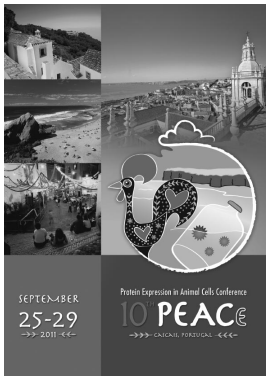


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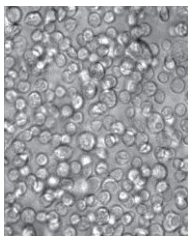

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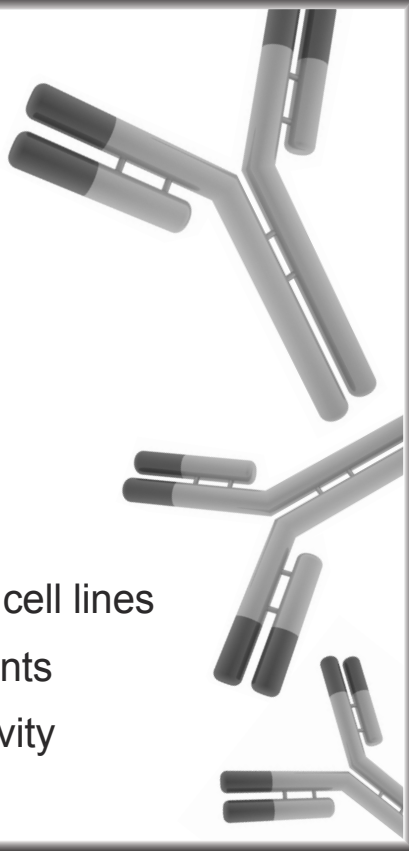

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## Message from the Chair - PEACe

Dear Participants

On behalf of the Organizing Committee it is my great pleasure to welcome you to the 10th PEACe Conference organized by the Protein Expression International Society (PEIS). It is the intention of the PEIS to promote conferences that allow the presentation and discussion of research in animal and insect cell technology, which includes the production of recombinant proteins, monoclonal antibodies and viral vaccines.

The present conference is the 10th of a series, which started in 1992. The first meeting in Interlaken, Switzerland specialized on Baculovirus and insect cell culture technologies. Since 1995, meetings have been held every second year and the topics have been expanded to include a wider scientific interest. In the 3rd conference organized in Jersey, Channel Islands the topics included gene delivery systems for recombinant protein expression. By the 6th meeting in Mont Tremblant, Canada the organizing committee was expanded significantly, additional topics introduced and the present meeting title "Protein Expression in Animal Cells" (PEACe) was created. The planning of future meetings was put on a firm footing following the Crete meeting by incorporating PEIS as a non-profit organization. For this and other aspects of meeting planning we have had the invaluable help of Bruno Bégin through his company, Hospitalité Québec. We are pleased to acknowledge and thank Bruno for his untiring efforts in organizing the conferences.

Following the tradition of past conferences in the series the location, scientific program and social events have been designed to enhance scientific discussion and interaction amongst conference participants. Past attendees have always commented favorably on both the science and the ease of meeting new colleagues. To celebrate the 10th PEACe the Organizing Committee decided to start the meeting with a special one day Symposium on Baculovirus Expression Technology.

The organizing committee would like to thank you for participating in the Conference and wishes you a pleasant and productive stay in Cascais.

Kenneth Lundstrom

## Message from the Chair - Baculovirus Symposium

Welcome to the Baculovirus Symposium. Building on the original demonstration from Max Summer's and Lois Miller's laboratories that baculovirus vectors could be used for recombinant protein expression in insect cells, Just Vlak, Jürgen Schlaeger and Alain Bernard organized "The Baculovirus and Recombinant Protein Production Workshop" in Interlaken, Switzerland in 1992. This was followed in 1995 by the "Baculovirus and Insect Cell Gene Expression Conference" in North Carolina, USA organized by Laurie Overton.

Since these conferences, significant advances have been made in baculovirus technology. Clinical biotherapeutics have been approved in the form of GlaxoSmithKline's Cervarix vaccine and Dendreon's Provenge cancer treatment. Modified baculoviruses have been developed that can be used to effectively deliver genes into a wide variety of mammalian cell types. The viruses have been used to produce adeno-associated virus, hepatitis B virus and lentiviral vectors and are showing promise as potential vaccine vectors. Amazingly, a PubMed search using the term baculovirus yields over 11,000 publications, highlighting the utility of this powerful technology.

The organizing committee thanks you for your participation in the Symposium. Enjoy the science, make new friends and have a great stay in Cascais.

Tom Kost

## Organizing Committee PEACe 2011

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Canada

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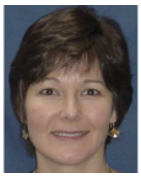

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# PROGRAM 10TH PEACE

**SUNDAY, SEPTEMBER 25 2011**

## Baculovirus Symposium

**07:00** **Registration**

**08:00 - 08:15** **Tom Kost** **GlaxoSmithKline , North Carolina,** **USA**
*Opening Remarks*

**08:15 -09:00** **Loy Volkman** **University of California, Berkeley** **USA**
*BAC.1 Baculovirus nucleocapsid transport to the nucleus and beyond*

**09:00 - 09:30** **Gary Blissard** **Cornell University, Ithaca, New York** **USA**
*BAC.2 Baculovirus Entry Mediated by Envelope Protein GP64*

**09:30 - 10:00** **Don Jarvis** **University of Wyoming, Laramie,** **USA**
*BAC.3 Insect Protein Glycosylation Pathways*

**10:00 - 10:30** **Break**

**10:30- 10:50** **Gorben Pijlman** **Wageningen University, Wageningen** **The Netherlands**
*BAC.4 Salmonid alphavirus envelope glycoprotein processing determines virus-like particle production in insect cells*

**10:50 - 11:10** **Sue Kadwell** **GlaxoSmithKline, North Carolina** **USA**
*BAC.5 Use of bacmam for recombinant protein production*

**11:10 - 11:30** **Chris Kemp** **KempBio, Gaithersburg, Maryland** **USA**
*BAC.6 Concentration and diafiltration of baculovirus and bacmam particles*

**11:30 - 12:00** **Linda King** **Oxford Brookes University, Oxford** **UK**
*BAC.7 Optimising gene expression and protein production in insect cells using baculovirus vectors*

**12:00 -13:30** **Lunch**

**13:30 - 14:00** **Kari Airenne** **Virtanen Institute, University of Eastern Finland** **Finland**
*BAC.8 Baculovirus-mediated gene delivery into mammalian cells and tissues*

**14:00 - 14:30** **Amine Kamen** **Biotechnology Research Institute, Montreal** **Canada**
*BAC.9 High yield production of viral vectors and subunit vaccines using the baculovirus expression system*

**14:30 - 15:00** **Break**

**15:00 - 15:30** **Paula Alves** **IBET** **Portugal**
*BAC.10 Systems biotechnology of baculovirus-producing insect cells*

**15:30 - 16:00** **Jimmy Kwang** **National University of Singapore** **Singapore**
*BAC.11 Baculovirus surface displayed hemagglutinin as a mucosal vaccine vector against H5N1 in mice*

**16:00 - 16:30** **Amine Kamen** **Biotechnology Research Institute, Montreal** **Canada**
*BAC.12 Baculovirus Reference Material Initiative*

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| **15:00 - 18:00** | **Registration to the PEACe conference** |

**18:00 - 19:00** **Welcome Reception**

**19:00 - 19:15** **Welcome - Kenneth Lundstrom Pan Therapeutics** **Switzerland**

**19:15 - 20:00** **Fernando Bianchi-de-Aguiar Intl. Org. of the Vineyard and Wine** **Portugal**
*Welcome presentation on regional wines*

## MONDAY, SEPTEMBER 26, 2011

**Session 1** **Vector Design - Chairs: Tom Kost & Kenneth Lundstrom**

**09:00 - 09:30** **Niall Barron** **Dublin City University** **Ireland**
*T.1.1 Engineering CHO cell growth by stable expression of miRNA expression*

**09:30 - 10:00** **Martin Schleef** **Plasmid Factory** **Germany**
*T.1.2 Minicircle vector design - new tools for non-viral gene transfer*

| | |
|---|---|
| **10:00 - 10:30** | **Break** |

**10:30 - 11:00** **Ignacio Izeddin** **Ecole normale supérieure** **France**
*T.1.3 One molecule at a time: from super-resolution imaging to single particle tracking of nuclear factors*

**11:00 - 11:30** **Noriaki Shimizu** **Hiroshima University** **Japan**
*T.1.4 Novel, convenient and efficient 'IR/MAR gene amplification method' for the production of recombinant protein in animal cells*

