

# 12<sup>th</sup> PEAC<sup>e</sup>

Protein Expression  
in Animal Cells Conference

Photos: Joanne DiBona, SanDiego.org



September 20-24, 2015  
SAN DIEGO, CALIFORNIA, USA





# **12th Protein Expression in Animal Cells**

**Loews Coronado Beach  
San Diego  
USA**

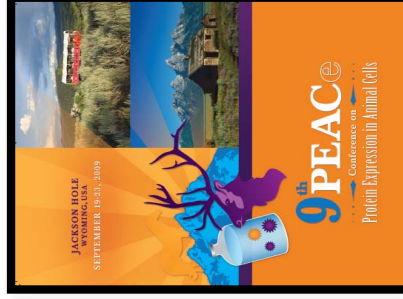
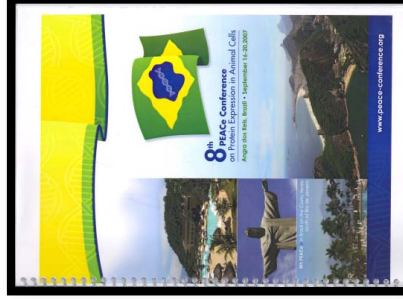
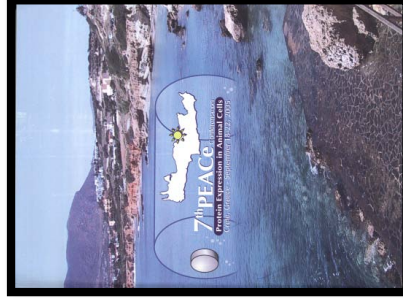
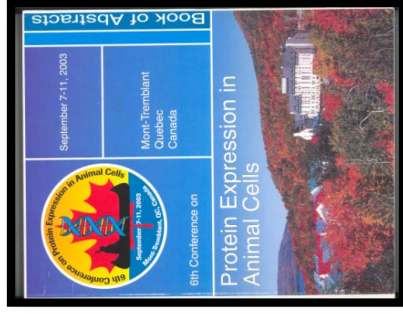
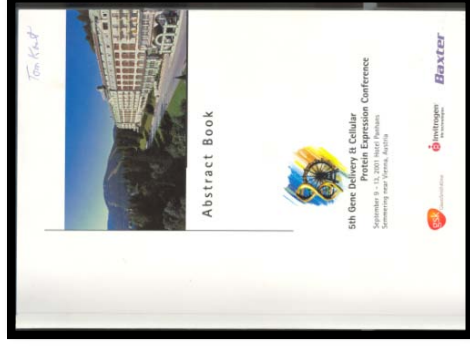
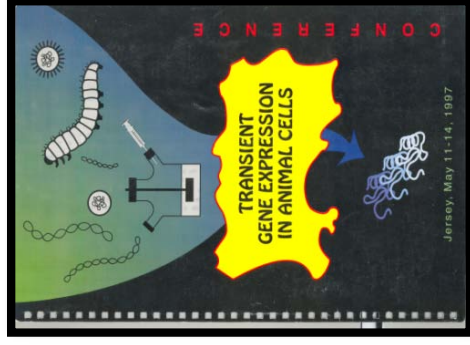
**September 20 - 24, 2015**



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## PEACe Conferences 1992 - 2013

## Message from the Chair - PEACe

Dear Participants,

It is with great pleasure that we welcome you to the 12th Protein Expression in Animal Cells (PEACe) Conference. We will have six scientific sessions plus an industrial workshop, covering a wide range of animal cell and protein expression technologies. The conference committees have done a fantastic job putting this program together by identifying and inviting leading-edge scientists who have made recent and significant contributions to the field. The quality of the submitted abstracts has also enabled the committee to fill out the program with additional oral presentations. As in past PEACe conferences, a large number of attendees will present posters. We have allotted significant time to attend the poster sessions and we encourage you to take advantage of this opportunity to discuss the many new and exciting results that will be presented.

The PEACe conference series started in 1992 and since then meetings have been held every two years. Over the past 23 years, the conference focus has adapted to address evolving developments in cell culture and recombinant protein expression technologies. This year, several changes have been made to the organizational structure of the conference. We have added an additional In-Country Representative committee, asking those committee members to help increase awareness of the PEACe conference to the scientific community of their country, recommend speakers, identify sponsors, and encourage attendance at PEACe. We have also asked recognized scientific leaders in the field to serve as Session Co-Chairs. 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 deeply appreciated. Finally, while the PEACe conference has always operated as a not-for-profit organization, this year the conference has applied for non-profit status in Maryland as the Protein Expression Conference Foundation. This will provide a solid basis for the planning and organization of future PEACe conferences. More details will be discussed at the General Assembly meeting after Session 5.

We 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 allowed us to provide student bursaries for a number of graduate students to attend and present their research at the conference. I would like to especially thank Bruno Begin, who has been the conference manager since 2003 for expert guidance and organization to keep the committee on track.

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. We thank you for participating in the conference and wish you a successful and productive PEACe meeting in San Diego.

Kim Stutzman-Engwall

Chair



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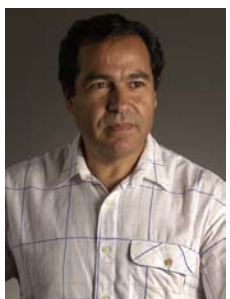
## Organizing Committee PEACe 2015



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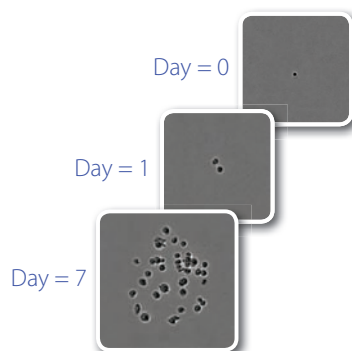


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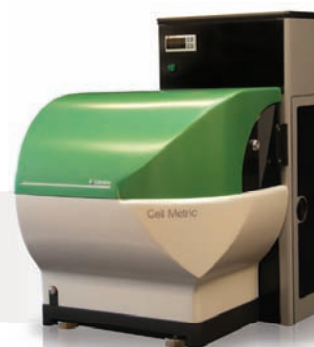
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**PROGRAM 12<sup>TH</sup> PEACE  
SUNDAY-SEPTEMBER 20, 2015**

<b>03:00</b>	<b>Registration opens</b>	<b>Foyer of the conference center</b>
<b>06:00</b>	<b>Welcome Reception</b> <b>This 1h30 minutes reception will offer food in a generous enough quantity to replace dinner</b>	<b>Bay Terrace</b>
<b>07:30</b>	<b>Opening presentation</b> <b>Not to be missed to start the conference on a humourous note</b>	



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**KARTAUSE ITTINGEN WORKSHOP**



**PROGRAM 12<sup>TH</sup> PEACE  
MONDAY-SEPTEMBER 21, 2015**

**08:30 Opening**

**Chair: Kim Stutzman-Engwall, Consultant**

**Maximizing Production from Cell Hosts - 1**

**Co-Chairs: Henry George, Sigma Aldrich , USA  
David Shaw , Genentech, USA**

08:45	Trent Munro	Amgen	USA
1.1	A high-throughput multi-parametric clone screening approach for the generation of tailored production cell lines		
09:15	Jamey Young	Vanderbilt University	USA
1.2	Application of 13c flux analysis to identify high-productivity CHO metabolic phenotypes		
09:45	Jennifer Lin	Pfizer, Inc.	USA
1.3	The effect of culture conditions on sequence variant levels in antibody biotherapeutics		

**10:05 Coffee Break**

**Maximizing Production from Cell Hosts - 2**

**Co-Chairs: Henry George, Sigma Aldrich , USA  
David Shaw , Genentech, USA**

10:35	Joseph Shiloach	NIDDK/NIH	USA
2.1	Genome-wide mai screen for improved functional expression of neurotensin receptor and other proteins		
10:55	Valentina Ciccarone	MacroGenics, Inc.	USA
2.2	Optimization of stable cell line development for expression of bispecific DART® Molecules		

**PEACe Lecture**

11:15	Dennis Burton	The Scripps Research Institute	USA
2.3	Toward a rationally designed HIV vaccine		

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12:15 Lunch

## Transient Technologies - 1

**Co-Chairs: Raj Haldankar, NGM Biopharmaceuticals , USA**  
**Rene Hubert, Agensys , USA**

13:45 Sarah Dunn MedImmune UK  
3.1 Developing transient expression tools to predict stable monoclonal antibody expression

14:15 Christopher Kemp Kempbio USA  
3.2 Baculovirus mediated transduction of HEK-293 and CHO cells for the gram-scale production of recombinant antibodies

14:45 David Fisher AstraZeneca UK  
3.3 Factorial Experimental Optimisation of Transient Heterologous Gene Expression in Mammalian Hosts

15:05 Coffee break

## Transient Technologies - 2

**Co-Chairs: Raj Haldankar, NGM Biopharmaceuticals , USA**  
**Rene Hubert, Agensys , USA**

15:35 Bram Estes Amgen USA  
4.1 Transient expression profiles at various transcription levels provide a greater understanding of how difficult recombinant proteins affect host cells and enable an early assessment for candidate selection

15:55 Markus Neubauer Roche Pharmaceutical Research Germany  
4.2 Development of efficient transient transfection processes for different cell lines using an automated and miniaturized bioreactor system

16:15 Jonathan Zmuda Thermo Fisher Scientific USA  
4.3 Surpassing 293-Based Protein Production with the ExpiCHO Transient Expressio System

17:00 Poster session

19:30 Dinner



A futuristic robotic hand, rendered in a translucent blue and black style, holds a dandelion. The dandelion's stem is a simple yellow line, and its seed head is a complex, multi-colored sphere of protein structure, with various colors (red, green, blue, orange) representing different parts of the protein. The background is a smooth gradient from light blue at the top to dark blue at the bottom.

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**PROGRAM 12<sup>TH</sup> PEACE**  
**TUESDAY-SEPTEMBER 22, 2015**

**Assembly of Protein-Protein Complexes - 1**

**Co-Chairs: Lorenz Mayr, Astra Zeneca , USA**  
**Linda Lua, IBN , Australia**

- |              |   |
|--------------|---|
| 08:30<br>6.1 | Joop van den Heuvel      Helmholtz Centre for Infection Research      Germany<br>Generation of stable CHO multi RMCE expression cell lines for recombinant multi-subunit protein complex production |
| 09:00<br>6.2 | René Assenberg      Novartis      Switzerland<br>Producing Protein Complexes for Small Molecule Drug Discovery at Novartis Strategies & Challenges  |
| 09:30<br>6.3 | Linda Lua      The University of Queensland      Australia<br>Approach for Large Antigen Display on a Virus-like Particle   |

**10:00      Coffee break**

**Industrial Workshops**

**Chair: Tom Kost, USA**

- |              |   |
|--------------|---|
| 10:30<br>7.1 | Henry Chiou      Thermo Fisher Scientific      USA<br>System-based strategies for maximizing HEK293 and CHO transient expression                                    |
| 11:00<br>7.2 | Kate Caves      DNA2.0      USA<br>A Systematic Approach to Engineering Antibody Expression   |
| 11:30<br>7.3 | John J. Stewart Cadwell      FiberCell Systems Inc.      USA<br>Recombinant Protein Production in Hollow Fiber Bioreactors: From Laboratory Scale to Process Scale. |

**12:30      Departure for Excursion with box lunch**

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**PROGRAM 12<sup>TH</sup> PEACE**  
**WEDNESDAY-SEPTEMBER 23, 2015**

**Cell Culture for Virus Production - 1**

**Co-Chairs:**     **Laura A. Palomares, IBT UNAM , Mexico**  
**Xin Swanson, Lonza , USA**

- |              |  |                               |         |
|--------------|--|-------------------------------|---------|
| 08:30<br>8.1 | Timo Frensing<br>Impact of virus-host cell interactions on cell culture-based influenza vaccine production                 | Max Planck Institute<br><br>  | Germany |
| 09:00<br>8.2 | Medhi Gasmi<br>Development of a highly scalable, cGMP compliant manufacturing process for an adeno-associated virus vector | Avalanche Biotech<br><br>     | USA     |
| 09:30<br>8.3 | Kendra Steele<br>Improved recombinant protein production with the vankyrin-enhanced baculovirus expression technology      | ParaTechs Corporation<br><br> | USA     |

**09:50     Coffee break**

- |              |  |                  |        |
|--------------|--|------------------|--------|
| 10:20<br>8.4 | Laura Palomares<br>Single cell analysis and fluorescent probes for the characterization of insect cell responses towards infection | IBT UNAM<br><br> | MEXICO |
| 10:40<br>8.5 | Steve Pettit<br>Development of a robust, defined, animal-free virus production medium optimized for microcarrier culture           | InVitria<br><br> | USA    |

**PEACe Lecture**

- |              |  |   |         |
|--------------|--|---|---------|
| 11:00<br>9.1 | Helene Fastrup Kildegaard<br>Application of CRISPR/Cas9-mediated genome engineering for improved protein production in mammalian cells | Technical University of Denmark<br><br> | Denmark |
|--------------|--|---|---------|

**12:00     Lunch**



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## Monitoring and Controlling Post-translational Modifications - 1

**Chairs: Carlos Bosque, Momenta Pharma , USA**  
**Claudia Buser, Genzyme , USA**

13:30 10.1	Mike Betenbaugh TBA	John Hopkins University	USA
14:00 10.2	T. Shantha Raju Fc glycans heterogeneity impacts the biological functions of therapeutic antibodies	Janssen Research & Development	USA
14:30 10.3	Nathan Lewis A genome-scale systems biology approach to protein expression	University of California, San Diego	USA
14:50	Coffee break		

## Monitoring and Controlling Post-translational Modifications - 2

**Chairs: Carlos Bosque, Momenta Pharma , USA**  
**Claudia Buser, Genzyme , USA**

15:20 11.1	Shivani Gupta Omics guided cell line engineering: reducing high mannose by over expressing n-glycosylation pathway regulators	Amgen Inc	USA
15:40 11.2	Natalie Krahn A camelid heavy chain monoclonal antibody displays unique fc'ri binding properties	University of Manitoba	Canada
16:30	General Assembly PEIS (Protein Expression International Society)		
17:30	Poster session		
19:30	Gala Diner		



**PROGRAM 12<sup>TH</sup> PEACE**  
**THURSDAY-SEPTEMBER 24, 2015**

**Trends in Bioprocessing - 1**

**Co-Chairs: Jams Piret, UBC, Canada**  
**Rodney Combs, Pfizer, USA**

08:30	James Piret	University of British Colombia	Canada
12.1	Effets of selected amino acid limitations in cho cell fed-bach cultures		
09:00	Govind Rao	Center for Advanced Sensor Technology, UMBC	USA
12.2	Manufacturing biologics at the bedside		
09:30	Tharmala Tharmalingam	Amgen	USA
12.3	Real-time and At-line monitoring for culture control		

**09:50 Coffee break**

**Trends in Bioprocessing - 1**

**Co-Chairs: Jams Piret, UBC, Canada**  
**Rodney Combs, Pfizer, USA**

10:20	Yuval Shimoni	Bayer Health Care	USA
13.1	Novel cell culture and assay platforms in support of continuous improvement and manufacturing support activities		
10:40	Jennitte Stevens	Amgen	USA
13.2	Comparison of manufacturability attributes, biophysical properties and post-translational modifications of molecules produced by transient vs stable expression methods		
11:00	Jill Cai	WuXi Biologics	China
13.3	Maximizing protein production in CHO cells to support protein Therapeutics Discovery and Development		
11:00	George Lovrecz	CSIRO Manufacturing Flagship	Australia
13.4	Improving production economy: the role of continuous cultures and on-line monitoring of fermentation processes in the pharmaceutical industry		





## **ORAL PRESENTATIONS**



O 1.1

## **A high-throughput multi-parametric clone screening approach for the generation of tailored production cell lines**

Kim Le, Huong Le, Szilan Fodor, Christopher Rollins, Hannah Victor and Trent Munro

Drug Substance Technologies, Amgen Inc., Thousand Oaks

*\*Corresponding Author:* Trent Munro, One Amgen Center Drive, Thousand Oaks, CA 91320 Tel: (805) 313 5456, Email: [tmunro@amgen.com](mailto:tmunro@amgen.com)

### **Abstract**

A key challenge for a successful biologic is the creation of clonal cell lines that produce proteins that closely match to a predefined product quality profile while maintaining manufacturing suitability. To address this challenge, Amgen has been developing key capabilities, such as understandings of development platforms and extensive experience in commercial manufacturing of monoclonal antibodies. We present a case study the development of a clonal cell line for the production of a monoclonal antibody designed to fit within a tight established range of product quality attributes. We demonstrate the utility of scale-down automation combined with state-of-the-art analytics for production of an antibody with characteristics that fall within this range. Using this approach we thoroughly analyzed large numbers of stable pools and clones from bench scale to manufacturing scale bioreactors. We highlight those attributes which can be matched via clone screening and those which require bioprocess control during scale-up. We also perform a retrospective statistical analysis that establishes the depth of clone screening required to achieve a pre-determined product quality profile that may be used for future second generation cell line development programs.



