transfection.

Discussion: The proof of concept experiments, circularization of human exonic sequence, was very promising. The proposed back-splice theory outlined in (Jeck et al, 2012; and Memzcak et al, 2013) proved true. Initial data from the circEPO expression plasmids revealed the expected result, but work is needed to refine the experimental design.

P-39

Expression of Difficult to Express Proteins using the Drosophila S2 based ExpreS2 system

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Drosophila S2 insect cell expression is less known than the extensively used Spodoptera or Trichoplusia ni (Hi-5) insect cell based Baculovirus expression system (BEVS). The cell line was derived from late stage Drosophila melanogaster (Fruit fly) embryos by Schneider in the 1970s, who named the cell line Drosophila Schneider line 2 (synonyms: S2, SL2, D.mel. 2). S2 cells have proved to be highly effective for the production of proteins from a great variety of protein classes (1), such as: viral proteins, toxins, membrane proteins, enzyme, etc. Recent publications have also shown the strength of the S2 system in expression of Virus Like Particles (VLPs) (2).

ExpreS2ion has developed the ExpreS2, Drosophila S2 platform to achieve improved yields for difficult to express proteins. Furthermore, several technologies have been developed to improve the ease of use of the system, as well as enable fast and efficient screening of multiple constructs. For example: An efficient transient transfection screening method using suspension cultures will be presented. This method can be applied to 1ml volumes in 96-well deep-well plates or larger volumes in shake flasks. Examples of a range of proteins expressed in the system will be presented, including immune modulatory proteins (IDO and PD- L1), glycoproteins from emerging diseases (Ebola, Zika, Dengue, and Marburg), malaria antigens (Rh5, VAR2CSA, and Pfs variants), and VLPs (HIV GAG, Zika, and Dengue).

The Drosophila S2 expression system has been used for antigen manufacture up to Phase II clinical trials. ExpreS2ion have developed S2 based production processes for two malaria vaccine clinical trails with Oxford University (Rh5) and Copenhagen University (VAR2CSA). Currently ExpreS2ion is developing a production process for Ebola antigen for generating hyperimmune anti-serum, containing neutralising antibodies in collaboration with AbiVax. The system is well suited to both R&D and clinical development, with particular advantages for difficult to express secreted proteins.

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P-40

Development of a Platform Expression System Using Targeted Integration in Chinese Hamster Ovary Cells

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In recent years the biomanufacturing industry has seen significant improvements in recombinant protein production titers due to advances in protein expression technologies as well as media, feed and manufacturing process development. However, the standard methods of recombinant cell line development have remained relatively unchanged. The majority of the BioPharma industry introduces transgenes into Chinese Hamster Ovary (CHO) cells using mechanical or chemical transfection processes followed by metabolic or antibiotic selection of stable recombinant pools. Through this process, the transgene(s) is randomly integrated into the genome, often resulting in significant heterogeneity within the stable pools. Individual recombinant CHO cells within the pools can vary greatly in their growth and productivity profiles, product quality attributes, and genetic stability. To isolate and identify the best performers, the time and resource consuming process of single cell clone generation and characterization is used, commonly requiring hundreds to thousands of clones to be characterized in order to find those suitable for manufacturing processes. Here we describe our strategy for developing a CHO expression platform that enables targeted and site specific integration of transgenes at well characterized and stable hot spots in the CHO genome. We have generated clonal cell lines in which a landing pad has been randomly integrated, and following RMCE with an expression vector for a therapeutic IgG, stable pools and clones were generated and characterized for productivity, stability and transgene copy number. Following this approach, we have identified and characterized several high performing landing pad clones with performance characteristics suitable for commercial manufacturing processes. We also describe our efforts to validate reagents and protocols that will enable integration of transgenes into our landing pad cell lines with high efficiency and reduced off-target effects, leading to the rapid generation of more homogeneous stable pools and consistent, predictable performance across single cell clones.