**11:30 - 12:00** **Kenneth Shea** **University of California, Irvine** **USA**
*T.1.5 Plastic antibodies: synthetic polymer nanoparticles with antibody-like affinity that function in vitro and in vivo*

| | |
|---|---|
| **12:00 - 13:30** | **Lunch** |

**Session 2** **Gene Regulation - Chairs: Chris Kemp & Ana Maria Moro**

**13:30 - 14:00** **Joseph Shiloach** **NIDDK/NIH** **USA**
*T.2.1 Identification of genes and micro-RNAs responsible for growth properties of mammalian cells - application for enhanced biological production*

**14:00 - 14:30** **Randall Kaufmann** **Sanford-Burnham Medical Research Institute** **USA**
*T.2.2 Protein misfolding in the ER and oxidative stress*

| | |
|---|---|
| **14:30 - 15:00** | **Coffee Break** |

**15:00 - 15:30** **Scott Tenenbaum** **University at Albany-SUNY** **USA**
*T.2.3 RIP-Chip: Using RNA-binding proteins and microRNA targeting to study the human regulatory code*

**SHORT TALK SESSION Chair: Kenneth Lundstrom**

**15:30 - 15:45** **Tohsak Mahaworasilpa The Stephen Sanig Research Institute** **Australia**
*S.1 Development of hybrid cell bank expressing natural tumour-specific monoclonal antibodies*

**15:45 - 16:00** **Yvette H. Tang** **BioMarin Pharmaceutical** **USA**
*S.2 Detection and elimination of N-glycolyneuraminic acid during CHO cell line generation and process development*

**16:00 - 16:15** **Joanne Nettleship** **OPPF-UK Oxford University** **UK**
*S.3 Antibody fabs as co-crystallization chaperones: crystal structure of SIRP*

**16:15 - 16:30** **Break**

**16:30 - 16:45** **Jessica Stevenson** **MedImmune** **UK**
*S.4 Development of a CHO-based cell line for high titre, scalable, transient gene expression.*

**16:45 - 17:00** **David James** **University of Sheffield** **UK**
*S.5 Cell line-specific control of recombinant monoclonal antibody production by CHO cells*

**17:00 - 17:15** **Ana Teixeira** **IBET** **Portugal**
*S.6 Development of fluorescence-based PAT enabling tools for animal cell processes*

**17:30 - 19:00** **Poster Session**

**19:00** **Dinner**

## TUESDAY, SEPTEMBER 27, 2011

**Session 3** **CELL ENGINEERING - Chairs: Kim Stutzman-Engwall & Mark Smales**

**08:30 - 09:00** **Carlos Bosques** **Momenta Pharmaceuticals** **USA**
*T.3.1 Chinese Hamster Ovary Cells Can Produce Galactose-a-1,3-Galactose Antigens: Considerations During Biologics Development*

**09:00 - 09:30** **Christine Mummery** **University Leiden** **Netherlands**
*T.3.2 Cardiac Derivatives of Human Pluripotent Stem Cells in Drug Discovery and Disease*

**09:30 - 10:00** **Ho Ying Swan** **Bioprocessing Technology Institute** **Singapore**
*T.3.3 Integrated strategies for enhancing yield and quality of recombinant proteins in chinese hamster ovary cells*

**10:00-10:30** **Coffee Break**

**INDUSTRIAL WORKSHOP - Chair: Girish Shah**

**10:30 - 11:00** **Life Technologies**
*W.1 New tools to provide greater freedom and capability in mammalian protein expression*

**11:00 - 11:30** **DNA2.0**
*W.2 Production of proteins as therapeutic agents, research reagents and molecular tools frequently depends on expression in heterologous hosts*

**11:30 - 12:00** **Mirus Bio**
*W.3 Maximizing protein expression in suspension CHO cells*

**12:00 - 12:30** **Expres2ion Biotechnologies & CELLution Biotech**
*W.4 A new plug-and-play disposable insect cell based protein production system*

**12:30** **Lunch**

**FREE AFTERNOON FOR SIGHTSEEING**

## WEDNESDAY, SEPTEMBER 28, 2011

**Session 4** **ENVIRONMENTAL CONTROLS / BIOPROCESSING - Chairs: Paula Alves & Mike Butler**
***Sponnsored by Roche Diagnostics***

**09:00 - 09:30** **Robert Freedman** **University Warwick** **UK**
*T.4.1 The oxidative protein folding pathway of the endoplasmic reticulum: a target for cell engineering?*

**09:30 - 10:00** **Govind Rao** **University Maryland** **USA**
*T.4.2 Peaceful cell culture processes*

**10:00 - 10:30** **Coffee Break**

**10:30 - 11:00** **Greg Hiller** **Pfizer** **USA**
*T.4.3 High-end pH-Controlled delivery of glucose effectively suppresses lactate accumulation in CHO Fed-Batch cultures*

**11:00 - 11:30** **Brian Lee** **PBS Biotech** **USA**
*T.4.4 Pneumatic mixing mechanism for single-use bioreactors*

**11:30 - 11:50** **Konrad Büssow** **Helmholtz Centre for Infection Research** **Germany**
*T.4.5 Establishing production cell lines for structural biology by targeted integration and cell sorting*

**12:00 -13:30** **Lunch**

**Session 5** **ALTERNATIVE EXPRESSION SYSTEMS - Chairs: Reingard Grabherr & Devender Sandhu**

**13:30 - 14:00** **Sachdev Sidhu** **University of Toronto** **Canada**
*T.5.1 Synthetic antibodies: new tools for new biology*

**14:00 - 14:30** **Reinhard Breitling** **Jena Bioscience** **Germany**
*T.5.2 LEXSY: Eukaryotic Protein Expression in Leishmania tarentolae*

**14:30 - 15:00** **Coffee Break**

**15:00 - 15:30** **Johanne Deman** **GSK** **Belgium**
*T.5.3 GSK's cervical cancer vaccine manufactured with the baculovirus expression vector system (BEVS)*

**15:30 - 16:00** **Stefan Schmidt** **ERA Biotech** **Spain**
*T.5.4 Zera fusions and StorPro: A versatile expression technology bridging the gap between animal cells and other hosts*

**16:15 - 17:00** **General Assembly**

**17:00 - 18:00** **Poster session**

**19:00** **Gala Dinner**

**THURSDAY, SEPTEMBER 29, 2011**

**Session 6** **EXPRESSION SYSTEM DEVELOPMENT - Chairs: Amine Kamen & Georg Schmid**

**09:00 - 09:45** **John Birch** **Lonza** **UK**
*T.6.1 Technology drivers in the development of animal cell processes*

**09:45 - 10:15** **Sabine Geisse** **Novartis** **Switzerland**
*T.6.2 Transient gene expression; gambling with approaches for success*

**10:15 - 10:45** **Coffee Break**

**10:45 - 11:15** **Andy Lin** **Genentech** **USA**
*T.6.3 The lessons learned for platform monoclonal antibody production*

**11:15 - 11:45** **Yvonne Genzel** **Max Planck Institute** **Germany**
*T.6.4 Influenza virus production: characteristics of different producer cell lines*

**11:45 - 12:05** **Stephanie Chen** **GSK** **USA**
*T.6.5 Strategies for expression of multi-component protein complexes in Sf9 cells*

*End of Conference*
*"We will see you in 2 years!"*

# BACULOVIRUS SYMPOSIUM

# Oral Presentations

## BAC.1 Baculovirus nucleocapsid transport to the nucleus and beyond

Loy Volkman, University of California, Berkeley, USA

Phylogenetic evidence indicates baculoviruses have been tracking their juvenile insect hosts for 230 million years or so, since the radiation of the Lepidoptera. Lepidopteran larvae developed a formidable defense against pathogens during this period by forming an exoskeleton that covers their exterior surfaces including their respiratory tubes, foreguts and hindguts. All viruses need to interact with live cells in order to establish infection, making this defensive tactic especially effective against viruses. Nonetheless, Baculoviruses countered this measure by targeting larval midgut columnar epithelial cells via their microvilli, an extraordinary biological feat. Microvilli, devoid of microtubules, are packed with polarized actin microfilaments aligned in the opposite direction needed for incoming nucleocapsid transport via myosin motors. Yet bioassays conducted with baculovirus Autographa californica M nucleopolyhedrovirus (AcMNPV) carrying a hsp-lacZ reporter gene reveal that the virus infects midgut cells relatively rapidly (3 hr -10 hr). These results suggest the involvement of a novel actin-based nucleocapsid transport mechanism. AcMNPV nucleocapsids contain an essential protein at their blunt terminus, P78/83. P78/83 is an actin-polymerization promotion factor: it binds actin and the Arp2/3 complex and thereby facilitates actin polymerization, which, in turn, generates the force needed to propel nucleocapsids to the nucleus. This actin-based mode of establishing nuclear infection is novel among viruses. Fluorescence microscopy studies reveal that upon bumping into the nuclear membrane, nucleocapsids undergo a corkscrew motion until they connect with nuclear pores and enter the nucleus. Upon early gene expression, nucleodcapsids not yet nuclear, are driven to cell surface spikes where they may connect with newly-expressed gp64 and bud, thereby spreading infection prior to viral replication. These novel modes of nucleocapsid transport and pass-through infection strategy offer opportunities for biotechnology not available with other virus platforms.