## O 1.2 APPLICATION OF <sup>13</sup>C FLUX ANALYSIS TO IDENTIFY HIGH-PRODUCTIVITY CHO METABOLIC PHENOTYPES

Jamey D. Young, Vanderbilt University

Industrial bioprocesses place high demands on the intermediary metabolism of host cells to meet the bio-synthetic requirements for maximal growth and protein expression. Identifying host cell metabolic phenotypes that promote high recombinant protein titer is a major goal of the biotech industry. <sup>13</sup>C metabolic flux analysis (MFA) provides a rigorous approach to quantify these metabolic phenotypes by applying isotope tracers to map the flow of carbon through intracellular metabolic pathways. We have conducted a series of <sup>13</sup>C MFA studies to examine the metabolic impacts of recombinant IgG expression using two common CHO expression systems, the glutamine synthetase (GS) and dihydrofolate reductase (DHFR) systems.

First, we performed <sup>13</sup>C MFA to characterize the metabolism of a IgG-expressing DHFR host (Amgen) during four separate phases of a fed-batch culture. We found that peak specific growth rate during early exponential phase was associated with high lactate production and minimal citric acid cycle (CAC) flux. Conversely, we found that lactate metabolism switched from net production to net consumption as the culture transitioned from peak growth to peak IgG production. During stationary phase when IgG production peaked, energy was primarily generated through CAC and oxidative phosphorylation.

Second, we examined ten CHOK1SV (Lonza) clones cultured in 3-liter fed-batch bioreactors, to assess their metabolism during stationary phase. Six of the clones used the GS system to express one of three different IgGs. Four of the clones were genetically manipulated to be apoptosis-resistant by expressing Bcl-2Δ. The six antibody-producing clones clustered together and were separated by host background (Bcl-2Δ or CHOK1SV). The lactate dehydrogenase (LDH) flux was most closely associated with specific IgG productivity: as IgG productivity increased, lactate production decreased. Additionally, elevated CAC fluxes corresponded strongly with increased specific productivity.

Taken together, these studies indicate that oxidative metabolism is enhanced in high-producing CHO cell lines. This presentation will discuss the central metabolic trends observed among both GS and DHFR expression systems as a means to provide potential metabolic engineering strategies to further enhance IgG productivity and titer of industrial CHO hosts.





### **O 1.3 The Effect of Culture Conditions on Sequence Variant Levels in Antibody Biotherapeutics**

T. Jennifer Lin<sup>1,2</sup>, Kathryn Beal<sup>1</sup>, Lisa A. Marzilli<sup>2</sup>, and Karin Anderson<sup>1</sup>

BioTherapeutics Pharmaceutical Sciences

<sup>1</sup>. Cell Line Development, BioProcess Research & Development

<sup>2</sup>. Mass Spectrometry and Biophysical Characterization, Analytical Research & Development  
Pfizer, Inc  
Andover, MA 01810

Sequence variants (SVs) are protein isoforms that contain unintended substitutions in the amino acid sequence. Detection and prevention of SVs in protein products are of utmost importance due to the risk of unwanted immunogenic responses. In the last few years, orthogonal genomic and mass spectrometric methodologies have revealed the presence of SVs in several early development products in the form of single amino acid changes. In this study, we examine four different sequence variant-containing cell lines with either a high or low level of SV at various culture conditions including scale, process and generational age. The effect of culture conditions on the RNA and DNA variant levels as well as the expressed protein were determined and compared by Extensive Clonal Sequencing (ECS) or Droplet Digital Polymerase Chain Reaction (ddPCR) and peptide mapping by LC/MS, respectively. DNA, RNA and protein SV levels in all four cell lines were not affected by the scale or culture process. However, generational age did show a change in SV level for several sequence variant-containing cell lines. This data allows us to start modelling population dynamics leading to better understanding of SVs and the clonality of cell lines.



### **O 2.1     Genome-Wide RNAi screen For Improved Functional Expression               of Neurotensin receptor and other proteins.**

Su Xiao<sup>1,2</sup>, Yu-Chi Chen<sup>3</sup>, Michael J. Betenbaugh<sup>2</sup>, Scott E. Martin<sup>3</sup>, Joseph Shiloach<sup>1</sup>

<sup>1</sup> Biotechnology Core Laboratory, National Institute of Diabetes and Digestive and Kidney Diseases,  
National Institutes of Health, Bethesda MD 20892, USA

<sup>2</sup> Departments of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore MD  
21218, USA

<sup>3</sup> NIH Chemical Genomics Center, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville MD 20850, USA

Genome-wide RNA interference screen is emerging as a powerful methodology for deducing gene functions in various diseases. We applied this technology to generate genome-wide profile of genes related to the recombinant protein expression process from HEK293 cells. We utilized human microRNA library composed of 875 microRNA mimics, and human siRNA library targeting 21,000 genes. By the application of high-throughput screening method, we identified miRNAs/siRNAs that significantly increased expression of membrane protein, cytosolic protein and secreted protein. In addition, up to 150 genes associated with enhancement of recombinant protein expression were validated and important pathways where these genes enriched in were also identified. We will describe the screening methodology as well as the identified genes and the expression results.

MiRNA mimic screen for improved functional expression of neurotensin receptor

Xiao et al. Biotechnology and Bioengineering 2015 in press



## **O 2.2 Optimization of Stable Cell Line Development for Expression of Bispecific DART<sup>®</sup> Molecules**

Valentina C. Ciccarone, Ph.D.  
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Rockville, MD 20850 USA

Dual-Affinity Re-Targeting (DART) molecules are antibody-like therapeutic proteins capable of targeting multiple different epitopes with a single recombinant molecule. These molecules are engineered to have excellent product stability, optimal chain pairing, predictable antigen-recognition, and customizable half-life in vivo. Several protein and cell-line engineering strategies, including vector selection and cell line screening, have been developed to achieve correct molecule assembly, high expression levels, and biological activity of these complex, multi-chain proteins. Data will be presented from case studies for the expression of DART proteins with different structures and target specificities.





### **O 2.3 Toward a Rationally Designed HIV Vaccine**

Burton, Dennis R

Dept of Immunology and Microbial Science, Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, and IAVI Neutralizing Antibody Center, The Scripps Research Institute, La Jolla, CA, USA

Ragon Institute of MGH, MIT and Harvard, Boston, MA, USA

Highly antigenically variable viruses such as HIV present huge problems for vaccine design. Broadly neutralizing antibodies to HIV generated during natural infection can identify weaknesses in the surface structures of the virus. These weaknesses can help guide vaccine and drug design and reveal fascinating aspects of the interplay between two highly mutable systems-the virus and antibody.



### **O 3.1 Developing transient expression tools to predict stable monoclonal antibody expression**

Sarah Dunn, Gary Pettman, Claire Hughes, Vidhi Shah, Jenny Keen, Ben Kemp, Diane Hatton, Lekan Daramola

MedImmune, Cambridge, UK

MedImmune's industry-leading transient and stable expression platforms are essential for the efficient progression of our pipeline. Transient protein production used to support in vitro biology studies during drug development is also important in the early assessment of the expression levels of lead candidates. Poor transient expression can impact early material supply, increasing delivery timelines for developability studies (investigations into the biophysical properties of lead molecules). Moreover stable manufacturing cell line development, a resource intensive process, can also be detrimentally impacted by poor expression. Therefore, the development of tools for the early prediction of low expression offers the potential to mitigate risk and enable the timely progression of candidate molecules.

CHO transient expression vectors and methods were developed to identify difficult to express mAbs. Correlation of transient data to expression in a stable platform was confirmed using both CHO pools and clonal cell lines. This newly developed transient expression assay for predictability has been further interrogated to determine the resolution of differences in expression levels and to investigate the effect of sequence optimisation of lead candidates.

The data generated indicates that it is possible to identify potential expression issues early using a transient system, which can then aid in the selection of developable lead candidates to progress into full cell line development.



### **O 3.2 Baculovirus mediated transduction of HEK-293 and CHO cells for the gram-scale production of recombinant antibodies.**

Christopher Kemp  
Kempbio, Inc. Frederick, MD 21704 USA

Recombinant baculovirus particles carrying genes of interest under the control of mammalian promoters (BacMam's) are an efficient and cost-effective tool for the production of proteins in mammalian host cell lines. This technology has been applied to the production of various proteins and it has proven to be particularly useful for the production of recombinant antibodies. Routine yields of IgG produced using this system exceed 150 mg/L in both HEK-293 and CHO-S cells without process development. The ease of virus amplification, efficiency of gene transfer and scalability of the process provide the basis for the development of a powerful new expression platform for antibody production.





### **O 3.3 Factorial Experimental Optimisation of Transient Heterologous Gene Expression in Mammalian Hosts**

David Fisher, Mark Abbott\*

Reagents and Assay Development, Discovery Sciences, AstraZeneca, UK

\*Now at Peak Proteins, BioHub, Alderley Park, UK

Transient expression of heterologous proteins in mammalian systems is a powerful way to generate protein reagents quickly. High expressing protocols for transient methods have been well described for HEK293 and CHO-based expression systems. However, these protocols may not always be suitable for different types of processes; there may be licensing costs, scale issues or logistical challenges. Factorial experimental screening via a design of experiments (DoE) approach enables the investigation of a wide experimental space, efficiently measuring the effect of a range of different parameters on protein expression. We show that DoE can be used to empirically interrogate transfection variables to design high-yielding, high-throughput expression systems. We show that HEK293 and CHO-based systems can be transfected with polyethylenimine (PEI), either with a priori complex formation (advance mixing of the DNA and transfection reagent prior to addition to the cell culture) or in situ complex formation (independent, sequential addition of DNA and transfection reagent to the cell culture). We describe our optimisation approach and experimental designs, along with protein expression data. In addition, we demonstrate that the designed processes are transferrable from 3ml cultures in deep 24-well plates through cultures in CultiFlask bioreactors, shake flasks and up to 25L culture in Wave Bioreactors. Data are shown to illustrate the utility of our optimisation approach with a number of different classes of protein in both HEK293 and CHO-based expression systems. We suggest that by using this type of approach any transfection system can be further optimised.



#### **O 4.1 Transient expression profiles at various transcription levels provide a greater understanding of how difficult recombinant proteins affect host cells and enable an early assessment for candidate selection**

Bram Estes<sup>1</sup>, Lisa Renshaw<sup>1</sup>, Jennitte Stevens<sup>1</sup>

<sup>1</sup>Department of Biologics Optimization, Amgen, Thousand Oaks, CA

In transient expression systems, the rate-limiting step for many molecules has been found to take place during the post-translational modifications of the protein. One technique to improve the quality of difficult proteins and the titers of low expressing proteins is to lower the level of transcript to match the rate-limiting step in the secretory pathway, also referred to as gene dosing. To gain a better understanding of this phenomenon, we have titrated the gene dose of a set of molecules being transiently transfected into 293-6E cells and measured the effects on mRNA, Russell body formation, unprocessed protein responses (UPR), cell growth during production, and protein expression. mRNA of the gene of interest, UPR markers, and GAPDH was measured from cell lysate using a branched DNA assay. Russell bodies and protein in cell lysate were measured using fluorescent microscopy coupled with computational analysis on a Cell Insight instrument. Cell growth was measured as viable cell density and viability on a cell counter. Protein expression was measured in conditioned media (CM) using a Forte Bio instrument. Results of the experiment provide contrasting profiles of high and low expressing molecules. They also reveal the progression of changes that take place within the cell when a titration of gene doses is used and correlations between these changes. Thresholds of RB levels and UPR indicators differentiate high and low expressing molecules and can help to recognize when alternative expression approaches such as lower gene doses will result in improved productivity. These expression profiles can also be applied as a screening method to identify variants within a panel of engineered molecules with a strong propensity to misfold and/or aggregate within the cell with a resulting probability of low titers in transient and stable expression systems.



#### O 4.2     **Development of efficient transient transfection processes for different cell lines using an automated and miniaturized bioreactor system**

Markus Neubauer, PhD, Principal Scientist

Roche Pharmaceutical Research and Early Development, Large Molecule  
Research, Roche Innovation Center Penzberg, Germany

Transient gene expression (TGE) represents an enabling technology for rapid expression of proteins in the milligram to gram range. In pharmaceutical development of therapeutic proteins, TGE plays a central role in generation of material for *in vitro* and *in vivo* studies in early-stage projects as long as no stably transfected Chinese Hamster Ovary (CHO) cell lines are available.

In this study, we developed transient transfection processes using an automated system (ambr15™, TAP Biosystems) in small-scale bioreactors (15 mL) aiming at high-yield systems. The ambr15 system has been reported to be a representative scale-down model for CHO fermentations but its suitability for development of transient transfection processes is virtually untried. Transfection processes based on human embryonic kidney (HEK) 293F, Expi293, and CHO cell lines could be transferred from shaker flask processes to the ambr15 system. With 24 bioreactors in parallel, a wide variety of crucial process parameters including DNA amount, DNA-polyethylenimine (PEI) ratio, polyplex formation conditions, and temperature shifts were optimized using the CHO-S cell line which resulted in titers of antibodies and complex antibody formats of up to 140 mg/L. Unexpectedly, titers using CHO-S cells in conjunction with the ambr15-optimized protocol were comparable to or clearly exceeded titers obtained with the HEK293F system as benchmark. This demonstrates the high efficiency of developed CHO transient transfection processes. The ambr15-optimized protocols were, again, transferable to shaker flasks for large-scale protein supplies. The key to success is thought to be the high precision, reproducibility, and control of the ambr15 system translating changes to process parameters in predictable titer changes.

In conclusion, development of highly efficient transient transfection processes for industrial applications were successfully developed by using a miniaturized and automated fermentation system.



### O 4.3 Surpassing 293-Based Protein Production with the ExpiCHO Transient Expression System

Jonathan Zmuda<sup>1</sup>, Chao Yan Liu<sup>1</sup>, Virginia Spencer<sup>1</sup>, Shyam Kumar<sup>1</sup>, Jian Liu<sup>1</sup>, Henry Chiou<sup>2</sup>

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CHO cells are the predominant host for biotherapeutic protein expression, with roughly 70% of licensed biologics manufactured in CHO. Multiple attributes make CHO cells desirable for bioproduction including the ability to adapt to high-density suspension culture in serum-free and chemically-defined media and the incorporation of post-translational modifications that are biologically-active in humans. For these reasons, the ability to produce transient CHO-derived proteins early on during drug development is highly advantageous to minimize, as much as possible, changes in protein quality/function observed when moving from R&D to bioproduction. Unfortunately, CHO cells express lower levels of protein than HEK293 cells in existing transient systems, in some instances 50-100 times less than the best 293-based systems, and only modest titer improvements are obtained through the optimization of individual components of existing transient CHO workflows. To address the significant unmet need for higher transient CHO protein titers, systems-based approaches were employed whereby the latest advances in cell culture media, feeds, transfection reagents and expression enhancers were optimized in conjunction with a new high-expressing CHO cell clone to generate a simple and robust workflow for transient protein expression in CHO cells capable of generating gram per liter protein titers in 10-14 days. These advances will allow for unprecedented access to CHO-derived proteins early on during candidate selection and may serve to revolutionize the use of CHO cells for transient protein expression during the drug development process.





### **0 6.1 Generation of stable CHO multi RMCE expression cell lines for recombinant multi-subunit protein complex production**

Joop van den Heuvel  
Helmholtz Centre for Infection Research  
Germany

In this presentation conventional and emerging new technologies for the production of complex biologics in mammalian expression systems are summarized. The essential features of the most relevant methods to generate stable production cell lines for the expression of recombinant multi-protein complexes are described.

Stable mammalian production cell lines in suspension culture enable the reproducible expression of target genes in any desired scale using bioreactor technology. Targeted integration methods have been developed to cut down timelines for the generation of stable producer cell lines. Especially the promising multiple targeted integration strategy by Flp mediated recombination and its future impact on multi-protein expression will be highlighted



## **O 6.2 Producing Protein Complexes for Small Molecule Drug Discovery at Novartis - Strategies & Challenges**

René Assenberg , Ian Hunt, Bernd Gerhartz

Center for Proteomic Chemistry, Novartis Institutes for Biomedical Research, Novartis.

Producing protein complexes for small molecule drug discovery often constitutes a formidable challenge, requiring significant investment and commitment to succeed. These challenges include the assembly of expression vectors, determining the optimal expression and purification strategies to achieve the correct stoichiometry, quality & homogeneity, overcoming limitations in biophysical analysis of large assemblies and more. This presentation will outline these challenges and, through a number of case studies, how to overcome some of these. Particular emphasis will be placed on the differences between co-expression versus co-transfection/infection versus co-purification, and the increasing role of synthetic biology in generating co-expression vectors.