P-41

Surfactants in cell culture media: impact of raw material quality on growth performance

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Surface active agents (surfactants) are commonly used cell culture medium components for reducing shear stress in non-static suspension culture. Within the cell culture community, there is an ongoing discussion about surfactant-related process deviations. Furthermore, surfactants have shown to interact with polyplexes as well as polymer nanoparticles within various applications (e.g. transfection, encapsulation). Better understanding the related mechanisms of action will facilitate finding alternative components for progressive cell culture media formulations.

HEK 293-F and CHO-K1 cell lines were cultivated in plain and baffled shake flasks using different poloxamers. The surfactants were also evaluated regarding their impact on transient gene expression and cellular localization. Variations in raw materials and different lots thereof were analyzed by size exclusion chromatography (SEC) and nuclear magnetic resonance spectroscopy (1H-NMR).

Cultivations of HEK and CHO cells under low shear stress conditions led to comparable growth with a maximum viable cell density (vcd) in the range of 1·107 cells/mL (CHO- K1) and 1.2·107 cells/mL (HEK 293-F), independent from respective surfactant. In contrast, experiments in baffled shake flasks revealed differences in peak vcd within a range of 0.2 – 1·107 cells/mL. Transient transfection efficiency was comparable for all tested poloxamers, independent from the respective lot. Tracking of poloxamers in the culture revealed a time-dependent uptake by cells as well as a colocalization with cell membrane and lysosomes. SEC of surfactants showed lot-to-lot variation in the low molecular weight fraction, especially for the tested lots of Pluronic® F-68. This fraction represents mostly the PEO block (revealed by 1H-NMR), a remnant from synthesis. Overall, results indicate that the use of poloxamers in cell culture media impacts growth performance and preliminary analysis of raw materials is advised. Additional experiments will focus on the investigation of specific fractions from SEC to elucidate poloxamer performance.

P-42

Fast Predictive Expression Platform – CHO-K1 with Transposase

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The Mammalian Expression group in Therapeutics Discovery Research produces panels of therapeutic candidates in high-throughput (HT) for early stage screening in addition to generating larger productions that are used for manufacturability assessment, pre-clinical PK, and efficacy studies. As our molecule types expand from monoclonal antibodies to include various bispecific modalities, the ability to predict how a molecule will behave in our stable manufacturing host becomes more difficult. To more quickly predict characteristics and manufacturability of our candidate therapeutics, we are developing in-house stable expression systems. The transposase is a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut and paste" mechanism. Because the transposase facilitates non- random, efficient genetic integration, we investigated the possible incorporation of this technology into our current in-house transient expression vector system. We have observed that its stable-like integration properties provide us with a foundation for enabling a short production time, comparable with a transient expression system, while generating proteins with attributes that are predictive of our manufacturing system. This approach has been validated and implemented with our CHO-K1 stable expression system. With this technology, we are able to reliably generate the diverse array of therapeutic candidate modalities in a high-throughput format while achieving higher predictability of material derived from our manufacturing hosts.

P-43

Role of KRAS on Exosome Formation and Dissemination

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Exosomes derived from KRAS mutated colon cancer cells were reported to possess greater invasive potential. This study aimed to investigate the effect of KRAS mutation in regulating exosome formation and dissemination. Two colon cancer cell lines with different statuses of KRAS gene, HT29 (wild type) and SW480 (G12V mutation) were transfected to express GFP-tagged CD63, a signature protein commonly used to mark the distribution of exosomes. In a flow cytometric analysis using BD FACSCalibur system, significant expression of GFP-tagged CD63 was observed in 80% of SW480 cells while only 36% of HT29 cells were found with similar expression. Later another flow cytometric analysis was carried out using Amnis ImageStreamX system, a new approach enables visualization of submicron size of nanoparticles. Exosomes derived from CD63- GFP transfected SW480 were labelled with membrane dye PKH26. Flow cytometry analysis showed only 2% of PKH26-labelled exosomes carried GFP tagged CD63, implicates that CD63 may not be an appropriate protein marker used in this study for marking of secreted exosomes. Therefore, proteomic analysis was performed to profile the proteins in SW480-derived exosomes. The list of identified proteins include (i) soluble signaling molecules (TRAIL, TNF) and (ii) regulatory proteins (Arf6-, Cdc-42- and RhoA-) facilitating exosome uptake via raft-mediated endocytosis. Next, a representative protein which uniquely expressed in exosome only will be selected as exosomal marker for the following imaging study. To further investigate the effect of KRAS mutation in regulation of exosomes, farnesylthiosalicylic acid (FTS) was used to inhibit RAS-mediated signaling in SW480 cells. Surprisingly in FTS treated cells, increased production and uptake of exosomes was observed as compared to untreated cells. In a nutshell, colon cancer cells with mutated KRAS may produce more exosomes in compare to those with wild type KRAS. However, inhibition of KRAS signaling may cause severe cell stress in which biogenesis and uptake of more exosomes are executed as a protective act in colon cancer cells bearing mutated KRAS.