## BAC.2 Baculovirus Entry Mediated by Envelope Protein GP64

Gary W. Blissard, Boyce Thompson Institute, Cornell University

Budded virions (BV) of the baculovirus AcMNPV enter cells by a process that involves initial interactions at the cell surface, clathrin-mediated endocytosis and transport, and a membrane fusion event that releases viral nucleocapsids into the cytoplasm. Late in the infection cycle, production of progeny BV and viral egress involves transport of nucleocapsids from the nucleus to the plasma membrane, where BV buds by an unknown process. GP64 is the major envelope glycoprotein of AcMNPV BV, and is critical for viral entry and important for efficient BV egress. To examine virion entry, we have examined the functions of GP64 in receptor binding and membrane fusion, with a specific focus on critical GP64 residues that interact with host cell membranes, and on the nature of interactions before and during membrane fusion. To study virus entry and egress, we have also examined the roles of host cellular pathways. Recently, we examined the possible roles of cellular protein sorting pathways (ESCRT complexes) in these processes. We studied viral infections in the presence of dominant negative forms of the VPS4 protein, a key cellular regulator of ESCRT function. Our studies indicate that ESCRT function plays critical roles in both virion entry and egress from infected cells.

**BAC.3 Insect Protein Glycosylation Pathways.**

Donald L. Jarvis, University of Wyoming, Laramie, WY

A widely recognized advantage of the baculovirus-insect cell system is its ability to produce recombinant proteins with eukaryotic modifications, such as glycosylation. However, insect cells have less extensive protein glycosylation pathways than higher eukaryotes and the insect cell lines used as hosts for baculovirus expression vectors are unable to produce sialylated glycoproteins. The inability of the baculovirus-insect cell system to produce recombinant glycoproteins with native carbohydrate side chains can be a serious problem because these side chains can influence glycoprotein behavior in various ways.

Over the past decade, my group has addressed this problem through glycoengineering efforts that have yielded transgenic lepidopteran insect cell lines with increasingly humanized protein glycosylation pathways. In parallel, we have worked alongside other labs to elucidate the endogenous glycoprotein glycan processing pathways in insect cell systems. In this presentation, I will report the results of our latest glycoengineering efforts and place these results in the context of recent findings that reveal a surprisingly extensive, yet ineffective capacity for glycoprotein glycan processing in lepidopteran insect systems.

**BAC.4 Salmonid alphavirus envelope glycoprotein processing determines virus-like particle production in insect cells**

Stefan W Metz[1], Femke Feenstra[1], Stephane Villoing[2], Marielle C van Hulten[3], Jan W van Lent[1], Sjo Koumans[3], Just M Vlak[1] & Gorben P Pijlman[1]

[1]Wageningen University, The Netherlands, [2]Intervet Norbio, Bergen, Norway, [3]Intervet International BV, The Netherlands

Pancreas disease (PD) and sleeping disease (SD) are important viral diseases in aquaculture of Atlantic salmon and rainbow trout, respectively. The etiological agent of PD and SD is Salmonid alphavirus (SAV), an unusual member of the Togaviridae (genus Alphavirus) that replicates at low temperature in cultured fish cells. Outbreaks of SAV are associated with large economic losses and current control strategies rely on vaccination with killed virus formulations. SAV virions are enveloped and display two major viral glycoproteins, E1 and E2, at their surface. In mammalian cells, recombinant, non-infectious virus-like particles (VLPs) of other alphaviruses can be produced upon expression of the entire alphavirus structural open reading frame. In this research we investigated the production of SAV VLPs in Spodoptera frugiperda Sf9 insect cells using recombinant baculoviruses. When baculovirus infection was carried out at the standard temperature of 27ºC, expression of SAV capsid, E1 and E2 was clearly observed on polyacrylamide gels and Western blots. Surprisingly, however, precursor E2 (PE2) was not processed by host furin into mature E2. Furthermore, no E2 could be detected on the surface of infected cells nor in the culture fluid, suggesting that E2 was not transported to the plasma membrane and that no VLPs were formed. However, when temperatures during expression were lowered, PE2 was processed into mature E2 in a temperature-dependent manner and E2 surface localization and secretion were rescued. An optimized temperature-shift expression process was designed that increases viral glycoprotein processing efficiency and significantly shortens the production time of this novel VLP-based SAV vaccine candidate.

## BAC.5 Use of BacMam for Recombinant Protein Production

Sue Kadwell, GlaxoSmithKline, NC, USA

For many years our fermentation group has routinely used two key expression systems for protein production to support screening and protein crystallography: E. coli and baculovirus expression in insect cells. However, in the past few years, this has changed to include a third system: BacMam transduced mammalian cells grown in suspension culture. This approach has proven to be a successful and important addition, especially for difficult to express proteins.

In this presentation, a review of BacMam virus biology will be given along with an overview of how these viruses are used. It will highlight our work to optimize BacMam transduction as an excellent choice for large scale protein production in a mammalian cell background. The in-house development of a robust serum free suspension CHO cell line for enhanced BacMam transduction and expression will be described, along with vector design modifications. Optimization of cell culture expression parameters such as multiplicity of transduction (MOT), temperature, addition of sodium butyrate, and harvest time will be discussed. Special emphasis will be given to secreted protein expression and the choice of signal sequence used in construct design. Our large scale process using Wavebags will be included.

**BAC.6 Concentration and Diafiltration of Baculovirus and BacMam Particles.**

Chris Kemp, Kempbio, Inc., MD, USA

Efficient concentration and buffer exchange of baculovirus and BacMam high-titer stocks has applications in two diverse fields, high-density insect cell expression protocols and BacMam mediated transduction of mammalian cells. High-density insect cell infections, (cell densities close to 1x107 cells per mL) require the addition of virus stock at concentrations greater than 10% of the culture volume which may inhibit the metabolism of the host cells and negatively affect protein expression. The transduction of mammalian cells by recombinant BacMam particles may be influenced by virus titer and the insect cell medium used to prepare the high-titer stock. Many cell lines including primary neurons are negatively affected by exposure to insect cell culture medium. The ability to produce a virus stock in a buffer formulation that is compatible with both insect cell and mammalian cell culture would be advantageous to both high-density insect cell processes and BacMam mediated transductions.

In this presentation we will discuss the development of a rapid and efficient method for the capture and elution of baculovirus and BacMam virus particles using membrane chromatography filters. We will compare the use of the membrane chromatographic process to hollow-fiber ultrafiltration for the concentration and buffer exchange of virus stocks by examining their ease of use, scalability, throughput and percent recovery of virus particles.

Bio:

Chris Kemp is President of Kempbio, Inc. located in Gaithersburg, MD. Kempbio specializes in the development of early stage processes for the expression of recombinant proteins using mammalian and insect cell systems. Dr. Kemp has developed processes for a wide variety of biopharmaceutical applications including VLP based vaccines, large-scale mammalian transient expression of r IgG and high-density baculovirus expression.

**BAC.7 Optimising gene expression and protein production in insect cells using baculovirus vectors**

Linda A King, Oxford Brookes University, Oxford, UK

The baculovirus expression system is often the system of choice for producing proteins in eukaryotic cells. However, as with all expression systems it has its advantages and disadvantages. This presentation will cover recent developments that been undertaken to improve the quality and quantity of difficult to express proteins, including improvements in transfer vector design and modification to the virus backbone as well as improved media and cell lines. The presentation will also explore the advances made, and still to be made, to enable the baculovirus system to be used with confidence in high throughput laboratories, such as those involved in structural genomics and drug discovery.

**BAC.8 Baculovirus-mediated gene delivery into mammalian cells and tissues**

Kari Airenne, University of Eastern Finland, Finland

Baculoviruses are arthropod specific enveloped DNA viruses that naturally infect predominantly only some butterfly larva. Since early 1980's the prototype baculovirus Autographa californica multiple nucleopolyhedrovirus (AcMNPV) has emerged as an versatile tool for many purposes beyond its initial use as an efficient vector for recombinant protein production in insect cells. The fact that an AcMNPV, if armed with an expression cassette compatible with the target cell, can direct gene expression in a wide range and origin of cells has open new avenues to apply baculovirus technology for protein production, vaccination, drug screening and therapeutic applications out of insect cell context. AcMNPV can direct gene expression also in vivo, naturally immunoprivileged tissues such as brain, eye or testis shown to being especially good targets for gene delivery. Therapeutic potential of AcMNPV in vaccination, tissue engineering, and cancer therapy has already been demonstrated in pre-clinical animal models. Notably, in non-target cells, AcMNPV is incapable for replication or proper viral gene expression making it gentle (non-toxic) to vertebrate cells and harmless for humans. Level 1 biosafety facilities are generally enough for propagation. In addition to safety, baculoviruses provide several other advantages over the other gene delivery systems. Working is easy and does not require particular skills or equipments. Transgene capacity of AcMNPV is in practice unlimited and multiple high-titer viruses can be generated and tittered in a short time frame. Insect cells are easy to cultivate in a serum free medium on plates or in suspension for virus generation and the produced viruses are easy to storage at the +4°C. The recent approval of the first baculovirus technology based drugs Provenge and Cervarix against prostate and cervical cancer, respectively, pave the way for the regulatory approval of other products in row by the regulatory agencies (EMA, FDA). In summary, AcMNPV is an excellent vector for gene delivery with which safe and efficient transduction of almost any cell from any origin can be achieved.