### O 6.3 Approach for Large Antigen Display on a Virus-like Particle

Linda Lua<sup>1</sup>, Cindy Chang<sup>1</sup>, Yuanyuan Fan<sup>2</sup>, Natalie Connors<sup>2</sup> and Anton Middelberg<sup>2</sup>

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Complementary use of both computational and experimental techniques to engineer modern virus-like particle (VLP) vaccines displaying large antigen is investigated. VLP is a class of safe and efficacious vaccine. The highly ordered and repetitive structure of a VLP has led to the development of a novel class of VLP-based vaccine candidates, displaying immunologically important antigens on their surfaces. These heterologous antigens could be peptide epitopes or protein domains. It is important to maintain the authentic structure of the heterologous antigen and avoid VLP structure instability during antigen modularization. Here we demonstrated the use of computational tools (e.g. homology modelling and molecular simulations) together with co-expression strategy via baculovirus-insect cell technology to overcome the challenges associated with large antigen display on VLP. Computational tools were able to predict the effect of linker design, flanking the 20kDa antigen module, on the VLP subunit stability. VLP assembly hampered by steric hindrance due to the large antigenic module was further overcome by module-titration approach using co-expression strategy in insect cells. Thus, VLPs displaying 20kDa rotavirus VP8\* antigens on the surface were successfully developed through a combination of computational and co-expression approach.



**O 7.1 System-based strategies for maximizing HEK293 and CHO transient expression**

Henry Chiou, Thermo Fisher Scientific, USA

Mammalian cell-based transient expression systems have become important tools for the rapid production of recombinant proteins for applications such as biotherapeutic drug discovery and development, production of antigen proteins for immunization, provision of reagent proteins, generation of mammalian proteins with “native” conformation and activity for structural studies and production of recombinant viruses and virus-like particles. Compared to expression systems based on host cells from other organisms, such as insect, bacteria and yeast, mammalian cell-based expression has often been characterized as low yield, costly and slow. Successful implementation of scalable transient transfection has greatly increased speed. Under the Gibco brand, Thermo Fisher Scientific has been able to synergistically apply advanced technologies in suspension culture media, feed and supplements, transfection reagents, expression enhancers and cell line development to multiplicatively increase yields from HEK293 and CHO transient expression systems to levels more commonly observed in stable cell lines. We will present in this talk a few key understandings about transient expression systems that we have learned during the development of the Expi293 and ExpiCHO systems.





## O 7.2 A Systematic Approach to Engineering Antibody Expression

Kate Caves, DNA2.0, USA

We describe a systematic engineering approach that combines machine learning methods with gene synthesis to explore vector element and codon optimization determinants on protein/antibody expression. This Design-of-Experiment approach allowed us to sample a large sequence-space without exhaustive testing. Combinations of vector components were designed so that elements are varied systematically and independently. We then use advanced machine learning algorithms to assess the contribution of each element to vector performance, allowing us to identify the optimal combination of vector elements for a given protein and host system. Our results display protein expression in mammalian cells is sensitive to the order, spacing and orientation of vector elements. Furthermore, we explore the use of novel transposases for fast, efficient, stable expression for generation of stable pools and stable lines. Additionally, we explored IRES elements to create and tune bi-cistronic messages for mammalian cell expression. A large variety of IRES elements have been assessed for the optimization of expression level and ratio of two polypeptides produced from a single vector.



### **O 7.3 Recombinant Protein Production in Hollow Fiber Bioreactors from Laboratory Scale to Process Scale.**

John James Stewart Cadwell

FiberCell Systems Inc. Frederick, Md. 21774

Hollow fiber bioreactors are not a new technology; they were first described in 1972. However, new high gross filtration rates fibers, improved fiber geometry and a better understanding of the correct protocols make them a powerful method for the production of recombinant proteins from both suspension and adherent mammalian cell lines. A hollow fiber bioreactor consists of thousands of small hollow fiber that are shaped like drinking straws, about 200 microns in diameter and have a defined molecular weight cut-off (MWCO). They are sealed at the ends so that cell culture medium entering the ends of the cartridges necessarily goes through the insides of the fibers, while the cells are cultured on the outside of the fibers. Hollow fiber bioreactors are fundamentally different from other reactor types in three ways: 1) the hollow fibers provide an extremely high surface area to volume ratio, 150cm<sup>2</sup>/ml. This permits the cells to be cultured at very high densities, in the range of 1-2X10<sup>8</sup> cells/ml. 2) the cells are bound to a porous support, not a non-porous plastic surface. This means the cells never require splitting and cultures can be maintained for many months of continuous production. 3) The MWCO can be controlled to allow small molecules such as nutrients and waste products to cross the fiber while secreted proteins are retained to high concentrations, as much as 100X higher than found in flask, roller and spinner culture. The high cell density in a hollow fiber bioreactor represents a unique cell culture environment that results in material benefits to the cells. The cells can easily adapt to growth in a simplified protein free, animal component free, chemically defined medium. The lack of shear means that surfactants are not required in the medium, with their concomitant reduction in KLA of the medium. Apoptosis is reduced so contamination with intracellular DNA and proteins is also reduced. Post-translational modifications are complete and uniform over a period of 3 months of production or longer.

The application of hollow fiber bioreactor technology to process scale manufacturing has been limited by the low solubility of gas in cell culture medium at 37C. FiberCell Systems has developed a novel large-scale hollow fiber bioreactor system that utilizes a unique method for the delivery of nutrients and oxygen and efficient removal of waste products and CO<sub>2</sub>. Gravity feed of medium is used rather than pumps to generate 6-8 liters per minute flow rates and the feed of medium is alternated with gas. 50% of the volume of the bioreactor is replaced with gas every 2 minutes. The prototype presented here has a volume of 500 mls and will equal the output from a 50 liter stirred tank type bioreactor, every day, and will do so for months of production. This unique design brings all the benefits of hollow fiber cell culture to the scales required for bioprocessing in a continuous production, single use apparatus.



### **O 8.1 Impact of virus-host cell interactions on cell culture-based influenza vaccine production**

Timo Frensing<sup>1,2</sup>

<sup>1</sup>Bioprocess Engineering, Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany, <sup>2</sup> Cellular Systems for Bioprocess Engineering, Otto von Guericke University Magdeburg, Germany

Seasonal influenza is a major public health burden and annual vaccination provides the best protection against this viral infection. For the production of influenza vaccines, cell culture-based processes have become an important alternative to the conventional production in embryonated chicken eggs. To optimize virus production in cell culture and to develop high-yield production cell lines, a thorough understanding of virus-host cell interactions is required. For instance, viruses have to overcome cellular defense mechanisms to replicate efficiently. In addition, the quality of seed virus stocks can have a significant impact on process yields since so called defective interfering viruses impede the propagation of infectious virus particles. For the analysis of the complex interplay between influenza viruses and their host cells sophisticated analytical methods such as quantitative real-time PCR and flow cytometry in combination with mathematical modeling are essential. Moreover, single-cell analysis is an important novel tool to characterize the requirements for highly efficient production processes. In particular, we observed that the majority of individual infected cells is non-productive or releases only few progeny virions. However, some cells produce very high virus titers. We were able to demonstrate that this high cell-to-cell heterogeneity in influenza virus infection is caused by stochastic fluctuations that are intrinsic to viral replication and by extrinsic noise, which can originate from cellular factors. In conclusion, the detailed investigation of virus replication in production cell lines using state-of-the-art analytical tools and mathematical modeling paves the way to optimize vaccine manufacturing by the targeted design of cell lines and vaccine virus strains.



**O 8.2 Development of a highly scalable, cGMP compliant manufacturing process  
for an adeno-associated virus vector**

Medhi Gasmi Avalanche Biotech, USA





### O 8.3 Improved Recombinant Protein Production with the Vankyrin-Enhanced Baculovirus Expression Technology

Kendra Steele<sup>1</sup>, Barbara Stone<sup>1</sup>, Bruce Webb<sup>1,2</sup>, and Angelika Fath-Goodin<sup>1</sup>

<sup>1</sup>ParaTechs Corporation, Lexington, KY; <sup>2</sup>Entomology Department, University of Kentucky, Lexington, KY

The baculovirus expression vector system (BEVS) is a powerful and versatile eukaryotic protein expression system. The BEVS is being used to express and produce antigens for vaccine development, to manufacture human therapeutics, to develop faster acting biopesticides, and as a protein expression system for a multitude of research projects. As a lytic viral expression system, the BEVS is limited by death and lysis of infected cells, which precludes protein expression that requires repetitive infection cycles. This results in decreased productivity levels and higher production costs to generate recombinant proteins.

We have identified a gene family (*vankyrins*) from an insect virus that significantly delays death and lysis of baculovirus infected cells while enhancing recombinant protein production. The vankyrin-enhanced BEVS or VE-BEVS increases and prolongs recombinant protein production significantly when the gene of interest is co-expressed with the *vankyrin* gene from a dual BEVS. An increase in cell viability and prolonged protein expression post-infection is also observed with VE-cell lines. When monoclonal Sf9 insect cell lines stably expressing vankyrin protein (VE-CL-01, 02, 03) are used to provide the protein activity in *trans*, western analysis and enzymatic assays show up to 5-fold increase in intracellular protein production and up to 9-fold increase in secreted protein production compared to expression from untransformed control cells. Tn5 cells and a Sf9 cell line developed with enhanced glycosylation pathways for mammalian glycoprotein expression were also stably transformed with the *vankyrin* gene, and enhanced protein expression is seen in these cell lines as well. Further increase in recombinant protein expression is achieved when VE-BEVS is used to infect VE-insect cells, showing a synergistic effect between the two systems. Taken together these results indicate that the vankyrin technology is versatile and can support and improve the utility of the BEVS for research and commercial applications.



#### **O 8.4 Single cell analysis and fluorescent probes for the characterization of insect cell responses towards infection**

Laura A. Palomares, David Hidalgo, Vanessa Hernández, Enrique Paz, Israel Montes de Oca, William A. Rodríguez-Limas and Octavio T. Ramírez  
Departamento de Medicina Molecular y Bioprocesos. Instituto de Biotecnología. Universidad Nacional Autónoma de México. Cuernavaca, Morelos, Mexico

Productivity of the insect cell-baculovirus expression vector system (IC-BEVS) depends on an efficient infection. It has been previously found that intrinsic cellular parameters determine the cell susceptibility to infection and the specific productivity of recombinant protein and baculovirus progeny. In order to investigate such parameters, a fluorescence microscopy system for monitoring, insect cells cultured under various set conditions, was developed. Three fluorescent biosensors were constructed, which allowed the real time monitoring of each infected cell. Sensors included roGFP, an EGFP variant with fluorescence dependent of redox potential, EGFP under de *polh* promoter and RFP under the *vp39* promoter, all incorporated into baculovirus vectors. The baculovirus life cycle (late and very late phases) and the effect of infection on the intracellular redox potential were monitored. Several populations of insect cells were found. Some cells had a high mobility and were resistant to baculovirus infection. Culture under oxygen limiting conditions resulted in cell clumping and a reduced susceptibility to baculovirus infection. Infection at low cell concentrations resulted in an oxidizing intracellular ambience, while the multiplicity of infection did not affect the intracellular redox state. The single-cell analysis approach of this work can complement traditional macroscopic population measurements for improved process development. The findings reported in this work are being integrated into improved infection and culture conditions that reduce population variability and that increase productivity of the IC-BEVS.

Economic support by PAPIIT- UNAM IT-201214 and IT-200315.



### O 8.5 Development of a Robust, Defined, Animal-Free Virus Production Medium Optimized for Microcarrier Culture

Steve Pettit<sup>1</sup>, Grishma Patel<sup>2</sup>, Mark Szcypka<sup>2</sup>, Bart Tarbet<sup>3</sup>, and Claire Huang<sup>4</sup>

<sup>1</sup>InVitria, Fort Collins, CO, USA; <sup>2</sup>Pall Corporation (SoloHill Engineering), Ann Arbor, MI; <sup>3</sup>Utah State University, Logan UT;

<sup>4</sup>Centers for Disease Control-DVBD Branch, Fort Collins, CO

The use of undefined components in animal product-free (APF) cell culture media could cause inconsistencies in cell growth and viral production. InVitria has developed a hydrolysate-free and APF Vero cell production medium that is ideal for use in bioreactors with microcarriers. Development was initiated in small-scale spinners prior to further optimization using bioreactors. We used dengue virus for the optimization of production optimization. Dengue virus typically achieves peak production 6-10 days post-infection in flask-based systems.

InVitria's medium supports robust cell growth on multiple types of APF SoloHill™ microcarriers (Pall Life Sciences) and others. However, two of these microcarrier types, ProNectin® F and Plastic, resulted in sustained, high-density cell growth. Vero cells achieve a maximum cell density of >3 M cells/mL in bioreactor without medium exchanges (batch culture) that is maintained for up to 10 days. In a mock infection process, utilizing a partial medium exchange, cell densities of up to 6 M cells/mL are achieved. The production of virus equals or exceeds other media options evaluated and the peak of dengue 2 virus production advances up to 3 days earlier in microcarrier culture compared to flask-based production. A model attenuated dengue vaccine produced from microcarrier culture was tested in a mouse vaccinate-challenge model system. Vaccine produced using InVitria's medium resulted in similar neutralizing titer and protection as vaccine produced using serum or the leading commercial medium.



### **O 9.1 Application of CRISPR/Cas9-mediated genome engineering for improved protein production in mammalian cells**

Helene Fastrup Kildegaard

The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark,  
Hørsholm, Denmark.

Chinese hamster ovary (CHO) cells are widely used in the biopharmaceutical industry as a host for the production of complex pharmaceutical proteins. Thus, genome engineering of CHO cells for improved product quality and yield is of great interest. Here, I describe the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) genome editing technology and demonstrate its applicability and efficacy for generating knock-in and knock-out CHO cell lines with desirable properties. Application of the CRISPR/Cas9 technology for CHO cell engineering facilitated the disruption of genes involved in apoptosis and glycosylation. Multiplexing and fluorescent enrichment of Cas9 expressing cells facilitated simultaneous generation of single, double and triple knock-out cell lines. Further characterization of selected triple knock-out cell lines confirmed improved resistance to apoptosis and removal of fucosylation as expected. In addition, site-specific integration of transgenes mediated by CRISPR/Cas9 and homology directed repair resulted in the generation of targeted integrants with improved clonal homogeneity compared to random integrants. The proven efficacy of genome editing mediated by CRISPR/Cas9 technology has a large potential to accelerate current CHO engineering efforts. Together with high-throughput technologies, computational models and systems biology approaches, genome editing can pave the way for accelerated generation of desirable CHO cell factories with predicted culture performance.





**O 10.1 TBA**

Michael Betenbaugh, John Hopkins, USA



**O 10.2      Structure-Function Studies of Daratumumab –  
A Breakthrough Therapy to Treat Multiple Myeloma**

T. Shanta Raju, Johnson & Johnson, USA

Recombinant antibodies (mAbs) are becoming major therapeutics to treat human diseases including life threatening diseases such as cancer. These mAbs exhibit multiple biological activities including binding to antigens, binding and neutralization of antigens, binding to antigens and elicit cell killing etc. The cell killing activities of antibodies include antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), apoptosis etc. Other biological activities of antibodies include antibody dependent cellular phagocytosis and trogocytosis. Glycosylation in the Fc has been shown to impact Fc effector functions. This presentation illustrates the structure-function studies to understand the multiple biological activities of mAbs using Daratumumab as an example.



**O 10.3 A genome-scale systems biology approach to protein expression**Nathan E. Lewis<sup>1,2</sup><sup>1</sup>Department of Pediatrics, University of California, San Diego<sup>2</sup>The Novo Nordisk Foundation Center for Biosustainability at the University of California, San Diego

The secretory pathway and related cell processes drive protein expression in biopharmaceutical manufacturing. Indeed, protein translation, folding, and post-translational modification are strongly influenced by the host cell genome, metabolism, and glycosylation pathways. Thus, it is essential to thoroughly consider these processes in efforts to control critical quality attributes. To guide cell line engineering efforts, bioinformatic and systems biology tools have been developed to study genomes, metabolism, and glycosylation. However, it has been difficult to concurrently account for the many genetic and environmental factors influencing protein expression.

For a holistic view of protein secretion in CHO cells, we are developing a computational framework that integrates transcriptomics, whole genome resequencing, glycomics, and metabolomics. For this, we sequenced the Chinese hamster and several CHO cell lines. With these data, we are constructing computational models metabolism, protein secretion, and glycosylation. These models couple media composition to protein synthesis and glycosylation, and account for transcriptional differences between cell lines. With these models, we identified cell-line specific effects of mutations and how media formulation impacts metabolism and protein glycosylation. Furthermore, these models predict changes in glycosylation following genetic manipulations.

Through these analyses we demonstrate that large systems biology models can integrate genomics, transcriptomics, and metabolomics to gain detailed insights into how diverse cell processes ultimately influence protein synthesis and glycosylation. Furthermore, these approaches show incredible promise for informing cell line development and glycoengineering, in an effort to better control biotherapeutic safety, efficacy, and affordability.



### **O 11.1 Omics Guided Cell Line Engineering: Reducing High Mannose by Over Expressing N-glycosylation Pathway Regulators**

Shivani Gupta, Kristi Daris and Trent Munro

Process Development, Amgen Inc., Thousand Oaks, California. US-91320

High Mannose (HM) is a critical quality attribute (CQA) for recombinant therapeutic monoclonal antibodies (mAbs) that can impact biological activity by influencing Fc mediated effector functions, product stability, clearance rate and safety. In preliminary gene expression studies, we observed that increased endogenous mRNA levels of N-acetyl-glucosaminyltransferases (Mgat1 and Mgat2) and the UDP-Galactose transporter (Slc35a2) correlated with lower levels of high mannose glycans in mAb producing Chinese Hamster Ovary (CHO) cell lines. We sought to further characterize the role of these genes by altering their expression levels and examining the effect on glycan processing. Using RNAi mediated knockdown in a low HM cell line we show that reduced levels of Mgat1 can increase the levels of HM by ~70%. Furthermore, we have overexpressed Mgat1, Mgat2 and Slc35a2 genes in a CHO cell line which produces mAbs with high HM levels. Expression of both Mgat1 and Mgat2 genes was sufficient to reduce high mannose levels by ~70 %. However, overexpression of Slc35a2 did not significantly impact HM levels. Our results show that Mgat1 and Mgat2 may represent ideal targets for the engineering of new host cell lines that can produce products with increased levels of fully processed glycan structures and hence low levels of HM.