P-44

Protein Profiling of Exosomes from Oral Cancer Cells with Distinct Cisplatin Sensitivity

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Exosomes are 30-100nm nanovesicles produced by various cell-types. These vesicles are reported to function in various biological processes, however their role in modulating drug resistance in cancer cells are least investigated. This study aims to investigate the role of exosomes in regulating cisplatin sensitivity in oral squamous cell carcinoma (OSCC). We analyzed the responses of eight OSCC cell lines derived from oral cancer patients to cisplatin treatment and found that the concentrations of cisplatin that inhibited 50% cell viability (IC50) were varied in these cell lines. H103, a cell line derived from stage I well- differentiated OSCC tumor was observed as cisplatin-sensitive cell line with IC50 of 15 μM while H314, from stage II tumor as cisplatin-resistant cell line with IC50 of 200 μM. Next, a resistant variant H103/D2 with 10-fold higher resistance in cisplatin treatment (IC50 150 μM) than its parental cells (H103) was successfully established. Exosomes were isolated from these OSCC cell lines and significant increased exosome production was observed in both cisplatin-resistant cell lines compared to sensitive H103. All three cell lines secreted more exosomes in response to cisplatin treatment. Interestingly, a lack of beta actin in exosomes isolated from H314 was found in the process of marker protein verification. Proteins of the exosomes isolated from the three cell lines were then profiled using nanoLC ESI MS/MS and Byonics (Protein Metrix). A total of 102 differentially expressed exosomal proteins was observed between H103 and its resistant variant H103/D2 while 154 was observed between H103 and de novo resistant cell line H314. Only 4 exosomal proteins were differently expressed in H103/D2 and H314 suggesting exosomes from both resistant cells contained similar proteins. Among the proteins identified in exosomes of resistant cells, those from integrin family (ITGB4, ITGA6, PLEC) may provide insight intothe drug resistance mediated by exosomes secreted from OSCC cells.

P-45

Improved CHO Cell Line Development: Combined UCOE® Technology and CHOZN® Platform

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The process of identifying highly productive clones is a time-consuming, albeit necessary, step in therapeutic protein production. In recombinant cell line generation, the site of chromosomal integration greatly influences transgene expression. When using a random integration approach most plasmid DNA integrates into transcriptionally inactive heterochromatin and the transgene is silenced. The addition of chromatin opening elements, which create a transcriptionally active open chromatin environment around the integrated transgene, improves the efficiency of identifying the highly productive clones.

In this study, we tested the combination of UCOE® (Ubiquitous Chromatin Opening Element) technology with the CHOZN® GS cell line development platform. Two different chromatin opening elements were introduced into the expression vector and recombinant cells were selected by both minipool and bulk pool selection methods. Titers and total cell line development times were evaluated.

Clones containing chromatin opening elements which were isolated using a bulk pool approach had comparable titers to those isolated from the standard vector using the minipool approach. However, clones derived using the minipool approach required an additional two months of development time. Additionally, the best clones containing chromatin opening elements derived from minipools expressed greater than 2g/L of recombinant antibody.

The ability to isolate highly productive clones from bulk pools suggests that UCOE® technology is preventing transgene silencing. Since clones derived from UCOE® minipools produce twice the titer of clones from control minipools, it is likely that chromatin opening elements aid in creating a larger population of highly productive clones. The combination of the CHOZN® GS cell line development platform with UCOE® technology enables rapid and robust cell line development.