**BAC.9 High yield production of viral vectors and subunit vaccines using the baculovirus expression system**

Amine Kamen, National Research Council, Canada

A high yielding production process using low MOIs and a feeding strategy successfully addresses several limitations of current recombinant adeno-associated virus (rAAV) production in insect cells and contributes to position the baculovirus expression vector system (BEVS) as one of the most efficient platforms for large scale manufacturing of not only recombinant proteins and viral like particles as sub-unit vaccines but also rAAV vectors as gene delivery system.

In this presentation we will review the most recent advances in bioprocessing of insect cell technology and we will use the example of rAAV as a case study. Recombinant AAV are the most promising vectors for gene therapy. BEVS has been engineered to produce high rAAV titers in serum-free suspension cultures of Spodoptera frugiperda insect cells. To increase the rAAV titer, a low MOI (0.1 plaque forming units/cell) triple infection was combined with a fed-batch feeding strategy to support productive infection at an initial cell density of 5.0 x $10^6$ cells/mL. This approach resulted in a 7-fold increase of rAAV yield as compared to the typical synchronous infection at 1.0 x $10^6$ cells/mL. The production strategy was further evaluated in 3-liter bioreactor runs to achieve serotype 2 rAAV yield of 2.8 x $10^{14}$ vector genomes/L. Comparable results were achieved with modified baculovirus constructs and other serotypes including serotypes 8 and 9. The process has been validated and transferred to a cGMP manufacturing site for production of clinical material.

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## BAC.10 Systems biotechnology of baculovirus-producing insect cells

Nuno Carinhas[1], Vicente Bernal[2], Paul Jenoe[3], Manuel JT Carrondo1[4], Rui Oliveira[5], Paula M Alves[1]

[1]Universidade Nova de Lisboa / Instituto de Biologia Experimental e Tecnológica, Portugal; [2]Departamento de Bioquímica y Biología Molecular B e Inmunología, Facultad de Química, Universidad de Murcia, Spain; [3] Biozentrum of the University of Basel, Switzerland; [4] Universidade Nova de Lisboa, Portugal; [5]Universidade Nova de Lisboa, P 2829-516 Caparica, Portugal.

The relatively recent developments in Systems Biology and computational methodologies present a paradigm-shifting opportunity to develop bioprocesses with improved productivities. The present work focuses on optimizing baculovirus vectors (BV) production in Sf9 insect cell cultures, where a significant challenge is the tendency for specific product yields to decrease when cell cultures are infected at later stages of growth. Based on a stoichiometric model of the metabolism of Sf9 cells, it was observed that cells undergo a progressive inhibition of their central energy metabolism when grown to high densities, which was only up regulated after infection at low cell concentrations (1). To test the hypothesis that the energetic state is a key determinant of system productivity, the effect of a variety of nutrients and energetic intermediates was screened. Specific BV yields could be increased up to 7 fold after supplementation of pyruvate or α ketoglutarate at the time of infection, representing a cost efficient strategy for high cell density production of recombinant BVs (2).

Though based on a rational study, this early work does not represent a systematic means to optimize bioprocesses. To tackle this, a hybrid framework combining the stoichiometric model with a statistical sub model was developed to bridge the estimated metabolic fluxes with measured productivities. The output of this framework is a description of which metabolic reactions are positively, negatively or uncorrelated with productivity, translating into a list of rules to develop metabolic and/or bioprocess engineering strategies. Based on the data accumulated, the importance of the energetic state for viral replication was confirmed by using only an unbiased subset of preliminary experiments, while the productivity of independent cultures could be predicted within experimental error (3). Finally, to explore the application of the "omics" as high throughput hypotheses generating tools, we surveyed the quantitative proteomic response of Sf9 cells to culture growth and BV infection. This was accomplished by stable isotope labeling with amino acids in cell culture (SILAC) and high-resolution mass spectrometry. Our findings suggest a possible mechanism for the cell density effect that is related with the differential accumulation of BV encoded proteins during the initial stages of infection, which is influenced by the cell physiological state. It is likely that a complete understanding can only come from an integrated view of the complex cell, a fundamental scientific goal yet to be accomplished.

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**BAC.11 Baculovirus surface displayed hemagglutinin as a mucosal vaccine vector against H5N1 in mice**

Jimmy Kwang, National University of Singapore, Singapore

Recent reports of baculovirus-mediated transductions of viral glycoprotein genes into mammalian cells and their efficient gene delivery in animal models have gained special attention. Here, we described the construction of baculovirus expressing Heamagglutinin (HA) under the control of the immediate-early promoter 1 (ie1) derived from the white spot syndrome virus (WSSV) genome, which enables the expression of hemagglutinin at the early stage of infection in insect cells, thereby enhancing the display of HA on the baculovirus envelope. Along with this suitable vaccine, the route of administration of the vaccine has a profound effect in controlling mucosally acquired infections such as influenza. Induction of mucosal immunity through nasal/oral immunization is an effective way to control influenza infection. In this study, we evaluated the oral vaccination of ie1 promoter based baculovirus displayed hemagglutinin (BacHA) against highly pathogenic H5N1 virus infection in a mouse model. Oral administration of live BacHA significantly induced strong cross-clade neutralization antibodies, both systemic and mucosal immune responses, against a challenge of 100 TCID50 of heterologous H5N1 strains (clade 1.0, 2.1, 2.3 and clade 8.0) infections. As an alternative approach, inactivated baculovirus was encapsulated within a reverse-micelle structure of phosphatidyl choline and delivered into the gastrointestinal tract of mice. Interestingly, oral administration of encapsulated BacHA significantly enhanced both systemic and mucosal immune response. The level of immune response obtained with encapsulated inactive form of baculovirus was equivalent with those obtained with live baculovirus, suggesting the potential of baculovirus as live and inactivated vaccine. Also, intranasal administration of baculovirus surface displayed HA showed that high level of HA specific mucosal and systemic immunity in mice. Moreover, the combination of BacHA with recombinant CTB mucosal adjuvant forms an effective mucosal vaccine and which provided 100% protection against 10 MLD50 of H5N1 infections. The baculovirus surface-displayed HA vaccine is efficacious in inducing mucosal immune responses as well as systemic immune responses and does not require either sophisticated biocontainment infrastructure or downstream purification processes for mass production. Further, oral or intranasal vaccinations are non invasive, pain free and affordable with improved logistics and immunization coverage during pre pandemic or pandemic situation.

Keywords: Influenza H5N1, baculovirus, recombinant baculovirus, immediate-early promoter 1, Hemagglutinin, mucosal vaccine

**BAC.12 Baculovirus Reference Material Initiative**

Amine Kamen, National Research Council, Canada

The short presentation will provide updates on the initiative to develop a baculovirus reference material. To be successful this initiative needs the support of a broad panel of researchers working with baculovirus vectors for recombinant protein production and gene delivery for either therapy or vaccination. First there is a need to reach a consensus on the nature of the reference material, the production protocols and the baculovirus characterization methods. It will also be important to define repository and distribution procedures so that the reference material is available to any researcher for calibrating experimental data and to compare experiments performed in the various laboratories. As more and more baculovirus-based products are licensed or in the final stages of development, the development of a baculovirus reference material is timely. Audience is invited to discuss the requirements for the reference material and for the project as a whole to be successful. Also mechanisms on establishing partnership that would involve academic, industrial laboratories and governmental organizations to support this international initiative will be discussed.

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# VECTOR DESIGN

## T.1.1 Engineering CHO cell growth by stable manipulation of miRNA expression

Sanchez, N, Gallagher M, Lao N, Gallagher C, Meleady P, Clynes M, Barron N.

Dublin City University, Ireland

MicroRNAs are small non-coding RNAs involved in many biological functions such as cell proliferation, apoptosis and cell cycle. They are capable of regulating hundreds of genes in a post-transcriptional manner by translation repression and/or mRNA degradation. These characteristics make miRNAs attractive tools for CHO cell engineering as multiple genes, and possibly entire biological pathways, may be targeted simultaneously.