### **O 11.2 A Camelid Heavy Chain Monoclonal Antibody Displays Unique FcγRI Binding Properties**

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Monoclonal antibodies (mAbs) are commonly used as biotherapeutic treatments in cancer and other autoimmune diseases. Engineering these mAbs to have a high affinity for their receptor is crucial for effective biotherapeutic use. One method to increase the affinity of the antibody to its receptor is by altering the N-linked glycosylation pattern in the Fc region (Asn297). This can be accomplished by using various glycoprotein processing inhibitors in the growth media. The inhibitors used in this study to grow an engineered 80 kDa chimeric antibody include kifunensine, swainsonine, and castanospermine. This heavy chain mAb (EG2-hFc) is designed with a camelid fragment variable (Fv) that is attached to a human fragment crystallisable (Fc) region. This antibody's small size makes it an ideal candidate for therapeutic use as it can probe areas too small for current immunoglobulin G (IgG) (150 kDa) biotherapeutics on the market. The effect of the N-glycan composition on the size distribution, secondary structure, and sedimentation rate of the antibody was analyzed via dynamic light scattering (DLS), circular dichroism (CD), and analytical ultra centrifugation (AUC), respectively. Furthermore, analysis of the effect of N-glycosylation on the binding of the antibody with FcγRI using surface plasmon resonance (SPR) and microscale thermophoresis (MST) was performed. It was found that upon alteration of the glycan by glycoprotein processing inhibitors, the secondary structure and sedimentation rate of the antibodies was not affected; however the degree of higher order oligomerization of EG2-hFc was increased. Comparing the effect of N-glycosylation on mAb binding to FcγRI for both EG2-hFc and a full IgG1, it was discovered that EG2-hFc has unique binding properties. Aside from slight differences observed in the correlation between the glycans attached and the affinity, removal of the glycan from Asn297 only reduced the affinity about 6 fold compared to a 118 fold decrease in a full-sized IgG1.



**O 12.1 Effects of Selected Amino Acid Limitations in CHO Cell Fed-Batch Cultures**

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Fed-batch cell proliferation, productivity and protein product quality can be compromised by cellular stress responses to nutrient limitations. This work investigated the effects of limiting glutamine, asparagine or cysteine on cell growth, metabolism, productivity and product quality of three antibody expressing CHO cell lines. CHO cell autophagy was induced by glutamine limitation, targeting intracellular components for lysosomal degradation as part of a survival response to stress. Adding a chemical inhibitor of autophagy increased monoclonal antibody and tissue plasminogen activator fed-batch production 2-fold or more, without impairing the protein glycosylation. Asparagine limitation had much lesser effects and cysteine limitation had the greatest negative impact, severely reducing cell productivity and survival. In addition, protein fucosylation was reduced in the absence of cysteine. Overall, these results illustrate CHO cell line requirements for particular amino acids in fed-batch cultures as well as how understanding physiological mechanisms can provide opportunities to improve industrial production.



**O 12.2 Manufacturing Biologics at the Bedside**

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The current biopharmaceutical production paradigm is a throwback to the industrial age, where large centralized facilities continue to be the main source for biologics. As healthcare becomes more decentralized and personalized, there is a need for bioprocess technologies to also evolve. We present a new paradigm that shows a path forward. Using CHO cell extracts, we demonstrate the production of several model biologics (Steptokinase, tPA and Erythropoietin) in a matter of hours with high activity. This breakthrough enables the possibility of a small briefcase size device that can produce any biologic at the point-of-care. Prototypes of the device components will be displayed. However, such technology leads to new issues such as the validation and regulatory path for such an approach. We will present a comprehensive approach that will demonstrate our approach and highlight potential solutions.



**O 12.3 Real-time and At-line monitoring for culture control**

Tharmala Tharmalingam<sup>1</sup>, Richard Wu<sup>1</sup>, Susan Callahan<sup>1</sup>, Robert Hong<sup>1</sup>, Sabrina Benchaar<sup>1</sup>, Siowfong Wee<sup>1</sup>, and Chetan Goudar<sup>1</sup>

<sup>1</sup>Agmen, USA

Controlling quality of recombinant protein production by maintaining and adjusting bioreactor performance throughout its production duration has become important and desirable. Current practice typically involves glycosylation and protein characterization at the end of a fed-batch culture, which in addition to being an aggregate of the process, reflects a bias towards the end of the culture where a majority of the product is made. In order to address this gap, we have developed a compact and robust sample preparation platform, using a micro-sequential injection system, capable of interfacing with analytical instruments to monitor key metabolites (glucose and amino acids) and recombinant protein produced within the bioreactor. In a recent publication, we have monitored the effect of medium components on glycosylation of a monoclonal antibody. Furthermore, using a similar technology platform, coupled with mass spectrometry, we are now monitoring peptides and glycopeptides for other posttranslational modifications such as deamidation and C-terminal lysine. The development of these technologies enables the ability to make process adjustments based on real-time monitoring data referenced to the targeted product specifications. These technologies help manufacture product with the desired quality attributes and also improve manufacturing robustness by allowing rapid decision making to mitigate cell culture related risks should adverse events occur.





### **O 13.1 Novel Cell Culture and Assay Platforms in Support of Continuous Improvement and Manufacturing Support Activities**

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Manufacturing Sciences organizations typically support commercial production activities. This is done through activities that include investigations of deviations as well as continuous process improvements and preparations for new product launches.

With any process improvement/change it is essential to verify that neither the cell culture performance nor product quality attributes have been affected. This verification would ideally be performed at early phases of the projects. The data generated would then serve to justify further work/ validation towards implementation (or alternatively, a no-go decision).

To this end, we embarked on establishing an infrastructure that includes two key platforms: small scale (perfusion) cell culture and rapid product quality assays - requiring no or minimal protein purification and low sample volumes.

In this talk we describe the development and qualification of these platforms. We further demonstrate how these platforms are being utilized in various process improvement projects and how they are used during investigations to help facilitate critical decision making.

Specific examples are shared through case studies including: serving as an early product quality diagnostic during an investigation into an unplanned deviation (that can occur during commercial manufacturing); assessments during cell culture medium and process improvements (for increased efficiency and yield); evaluation of changes to raw materials and qualification of new (e.g., dry powder medium) suppliers; and assessment of state-of-the-art virus containment systems (such as virus-removal filtration and high temperature short time [HTST] medium treatment).

In sum, these platforms and assays provide significant advantages such as flexibility and early detection; they extend the testing capabilities of our QC labs and in the future may also be used in production.



**O 13.2 Comparison of manufacturability attributes, biophysical properties and post-translational modifications of molecules produced by transient vs stable expression methods**

Jennitte L. Stevens

Therapeutic Discovery, Biologics Optimization. Amgen, Thousand Oaks.

Manufacturability assessment is a critical component of the candidate selection process for protein molecule therapeutics. In addition to choosing candidates based on their biologic activity, it is also critical to understand if they have drug like properties and will perform well in a clinical and commercial manufacturing setting before progressing them into development. Molecules with poor manufacturability attributes can cost significant delays in the downstream. We have moved developability assessment very early on in the drug selection process when there are a large number of candidates to assess. We typically rely on transient 293 productions to deliver protein in a rapid timeframe for a large number of protein molecules. To ensure that the proteins made by this production method were representative of material produced from the manufacturing host, we performed systematic comparison of material produced from CHO and 293 transients to material produced from 2 different CHO stable hosts. We ran this material through a series of stability, viscosity and analytical assays. Glycosylation, signal peptide analysis pharmacokinetic (PK) assessments were also measured, as well as comparisons between transient and stable titers. We find that 293 transient produced material can be confidently used to assess biophysical attributes, and to predict O-linked glycosylation sites, but not occupancy or levels. Transient performance from CHO or HEK and performance can also be predictive of relative stable cell line outcomes. However, host cell selection is important in considering PK, signal peptide processing and proteolysis sensitivities. Such considerations need to be taken into account when planning an expression strategy.



### **O 13.3 Maximizing Protein Production in CHO Cells to Support Protein Therapeutics Discovery and Development**

Jill Cai and Weichang Zhou

Biologics and Bioprocess Dept, WuXi Biologics, Shanghai, China

WuXi Biologics is a premier CDMO for discovery, development and manufacturing of protein therapeutics in mammalian cells, which include monoclonal antibodies, recombinant and fusion proteins. We have established a versatile and efficient protein production platform in CHO cells ranging from microgram to multiple grams during different stages of discovery and development. Several case study examples will be presented to highlight our approach to optimizing signal peptides to improve expression levels of difficult to express proteins and product quality; how we developed an antibody stable pool platform with an average expression level of 1.5g/L. And finally by using our targeted integration platform, high expressing recombinant proteins and stable cell lines for membrane proteins can be rapidly generated.



### **O 13.4 Improving production economy: the role of continuous cultures and on-line monitoring of fermentation processes in the pharmaceutical industry**

*George Lovrecz, Louis Lu, Burkhard Raguse and Lech Wiczorek*

CSIRO Manufacturing Flagship

Clayton & Lindfield, Victoria, Australia

**Keywords:** *intelligent bioreactors, continuous cultures, on-line monitoring, lab-on-a-chip, chemiresistors*

The last few years showed an unprecedented improvements in mammalian cell culture yields used for the large-scale production of pharmaceuticals, especially of monoclonal antibodies. However ongoing cost-cutting measurements from the governments demand the introduction of new methods to further reduce production costs.

Potentially new techniques in genetic engineering and clone selection offer the most significant improvement in final yield but maintaining optimal environment for the cells might also have a major role too. Using continuous cultures and novel on-line monitoring and control techniques would be one of the most obvious way to ensure that the cells are subjected to optimal conditions and therefore maximum yield is expected.

In the past continuous fermentation processes were typically employed when the cells were anchored as cell recycling from suspension cultures are rather difficult in large-scale situations. Furthermore, the lack of reliable and low cost in-situ sensors are posing a great limitation to on-line monitoring and control. In the past few years our laboratory at CSIRO started to develop novel chemiresistors which have the potential to be used as reliable on-line sensors and thus help to maintain optimal culture parameters. Our patented chemiresistor technology was already used for the detection of various biochemicals in typical fermentation broth to follow the growth of different bacteria. Our current focus is to make this technology available for measuring the concentration of typical media components such as glucose, glutamine and by-products such lactic acid.

This paper will discuss the current status of our chemiresistor development work and summarizes the advantages and disadvantages of continuous fermentation processes.





## POSTER PRESENTATIONS



### **P 5.01 Expression of Viral Surface Proteins Using the Multi-Host Expression System for Functional and Structural Analysis**

Margitta Schürig<sup>1</sup>, Christian Schinkowski<sup>1</sup>, Ruth Stricker<sup>1</sup>, Jörn Krauß<sup>1</sup>, Tanvi Khera<sup>2</sup>, Thomas Pietschmann<sup>2</sup>, Klaus Schughart<sup>1</sup>, Joop van den Heuvel<sup>1</sup>

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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 glycoproteins E1 and E2 of HCV. Among the eukaryotic expression systems allowing post-translational modifications HEK, CHO and insect cells are widely used. On account of this the multi-host expression system can be used in these three cell lines for transient or stable protein expression. 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 for the subtypes H1 and H3. For H1 large scale production and further purification were performed. This included cleavage by thrombin of the tagged heterologous trimerization domain and subsequent purification by gel filtration chromatography to isolate the trimeric H1. Crystallization experiments of H1 trimer are in progress.



**P 5.02 The Use of Lumino Tags to Quantify Proteins in High Throughput**

Huizhen Zhao and Benjamin Schwartz

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We previously developed methods for high throughput, parallel expression, purification, and analysis of proteins (nicknamed HEAT). These protein libraries are useful for many areas of discovery, including mapping of compound binding sites, functional testing disease associated SNPs, and development of new enzyme catalysts for manufacturing. However, a limitation in this method is the ability to accurately determine the concentrations of each protein variant in an array due to sensitivity limitations in the typically used Bradford assay.

To overcome this issue, we developed the use of a small, peptide luminescent tag (Lumino) for protein detection. The tag contains a tryptophan flanked by a span of basic aspartate residues and can be engineered onto the N or C-terminus of a protein. Once expressed and purified, Terbium is added to the protein sample and is chelated by the aspartate residues. Protein is analyzed by excitation of the tryptophan residue and detection of the time-resolved fluorescence by the neighboring Terbium after energy transfer. Compared with the Bradford assay, this luminescent format offers >50-fold increase in sensitivity and costs 10,000 times less per well. We demonstrate that the Lumino tag works equally well across several different proteins, also can be used to screen soluble constructs and expression conditions via 96-well plate format without running the gel. The method is compatible with production of protein arrays by HEAT.



**P 5.03 Baculovirus mediated protein production in insect cells - a new dimension**

*Sebastien Rieffel, Julia Klopp, Susan Roest, Bernd Gerhartz, Binesh Shrestha*

Baculovirus mediated insect cell expression system has been considered as one of the efficient systems for expression of human proteins of therapeutic interest. The conventional way of handling insect cell culture gives an impression of system as an expensive and labor intensive. Here we present the recent developments from our lab where an effort was put to make it as simple and inexpensive as possible. The changes introduced can be easily adapted without much alteration in any standard lab facilities.

Outcome of the study:

- New culture vessel is identified in which cells can be grown with less as well as more air space.
- Expression parameter identified in 24-deep well block can be directly translated in these vessels (with working volume from 0.8L to 4.7L).
- Commercially available insect cells are not fragile as original isolates. Hence cells can be grown using higher shaking speed that are originally considered not possible.

The innovative method that changed the way we run insect culture has recently been published (See <http://www.sciencedirect.com/science/article/pii/S2215016114000077>). Furthermore, we have also introduced the effective and efficient method of transfecting cells in suspension, virus amplification in shorter time, expression optimization in 24-deep well block to identify the parameters for scale up and effective way of harvesting culture. The poster will highlights above mentioned development as a hands-on guidelines for running insect cell culture lab.





**P 5.04    Viral engineering for better secretion of Hemagglutinin virus like particles in insect cells**

Krisztina Koczka<sup>1</sup>, Monika Wilde<sup>1</sup>, Dieter Palmberger<sup>1</sup>, Wolfgang Ernst<sup>1</sup> and Reingard Grabherr<sup>1</sup>

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The baculovirus insect cell expression system is an integral component of recombinant protein expression technologies. A variety of complex eukaryotic proteins have been successfully expressed in insect cells *Spodoptera frugiperda* (*Sf9*) at large scale, including commercialized vaccines. Recently, *Trichoplusia ni* (*T. ni*) cell lines (High5, TnAO38) have been recognized as an alternative host for their superior capacity in secreting heterologous proteins at high yields. These cell lines have been shown to be feasible to express complex glycosylated proteins, e.g. viral surface proteins and cellular membrane proteins as well as multi-subunit complexes. In particular, the successful expression of multi-subunit vaccines such as virus like particles has proven the potential of this.<sup>1</sup> To date, for the generation of many products, also at large scale, mostly *Sf9* cells are being used. Thus, little is known about process engineering of up-scaled insect cell based systems based on *T. ni* derived cell lines. The focus of our work is the characterization of the above mentioned cell lines and maximizing their yield. To this end, we conducted time course experiments that proved the superior protein expression capacity of High5 cells over *Sf9*. To overcome the widely experienced problem of low yield for many secreted glycoproteins, certain chaperones (e.g. the BIP from... and the PDI from...) were over-expressed in High5 cells in order to test their capability to increase the heterologous protein production. By precisely adjusting the multiplicity of infection (MOI) of the co-infecting viruses, expressing the target protein or the chaperones, we managed to increase the expression rates for influenza A virus hemagglutinin as well as for influenza A virus like particle yield in small scale. Our strategy will further be tested in large scale production processes and downstream processing.

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1        Krammer, F., Grabherr, R. Alternative influenza vaccines made by insect cells. Trends in molecular medicine 16, 313-320 (2010).