P-46

Evaporation and aeration in shaken bioreactors

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pCO2 and osmolarity are known to have a significant impact on the growth rate and production formation of a cell cultivation. Both parameters are influenced mainly through the transfer processes of ventilation and evaporation. If not taken into account during screening for an optimal clone, these processes could influence our decision. Especially in small scale systems (such as shake flasks, microtiter plates and tubes) these parameters/ processes are unknown.

The poster being presented will show ventilation and evaporation data for different shaken vessels and humidity levels. This data will help users to classify the shaken vessels and to estimate if these effects have an influence on the cell culture cultivation. This will lead to a more robust and valid screening phase.

P-47

New and Improved System for Transient Transfection of Suspension CHO Cells

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Suspension Chinese hamster ovary (CHO) cells are a desirable host system for biotherapeutic protein production due to their history of regulatory approval, adaptability to various culture conditions and the infrastructure that has been created through academic and pharma researchers. Transient transfection of suspension CHO cells affords the rapid generation of milligrams to grams of protein early in the drug discovery process. This, in turn, allows researchers to determine if drug candidates have desirable attributes and warrant the generation of stable clones.

With a continued commitment to the field, Mirus Bio has improved upon the CHOgro® Expression System for transient transfection, by: (1) identifying components (i.e. enhancers) to significantly increase titers and (2) developing a simple, single day protocol that includes transfection, addition of enhancer, and temperature shift. Within this new system, the enhancers act in synergy with the TransIT®-PRO Transfection Reagent and the CHOgro® Expression Medium for increased antibody production in multiple derivatives of suspension CHO cells including CHO-S and CHO-K1. By adding the enhancers at the time of transfection and immediately shifting to 32 °C, the optimized user-friendly protocol allows researchers more flexibility in timing the initiation of experiments and reduces the risk of contamination by eliminating the need for repeated handling of the culture.

To demonstrate the robustness of the improved CHOgro® Expression platform we expressed a panel of five commercial IgG1 therapeutic antibodies with identical vector backbones and compared titers. Additionally, we examined titers from representative Fc- fusion proteins. Competitor benchmarking against commonly used transient CHO transfection methods were performed and scalability of the transient transfection was assessed from culture sizes ranging from 2.5 milliliters up to 2.5 liters in shake flasks using a representative IgG antibody. Our results indicate that the attributes of the new and improved CHOgro® Expression System will help researchers obtain gram quantities of protein and realize their research goals with ease.

P-48

Expression and neutralizing evaluation of anti-tetanus human monoclonal antibodies

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Infectious diseases can be treated with antibodies since 1890; today the immunotherapy can move towards to monoclonal antibodies (mAbs). MAbs derived from human B cells are especially important, as generated and tolerized in humans, their safety and efficacy are potentially higher than mAbs from other origin. Tetanus is a severe neuromuscular disease with approximately 12,000 new cases each year (WHO), despite the existence of a safe and low cost vaccine. Recently, our group obtained a series of human anti-tetanus monoclonal antibodies (mAbs) sequences from single sorted B lymphocytes, which were submitted to RT-PCR followed by amplification of the genes of the variable regions by nested PCR. After analysis of the sequences of the genes, samples with the same V(D)J classification and similar CDR3 amino acid residue sequences had their fragments amplified and cloned into expression vectors encoding the constant region of one of the human immunoglobulin chains. The vectors obtained were transformed into E. coli DH5α and used for the expression of mAbs by transient cotransfection into HEK 293F cells. We started the evaluation of tetanus toxin (TT) neutralizing capacity of expressed mAbs following a Pharmacopeia protocol. In accordance to the potency test used for hyperimmune sera, an appropriate level of TT was incubated with the mAbs at 5 different concentrations (125, 62.5, 31.25, 15.62 and 7.81μg/kg) and injected subcutaneously into Swiss mice, after which were observed for 4 days for survival rates. Due to limitations imposed to animal testing only a small part of different clonally related mAb groups was screened so far. A preliminary preclinical in vivo testing has demonstrated neutralization of tetanus toxin with a cocktail of 3 mAbs chosen according to in vitro testing results. To understand the contribution of each mAb to TT neutralization the 3 mAbs were tested individually, in pairs and triplet. In the mice group receiving the 3 mAbs cocktail, 100% of the animals survived to 125 and 62.5μg total mAbs/kg. One pair of 2 mAbs resulted in 100% survival at a dose of 125μg/kg. Blocking of multiple epitopes must be needed to neutralize the TT completely. The project was conducted following human and animal ethical protocols. Alternatives to mice testing are in course to evaluate the mAbs panel.