MiRNA expression profiling of CHO cells after temperature-shifting the culture from 37°C to 31°C, identified several differentially expressed miRNAs including miR-7, which was found to be downregulated. Transient overexpression of miR-7 led to a significant decrease in CHO cell growth whereas transient inhibition enhanced cell growth, though to a lesser extent.

To achieve more complete knockdown of miR-7 in cells, stable CHO cell lines were engineered to express a miR-7 decoy gene (‘sponge’) consisting of a destabilised enhanced GFP with four tandemly repeated miR-7 binding sites downstream to sequester the mature endogenous miR-7. These cells were found to have reduced GFP expression and improved growth rate compared to controls demonstrating that stable miRNA knockdown may be used to manipulate CHO cell phenotypes in culture.

## T.1.2 Minicircle vector design – new tools for non-viral gene transfer

Martin Schleef, PlasmidFactory GmbH & Co. KG, Bielefeld, Germany

Plasmid-DNA is frequently used particularly for non-viral gene therapy but also for protein (e.g. Ab), virus (e.g. AAV or lenti viral vector), or RNA production. The dissemination of antibiotic resistance genes, as well as the uncontrolled expression of backbone sequences present in plasmid DNA may have profound detrimental effects. Additionally, CpG motifs have been shown to contribute to silencing of episomal transgene expression. Hence, these components are mostly needless for gene transfer, rather reducing transfer efficiency and gene expression. They also represent a potential risk especially for safe clinical applications. The minicircle technology is removing needless sequences. The resulting minicircle DNA consists almost only of the gene of interest, leading to significant size reduction and improved performance. Here we show the effective production of minicircle DNA with high yield and reproducible quality.

Comparison of plasmid and minicircle-mediated gene expression shows improved performance of the minicircle in different cell lines, i.e., minicircle vectors improve transfection efficiency and transgene expression in different cell lines, mainly due to size reduction, but also due to the high purity of such DNA product. In combination with S/MAR elements the duration of expression is significantly higher (higher or longer?).

Availability of minicircles at reproducible quality and sufficient amount makes this system an applicable and effective alternative to conventional plasmid vectors.

## T.1.3 One molecule at a time: from super-resolution imaging to single particle tracking of nuclear factors

Ignacio Izeddin, Ecole normale supérieure, France

Progress in optical microscopy, combined to the emergence of new fluorescent probes and advanced instrumentation, now permits the imaging of single molecules in fixed and live cells. This extreme detection sensitivity has opened new modalities in cellular imaging. On the one hand, optical images with an unprecedented resolution in the 10-50 nm range, well below the diffraction limit of light, can be recorded. These super-resolution images give new insights into the properties of cellular structures. On the other hand, proteins, either in the membrane or intracellular, can be tracked in live cells and in physiological conditions. Their individual trajectories provide invaluable information on the molecular interactions that control their dynamics and their spatial organization. Single molecule imaging is rapidly becoming a unique tool to understand the biochemical and biophysical processes that determine the properties of molecular assemblies in a cellular context.

We explored new conditions of single particle tracking using photoactivated localization microscopy that enables the study of protein dynamics inside live eukaryotic cells. Applying this method to several nuclear proteins, we captured the wide range of diffusive behaviors from very rapid diffusion (~ 10 μm2/s) for the free fluorophore Dendra2, to anomalous or corralled motion for transcription factors such as the P-TEFb, c-Myc and the Fibrillarin, and the bound states of H2B histone proteins. Our results suggest that nuclear exploration is not governed solely by the nucleoplasmic geometry, but also by the nature of the protein interactions. This approach opens the road to single-molecule biochemical studies inside living eukaryotic cells.

## T.1.4 Novel, Convenient and Efficient "IR/MAR Gene Amplification Method" for the Production of Recombinant Protein in Animal Cells

Shimizu, N.[1], Araki, Y.[1], Noguchi, C.[1], Hamafuji, T.[2], and Miki, D.[3]

[1]Hiroshima University, Higashi-hiroshima, JAPAN,[2]Transgenic Inc., Kobe, JAPAN, [3]Tosoh Co., Tokyo, JAPAN

Amplification of dihydrofolate reductase (DHFR) gene in Chinese hamster ovary (CHO) cells by selection of methotrexate (MTx) has been widely used for the establishment of cell lines that produce recombinant protein pharmaceuticals. However, the DHFR/MTx method is highly time-and labor-consuming. On the other hand, we found that a plasmid bearing a mammalian replication initiation region (IR) and a matrix attachment region (MAR) initiates gene amplification in mammalian cells, and it quite efficiently generate the chromosomal homogeneously staining region (HSR) and/or the extrachromosomal double minutes (DMs). This is a novel gene amplification method that enables us to amplify the desired genes up to thousands copies per cell, by only one step drug selection. We have used this method in the basic biology of chromosome science, and also revealed the mechanism of gene amplification. We now show that the method also provides a new and a powerful platform for the production of recombinant protein. Namely, we have examined mainly antibody (Ab) protein expression, and also green fluorescence protein (GFP) or Fc receptor protein expression, in mainly CHO DG44 cells or human COLO 320 cells. As a result, we found that the Ab gene that was co-transfected with the IR/MAR plasmid was amplified to few thousands copies per cell in COLO 320 or few hundreds copies per cell in CHO DG44 cells after one step drug selection for 3 to 4 weeks. The introduced sequence generated multiple DMs or long and homogeneous HSR in COLO 320, or the ladder-shaped or fine-ladder-shaped HSR in CHO DG44 cells. The Ab protein expression increased few tens-fold in COLO 320 cells, or more than a hundred-fold in CHO DG44 cells by co-transfection with the IR/MAR plasmid compared with the control plasmid that did not have IR/MAR sequence. From such polyclonal CHO DG44 transformants, a clone that showed highest Ab production was selected from a hundred clones. This clone, after adaptation to suspension batch culture in shaking flask showed the specific production rate of 45 pg Ab protein /cell/day that was almost the highest reported for antibody protein. The integrity and the functionality of the Ab protein was no problem. The amplified structure and the productivity of Ab protein did not change during at least 2 months culture of cloned cell. In addition to that, we have found that the combination of the IR/MAR method with the DHFR/MTx methods synergistically works, and far more rapidly generates the cells of higher production rate compared with each of the solitary method. In conclusion, the IR/MAR gene amplification method provides a novel highly-competitive platform for use in recombinant protein production.

**T.1.5 Plastic Antibodies Synthetic polymer nanoparticles with antibody-like affinity that function in vitro and in vivo.**

K. J. Shea, University of California, Irvine, California

General methods for the recognition of specific peptide sequences, proteins and related biological macromolecules remain a significant challenge. The talk will describe general protocols for creating synthetic polymer receptors for peptides and proteins with antibody-like affinity for biological macromolecules. These nanoparticles have been found to function in vivo and are now being evaluated for therapeutic applications, in bioprocessing and diagnostics.

# GENE REGULATION

## T.2.1 Identification of genes and micro-RNAs responsible for growth properties of mammalian cells - application for enhanced biological production

Joseph Shiloach, Biotechnology Core Laboratory NIDDK/NIH

Modifying growth properties of mammalian cells is important for the design and the performance of biotechnology-related processes. Efforts have been devoted to altering cell properties such as growth rate, lactate production, glucose consumption and sensitivity to various media components and environmental conditions. The traditional approach for achieving desired growth properties is by adaptation and selection; with the developments in genomics and bioinformatics, it can also be done by identifying and modification of specific genes. The latter approach, although more involving, can potentially produce more stable cell lines. Two examples will be presented: one is the process and possible mechanism for transforming an adherent cell line to grow in suspension by modifying a specific gene, and the application for producing influenza virus. The second example for improving production will describe an attempt to utilize micro-RNAs, which are non-coding RNAs, to delay the apoptosis process.

## T.2.2 Protein Misfolding in the ER and Oxidative Stress

Randal J. Kaufman, Sanford-Burnham Medical Research Institute, La Jolla, CA

Background: Protein misfolding in the endoplasmic reticulum (ER) contributes to the pathogenesis of many diseases. Although accumulation of misfolded protein within the ER is a central event in cell death the relationship between protein misfolding in the ER, ER calcium release, oxidative stress and mitochondrial dysfunction is not explored. We have analyzed expression of coagulation factor VIII (FVIII), the protein deficient in hemophilia A, to elucidate the relationship between protein misfolding, mitochondrial function and oxidative stress. Newly synthesized FVIII is subject to misfolding in the ER lumen, aggregates, activates the unfolded protein response (UPR), causes oxidative stress and induces apoptosis in vitro and in vivo in mice. Strikingly, antioxidant treatment reduces UPR activation, oxidative stress and apoptosis, and increases FVIII secretion in vitro and in vivo (1). The findings indicate that reactive oxygen species (ROS) are a signal generated by misfolded protein in the ER that cause UPR activation and cell death. Genetic or chemical intervention to reduce ROS improves protein folding and cell survival and may provide an avenue to treat and/or prevent diseases of protein misfolding. Although accumulation of misfolded protein within ER, ER calcium release, oxidative stress and mitochondrial dysfunction is not explored.