**P 5.06      A recombinant-Baculovirus/Replicon Model for Efficient RNA Packaging and Production of Infectious HEV Particles in Hepatoma Cells**

Mohammad Khalid Parvez

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Hepatitis E virus (HEV) is an emerging hepatotropic RNA virus that causes acute and chronic liver disease with a global mortality rate of ~2%. Despite milestone developments in understanding HEV biology, there is still a lack of an experimental animal model or robust culture system. Therefore, in a novel approach, two recombinant-baculoviruses: vBacORF2 and vBacORF3 that could overexpress HEV ORF2 (capsid) and ORF3 proteins, respectively were constructed in pTriEx1.1 vector and insect cells, Sf9 (BacVector Kit). Co-transduction of human hepatoma cells S10-3 with the baculoviruses ( $10^8$  pfu/ $\mu$ l, each) produced high amounts of ORF2 and ORF3 proteins in ~60% of the cells (IFM: 1<sup>o</sup>ORF2/2<sup>o</sup>AlexaFluor 488-conjugate; 1<sup>o</sup>ORF3/2<sup>o</sup>AlexaFluor 568-conjugate). The Western blot analysis further confirmed the two viral proteins expressions that were of native sizes. The HEV-SAR<sub>55</sub> (genotype 1) replicon that contained GFP gene, in place of ORF2/3 sequences (nt. 5130-7204) was in vitro transcribed, and GFP production in transfected cells was scored by FACS. To determine if the exogenously-supplied ORF2 could transencapsidate viral RNA, and if ORF3 could enhance virion infectivity of naïve HepG2 cells, RNA was transfected into S10-3 cells a day before baculovirus transduction(s). Cell lysates were prepared on day 6 post-transfection or day 5 post-transduction, and virion infectivity assay was performed. FACS scoring indicated that lysates from S10-3 cultures receiving the replicon plus vBacORF2 were capable of producing HEV particles with ~4% infectivity in HepG2 cells. However, lysates of cultures that were co-transduced with vBacORF3 were found to further enhance virion infectivity by ~17%. This supported a previously proposed role of ORF3 as a minor structural protein in infectious virion assembly. In conclusion, the present model for efficient genomic RNA packaging and production of infectious virions could be a valuable tool to study various aspects of HEV molecular biology, ex vivo.



**P 5.07 Using the endoplasmic reticulum as a physiological test tube:  
predicting undesirable solution properties of mAb clones by cell phenotype analysis**

Haruki Hasegawa <sup>1</sup>, Neha Patel <sup>2</sup>, Janelle Stoops <sup>2</sup>, Samantha Byrd <sup>2</sup>,  
Christopher Woods <sup>2</sup>, Francis Kinderman <sup>3</sup>, Feng He <sup>2</sup>, Ai Ching Lim <sup>1</sup>

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The underlying reasons for why some mAb (monoclonal antibody) clones are much more inclined to induce a Russell body (RB), or Mott cell phenotype, during immunoglobulin biosynthesis remain elusive. Although RBs are morphologically understood as enlarged globular aggregates of immunoglobulins deposited in the endoplasmic reticulum (ER), little is known about the properties of the RB-inducing mAb clones as secretory cargo and their solution behaviors in the extracellular space. To elucidate how RB-inducing propensities, secretion outputs, and the intrinsic physicochemical properties of individual mAb clones are interrelated, a HEK293 cell transient expression system was used to study the biosynthesis of human IgG mAbs for which prominent solution behavior problems were known a priori. All model mAbs with inherently high condensation propensities (i.e., low solubility, high agitation sensitivity, etc.) resulted in low secretion titers and induced RB phenotypes (both at steady state and under ER-to-Golgi transport block). By contrast, a reference mAb that readily crystallized at neutral pH in vitro produced rod-shaped “crystalline body” phenotypes. Another easy-to-crystallize mAb clone also induced grain-shaped crystalline bodies in the ER. Similarly, a single-chain antibody clone with cryoglobulin-like propensities induced morular cell phenotypes via intracellular liquid-liquid phase separation event. Lastly, selected IgG clones without prominent solution behavior issues did not induce notable cellular phenotypes and were secreted robustly. These results implicated that intrinsic physicochemical property of individual mAb clones directly affected the biosynthetic steps in the ER, and thereby produced distinctive cellular phenotypes and also influenced IgG secretion output. The apparent correlations between solution behaviors in vitro and biosynthetic events in the ER can be leveraged to identify unfavorable mAb clones that are not suitable for high-level expression and high concentration liquid formulation.



**P 5.08 Protein Production for Biologics Drug Discovery**

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<sup>1</sup>Global Biologics, AbbVie Bioresearch Center, Worcester, MA, <sup>2</sup>AbbVie Biotherapeutics, Inc. Redwood City, CA

Antibodies and related biological products are the fastest growing class of therapeutic agents. To date, over 200 monoclonal antibodies are in various stages of clinical development. Biological programs require large quantity of high quality antigens, of various species, during the research and development stage. Generation of antigen reagents is typically more challenging than antibodies. Numerous constructs and expression hosts are explored to identify a viable path for rapidly producing adequate quantities of antigens to enable screening, optimize affinity and generate proprietary antibodies. This talk will focus on the expression and characterization of antigens in order to generate functional therapeutic antibodies.

Disclosures:

All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.





**P 5.09      Addressing key steps along the path to obtaining the ‘best’ recombinant CHO cell lines**

Alison Porter, FUJIFILM Diosynth Biotechnologies

The keys to developing biomanufacturing processes which are efficient, robust and of high quality start during cell line and process development. It is therefore imperative that the expression system and the process to isolate and identify the best clonal cell line are of the highest quality. Getting it ‘right first time’ at the start of development can allow one to avoid later challenges. In this presentation we will discuss key steps in the cell line development (CLD) process, including the development and integration of: (i) a host cell line with improved growth and productivity capability; (ii) a high expression vector; and (iii) state-of-the-art screening technologies enabling better decisions to be made during cell line selection.

Directed evolution is an approach which can be taken when developing a new host cell line. Four methods were trialled when such an approach was used at FUJIFILM Diosynth Biotechnologies (Fujifilm): continuous culture in altered subculture regimes; enrichment of cells with extended viability via flow cytometry assisted sorting; cloning; and growth of cells in chemostat culture. A hierarchical screening strategy was then employed to gradually decrease the large number of potential new host cell lines generated and identify those with superior biomanufacturing characteristics. During this process, observations from an assessment of 25 host cell lines included identification of cell lines which achieved up to a 3-fold increase in transient mAb expression or a 2 fold increase in viable cell density at sub-culture when compared to the original host cell line.

A challenge during the development of an expression vector is how to take into account the multiple components which can affect expression. Development work can quickly become impractical with large numbers of possible component combinations. Similar to the host cell line development, Fujifilm employed a hierarchical screening strategy to allow the assessment of multiple variants of a number of vector components individually and then in combination.

Finally, the optimisation of steps to isolate the best recombinant cell lines created using the new host cell line and vector will be discussed. This includes the introduction of platform screening procedures which are more relevant to platform production processes. An example of this is a shaking fed-batch 24 deep-well plate screen (using un-optimised basal media and feeds) where titres of up to 3 g/L have been observed.

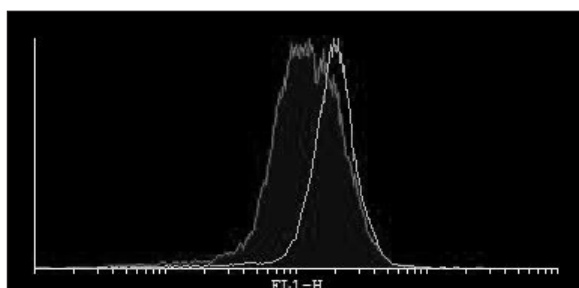


**P 5.10 Cell and Genetic Engineering Techniques for Enhanced Membrane Protein Expression in Mammalian Cells**

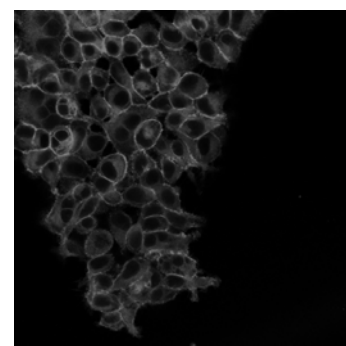
Joseph J. Priola &amp; Michael J. Betenbaugh

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Membrane proteins are a medically relevant class of proteins that play a key role in a multitude of diseases and disorders, such as depression, Alzheimer's, and anxiety. In order to most accurately design potential therapeutics to treat membrane protein associated diseases and disorders, the structure of the membrane protein of interest must be known. However, due to the typically low native expression level and large size of membrane proteins, they are underrepresented in the protein structure database, and pose several challenges to researchers aiming to elucidate the structure of these large, toxic proteins. As a result, membrane protein expression is one of the greatest bottlenecks in structure determination via techniques such as x-ray crystallography. In order to combat the expression limitations of membrane proteins, our lab has applied cell and genetic engineering techniques in an attempt to optimize mammalian cells, particularly HEK293, for increased expression of membrane proteins. We have studied the use of different mammalian cell lines (HEK293, CHO-K1, etc), different expression systems (Tetracycline-inducible, for example), different plasmid constructs and promoters, as well the effect of overexpression of the Endoplasmic Reticulum folding chaperone Calnexin, and the anti-apoptosis gene Bcl-Xl. In addition, we have performed miRNA and siRNA screens to identify candidates that increase membrane protein expression in HEK293 cells. Techniques such as flow cytometry, fluorescence-activated cell sorting, SDS-PAGE, ELISA, confocal microscopy, and radioligand binding assays have been employed in order to assess membrane protein expression in a variety of engineered cell lines. We have identified a number of methods to increase membrane protein expression in mammalian cells, and look to continue to optimize membrane protein expression and purification, and also to begin structural studies using our optimized cell lines as expression hosts.

**Relevant Figures**

**FIGURE 1:** FLOW CYTOMETRY RESULTS INDICATING INCREASED EXPRESSION OF A MEMBRANE PROTEIN – EGFP FUSION IN AN ENGINEERED CELL LINE. RED: ORIGINAL CELL LINE EXPRESSING A MEMBRANE PROTEIN-EGFP FUSION. YELLOW: ENGINEERED CELL LINE.



**FIGURE 2:** CONFOCAL IMAGE OF AN ENGINEERED CELL LINE EXPRESSING A MEMBRANE PROTEIN – EGFP FUSION PRIMARILY AT THE CELL MEMBRANE.



**P 5.11      Engineering a CHO Dual Selection Knockout Host for Increased Productivity**

Shelly Martin, Eric Lee, Scott Estes

Biogen, Cambridge MA, U.S.A

Concurrent with increasing demand for biopharmaceuticals is the necessity for continual reduction in cell line development (CLD) timelines to accelerate clinical submission. Chinese hamster ovary cells (CHO) are the most widely used host cells for recombinant therapeutic protein production, in particular monoclonal antibodies (mAbs). The two most successful selection systems for the production of therapeutic proteins utilize deficiencies in dihydrofolate reductase (DHFR) or glutamine synthetase (GS). In our continuing effort to explore more efficient generation and selection of high productivity cell lines, we combined the power of both systems and developed a dual selection GS (-/-) and DHFR (-/-) double knock-out (DKO) CHO host cell line. Engineered using SAFC's zinc finger nuclease technology (zfn's) the new DKO host was compared to our highly productive GS-deficient CHO host for the production of mAbs. Stable, unenriched pools from each host were evaluated in a plate based production fed-batch. Our newly developed DKO host yielded up to 2X increase in titer compared to our traditional host. These data suggest our more stringent dual and amplifiable selection system results in higher stable transfection pool titers (3.6 g/L, 15 pcd) in 6 weeks and amplified stable pools (5.1 g/L, 26 pcd) in 12 weeks. Based on these promising results, the DKO host is being further analyzed in cell line development efforts to evaluate amplified, enriched, clonal cell lines.



**P 5.12 Improved recombinant protein production with a novel *cis*-regulatory DNA element in Chinese hamster ovary cells**

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Incorporation of *cis*-regulatory DNA elements into an expression vector is one of the methods to achieve high productivity. Although Chinese hamster ovary (CHO) cells are the most widely used mammalian cell line in the production of biopharmaceuticals, there are still regulatory elements in CHO cells that are not well-known. Thus, we isolated novel *cis*-regulatory elements from a CHO cell line that enhance transgene expression. Genomic DNA fragments from CHO-K1 cells and a plasmid vector expressing GFP regulated by the CMV promoter were used to construct a genomic library, and after screening, the putative *cis*-regulatory element E77 was isolated from a cell with the highest expression level of GFP. The enhancing effect of E77 was evaluated by the generation of stable cell pools transfected with vectors that included E77 and the genes for GFP and an antibody. The protein expression levels of GFP and the antibody increased by about fifty percent and five-fold, respectively, compared to the CMV promoter alone. The sequence of E77 consisted of two fragments, and its location and orientation in the vector system were related to the regulation of gene expression. This study suggests that incorporating E77 into a vector could be useful in the production of recombinant proteins in a CHO expression system.





**P 5.13 Antibody Sequence Variant Detection using Next Generation Sequencing**

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<sup>1</sup>Cell Culture Development, <sup>2</sup>Computational Biology and Genomics, Biogen Cambridge, Massachusetts, USA

Next-Generation Sequencing (NGS) has greatly impacted the Biologics industry. Advancements in the NGS field have reduced cost and complexity of operation for this technique, leading to more groups benefiting from bioinformatics data. These big datasets harbor rich information and with the help of benchtop sequencers, multiple disciplines now have the ability to expand their research. Utilizing this technology, we created a workflow to verify the mRNA sequence of top antibody expressing clones to ensure that they are free of mutations during clone screening. This method takes advantage of the multiplexing and high-throughput capability of NGS to confirm the mRNA sequence from multiple antibody producing clones in one experiment. The workflow is designed to screen the heavy and light chain mRNA, similar to amplicon sequencing. Heavy and light chain amplicons were generated using specific primer sets for each transcript using cellular cDNA as a template. These PCR products were then fragmented, barcoded and loaded onto the MiSeq. For bioinformatics, we were assisted by our computational biology group, which assessed the quality of reads generated by the sequencer, aligned them to our heavy and light chain reference, and then formatted the results for evaluation. To determine the limit of detection (LOD) of this workflow, we created a point mutation in the heavy chain expression plasmid and performed a dosing study with various amounts of this mutated plasmid mixed with the correct plasmid. The results of this experiment confirmed that a mutation can be detected at various percentages down to 1-2%, which is the error rate for a high number of NGS experiments. Using NGS, we are able to process and sequence-confirm heavy and light chain mRNA from 30-50 antibody producing clones in 6-8 business days. This early screening method has identified clones with synonymous and non-synonymous mutations at the clone screening stage, allowing us to focus our efforts on top-quality clones. With this method in place, we have confidence in the sequence integrity of these clones moving forward in our cell line development process. In summary, we have successfully implemented a NGS application to verify production clones sequences, improving the quality of clones moving forward in our cell development workflow and maintaining the overall throughput.



**P 5.14 Chemical Inhibition of Autophagy: Examining its Potential to Increase the Specific Productivity of Recombinant CHO Cell Lines**Eric Baek<sup>1</sup>, Che Lin Kim<sup>1</sup>, Mi Gyeom Kim<sup>1</sup> and Gyun Min Lee<sup>1</sup><sup>1</sup>Department of Biological Sciences, KAIST, 335 Gwahak-ro, Yuseong-gu, Daejeon, Republic of Korea

Cell death is undoubtedly one of the most important issues to consider during Chinese hamster ovary (CHO) cell culture. In order to delay the onset of cell death and thereby increase the duration of culture, many studies have been focusing on the manipulation of programmed cell death (PCD); namely, apoptosis and autophagy. While the strategies of anti-apoptosis engineering have been numerous studied, the researches to increase the therapeutic protein production by targeting the pathway of autophagy are limited. Autophagy is an evolutionarily conserved catabolic process that is responsible for the degradation and recycling of cytoplasmic organelles and for the removal of damaged organelles. It has been reported that upon various environmental stresses, CHO cells activate and undergo the process of autophagy. Only few studies have modified the autophagic flux in recombinant CHO cells by means of chemical treatment and investigated its effect on the specific productivity. In this study, three different phases of autophagy were arbitrarily determined by their role in autophagy; initiation/nucleation, elongation, and autophagosome fusion. Along with well-known autophagy inhibitors 3-MA and bafilomycin A1, seven chemicals from three different phases were selected to inhibit autophagy in three different recombinant CHO cell lines, which include Fc-fusion protein producing DUKX and DG44 cell lines and antibody producing DG44 cell line, and their effects on the specific productivity were analyzed. Among various autophagy inhibitors tested, only 3-MA, dorsomorphin, and sp600125 significantly increased the specific productivity of Fc-fusion protein producing DUKX CHO cell line. However, for Fc-fusion protein producing DG44 CHO cell line and antibody producing DG44 cell line, only 3-MA significantly increased the specific productivity. Taken together, only 3-MA showed positive effects on the specific productivity in all of the cell lines tested, while the positive effect of dorsomorphin and sp600125 were cell-line dependent.