P-49

Production scheduling of the rhPDGF-BB protein in mammalian HEK cells

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Due to its great mitogenic and angiogenic potential, platelet-derived growth factor (PDGF) has been studied over the years. One of its isoforms, PDGF-BB plays a large role in bone, dental, tissue repair in diabetic patients, but its production involves high costs, and low amounts. Then this article presents a way to increase the production of PDGF- BB for pharmaceutical industries. The focus of this article is the process of scheduling rhPDGF-BB production in spinner and Wave bioreactor using HEK 293T cells with growth adhered to the microcarriers aiming for greater production of rhPDGF-BB. To obtain PDGF-BB cDNA was used in 293T lineage obtained from fibroblast RNA by reverse transcription reaction in HEK cells. Dulbecco's Modified Eagle's Medium (DMEM) was enriched with Fetal Bovine Serum (FBS) [10-0.2%] and addition of microcarriers in the ratio 50/100 mL / g of Cytodex for 3 h at room temperature with agitation. Three useful volumes were used during the scaling stages: (1) Vinitial = 75mL; (2) Vspinners = 100mL containing: [2.0 g / L] microcarriers, Number of cells: 1.0 x105 cells / ml, 40 rpm, 37 °C, 5% CO2 saturated in moisture in a period of 12 days (288h); (3) Vbags = 1- 5L coupled to Wave Bioreactor TM 20/50 (GE®) in batch and cell densities between 5 × 106 and 1 × 107 mL-1 cells. Heparin (HiTrap TM 5mL, Heparin HP-General Electrics-GE®) column was coupled to Purifier TM UPC 100 (General Electrics-GE®) for downstream process, followed by polyacrylamide gel SDS- PAGE and Western Blot techniques and ELISA and other in vitro biological assay were used to quantify the activity of PDGF. Until now, cells adhered to microcarriers were capable to produce 350- 700 ng/mL of PDGF with biological activity.

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P-50

Evaluation of two alternative downstream process of rh-PDGF-BB produced via mammalian cells

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Recombinant human platelet-derived growth factor-BB (rhPDGF-BB) is used to treat full-thickness diabetic ulcers and is being investigated for use in other chronic ulcers, non-healing wounds, and periodontal defects. This article presents a way to improve downstream processes of PDGF-BB produced via HEK 293T cells. The focus of this article is the purification process of rhPDGF-BB production obtained in spinner flasks. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), enriched with Fetal Bovine Serum (FBS) [10-0.2%] and addition of microcarriers in the ratio 50/100 mL / g of Cytodex for 3 h at room temperature with agitation. Process condition: Vspinners = 100mL containing: [2.0 g / L] microcarriers, number of cells: 1.0 x105 cells / ml, 40 rpm, 37 °C, 5% CO2 saturated in moisture, 12 days (288h). Two alternative downstream process were tested: (1) Heparin (HiTrap TM 5mL, Heparin HP- General Electrics-GE®) column coupled to Purifier TM UPC 100 (General Electrics-GE®) and (2) RP-HPLC (Reversed-Phase High Performance Liquid Chromatography) - Source-30 column (30 mm polystyrene/divinylbenzene matrix; GE Healthcare) equilibrated with 0.1% TFA in water. Affinity chromatography showed higher levels of recovery (70-80%) when compared to RP-HPLC (50-60%).