Methods: We have studied expression of two proteins prone to misfolding clotting factor VIII and VII by inducible expression in Chinese hamster ovary (CHO) cells and by gene delivery to hepatocytes in mice.

Results: Analysis of superoxide production by MitoSox staining demonstrated that expression of FVIII and FVII cause mitochondrial oxidative stress. This oxidative stress required calcium release from the ER, as it was blocked by either BAPTA-AM or Bcl2 overexpression. Calcium release likely occurred through the IP3 receptor as IP3 antagonists reduced the oxidative stress upon misfolded protein accumulation. The oxidative stress required cis-trans prolyl isomerase as it was blocked by cyclosporine A. In addition, the oxidative stress required cyclophilin D, a component required for calcium uptake into the inner mitochondrial matrix. Finally, protein misfolding in the ER lumen depletes cellular ATP.

Conclusions: The results demonstrate that protein misfolding in the ER lumen initiates a cascade of events that include calcium leak and uptake through the calcium transporter into the mitochondrial matrix leading to membrane depolarization, depletion of cellular ATP, mitochondrial-derived oxidative stress. Cyclophilin D may be a critical mediator of the oxidative stress and apoptotic response that occurs upon protein misfolding in the ER. These findings may provide an avenue to novel therapies to treat diseases of protein misfolding.

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## T.2.3 RIP-Chip: Using RNA-binding proteins and microRNA targeting to study the human regulatory code

Scott A. Tenenbaum, The University at Albany-SUNY

Unlike DNA, RNA has tremendous potential to form complex and elegant structures. This may be one of the most important attributes of RNA that permits it to both serve as a template for the genetic code while simultaneously containing essential regulatory information that determines where, when and how much of the encoded gene will be expressed. It is very unlikely that mRNA, or any cellular RNA for that matter, exists in a truly naked state, but rather, is likely bound by multiple molecules, existing as RNA-protein complexes (mRNPs). Many of these molecules including microRNAs and RNA-binding proteins (RBPs) can regulate gene expression most notably through their actions with the untranslated-regions (UTRs) of mRNA. Accordingly, eukaryotic organisms depend integrally on the actions of RBPs for successful post-transcriptional control of gene expression.

We are using methods for purifying endogenously formed RNA-Protein (RNP) complexes and identifying the associated RNA targets with microarray and next-gen sequencing technologies (RIP-Chip/Seq or ribonomic profiling). This approach has enabled the genomic-scale identification of targets of RBPs. In conjunction with the NIH/NHGRI ENCODE project we have been using tiled-microarray platforms and next-gen sequencing to determine the associations of both coding and non-coding RNAs for several RBPs. This advance has enabled the large-scale identification of many mRNA targets of RBPs and provides new insight into the principles governing post-transcriptional gene regulation. Our studies reveal that analogous to transcriptional regulation, groups of functionally related RNAs are coordinately regulated in a combinatorial manner by distinct classes of RBPs targeting related cis-regulatory elements located in the transcripts.

Our data also suggests that non-coding RNA such as miRNAs are modulating RBP binding sites in a dynamic manner, which implies that the cis-regulatory code targeted by miRNAs is at least in part, the same as that read by mRNA-binding proteins. This model predicts that miRNAs indirectly or directly bind to RBP binding sites. This model also suggests that multiple mRNA regulatory elements can simultaneously be influenced by miRNA-mRNA interactions such that the binding of one or more miRNA results in conformational changes in the structure of the mRNA, thereby, either revealing or masking a second regulatory element.

By using RIP-Chip/Seq profiling to explore the post-transcriptional infrastructure of the cell, our studies reveal that many of the non-coding RNAs expressed from the genome also appear to have an interesting regulatory association with RBPs in a specific and selective manner and could modulate RNA-binding protein binding sites in a dynamic manner.

# SHORT TALK SESSION

## S.1 Development of hybrid cell bank expressing natural tumour-specific monoclonal antibodies

Mahaworasilpa T.L., Kaseko G., Liu M., Li Q., Hoe E.

The Stephen Sanig Research Institute, Sydney, Australia

The analysis of the natural antibody (Ab) repertoire of tumour infiltrating B lymphocytes (TIL-B) could lead to an understanding of the mechanisms underlying natural Ab responses to tumour antigens (Ags) as well as an identification of novel tumour-related Ags. However, the progress in this direction has been hindered, in part, by the relatively small quantity of these B cells extracted from individual tumour tissues. Here, we report a method for the development of a unique hybrid cell bank derived from hybridising an immunoglobulin-producing TIL-B lymphocyte with a human cell of an in-house established human hematopoietic hybrid cell line (as a partner) by using the one-on-one electrical cell hybridisation technique developed in-house. That is, after a small number of CD40+ B cells were extracted from the specimens of ductal breast adenocarcinoma, each CD40+ TIL-B cell was hybridised with a cell of the partner human cell line followed by the recovery and culture of individual newly created hydrid cells in a B lymphocyte promoting medium. Out of each tumour specimen, on average 74% of the hybridisation attempts resulted in stable hybrids, of which up to 64% expressed and secreted monoclonal Abs of different isotypes resulting in mAb library for ductal breast adenocarcinoma. This approach allows a very fast and efficient establishment of a unique TIL-B cell-derived hybrid cell bank of various tumors for further structural and functional analysis of natural tumour Ab repertoire. Furthermore, the immunoglobulin producing hybrids were generated from TIL-B sample size as small as 50 cells and also eliminated laborious screenings for hybrids and immunoglobulin producing clones.

## S.2 Detection and elimination of N-glycolyneuraminic acid during CHO cell line generation and process development

Jim Liu, Jeff Norman, Shilpa Shroff, Jim Michaels, and Yvette Tang

BioMarin Pharmaceutical Inc, Novato, California, USA

The presence of N-glycolyneuraminic acid (NGNA or Neu5Gc), which is not typically found in adult humans on therapeutic glycoproteins could potentially post a risk of immune responses in patients. The "expression" of Neu5Gc seemed to be cell type and clone specific. Therefore the ability to identify and eliminate clones with high levels of Neu5Gc glycosylation early in development could allow us to focus only on the high quality clones. Traditionally, the level of Neu5Gc can only be detected by a HPLC assay with purified protein samples. Using an antibody specific to Neu5Gc allowed us to implement a high throughput and robust method to identify the presence of N-glycolylneuraminic acid in the therapeutic proteins produced from different clones. In addition, the level of Neu5Gc glycosylation can be greatly impacted by the cell culture media and culture conditions. A case study will be reported on how to control Neu5Gc level through clone selection and media formulation for a non-antibody therapeutic glycoprotein.

## S.3 Antibody Fabs as co-crystallization chaperones: Crystal structure of SIRPγ.

Joanne E Nettleship[1,2], Jingshan Ren[2], Nahid Rahman[1,2], Yuguang Zhao[2], Deborah Hatherley[2], A Neil Barclay[2] and Raymond J Owens[1,2]

[1]R92 Rutherford Appleton Laboratory, Oxford, UK, [2]University of Oxford, Oxford, UK

Fabs have been used for (co)-crystallization and structure determination for over 15 years. There are a number of examples where binding to a Fab fragment has enabled the crystallization of a protein or improved the resolution of its structure. In addition to aiding crystallization, the structure of the antibody-antigen complex may offer insight into the structure-function relationship of the antigen.

The OPPF-UK has established a rapid method of Fab production using secretion from mammalian cell lines. The heavy chain variable (VH) domain and light chain variable (VL) domain are cloned, from the parent hybridoma, into vectors containing the heavy chain constant region (CH1) and light chain constant (CL) domain respectively. Production of secreted product is by automated transient transfection in human embryonic kidney (HEK) 293T cells using a CompacT SelecT robot (The Automation Partnership) followed by semi-automated protein purification.

Signal Regulatory protein (SIRP) γ has shown a low propensity for crystallization falling at the very edge of group A in a grand average of hydropathy (GRAVY) v's pI plot and with a "difficult" rating using the XtalPred server (NIH). We have recently solved the structure of SIRPγ to 2.5 Å using FabOX117 as a co-crystallization chaperone.

## S.4 Development of a CHO-based cell line for high titre, scalable, transient gene expression.

Jessica Stevenson, Scientist 1, CMC, MedImmune Cambridge, UK

In order to meet the ever-increasing demands for material for preclinical studies, a high yielding transient expression system is required. Historically, the Human Embryonic Kidney cell (HEK 293) system has been used extensively for transgene expression. At MedImmune, we have developed a CHO-based transient expression system. Using CHO cell lines for production of preclinical material means that the same host is used throughout drug development process, from pre-clinical development through to manufacturing and clinical supply. This minimises the potential risk of product quality issues early in the process, and allows insights into manufacturability.