**P 5.15 Improved recombinant protein production with a novel *cis*-regulatory DNA element in Chinese hamster ovary cells**

Shin-Young Kang<sup>1</sup>, Kyoung-Hwa Ryu<sup>2</sup>, Yeon-Gu Kim<sup>1,2</sup>,  
Hong-Weon Lee<sup>2</sup>, Eun Gyo Lee<sup>1,2\*</sup>

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<sup>2</sup>Biotechnology Process Engineering Center, KRIBB, Ochang, Republic of Korea

Incorporation of *cis*-regulatory DNA elements into an expression vector is one of the methods to achieve high productivity. Although Chinese hamster ovary (CHO) cells are the most widely used mammalian cell line in the production of biopharmaceuticals, there are still regulatory elements in CHO cells that are not well-known. Thus, we isolated novel *cis*-regulatory elements from a CHO cell line that enhance transgene expression. Genomic DNA fragments from CHO-K1 cells and a plasmid vector expressing GFP regulated by the CMV promoter were used to construct a genomic library, and after screening, the putative *cis*-regulatory element E77 was isolated from a cell with the highest expression level of GFP. The enhancing effect of E77 was evaluated by the generation of stable cell pools transfected with vectors that included E77 and the genes for GFP and an antibody. The protein expression levels of GFP and the antibody increased by about fifty percent and five-fold, respectively, compared to the CMV promoter alone. The sequence of E77 consisted of two fragments, and its location and orientation in the vector system were related to the regulation of gene expression. This study suggests that incorporating E77 into a vector could be useful in the production of recombinant proteins in a CHO expression system.



**P 5.16 Improved human erythropoietin expression by IRES-mediated multicistronic vector in Chinese hamster ovary cells**

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The use of internal ribosome entry site (IRES) in multicistronic vectors enables the expression of multiple genes controlled by one promoter in target cells. Multiple gene expression using a multicistronic vector has been thought to be useful to improve the production of recombinant proteins in mammalian cells. Therefore, we have constructed the multicistronic vectors expressing erythropoietin (EPO) with three kinds of different tags to evaluate the specific EPO productivity ( $q_{\text{EPO}}$ ) and their expression level by specific insertion site. The  $q_{\text{EPO}}$  has been increased by an addition of IRES linked EPO, but the rate of increase was not proportional to the gene number of EPO. To find relationships between  $q_{\text{EPO}}$  and length of transcript unit, detectable fusion tags in C-terminus of EPO gene were introduced and analyzed by using ELISA. The expression level of individual fusion EPOs was affected by its position and length of transcript unit in multicistronic system. This study demonstrated that the use of multicistronic vectors could be available to enhance the specific protein productivity, and it was not proportional to the integrated gene number due to different expression levels of individual protein driven by each IRES.





**P 5.17 Relationship between tissue plasminogen activator (tPA) productivity and cell cycle phases of CHO cells growth in chemostat culture.**

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The production of therapeutic proteins by mammalian cell cultures is a currently intensely developed area. Previous studies point to mild hypothermia (28-35°C) as a recognized strategy to increase the production of heterologous proteins in mammalian cell lines. These methodologies focus on key modular regulators of cell growth and survival during production phases. In this study, we evaluate the ability of these strategies to increase tPA productivity in chemostat culture of CHO cell lines, and the relation between specific production and the cell cycle phase when the cell is most productive. For this, we used a chemostat culture of cell line CHO TF 70R producer of ht-PA. This culture was grown at 37°C and at 33°C (previously acclimatized at this temperature). Specific growth rates ( $\mu$ ) were measured as 0.01 h<sup>-1</sup> and 0.018 h<sup>-1</sup>. We determined specific productivity of tPA and how this was associated with the phase of cell cycle through flow cytometry (Beckman Coulter FC500). The results showed a relation between the increase of specific productivity and mild hypothermia (33°C). Previous studies link the increase of production of the heterologous protein with decrease of temperature and an increase of percentage of cells in G1 phase. However, for this experiment, the percentage of cells in phase G1, was always higher than those in phase S and G2/M, for all conditions at the steady state. Moreover, we observed that the cell population in the G1 phase increases at slower rates, like was pointed out by Ti, 2005. Additionally, in cultures with the same  $\mu$  the condition of mild hypothermia does not affect the percentage of cells in the G1 phase. However, cultures grown at 0.018 h<sup>-1</sup> have higher specific productivity of tPA than cultures growth at 0.01 h<sup>-1</sup>. Finally we observed that mild hypothermia promotes the increase of productivity of tPA through a different mechanism than arrest of cell in G1 phase. This discovery can contribute to the knowledge about production of recombinant protein, specifically, regarding the characterization of the animal cell production system.



**P 5.18      Stable cell pools as alternative to transient transfection  
to boost HIV-1 antibodies production**

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NIAID/VRC, USA

Large-scale transient protein expression in mammalian HEK293 cells is usually employed to produce recombinant HIV-1 proteins and antibodies for the structural studies of HIV-1 since it provides the speed and versatility for protein production needs. However, substantial quantities of commonly produced HIV-1 envelop trimers and broadly-neutralizing antibodies (bNabs) need to be generated to facilitate crystallization, antigenicity, and immunogenicity studies of HIV-1 trimer as well as its purification; these demands of production may be challenging for transient expression platform. Mammalian cell lines which stably produce bNabs and HIV-1 envelope proteins need to be explored to facilitate demands for commonly produced HIV-1 proteins. However, it is time and labor consuming to develop the stable cell line and the whole process can take 6-12 months. High-expressing stable cell pools provide an alternative to transient transfection and can be generated in several weeks. Conventional methods to generate stable pools employ antibiotic selection with gradual increase of antibiotic concentration allowing for selection of stable transfectants without introducing severe selection conditions which can kill the stably transfected cells via acute apoptotic signaling from neighboring cells. Here we developed a stable cell pool platform for expression HIV-1 proteins in CHO cells which combined gradual antibiotic selection with the enrichment of high-producing stable cell pools by Fluorescent Activated Cell Sorting (FACS). This enabled us to generate stable CHO pools producing 144mg/L in 38days or 184mg/L in 43 days of bNab, PGT122, cloned into dual-expression pCHO1.0 vector (HEK transient expression is 20mg/L). Furthermore, semi-solid single-cell cloning technology on the best stable pool allowed selecting the single CHO-PGT122 clone with 211mg/L production titer in additional 24 days. The latter production was scalable and consistent for several passages. Hence, the stable pool protein expression platform can be utilized alongside the large-scale transient system for the production of commonly used HIV-1 antibodies and envelope proteins to facilitate studies of HIV-1 envelope en route to vaccine development.



**P 5.19 Utilizing Cell Imaging Technology to Shorten Timelines and Reduce Development Resources by Earlier Identification of High Producing Recombinant CHO Cell Lines**

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Reducing cell line development timelines and resources is a key goal in the generation of biomanufacturing cell lines. In traditional cell line development workflows hundreds or even thousands of clones are scaled up to large static or shaken culture volumes so that accurate cell counts and recombinant protein titer measurements can be obtained. Not only does the scale up of these large quantities of clones require a significant amount of time, but it is also very resource heavy, often typically requiring multiple personnel and a significant amount of laboratory space. In this study we employed the Solentim<sup>®</sup> Cell Metric<sup>™</sup> imaging technology in our cell line development workflow with the aim of reducing development timelines and resources by earlier identification of lead producing recombinant clones. A CHOZN<sup>®</sup> GS stable pool expressing a recombinant human IgG was single cell cloned by limiting dilution in 96 well plates. Clonality was verified by imaging on D0, D1, D4 and D7 post plating. The clones were allowed to expand, and once they reached approximately 75% confluency, the verified single cell clones (SCCs) were consolidated onto a new plate. Post consolidation, the clones were split into replica 96 well plates, one for continued scale up and one for a 96 well assay. 7 days post plating, the confluencies of the clones in the assay plate were analyzed via the Cell Metric<sup>™</sup> imager, and cell culture supernatants were analyzed for IgG titers via ForteBio<sup>®</sup> interferometry. Titers were normalized to confluency and the rank order of the clones was established. The same set of clones were scaled up into shake cultures and characterized for growth and productivity in both a 7 day batch assay and a 14 day fed-batch assay. The data indicates that the rank order determined in the early 96 well assay correlates well with the rank order of the clones determined by the shake assays. Incorporation of this early normalized productivity screen into our cell line development process may prove to be invaluable, saving us a considerable amount of time and resources by significantly reducing the number of clones that need to be scaled up into shake cultures for further characterization.



**P 5.20 Improvements in the Expression and Correct Formation of Bispecific Antibodies Enables the Manufacture of Novel Drug Formats in CHO Cells**

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In recent years, bispecific antibodies have constituted a growing percentage of drug portfolios, becoming a valuable class of therapeutics owing to their ability to bind two distinct targets. Dual targeting by design is intended to enhance biological efficacy, limit escape mechanisms, and if the targets are cell surface expressed, increase drug selectivity via a strong avidity effect mediated by concurrent binding to both antigens. The production of this class of non-conventional mAbs poses significant challenges due to the increased size and complexity of coding sequences of the genes, the need for simultaneous and balanced expression of multiple proteins as well as the correct folding and assembly of the final product. Here we present multiple vector engineering and screening approaches to address these challenges. As a result, stable cell lines producing multiple grams per liter of several classes of clinical candidate bispecific molecules have been generated. In addition, approaches facilitating the development of a novel monovalent bispecific IgG platform ('DuetMab') will be discussed.





### **P 5.21 Modulation of Endogenous Glutamine Synthetase (GS) in CHO Cells Improves Selection and Characteristics of Expression Hosts**

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The generation of high yielding, stable manufacturing hosts for mAbs, bispecifics and non-Ab therapeutic proteins needs to be an efficient and predictable process in the biotherapeutic drug industry. Our current manufacturing platform utilizes an in-house derived CHOK1 suspension derivative that fits our current needs. However, in an effort to improve upon our results we wanted to investigate potential causes of isolating non-expresser cell lines during the initial parts of our glutamine synthetase (GS) cell line engineering process. From our experience with clonal host cell lines we observed differences in the transfection efficiency, outgrowth numbers, and sensitivity to methionine sulfoximine (MSX) upon cell line selection following transfection. When measuring endogenous GS expression the levels varied widely between cell lines and a non-clonal host. To investigate further we studied end-product regulation of GS by glutamine, and what effect manipulation of GS expression prior to transfection and selection had on cell line outgrowth and recombinant protein expression. This knowledge is important because non-engineered CHO hosts are selected with a vector encoded GS gene and background endogenous GS expression must be sufficiently inhibited with MSX for selection to occur. In this study we demonstrate that manipulation of endogenous GS can be beneficial for cell line development and more efficient isolation of high titer expression hosts.



**P 5.22 Selexis SUREcode™: De-risking Research Cell Bank Generation with Comprehensive Genomic Analysis**

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Research Cell Banks (RCBs) generated in CHO cell lines are not necessarily cell populations with identical genomes or single integration sites, even though they arise from a single isolated cell. These mixed populations can lead to unacceptable manufacturing variability. Selexis' SUREcode™, consisting of the detailed CHO-M genomic map and proprietary bioinformatics tools, decreases manufacturing risks by ensuring transgene integrity in RCBs and by surveying for the emergence of deleterious mutations either in the transgene sequence or in genes that are important for cell survival.



**P 5.23 A community genome-scale model of Chinese hamster ovary cell metabolism identifies differences in the efficiency of resource utilization for different bioprocesses**

Hooman Hefzi<sup>1\*</sup>, Kok Siong Ang<sup>2\*</sup>, Michael Hanscho<sup>3\*</sup>, Aarash Bordbar<sup>1</sup>, Jahir Gutierrez<sup>1</sup>, Deniz Baycin Hizal<sup>4</sup>, Henry Huang<sup>5</sup>, Sarantos Kyriakopoulos<sup>2</sup>, Meiyappan Lakshmanan<sup>2</sup>, Daniel Ley<sup>6</sup>, Shangzhong Li<sup>1</sup>, Anne Mathilde Lund<sup>6</sup>, Alyaa Mahmoud<sup>7</sup>, Harish Nagarajan<sup>5</sup>, Camilla Orellana<sup>8</sup>, Giuseppe Paglia<sup>9</sup>, Lake-ee Quek<sup>8</sup>, David Ruckerbauer<sup>3</sup>, Veronica Salazar<sup>8</sup>, Alex Thomas<sup>5</sup>, Juergen Zanghellini<sup>3</sup>, Daniel Zielinski<sup>1</sup>, Michael Betenbaugh<sup>4</sup>, Mikael Andersen<sup>6</sup>, Bernhard Palsson<sup>10</sup>, Lars Nielsen<sup>8</sup>, Nicole Borth<sup>11</sup>, Dong-Yup Lee<sup>2</sup>, Nathan Lewis<sup>12</sup>

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Genome-scale models of metabolism have successfully been employed in many microbial and eukaryotic metabolic engineering efforts by guiding pathway engineering and media optimization. They have also been used to explore the genotype-phenotype relationship in mammalian cells. With the recent publication of the genomic sequence for Chinese hamster ovary (CHO) cells, these metabolic modeling tools can now be used to explore similar questions in CHO-based recombinant protein production. Here we have developed a genome-scale, manually curated, community metabolic network reconstruction of the CHO-K1 cell line. The metabolic model is capable of integrating proteomic, transcriptomic, and metabolomic data and can accurately simulate experimentally measured growth and production phenotypes. We used the model to assess the metabolic limitations on recombinant protein producing lines subject to different cell line and process modifications and found that some alterations result in specific productivities up to 20-fold lower than computational predictions of metabolically feasible production rates. The results indicate a possible secretory bottleneck and implicate engineering the secretory pathway as a lucrative target to pursue in future CHO cell line engineering.



**P 5.25    Improvement of Baculovirus Transgene Expression in Mammalian Cells by  
             Silencing of Immune Pathways**

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Baculovirus is a versatile viral vector widely used for gene expression in insect cells. Although baculovirus cannot infect and replicate in mammalian cells, it can serve as an efficient gene delivery vector to many of these cells, and properly express target genes if these genes are driven by mammalian recognizable promoters. However, previous studies have found baculoviral transgene expression is low in some of the mammalian cells, especially in human lung cancer cell A549. To discern why the transgene expression level is low in this cell and make improvements, we performed an intensive screen in A549 cells using short-hairpin-RNA (shRNA) library highly enriched in human antiviral response pathways including Toll-like receptor, RIG-I-like receptor, NOD-like receptor, and cytosolic DNA sensing pathways. We identified several cellular genes which are effective repressors for baculoviral transgene expression that do not affect cell viability. These proteins are mainly involved in RIG-I-like and Toll-like receptors pathways. Silencing of these genes enhanced baculoviral transgene expression about five-ten folds, and the cell viability was not significantly affected in some lines. By viral entry efficiency assay, we found that baculoviral entry efficiency was not affected in stable gene-knockdown cells, rather, the activity of the CMV promoter was increased. These studies suggested that transgene expression is attenuated by cellular immune response induced by baculovirus infection, and the immune suppression can be removed by knocking down of the immune-related genes. These results will make baculovirus as suitable vector for enhanced transgene expression in some mammalian cells.





**P 5.26 Effect of temperature down shift on the transcriptome and proteome responses of CHO cells producers of recombinant tPA**

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Many recombinant proteins used as biopharmaceuticals are carried out on Chinese hamster ovary (CHO) cells. However, their manufacturing and processing are expensive. Hence, temperature down shift (TDS) has been implemented to increase the productivity. Here, we observed that TDS result in a 1.6-fold increase in specific productivity (q) of tissue plasminogen activator (tPA) in CHO cells, line TF70R. To explain the mechanisms associated with qtPA increase, biphasic cultures initially grown at 37°C, and downshifted to 30°C at the exponential growth phase were performed, determining differential expression by RNA-seq and using 2D gel-based proteomics. At 24 and 48 h after TDS a total of 416 (q>0.8) and 3,472 (q>0.9) transcripts were differentially expressed. TDS did not affect the transcription of the recombinant tPA gene, but induced the overexpression of genes coding for proteins related with temperature sensing (such as *Rbm3* and *Cirp*). Different transcripts encoding for proteins involved in the secretory machinery suffer fluctuations, like genes coding for sec61, Bip and calreticulin, and those associated with the quality control in the endoplasmic reticulum (ER) like ERManI. Furthermore, different transferases localized at ER and Golgi were affected, indicating that TDS could cause changes in glycosylation patterns. On the other hand, proteomic response shows a differential expression of 61 proteins related with ER, Golgi and vesicles. Furthermore, the TDS caused variation in recombinant tPA isoelectric points comparing with the protein obtained in control cultures at 37°C. The present study contributes to fill the gap of the information related with responses in CHO cells under TDS and its relation with recombinant protein productivity increase.