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P-51

Development of Rapid Point of Care Diagnostics for Infection in Cystic Fibrosis Patients

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By age eighteen, approximately 70% of cystic fibrosis patients suffer from Pseudomonas aeruginosa lung infections. In the early stages of these infections, the bacterium is receptive to antibiotic treatment and can be eradicated with relative ease. However, as this infection proceeds, P. aeruginosa develops a mucoid phenotype, forming bacterial biofilms and developing resistance to many antibiotics. This results in an accelerated decline in lung function among other symptoms which typically increase mortality rates among cystic fibrosis sufferers. By targeting secreted proteases of P. aeruginosa, we aim to develop a cheap, easy to use rapid point of care diagnostic assay to identify such infections before they reach a chronic stage, allowing treatment to begin sooner. Of particular interest is the P. aeruginosa virulence factor Elastase (LasB), a protease involved in the breakdown of components of the extracellular matrix such as collagen, elastin and fibronectin, as well as acting to initiate formation of the bacterial biofilm via an intracellular mechanism. To date we have developed plasmid based constructs with particular features for the expression of LasB and other biomarker recombinant proteins that allow their expression in different systems, depending on the synthesis, folding, assembly and post-translational requirements of the target protein. LasB has been expressed in the periplasm of E. coli, from where it is recovered as an auto-processed fragment that has been partially purified by affinity chromatography. This material has been characterised by mass spectrometry, whilst we are currently assessing the activity of the partially purified molecule using a peptide fluorescence based assay. The affinity purified LasB is being used to raise polyclonal antibodies in rabbits which we intend to use to develop into a lateral flow assay diagnostic chip for use at the point of care. These antibodies are currently being assessed via sandwich ELISA and a synthetic LasB peptide array to determine their sensitivity and specificity, with intent to establish a monoclonal antibody for incorporation into a diagnostic assay.

P-52

Case study on human α1-antitrypsin: Recombinant protein titers obtained by commercial ELISA kits are inaccurate

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Accurate titer determination of recombinant proteins is crucial for evaluating protein production cell lines and processes. Even though enzyme-linked immunosorbent assay (ELISA) is the most widely used assay for determining protein titer, little is known about the accuracy of commercially available ELISA kits. We observed that estimations of recombinant human α1-antitrypsin (rα1AT) titer by Coomassie-stained SDS-PAGE gels did not correspond to previously obtained titers obtained by a commercially available ELISA kit. This prompted us to develop two independent quantification assays based on biolayer interferometry (BLI) and reversed-phase high-performance liquid chromatography (RP-HPLC). We compared the rα1AT titer obtained by these assays with three different off-the-shelf ELISA kits. We found that the ELISA kits led to inconsistent results with up to a 17-fold difference in rα1AT titer between the three ELISA kits. Moreover, none of the three ELISA kits reported rα1AT titers similar to our three reference assays: Coomassie-staining, BLI and HPLC. Our case study on rα1AT suggests that absolute quantification of recombinant protein reported in the literature cannot be trusted per se if determined by a non-validated ELISA kit. Consequently, any ELISA kit to be used for determining recombinant protein titer must be validated by a different, preferably orthogonal method.

P-53

Comparison of two glycoengineering methods aimed at reducing fucosylation of a camelid heavy chain monoclonal antibody

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Core fucosylation on a Fc N-linked glycan affects antibody effector functions. Absence of fucose increases the antibody dependent cell cytotoxicity response with increased binding to the FcγRIIIA receptor. To this end, the work presented here compares two different approaches to reduce core fucosylation of a camelid heavy chain antibody, EG2-hFc expressed in CHO cells. We have also analysed fucosylation on the intact antibody to determine steps in loss of fucose using ESI-MS and MALDI. The first method uses a FUT8 inhibitor, 2F-peracetylated fucose, which was added to cell cultures expressing the EG2-hFc antibody in increasing concentrations of 5,10,15,20 and 50μM. The addition of the inhibitor did not have any effect on cell growth. Glycan analysis was performed on antibodies collected from these samples using HILIC-HPLC. The inhibitor reduced fucosylation from 80% (0μM) to 17.5% (20μM). There was no substantial difference observed between 20μM and 50μM inhibitor added samples. The second method involved co-expressing a plasmid with a prokaryotic GDP-6-deoxy-D-lyxo-4-hexulose reductase (RMD) gene in the EG2-hFc producing cell line, to manipulate the fucose de novo pathway into producing GDP-rhamnose instead. The RMD plasmid was transfected into the CHO-EG2-hFc cell line with increasing concentrations. After applying a selection pressure, a stably transfected pool of cells was generated. As with the previous method, HILIC-HPLC was used to analyse the glycans produced. It was observed that this method was not as efficient in producing non-fucosylated antibodies. We also compared the products of these two methods using MALDI. The results point towards use of a chemical inhibitor such as 2F-peracetylated fucose, as a more effective approach towards producing non-fucosylated antibodies.