A suspension-adapted variant of the CHOK1 (ECACC) cell line was created in-house; however, this required further development for desired expression levels. A multi-factorial approach was used to facilitate creation of a CHOK1 derivative which has significantly improved the productivity of the CHO transient system.

To generate the host cell line, a cell engineering strategy was applied. Two separate enhancer elements were stably transfected into the host to give a proprietary cell line suitable for use as a transient host. The use of the two enhancers gave a synergistic improvement in expression, demonstrated over a panel of different antibodies and with both early and late passage cells.

Using the engineered host, a Design of Experiments (DoE) approach was taken to further achieve target expression levels. Variables evaluated included transfection reagent, transfection conditions and general process parameters. This approach also allowed identification of key interactions between variables. Overall a ~100-fold improvement in transgene expression was observed compared with the wild type suspension cell line and the final process, and titres in the 1g/l range have been achieved.

Antibody product from the described transient system has been characterised and shown to be comparable to material from stable cell lines.

In summary, we have created a proprietary, scalable, suspension CHO transient expression system capable of producing industry leading titres of high quality protein.

## S.5 Cell Line-Specific Control of Recombinant Monoclonal Antibody Production by CHO Cells

Peter M. O'Callaghan[1], Jane McLeod[1], Leon P. Pybus[1], Clare S. Lovelady[1], Stephen J. Wilkinson[1], Andrew J. Racher[2], Alison Porter[2], David C. James[1]

[1]University of Sheffield, Sheffield, U.K. , [2]Lonza Biologics, Slough, U.K.

In this study we compare the cellular control of recombinant human IgG4 monoclonal antibody (Mab) synthesis in different CHO cell lines. Based on comprehensive empirical analyses of mRNA and polypeptide synthetic intermediates we constructed cell line-specific mathematical models of recombinant Mab manufacture in seven GS-CHO cell lines varying in specific production rate (qMab) over 350-fold. This comparative analysis revealed that control of qMab involved both genetic construct and cell line-specific factors. With respect to the former, all cell lines exhibited excess production of light chain (LC) mRNA and polypeptide relative to heavy chain (HC) mediated by more rapid LC transcription and enhanced LC mRNA stability. Downstream of this, cell lines differed markedly in their relative rates of recombinant mRNA translation, Mab assembly and secretion although HC mRNA abundance and the rate of HC translation generally exerted most control over qMab - the latter being directly proportional to qMab. This study shows that (i) cell lines capable of high qMab exceed a threshold functional competency in all synthetic processes, (ii) the majority of cells in parental and transfected cell populations are functionally limited and (iii) cell engineering strategies to increase Mab production should be cell line specific.

## S.6 Development of fluorescence-based PAT enabling tools for animal cell processes

Tiago M Duarte[1], Fabiana Fernandes[1,2], João Vidigal[1,2], Paulo Fernandes[1,2], Vanessa Bandeira[1], Carina Silva[1,2], Daniel Simão[1], Marcos Sousa[1], Carla Portugal[3], João P Crespo[3], Paula M Alves[1,2] and Ana P Teixeira[1,2]

[1]IBET, Oeiras, Portugal, [2]ITQB-UNL, Oeiras, Portugal, [3]Universidade Nova de Lisboa, Caparica, Portugal

Real-time cell culture monitoring is essential for the optimization and control of biotechnological processes. However, it is still largely limited to classical physicochemical parameters such as pH, dissolved oxygen and temperature. To improve the control options for bioprocesses, data from relevant biological variables need to be available in a real-time context. To this end, fluorescence- based techniques are non-invasive and able to capture information from multiple culture bulk components, being ideal for in-situ and real-time monitoring of cell culture processes. The fluorescence fingerprint of bioreaction bulks will include the contribution of different fluorophores and the effect of their interaction with fluorescence quenchers [1]. 2D fluorometry has long been applied to collect complete fluorescence signatures in microbial fermentations [1,2]. More recently, our group extended the application of 2D fluorometry to animal cell cultures [3], and compared its performance with a novel technique called synchronous fluorescence spectroscopy (SFS) [4]. SFS is able to provide data on multiple fluorophores in a single scan by keeping a constant interval between excitation and emission wavelengths, which can be recorded much faster than a complete map.

In the present study, our goal was to investigate the applicability of SFS to monitor several biotechnologically relevant cell lines used for the production of recombinant proteins, vaccines and viral vectors for gene therapy applications. Six cell lines growing in adequate culture media were followed over time by fluorescence and absorbance analysis. Each cell system presented a characteristic fluorescence fingerprint, and significantly different optical densities. Culture media with lower optical densities allow a more interpretable fluorescence profile over culture time. We will present results on the predictive power of SFS, coupled with absorbance measurements and chemometric tools, to monitor product titer and cell density in these different biological systems.

Acknowledgements: Funding from Fundação para a Ciência e a Tecnologia, Portugal (PTDC/EBB-EBI/102750/2008).

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# CELL ENGINEERING

## T.3.1 Chinese Hamster Ovary Cells Can Produce Galactose-α-1,3-Galactose Antigens: Considerations During Biologics Development

Carlos J. Bosques, Associate Director, Momenta Pharmaceuticals Research Affiliate, MIT , USA

CHO cells are widely used for the manufacture of biotherapeutics mainly because of their recognized ability to produce proteins with desirable properties, including "human-like" glycosylation profiles. For biotherapeutics production, control of glycosylation is critical since it can impact protein function, including half-life and efficacy. Additionally, specific glycan structures may adversely affect their safety profile. One of the best documented example of this is the terminal galactose-α-1,3-galactose (α-Gal) antigen due to high levels of anti-α-Gal antibodies present in humans. Available evidence to date indicates that while this is of concern for some biotherapeutics, specifically those produced in murine cell lines, it generally is accepted that CHO cells lack the biosynthetic machinery to synthesize glycoproteins with α-Gal epitopes. Contrary to this assumption, in this presentation we will discuss the identification of the active CHO ortholog of the N-acetyllactosaminide-3-α-galactosyltransferase-1, which is responsible for the synthesis of α-Gal in CHO. We further discuss the characterization of this antigen in a commercial protein manufactured in CHO and describe specific approaches to monitor and control the levels of α-Gal during biotherapeutic development.

## T.3.2 Cardiac Derivatives of Human Pluripotent Stem Cells in Drug Discovery and Disease

Christine Mummery[1], Douwe Atsma[2], Connie Bezzina[3], Cathelijne van den Berg[1], Stefan Braam[1], Simona Casini[1], Cheryl Dambrot[1,2], Richard Davis[1], Christian Freund[1], Robert Passier[1], Leon Tertoolen[1], Arthur Wilde[3]

[1]Dept. Anatomy and Embryology, Leiden University Medical Centre, [2]Dept Cardiology, Leiden University Medical Centre , [3]Dept Cardiology, Amsterdam Medical Centre, The Netherlands

Derivation of heart and vascular endothelial cells from human pluripotent stem cells (embryonic stem cells or HESCs and induced pluripotency stem cells or hiPS cells) is an area of growing interest both for potential cell therapy for the heart and as a platform for drug discovery and toxicity. Understanding the underlying developmental mechanisms that control differentiation of pluripotent cells to cardiac progenitors and their derivatives and mimicking these in defined culture conditions in vitro is now essential for moving the field forward. Culture conditions have now been sufficiently refined that cardiomyocyte and vascular differentiation is a fairly efficient and reproducible process. Genetically marked hESCs have been produced in which expression of the green fluorescent protein marker is expressed ubiquitously or driven by specific lineage markers. We have used these tagged lines to trace cardiomyocytes following transplantation into a mouse heart after myocardial infarction and select the progenitors of cardiomyocytes, endothelial cells and smooth muscle cells. Long term survival of the cells and integration into the host heart has been observed and early improvements in cardiac function although these are not sustained in our model. Cardiac repair using stem cell derived cardiomyocytes will likely require more than efficient cardiomyocytes production. More immediate applications of hESC- and hiPSC derived cardiomyocytes and vascular endothelial cells in drug discovery and disease are now close to implementation. Results of these studies, in particular drug responses of hESC-derived cardiomyocytes to a variety of cardiac and non-cardiac drugs and an hiPSC model for vascular disease in which Thalidomide has a therapeutic effect, will be shown. In addition, we show that iPSC derived cardiomyocytes with mutations in ion channel genes can accurately predict changes in cardiac electrical properties observed in primary cardiomyocytes despite being relatively immature.