**P 5.27 Production of recombinant FVIII with A1 and A2 domain mutations in order to increase secretion in human cell line HEK293**

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Hemophilia A is a bleeding disorder caused by deficient or absent production of factor VIII (FVIII). Nowadays, the only available therapeutic option is the replacement therapy using human plasma-derived protein or recombinant proteins produced in rodent cell lines. However, recombinant factor VIII (rFVIII) produced in hamster cells does not have the post-translational modifications identical to those of native human FVIII, which may lead to immunogenic reactions. Furthermore, expression of rFVIII in these systems is inefficiently because rFVIII is retained within the endoplasmic reticulum (ER) through interaction with various ER chaperones including immunoglobulin-binding protein (BiP). The main objective of this work was to produce a recombinant FVIII using a A1-domain mutation (F309S) for improving secretion and three more mutations in A2 domain (R484A /R489A /P492A) for reduced immunogenicity. For the production of FVIIIr was used a human cell line HEK293, and as a control we used a construct containing FVIIIΔB. Constructions were transferred into human HEK293 cells through repeated transductions at various multiplicities of infection (MOI) to achieve high yields of rFVIII production. rFVIII molecules were characterized *in vitro* as to production by chromogenic assay and the gene expression profile by real time PCR. Our results showed that after five transductions cycles, the cell line transduced with the construction containing the mutations (HEKMUT) was able to produce 7.5 IU/mL. On the other hand, the cell line transduced with the control vector (HEKΔB), which had the same integrated copy number, demonstrated biological activity equal to 2.5 IU/mL, in other words, three times less. Moreover, we analyzed the expression of the FVIII and BiP genes. While the FVIII levels were higher in HEKMUT cells than HEKΔB cells, the BiP mRNA levels were not altered in both cell lines, as well as by Western Blot. Besides the F309S mutation, we tested another strategy to improve FVIII secretion. Besides the mutation F309S, we hypothesized that using chemical drugs could alter FVIII secretion in cell culture. For this, we tested the drugs betaine and sodium butyrate at the modified cell lines. Surprisingly, only the HEKΔB cells had FVIII production increased, whereas the HEKMUT cells had no effect in betaine treatment and decreasing two times FVIII biological activity in sodium butyrate treatment. Finally, we evaluated the haemostatic potential of pLFVIIIΔB which was able to correct the bleeding in hemophilic A mice caused by total transection of the tail tip, whereas the control group died within 50 hours after injury. Taken together, these evidences emphasize the role of the F309S mutation increased FVIII secretion at human cell line HEK293.



**P 5.28      Viral engineering for better secretion of Hemagglutinin virus like particles in insect cells**

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The baculovirus insect cell expression system is an integral component of recombinant protein expression technologies. A variety of complex eukaryotic proteins have been successfully expressed in insect cells *Spodoptera frugiperda* (*Sf9*) at large scale, including commercialized vaccines. Recently, *Trichoplusia ni* (*T. ni*) cell lines (High5, TnAO38) have been recognized as an alternative host for their superior capacity in secreting heterologous proteins at high yields. These cell lines have been shown to be feasible to express complex glycosylated proteins, e.g. viral surface proteins and cellular membrane proteins as well as multi-subunit complexes. In particular, the successful expression of multi-subunit vaccines such as virus like particles has proven the potential of this.<sup>1</sup> To date, for the generation of many products, also at large scale, mostly *Sf9* cells are being used. Thus, little is known about process engineering of up-scaled insect cell based systems based on *T. ni* derived cell lines. The focus of our work is the characterization of the above mentioned cell lines and maximizing their yield. To this end, we conducted time course experiments that proved the superior protein expression capacity of High5 cells over *Sf9*. To overcome the widely experienced problem of low yield for many secreted glycoproteins, certain chaperones (e.g. the BIP from... and the PDI from...) were over-expressed in High5 cells in order to test their capability to increase the heterologous protein production. By precisely adjusting the multiplicity of infection (MOI) of the co-infecting viruses, expressing the target protein or the chaperones, we managed to increase the expression rates for influenza A virus hemagglutinin as well as for influenza A virus like particle yield in small scale. Our strategy will further be tested in large scale production processes and downstream processing.

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1 Krammer, F., Grabherr, R. Alternative influenza vaccines made by insect cells. Trends in molecular medicine 16, 313-320 (2010).



**P 5.29 Anti-apoptotic agents improve baculovirus cell specific yields.**

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**Introduction:** The baculovirus-insect cell system continues to be of interest to produce recombinant proteins, vaccines, vectors for gene therapy and biopesticides<sup>1</sup>. Maximizing yields is of interest to ensure this manufacturing platform is competitive against competing expression systems. Despite systems biology studies, insights into how to further increase yields with this expression system are not obvious<sup>2</sup>. In 2008 Blissard et al reported that the expression of the anti-apoptotic P35 gene from *Autographa californica* multi-capsid nucleopolyhedrovirus (AcMNPV) in Sf9, (*Spodoptera frugiperda*), cells resulted in a two fold increase in the cell specific yield of foreign protein following infection of P35 transfected Sf9 cells with rAcMNPVs<sup>3</sup>.

**Results:** In this work it is shown that transfection of the H. zea (HzAM1) cell line with the AcMNPV P35 gene results in a 1.6 fold increase in the cell specific yield of the wild type *Helicoverpa armigera* nucleopolyhedrovirus, (HearNPV), virus following infection of P35 transformed H.zea cells with HearNPV.

**Conclusions:** The Baculovirus expression system can express relatively simple (non- glycosylated) intracellular proteins at high levels if expressed under control of the polyhedral promoter. In our experience  $\beta$ -Galactosidase is produced at ~0.5 g/L in  $\beta$ -Gal-rAcMNPV infected Sf9 cells (SF900III medium, optimal batch culture), ~0.9 g/L in  $\beta$ -Gal-rAcMNPV infected Hi5, (BTI-TN-5B1-4), cells (Express Five medium, optimal batch culture), and polyhedral protein is expressed at ~0.5 g/L in wild type HearNPV infected H.zea cells (SF900III medium, optimal batch culture). While Hi5 cells have a superior cell specific productivity, the expression of virus derived anti-apoptotic agents in Sf9 and H.zea cells may allow these cell lines to match Hi5 cells in terms of productivity per unit biomass and subsequently per L of culture medium. Fed batch processes may improve productivities of all systems 2-4 fold.

<sup>1</sup>Felberbaum RS 2015, Biotechnology journal 10: 702-714.

<sup>2</sup>Chen YR, et al. 2014, Journal of Virology 88: 13781-13797.

<sup>3</sup>Blissard et al. 2008, Patent Number: US 7405038 B1





### **P 5.30 Enhancing Production in CHO Cells Using RNAi Technology**

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A key step in the process development of biologics is finding a high producing cell line. At the genomic level, one way that may increase the expression of the gene of interest is to silence host genes that are limiting the expression of the transgene. To find these potential host genes, a library of approximately 3,000 mouse/rat siRNA was evaluated to see which siRNAs significantly increase or decrease antibody titers of the CHO production cell lines. The screening identified 11 siRNAs that increased the antibody titers for multiple cell lines. In order to assess the long term silencing effects on antibody titers for one of these genes, we designed shRNAs to simulate the effect of siRNA. We found that knocking down the expression of this gene increased the specific productivity of the mAb production cell line; however the volumetric productivity did not increase, due to slower growth of the knocked down cell lines. Nonetheless, these results showed the proof of concept that we can increase cell line productivity by knocking down genes or pathways that may restrict expression or secretion of the product. And with some further development and optimization, we could potentially increase the volumetric productivity by increasing the growth of the engineered cells.



**P 5.31 A genome-scale reconstruction of the secretory pathway of CHO cells**Jahir M. Gutierrez<sup>1</sup>, Amir Feizi<sup>2</sup> and Nathan E. Lewis<sup>3</sup><sup>1</sup> Department of Bioengineering, University of California, San Diego, U.S.A, <sup>2</sup> Novo Nordisk Foundation Center for Biosustainability, Chalmers University of Technology, Gothenburg, Sweden<sup>3</sup> Department of Pediatrics, University of California, San Diego

The production of effective, functional monoclonal antibodies and other relevant biotherapeutics for human health requires the utilization of cell hosts that can process the necessary post-translational modifications (PTMs) to these polypeptides. Chinese hamster ovary (CHO) cells have emerged as the predominant cell factories in the biopharmaceutical industry due to their excellent secretory capabilities. Despite the advances in the field of CHO bioprocessing, there are still many challenges that need to be addressed as the demand for biotherapeutics continues to increase. Thus it has become very clear that a systems-level mechanistic understanding of the secretory pathway of CHO cells is necessary. Here we present the first genome-scale reconstruction of the protein secretion pathway in CHO cells along with its predictive capabilities. This reconstruction incorporates the main modules associated with protein secretion and accounts for the metabolic cost of each process. These modules include protein folding and basic glycosylation. By coupling our model with a CHO metabolic network, we constructed and analyzed six different CHO *in silico* models that secrete six different recombinant proteins that are relevant to the pharmaceutical industry. The metabolic cost for producing and folding each target protein is obtained from approximate stoichiometric reactions that are specific for each secreted product. We show our results obtained from Flux Balance Analysis (FBA) and Phenotypic Phase Planes (PhPP) that allowed us to identify the main pathways and reactions that serve as fundamental constraints for optimal secretion at different growth rates. These results suggest potential improvements that could be made to growth media as to maximize the efficiency of CHO cells for producing specific recombinant proteins with characteristic PTMs.



**P 5.315 A novel CHO cell expression platform (Apollo™) developed to deliver high monoclonal antibody titer and acceptable product quality in fed-batch cultures**

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Chinese Hamster Ovary (CHO) cells are used to manufacture a variety of recombinant therapeutic proteins. Performance characteristics of CHO cells including culture growth, volumetric productivity, and product quality, have rapidly improved through innovation of production media, process optimization and novel process technologies. Fujifilm introduces a novel CHO expression platform, Apollo™ that includes a newly selected CHO DG44-derived host cell line, optimized DHFR expression vector, chemically-defined (CD) and protein-free (PF) platform basal medium and feed, and cell culture bioreactor process. Using the Apollo™ CHO expression platform, we are able to achieve monoclonal antibody titers up to 5g/L in a typical 14 day fed-batch production culture process. Monoclonal antibody product quality attributes related to glycan structures, aggregates, and charge variants consistently meet standard criteria. Fujifilm developed a CHO DG44-derived host cell line with improved growth characteristics and expression capability and performs cell line development using state-of-the-art screening technologies. Techniques employed include use of a fed-batch shaken multi-well plate system to enable shortened development timelines allowing better decisions on which cell lines to move forward for clone-selection confirmation in an ambr15™ bioreactor system. To complement the CLD process, we have strategically developed platform basal medium and feed, and combine these with high-throughput, DOE-based approach to process optimization. Basal media and feeds were created using DOE-based methodology, in a sequential development strategy that was designed for continuous optimization based on Multi-Variant Analysis (MVA), and by leveraging media formulation knowledge and Spent Media Analysis (SMA) on key biochemical nutrients (i.e. amino acids, vitamins). Fujifilm's Apollo™ platform media and feed routinely support CHO peak densities of 30-40x10<sup>6</sup> viable cells/mL. Presented are examples of CHO cell culture growth performance, protein expression, and product quality using Fujifilm's Apollo™ platform CHO expression system at process scales ranging from shake flasks through bench-scale bioreactors.



**P 5.32      A New Expression Platform for Highly Glycosylated Proteins**

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Due to their clinical importance, the development of therapeutic proteins has accelerated immensely over the past years. However, the expression of highly glycosylated recombinant therapeutic proteins, like for example blood coagulation factors or serum proteins has remained a challenging task. Human cell lines, like HEK293 or our amniocyte-derived CAP cell line appear to be efficient in producing and secreting these proteins, however glycosylation can at times be incomplete. While we have found that CAP cells generate a more authentic human glycosylation pattern than HEK293, we have for some proteins, e.g. human C1 esterase inhibitor (hC1-Inh), also detected incomplete sialylation, resulting in reduced serum half-life of the recombinant protein. For this reason we have developed an array of novel CAP cell lines (CAP-GO cells) that confer optimal glycosylation to highly complex glycoproteins with several N- and O-linked glycans. Recombinant human alpha1-antitrypsin (rhAAT), human placental alkaline phosphatase (hPLAP) and rhC1-Inh expressed in different CAP-GO cell lines all show complete lack of terminal galactose, which is considered to be responsible for rapid clearance of glycoproteins in the liver. Consequently, the serum half-life of the produced protein is significantly longer than that of its counterpart generated on a conventional expression platform and indistinguishable from or even improved in comparison to the natural proteins. In addition, the glycans of recombinant proteins expressed in CAP-GO cell lines are more homogenous than the glycans found on conventional recombinant glycoproteins. Interestingly, the over-expression of just one particular sialyltransferase is not always sufficient to improve the pharmacokinetic profile, but different CAP-GO cell lines are required for optimal glycosylation of different recombinant proteins. In combination with our optimized approach for clone selection, we can now very quickly and efficiently generate cell lines, expressing complex glycosylated proteins with homogenous, fully sialylated glycans at high levels and have thus generated an optimized expression platform for this class of proteins.





### 5.33 Baculovirus based engineering approaches for minimizing fucosylation in insect cell

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The baculovirus/insect cell system has proven to be a very powerful tool for the expression of several complex proteins, especially multi-subunit vaccines. Nevertheless, these proteins sometimes suffer from reduced biological activity and unwanted side effects. Several studies have demonstrated that glycosylation can greatly influence the structure, function, half-life, antigenicity and immunogenicity of various viral glycoproteins. Yet, the glycosylation pattern of insect cell-derived products is not favorable for many applications. We therefore, investigated flexible, baculovirus based engineering approaches for improving immunogenicity of insect cell expressed Influenza A Virus-Like particles and at the same time for reducing their allergenic potential.

The presence of specific carbohydrate structures, mainly core  $\alpha$ 1,3-linked fucose, bears the risk of causing immediate hypersensitivity reactions in patients with allergy. To diminish the fucose *de novo* synthesis pathway of different lepidopteran insect cell lines, we integrated the *Pseudomonas aeruginosa* GDP-6-deoxy-d-lyxo-4-hexulose reductase gene in a SweetBac baculovirus backbone. This setup gives the possibility to produce VLPs almost free from fucose. A binding assay of non-fucosylated VLPs with sera from patient's with allergy showed a 10-fold decrease in IgE binding levels compared to wildtype variants. In another approach we adapted the CRISPR/Cas9 system for a baculovirus based knockdown of the *Spodoptera frugiperda* fucosyltransferase FUT8 by integrating Cas9 and specific guide RNAs in the backbone of a SweetBac virus.



**P 5.34 Effect of media components on the increase in the incorporation of the immunogenic N-Glycolylneuraminic acid.**

Carina Villacrés Barragan<sup>1</sup>, **Hélène Perreault<sup>1</sup>**, Michael Butler<sup>1</sup>

<sup>1</sup>University of Manitoba

Background and novelty

Glycosylation of recombinant proteins is known to play a critical role in the clinical properties of the final glycoprotein such as stability, solubility, secretion, *in vivo* clearance and immunogenicity. Thus, the post-translational machinery present in mammalian cells has become valuable for the production of recombinant therapeutics. However, murine-produced glycoproteins can contain non-human glycan epitopes that can result in immunogenic reactions (e.g. terminal Gal 1-3Gal and N-glycolylneuraminic acid) if used *in vivo* as a therapeutic agents. The objective of this study was to determine which media components can enhance the incorporation of N-glycolylneuraminic acid into glycoproteins.

Experimental approach:

For this purpose, NS0 cells were cultured in media containing different combinations and concentrations of manganese chloride (MnCl<sub>2</sub>), uridine and galactose. Cell growth, viability and substrate consumption were monitored daily. The glycosylation profile was analyzed using hydrophilic interaction liquid chromatography (HILIC) and N-glycolylneuraminic acid (NGNA) and N-acetylneuraminic acid (NANA) levels were determined by reverse-phase (RP) chromatography.

Results and discussion:

Using HILIC we showed that the addition of MnCl<sub>2</sub> (7μM) increased the galactosylation index (GI) of IgG produced by NS0 cells from 0.58 to 0.64. Further increases were observed when MnCl<sub>2</sub> was combined with uridine (GI=0.73) or galactose (GI=0.78). In addition, the percentage of sialylation was incremented in all media supplements. The highest increase in sialylation (34%) was observed in media containing both MnCl<sub>2</sub> and galactose. RP chromatography allowed the quantification of the proportion of the sialic acids, NGNA and NANA under the described conditions. We also detected an inverse correlation between the galactosylation index and the fucosylation index when MnCl<sub>2</sub> plus galactose were incorporated into the media. Thus, the fucosylation index decreased from 0.85 to 0.69. Cell growth and viability were not affected by MnCl<sub>2</sub> alone or when combined with uridine or galactose. However, there was a slight decrease in growth observed in cultures containing a combination of MnCl<sub>2</sub>, uridine and galactose along with a decrease in cell viability.



**P 5.36 Effect of Redox Potential on Glycosylation: Elucidating The Intracellular Redox Status of Glycosylation Machinery**

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Dissolved oxygen, redox status, pH and ammonia are suggested to be factors influencing the intracellular machinery; the endoplasmic reticulum (ER) and golgi where post-translational modifications such as glycosylation occur. Studies have focused on the effect of these biochemical parameters on monoclonal antibody (MAb) production and glycosylation. However, the interplay between the factors and the cellular machinery during MAb production and its influence on glycan processing pathway has not been explored extensively. Previous work in our group has shown a variation in glycosylation profiles due to change in culture redox potential (CRP) with a decrease in glycosylation index by ~50%. To this end, the study is designed to investigate the intracellular redox state using redox sensitive green fluorescent proteins (roGFPs) and its role in MAb production and glycosylation, by integrating roGFPs into the CHO cell line producing EG2-hFc chimeric monoclonal antibody variants. The roGFPs elicit a ratiometric response to changes in redox potential when targeted to various cellular compartments and can be used to study real-time changes in living cells using fluorescence microscopy. Results obtained from these studies will be compared to CRP (external redox potential) obtained from earlier studies to co-relate redox state in cellular compartments to the effect on glycosylation profile in production of MAbs.