P-54

Expression of Chikungunya proteins in Drosophila melanogaster S2 cells

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Chikungunya virus (CHIKV) has circulated throughout the globe arriving in America continent by 2014. It is a zoonotic disease transmitted mainly by Aedes spp mosquitoes, widely distributed through the tropical territory. The disease caused by CHIKV infection is clinically characterized by sudden-onset of fever and severe arthralgia, which may persist for weeks, months or years after acute phase of the infection. Because CHIKV circulates during epidemics in an urban mosquito–human cycle, control of transmission relies on mosquito abatement, which is rarely effective. Furthermore, there is no antiviral treatment for CHIKV infection and no licensed vaccine to prevent the disease. The objective of this study is to obtain and characterize recombinant Chikungunya structural proteins expressed in Drosophila melanogaster S2 cell system, so that it can potentially be used for the development of a vaccine. To generate stable S2 transfectants producing CHIKV antigens, S2 cells were cotransfected with CHIKV cDNA under the control of a Drosophila methalotionein promotor along with a selection vector, which contains the Hygromicin resistance gene. A recombinant cell population was selected after 3 weeks and cultivated in SF900-III medium. Cells showed morphologic characteristics and growth parameters similar to the native S2 population, showing that the heterologous genes were not toxic to the cells. For protein expression 106 cell/mL S2 recombinant cells were inoculated in Shott flasks with 20 mL of SF900-III medium at 28 °C and 100 rpm. The expression was induced with CuSO4, for 120 hours and the supernatant was harvested by centrifugation, clarified using a 0.45 μm filter and finally concentrated by ultracentrifugation in a sucrose gradient. CHIKV antigens were detected in the S2 cell supernatant and cell pellet fractions by Immunofluoresce and Western Blotting, using anti-CHIKV polyclonal antibodies (ATCC). A Nanoparticle Tracking Analysis showed vesicle-like structures in the supernatant samples, suggesting the formation of Virus-Like Particles, which has to be further confirmed. CHIKV antigens expressed in the S2 system are now been evaluated for antigenicity in mice.

P-55

Transient expression of recombinant human prolactin and thyrotropin in human embryonic kidney (Expi293FTM) suspension cells

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Human prolactin (hPRL) and human thyrotropin (hTSH) are pituitary polypeptide hormones with key functions in the physiological regulation of the human body. hPRL is highly secreted during lactation, has important action in reproduction and for immunoregulation, among other functions. hTSH is related to the control of thyroid gland. The Chinese Hamster Ovary (CHO) and Human Embryonic Kidney (HEK293) cells are the most used hosts for expression of recombinant human proteins because they can be easily cultured in suspension conditions, and express high levels of proteins that have a relative similarity in post-translational modifications compared to their human counterparts. Our laboratory has experience in the synthesis of these proteins in the Escherichia coli periplasm (hPRL), adhered CHO (hPRL and hTSH), suspension CHO (hPRL) and adhered HEK293T cells (hTSH). The aim of this work was to produce hPRL and hTSH in suspension Expi293FTM cells for their characterization. The hPRL and hTSH cDNA were introduced into the commercial plasmid pcDNATM 3.4-TOPO® and 30 μg of these plasmids were used to transfect 30 mL of suspension Expi293FTM cells (2.5 x 106 cells/mL) in a 125 mL erlenmeyer, using 81 μL of ExpiFectamineTM transfection agent. After 16 h of transfection, 150 μL of Enhancer 1 and 1.5 mL of Enhancer 2 were added and the culture was maintained in an incubator at 37 °C, 8% CO2, at 125 rpm in orbital shaker. Samples of conditioned media (Expi293TM expression medium) were collected during 4 days and stored at -80 °C. These were analyzed by SDS-PAGE, ELISA, Western blotting, and HPLC. For the first time, hPRL and hTSH, were transiently expressed in human (Expi293FTM) suspension cells, the expression levels reaching, on the 3rd day, 46 μg of hPRL/mL and 116 μg of hTSH/mL. These results show that the expression is clearly dependent on the characteristics of the protein and that this methodology is very efficient to obtain high levels of human glycoproteins in a short time and will allow us to purify them and compare their glycosylation profiles of these to CHO-derived and human native pituitary hormones.