**P 5.37    A Markov chain model for N-linked protein glycosylation – towards low-parameter tool for model-driven glycoengineering**

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Glycosylation is a critical quality attribute of most recombinant biotherapeutics. Consequently, drug development requires careful control of glycoforms to meet bioactivity and FDA requirements. However, glycoengineering can be extraordinarily difficult given the complex reaction networks underlying glycosylation and the vast number of different glycans that can be synthesized in a host cell. Computational modeling offers an intriguing option to rationally guide glycoengineering, but the high parametric demands of current modelling approaches pose challenges to their application. Here we present a novel low-parameter approach to describe glycosylation using flux balance and Markov chain modeling. The model recapitulates the biological complexity of glycosylation, but does not require user-provided kinetic information. We use this method to predict and experimentally validate glycoprofiles on EPO, IgG as well as the endogenous secretome following glycosyltransferase knock-out in two different Chinese hamster ovary (CHO) cell lines. Thus, this approach offers a flexible and user-friendly platform that can serve as a basis for powerful computational engineering efforts in cell factories for biopharmaceutical production.





**P 5.38 Cutting-Edge Transient Gene Expression: From DNA Prep via Media, Reagent and Cell Line Development to Holistic Process Optimization**

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InVivo BioTech Services has implemented a novel technology for efficient transient transfection and expression in HEK cells during the last years. In brief, InVivo BioTech Services developed in cooperation with emp Biotech, Germany and Xell AG, Germany a transfection reagent and culture medium that can be used for transfection and production. The establishment of a TGE optimized HEK cell line and a method for large scale plasmid preparation helped us to install a production platform for HTS approaches and large scale transfection for the production of gram quantities IgG within days. Transfections were performed according to standard protocols described in the literature. Briefly,  $5 \times 10^6$  cells/mL were transfected with 2 pg DNA/cell and INVect or 25 kDa PEI. Cultivations were carried out using shake flasks under standard conditions. Transfection efficiency was determined via flow cytometry and yields were quantified by SEAP assay or protein-A affinity chromatography. Bulk-sorts were performed using a Bio-Rad S3 cell sorter and DoE was used for process optimization. Several *E.coli* strains and media were screened for high productivity, high quality and flexibility for DNA preparation in comparison to commercial kits. Afterwards a purification process was implemented using a weak anion exchanger. Up-scaling this process to 50 L culture volume results in approx. 250 mg purified plasmid DNA. Starting from a simple basal medium we were able to generate a novel medium, which supports high titer transient gene expression. Improvements were achieved by stepwise screening and optimization of media ingredients due to higher transfection efficiency and productivity. To generate an optimized host cell line for TGE processes we utilized FACS-assisted evolution, which results in a threefold increase in IgG productivity. In combination with the developed transfection reagent, it was possible to generate a high yielding “pseudo” perfusion TGE production process enabling space time yields exceeding 200 mg IgG/L x day. Because of the low toxicity of INVect, transfection and cultivation at extreme-high densities (up to  $8 \times 10^7$  cells/ml) was possible. Furthermore we work on a simplified procedure of the production process using concentrated feed supplement. First screenings in which VCD, amount of DNA, feed volume and transfection enhancer were varied showed promising results with titers up to 680 mg/L.



**P 5.39 Development and Optimization of CHOgro™ Transient Expression Technologies for High Titer Antibody Production in Suspension CHO cells**

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During early stage drug development, obtaining relevant candidate proteins quickly through transient transfection can accelerate drug discovery. High titers can often be obtained from Human Embryonic Kidney (HEK) 293 derived cell types; however, the use of different host cells between early stage transient and later stable protein production is a concern and can lead to false-positive candidates being advanced. Chinese hamster ovary (CHO) cells are a desirable target cell type, but in the past have been hampered by low transient transfection expression levels. To address this need, we have created a transient protein expression system that is robust and simple and provides critical media attributes such as high density cell growth, quick adaptation and minimization of cell clumping post-transfection. The CHOgro Expression System was developed through systematic optimization of transfection protocol parameters including: cell density, transfection reagent, media formulation and culture temperature leading to a commercially accessible high titer CHO transient transfection platform. Six different representative antibody constructs were tested using the CHOgro Expression System, and antibody titers increased 2-10 fold over existing technologies with higher amounts of antibody secreted on a per cell basis. Notably, even CHO cells maintained in other commercially available media formulations (e.g. FreeStyle™ CHO Expression Medium) can be exchanged to the CHOgro Expression Medium 24 hours prior to transfection and yield multi-fold increases in transient expression levels at 3-6 days. High protein titers can be realized in suspension CHO cells through high density transient transfection using the CHOgro Expression System.



**P 5.40 Expression of Human Neurokinin-1 Receptor in CHO Cells to Evaluate Involvement with Vietnamese Medicinal Plant Extracts**

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Van Hong Thi Nguyen<sup>2</sup>, Lan Thuong Thi Vo <sup>2</sup>, Huyen Thanh Pham <sup>3</sup> & Kenneth Lundstrom <sup>4\*</sup>

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**Abstract**

More than 4000 Vietnamese plant species have been used for centuries in traditional medicine without much knowledge of their pharmacological mechanism and mode of action. We have prepared extracts and isolated compounds from 10 Vietnamese medicinal plants, which were subjected to studies on their effect of the binding activity of the human neurokinin-1 receptor (hNK1R) expressed from Semliki Forest virus (SFV) vectors in CHO cells. Extracts from *Piper nigrum*, *Stephania cambodica* and *Styphnolobium japonicum* were found to exert inhibition on agonist-induced hNK1R activity. Secondary assays and HPLC analysis of lead compounds addressed a possible association between pharmacological responses and these chemical compounds. Strong inhibition of hNK1R was observed for extracts revealing the highest inhibitory potency for rotundine (tetrahydropalmatine, THP) in *S. cambodica* extracts with IC<sub>50</sub> of 0.88 µM, followed by piperine and capsaicin in *P. nigrum* with IC<sub>50</sub> values of approximately 2 µM, whereas rutin in *S. japonicum* failed to inhibit hNK1R up to 100 µM. Individual compounds in the plant extracts will be subjected to further characterization and additional CNS receptors expressed from SFV vectors may be analyzed for interaction with plant extracts.



**P 5.41 Development of a kit for the rapid transient expression of recombinant proteins based on HEK-293 cells cryopreserved at high cell density.**

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The method of transient recombinant protein expression in mammalian cell lines has advanced from the micro-scale to bioprocess-scale over the past decade. The ability to produce significant quantities of protein to support applications including immunizations, structural studies and biological assays is based in part on the ease with which HEK-293 cells are transfected using polycation based reagents. The development of a kit that includes standardized reagents including cells frozen at high cell density offers numerous advantages including the ability to limit variability in repeat experiments and experiments performed at varying sites. We have developed a method to cryopreserve HEK-293 cells at a density of  $1 \times 10^8$  cells per mL and have combined the cells with media, buffers and a transfection reagent optimized for expression. Transfection of the cells may be performed in as little as 4 hours post-thaw and the serum-free cultures may be incubated in suspension or static flasks. The kit, Simple Screen™, has been successfully used to produce viral glycoproteins, virus-like particles and recombinant immunoglobulins at levels from 1 to 20 mg/L. Each kit includes a sufficient quantity of cells and reagents to provide up to a liter of expression volume in 100 mL increments. Data related to the development and performance of the kit will be presented.





**P 5.42 Expression Assessment Assays to Define Intracellular Bottlenecks in Therapeutic Molecule Production and Secretion in a 293-6E Transient Platform**

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Manufacturability plays a critical role in selecting quality protein therapeutic candidate molecules to move forward to process development. The earlier we can understand potential liabilities of antibodies or other large molecule candidates, the sooner we can work to address these issues, saving time to the clinic. One aspect our group's work is focused on is defining and understanding intracellular bottlenecks that lead to low expression, and finding work arounds to alleviate those bottlenecks. Low expression can signal issues in folding, instability, or other potential problems that may impact manufacturability down the line. In the past, we relied primarily on secreted protein titers to assess expression problems, since addressing such issues at a cellular level was low throughput and labor intensive. To this end, we are developing new assays to identify expression bottlenecks in high-throughput quantitative ways, and using this data to better understand potential liabilities that may impact manufacturability. Using the Cell Insight, we have developed novel assays which give rapid read outs for multiple aspects of cell culture performance during protein expression. These include a High Content screening plate-based assay for intracellular protein aggregates, known as Russell Bodies, which have been associated in antibodies with poor folding and lower stability; and high throughput counting methods which allow us to determine if cell growth is inhibited in certain molecule subsets. We have also developed ways to monitor UPR in our cultures, and to study the impact of gene dosing on final titer and quality. These new tools will allow us to screen more therapeutic candidates for expression issues up front and maximize the efficiency of our work flow.



**P 5.43 The Speed and Flexibility of Scalable Transient Protein Production: Meeting the Demands of Early Drug Discovery Efforts**

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The establishment of a robust and reproducible transient protein production facility provides critical support to drug discovery efforts.

The genesis of our scalable transient protein production efforts will be briefly reviewed. The direct impact to our overall drug discovery efforts will be discussed through case studies highlighting the variety of both applications (expression analysis, clonal selection, reagent production, etc.) and scale (96-well plate to WAVE bioreactor) that our system provides. In addition, the on-going efforts to further optimize the system to achieve even greater titers and increased throughput will be presented.



**P 5.44    Incorporating High Throughput Robotic Operation in Mammalian Tissue  
Culturing  
from Cell Line Development to End Assays**

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Agnes Hamburger<sup>3</sup>, Jennitte Stevens<sup>1</sup>

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Technologies  
Amgen, Thousand Oaks, CA, USA.

The mammalian expression groups in the Biologics Department are tasked to generate critical cell lines and proteins to enable development of therapeutic candidates. We rely on several optimized expression platforms to deliver these molecules. In the last several years, we have seen increases in number of challenging targets for discovery validation and have expanded engineering and screening efforts for optimized therapeutic candidates. To accommodate this increased demand yet retain flexibility for our different expression platforms, we developed a customized liquid handling workstation to automate much of our cell culture work. This robotic liquid handling platform accommodates workflows required for tool protein and cell line production generation for early discovery program to early therapeutic candidate screening and assessment for hand off to Process Development. This platform is uniquely equipped with a 96W head and multi-channel arms capable of picking up 300 µl, 1 ml and 5 ml tips. The robotic platform also controls a capper and de-capper that expedites higher throughput cell freezing. With this system, we have automated the development of up to 100 stable CHO cell lines from transfection, production and freezing. We have also utilized the platform for cherry picking single cell clones, and 96 well replica plating for parallel assay set up. For transient expression, the robotic platform enables 100s of parallel productions at scales from as small as 96 wells to 35 ml spin tubes with pipeline cell dispensing. Work lists also facilitate complex transfection set up and split calculations, minimizing human error and labor. This poster will describe how we have tailored automated processes for both flexibility and increased throughput to enable parallel approaches to move projects forward.



**P 5.45      Novel Small Scale TFF Cell Retention Device for Perfusion Culture of a Recombinant Mammalian Cell Line**

Jorge Padilla-Zamudio, Patrick McInnis, Mikhail Kozlov, Sarah Reynolds, Joe Parrella, Lee Madrid, Joe Orlando, Doug Rank.

EMD Millipore Corporation, Bedford MA

Biotechnology companies developing new therapeutics are facing the challenge of limited manufacturing capacity. A solution to this issue is required to increase yields without increasing the footprint. Perfusion is now gaining interest due to its ability to prolong high cell densities and increase overall yields; therefore it shows an advantage over batch and fed-batch processes. In the past perfusion processes were typically used for the generation of unstable proteins in cell culture systems due to the short residence time of the product in the bioreactor.

The critical components of a perfusion system are the bioreactor controller, cell culture vessel, and cell retention device. The cell retention device or system is often used to define the type of perfusion system with gravity-based and filtration devices being the most common.

Here we demonstrate the application of a small-scale tangential flow microfiltration device (prototype small scale ProstaK™) as a cell retention device in a perfusion test platform. We show that this device is a viable option for process development and seed bioreactor applications.

The performance of the small scale ProstaK™ device was evaluated using a recombinant CHO cell line and CellVento™ media. Various vessel volumes per day (VVD) media changes were evaluated. Performance was determined based on the measurement of viable cell density, culture viability, fouling profile, and titer. Cell density, stabilized by cell bleeds, was maintained daily at  $30\text{--}40 \times 10^6$  cells/mL and cultured for a period of 30 days. The perfusion rate was adjusted to maintain the target cell density. In the absence of cell bleeds, we obtained cell densities over  $70 \times 10^6$  cells/mL and thus demonstrated the use of this device for seed bioreactor applications.

Currently, ProstaK™ devices are used for production scale perfusion processes. Here, we demonstrate that the small scale ProstaK™ is a valuable tool for process development as well as seed bioreactor applications.





**P 5.46 Clones to Clinic- How do we get to the trials faster?**

Swati Dalvi, Christina Alves, Alan Gilbert, Scott Estes  
Cell Culture Development, Biogen Idec

The traditional process of getting a therapeutic molecule from discovery to the clinic and getting the result of the toxicology (tox) studies takes about 24 months after candidate selection. Any strategy to speed up this process will shorten the timelines to IND filings. We developed a 'fast- to tox' strategy that will generate drug substance for toxicological studies sooner in the drug development process, getting a quicker read out on the suitability of therapeutic molecules for further studies.

Some of the key factors in producing drug substance for toxicological and clinical studies are generation of enough material to supply studies and assurance that the material is representative of the final commercial drug substance with the desired product quality attributes of the molecule. To that end we assessed the potential to generate material for these studies using a pool of the top clones, which allows the tox material generation to take place in parallel with the lead clone selection process.

For these experiments we used a glycosylated monoclonal antibody as the test molecule. We ran our platform process in bench scale bioreactors using the top producing clones, a pool of the top clones and the uncloned pools from which the clones were derived. We monitored growth, titer, productivity and product quality of the various conditions. Product quality attributes that were assessed included charge variant, impurity profile, N glycan occupancy, as well as binding affinity. We found that the clones, uncloned pools and pool of clones follow similar trends in terms of growth and PQ characteristics over the course of the bioreactor run suggesting that it may be possible to generate tox material from either an uncloned pool or pool of the top clones. Subsequent studies using additional therapeutic molecules are being pursued to confirm that this strategy is applicable to a wide variety of proteins.



**P 5.47      Flocculation of CHO cells for separation of recombinant glycoproteins:  
Effect on glycosylation profiles**

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Centrifugation of mammalian cells is the operation most commonly used as the first step in the recovery of recombinant glycoproteins. However, large-scale centrifugation equipment is expensive and undesirable cell damage can occur under this operation. Accordingly, in this work the use of flocculants was studied as an alternative to centrifugation for the initial clarification of two different model recombinant glycoproteins, erythropoietin and a monoclonal antibody, from Chinese Hamster Ovary (CHO) cells. Ten different polymers, including poly-L-amino acids, polyacrylamide co-polymers and polyamides, were evaluated at concentrations between 60 to 100 ppm. The effect on cell viability, sedimentation rate and clarification efficiency was evaluated. The best clarification resulted when using polyethylenimine, whose effect on the glycosylation pattern (quality) of recovered glycoprotein was studied. Whereas no effect on the quality and interaction (as determined by dynamic light scattering) of the recovered recombinant monoclonal antibody was observed, polyethylenimine had a negative effect on the glycosylation pattern of recombinant erythropoietin. In particular, the flocculant removed high sialic acid glycoforms, and thus, affected the quality of the final product. The results that will be presented will show the cases and conditions where flocculants can successfully replace centrifugation as the first clarification step in production of recombinant glycoproteins and will also show the cases and conditions where flocculation is undesirable. To our knowledge, this is the first report of how flocculants can affect the glycosylation profiles of recovered recombinant glycoproteins.



**P5.48      A Systematic Approach to Engineering Antibody Expression**Kate Caves<sup>1</sup>, James Love<sup>2</sup>, Jeremy Minshull<sup>1</sup>, Mark Welch<sup>1</sup><sup>1</sup>DNA2.0, Menlo Park, CA 94025 <sup>2</sup>Albert Einstein College of Medicine, Bronx, NY 10461

We describe a systematic engineering approach that combined machine learning methods with gene synthesis to explore vector element and codon optimization determinants of protein/antibody expression. Expression elements explored include secretion signal sequences, transposases, viral amplifiers, and RNA export signals in addition to novel combinations of classical enhancer, promoter, intron, polyadenylation signal elements used in existing mammalian expression backbones. Combinations of vector components were designed so that elements are varied systematically and independently. This Design-of-Experiment approach allowed us to sample a large sequence-space without exhaustive testing. We then used advanced machine learning algorithms to assess the contribution of each element to vector performance, allowing us to identify high performing combinations of vector elements for any protein and cell-line. We found that protein expression in mammalian cells is sensitive to the order, spacing and orientation of vector elements. Optimal vectors varied depending on the target antibody; and different heavy light chain ratio preferences could be accommodated by selecting from a custom panel of IRES (Internal Ribosome Entry Site) sequences. Furthermore, we identified novel transposases for fast, efficient, stable expression for generation of stable pools (gram/L scale in 2 weeks) and stable lines (in 6 weeks).



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