P-56

High throughput screening of different Baculovirus infection strategies in a micro- bioreactor system for Insect cells

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Recombinant protein production based on the baculovirus insect cell system is fast and flexible and generally offers high yields. Expression of complex, multi-subunit structures like virus like particles (VLP´s) that pass through the secretory pathway can be a considerable challenge for the host cell.

For optimizing the productivity of VLPs in terms of bioactivity and quantity, the cell density and the multiplicity of infection (MOI) are very critical parameters. Furthermore, the capability of available and commonly used media is not fully exploited as well as medium replacement at the time of infection for supporting cell viability.

To identify the highest infectable cell concentration and the best cell to virus concentration the BioLector from m2p-labs was used. The BioLector enables continuous online monitoring of the cell density, fluorescence, dissolved oxygen level, and pH in a continuously shaken 2.5 mL microtiter plate format. These features confer a significant benefit as compared to the currently used method with shaker flasks where no major process parameters can be online monitored and the sample rate is lesser.

A scale-up of these BioLector results to 1 L and then to the 10 L stirred tank bioreactors was performed, where an additionally monitoring could be applied to analyze the process more deeply.

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High quality and Optimized PEI-based transfection reagents for production of clinical grade viral vectors

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Gene- and cell therapy-based medicines are experiencing resurgence due to the introduction of "next generation" transfer viral vectors, which have demonstrated improved safety and efficacy. Adeno Associated Virus (AAV) and Lentivirus are very commonly used in therapeutics and often produced using Polyethylenimine (PEI)-mediated transient transfection in HEK-293 or HEK-293T cells. The critical raw materials needed for cGMP vector production must be sourced from approved suppliers and should have gone through a rigorous testing program to reduce the risk of introducing adventitious agents into the production process. Polyplus-transfection now provides PEIpro® and its high-quality counterpart PEIpro®-HQ, the unique PEI-based transfection reagents suitable for use in process development and in cGMP biomanufacturing, respectively. Both reagents are particularly well suited for therapeutic virus production as they are PEI (Polyethylenime) based and free of components of animal-origin. They have been selected for their high transfection efficiency using low DNA amount. PEIpro® and PEIpro®-HQ undergo stringent quality controls for use in process development. In addition, impurity profile, residual organic solvent and heavy metal content are also tested in PEIpro®-HQ to assess the purity of the reagent. Moreover, each lot of PEIpro®-HQ is provided with an extensive documentation, making PEIpro®-HQ perfectly suitable for use as a qualified raw material for the production of clinical batches of viruses in GMP processes.

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A novel and powerful way to obtain high yield protein production using Transient Gene Expression in CHO and HEK-293 cells using FectoPRO transfection reagent

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Transient protein expression is a common bioproduction process used to produce milligram to gram quantities of recombinant proteins and antibodies for a wide range of applications. However, when compared to stable expression platforms, the overall yield of transient protein expression remains weak, limiting its use in bioproduction processes. One of the manufacturing strategies for improving the transient productivity is then to increase transfection efficiency. We have developed a novel technologically advanced transfection solution, FectoPRO®, which outperforms currently available PEI-based and lipid-based transfection reagents. This poster presents data and protocol leading to remarkable protein and antibody yields in different transient CHO and HEK-293 expression systems. FectoPRO leads to higher transfection efficiency than any other currently available reagent and to very high protein yields. Transient transfection is now reproducible and easily scalable. In addition FectoPRO is compatiblewith a number of different media, including the Expi293 and ExpiCHO systems.

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