## T.3.3 Integrated Strategies for Enhancing Yield and Quality of Recombinant Proteins in Chinese Hamster Ovary Cells

Ying Swan Ho, Bioprocessing Technology Institute, Singapore

Research at the Bioprocessing Technology Institute spans the generation of novel cell lines and biomolecules; optimisation of therapeutics production in prokaryotic and eukaryotic systems; expansion and characterization of stem cells; product purification and analysis; and profiling of bioprocesses using "-omics" technologies. Capabilities in these key research thrusts have been integrated for the production of both biopharmaceutical drugs and cells for therapy.

For enhancing recombinant protein production, IRES-based tricistronic vectors and anti-silencing strategies are currently being employed to enrich for high producing clones and to improve expression stability respectively. A panel of novel Chinese Hamster Ovary cell lines has been generated, including anti-apoptotic cell lines, as well as glycosylation mutants capable of producing non-sialylated or highly sialylated glycoproteins. We have also developed in-house proprietary protein-free chemically defined media and fed-batch strategies. Our "-omics" platforms enables the identification of gene targets for cell engineering and growth- or apoptosis-related metabolites for further media optimisation, leading to extended culture viabilities and improved product titres.

This presentation will provide snapshots of our research, with a focus on the design of tricistronic vectors and anti-silencing strategies, the generation of glycosylation mutant cell lines and the application of metabolomics to guide culture and media development.

# INDUSTRIAL WORKSHOP

## W.1 New tools to provide greater freedom and capability in mammalian protein expression

Henry Chiou, Life Technologies, USA

Life Technologies provides a broad range of molecular tools, reagents, services and instruments for life science research and development. Our latest developments in the area of mammalian protein expression include a new commercial cell line development platform with an innovative and simple commercial license option, incorporation of highly regarded GeneArt® gene synthesis capabilities into our expression services and development of a next generation transient expression system that significantly increases protein yields. The presentation will discuss these three areas:

Stable cell line development (CLD) is a critical phase of biotherapeutic development and manufacture. While several CLD platforms are widely marketed, they are costly to access. To enable everyone to perform stable CLD we have developed the Freedom™ CHO-S™ kitted platform. All components necessary to go from transfection to stable clone in 4 months are included in this kit. IgG titers achieved are ~1 g/L for an un-optimized simple glucose fed batch process and > 3 g/L when more complex nutrient feeds are employed. Research use rights are granted upon kit purchase. Commercial licensing is unprecedented and requires only a onetime fee.

Gene synthesis is an increasingly cost-effective method to obtain DNA constructs with 100% sequence accuracy. Using GeneArt® gene synthesis technology, nearly any gene sequence is quickly available and can be tailored to individual needs. Increased expression rates through gene optimization combined with advanced Life Technologies FreeStyle™ transient expression systems improves protein expression in terms of yield, reliability and production time. Starting with only the gene sequence, a custom protein can be available ready-to-use within 30 business days.

Life Technologies will debut a significant advance in 293-based transient expression systems early in 2012. The system is similar in configuration to current transient systems, but consists of a ground-breaking new Gibco medium for high density 293 culture, 293-F cells adapted to the new medium and a transfection kit for maximal transfection of high density cultures. Test in our hands, as well as by external alpha test sites have generated 2 to 10-fold higher protein yields per mL of culture transfected compared to current mammalian transient expression systems, exceeding 700 mg/L within 7 days in some cases.

**W.2 Production of proteins as therapeutic agents, research reagents and molecular tools frequently depends on expression in heterologous hosts.**

Louise Rafty, DNA2.0 Inc, USA

Synthetic genes are increasingly used for protein production because sequence information is easier to obtain than the corresponding physical DNA. Protein-coding sequences are commonly re-designed to enhance expression, but there are no experimentally supported design principles. This workshop addresses the recent development of novel gene redesign algorithms to ensure the synthetic genes encode high protein expression levels. We will also demonstrate the DNA2.0 developed free software application, Gene Designer. Gene Designer has been created from the ground up to help scientists create genes with the aid of a graphically-rich software tool that enables bio-engineers to easily manipulate and visualize DNA elements such as promoters, terminators, fusion tags and vector components. DNA2.0 (www.DNA20.com <http://www.DNA20.com> ) is the leading synthetic genomics company and the fastest provider of synthetic genes, based in the US with a global customer base encompassing industry, academia, and government. DNA2.0 has provided genes to thousands of customers, for whom it has synthesized many millions of base pairs. The tools and applications brought to market by DNA2.0 are transforming biology into an engineering discipline.

## W.3 Maximizing protein expression in suspension CHO cells

Scott Hayes, PhD, Mirus Bio LLC, Madison, WI, USA

Transient transfection allows drug discovery researchers to bridge the development bottleneck and shorten the time to usable protein. High efficiency methods for mammalian protein production are becoming essential tools to facilitate the production of biotherapeutic proteins with proper post-translational modifications. CHO suspension cells are used for protein expression, despite being refractory to commonly used transfection methods (e.g. linear PEI). Mirus Bio has developed a more effective transfection method for CHO cells, the TransIT-PRO™ transfection reagent. Maximum levels of transient expression are achieved when an effective delivery reagent is combined with optimized transfection parameters and growth conditions. The TransIT- PRO transfection kit uses animal origin free components designed for high and reproducible yield in suspension CHO cells across varied media formulations.

## W.4 A new plug-and-play disposable insect cell based protein production system

Wian de Jongh[1], Frank van Dongen[2]

[1]Expres2ion Biotechnologies, Hørsholm, Denmark, [2]CELLution Biotech B.V., The Netherlands

In this workshop we will introduce a new easy to use protein production system combining the ExpreS2 constitutive insect cell expression system with the CELL-tainer® disposable bioreactor technology. This solution allows for rapid production of complex proteins at 1.5L to 10L scale.

The CELL-tainer® is a single-use bioreactor with a 2-dimensional movement of the bag. This unique movement enables extremely efficient mass transfer and exceptional control over the culture. Built–in pH and dissolved oxygen (DO) censors can be employed for detailed process development, alternatively pre-saved parameters can be used to run the process without the need for a bioreactor expert.

The ExpreS$^2$ Drosophila insect cell expression system has been developed over the past 10 years for expression of complex proteins. We have run up to phase II clinical trials using the technology and shown it to be both a safe and efficient production system. The cells are robust, and easy to work with: No special $CO^{\neg}_2$ or humidified incubators are required and the cells grow at room-temperature. The system is simple to scale-up as the cells can be diluted over a wide range of cell densities and grow well between 1.5L and 10L scale in the CELL-tainer®.

Combining the two technologies we have been able to establish parameters which allows for batch production runs without the need for monitoring pH or DO. The parameters can be pre-loaded into the CELL-tainer® and allow for rapid 10L scale protein production. The ExpreS2 system allows for the use of stable polyclonal pools for protein production, which greatly reduced timelines and allows for up to 10L production runs in as little as six week after transfection.

Finally, we will use the Malaria protein antigen based vaccine currently under development using the new system as a case study to highlight the specific advantages of the combined system.

# ENVIRONMENTAL CONTROLS / BIOPROCESSING

## T.4.1 The oxidative protein folding pathway of the endoplasmic reticulum: a target for cell engineering?

Robert Freedman, University of Warwick, UK

The great majority of high-value proteins produced by expression in animal cells are secretory or cell-surface proteins containing disulphide bonds and other post-translational modifications that are introduced during the passage of the proteins through the secretory pathway. In such proteins, folding to the native conformation and the formation of correct disulphide bonds are inter-connected events that take place early in the secretory pathway, within the lumen of endoplasmic reticulum (ER). This process of 'oxidative folding' is crucial to the production of functional proteins for export, and the ER quality control system ensures that only correctly-disulphide-bonded and –folded proteins exit from the ER en route for secretion.

There is not yet an adequate 'whole system' understanding of the folding, modification and export machinery of animal cells, so the key steps which have greatest influence on yield and productivity have not been determined. Nevertheless, it is widely recognized that the capacity and efficiency of the oxidative folding system in cells may be critical factors in determining the productivity of those cells in generating secretory protein products. Hence several attempts have been made to determine and manipulate the cellular content of key elements of the cellular oxidative folding machinery initially focussing on protein disulphide-isomerase (PDI) the key catalyst and chaperone ensuring formation of correct disulphide bonds. Apparently there has been less effort in engineering the other crucial component of the oxidative folding machinery, the ER oxidases (Ero proteins) that transfer oxidising equivalents from molecular oxygen to PDI.

Early studies in yeast (S. cerevisiae) showed that genetic engineering to overexpress mammalian PDI or endogenous yeast PDI led to substantial increases in yield of heterologous disulphide-bonded secretory proteins (Schultz et al (1994) Ann NY Acad Sci 721 148-157). This finding is now exploited in the commercial production in yeast of human blood proteins (Finnis et al (2010) Microb Cell Fact 9 87). In a recent review of methods for improving S. cerevisiae as a host for high-value protein expression (Idiris et al (2010) Appl Microbiol Biotech 86 403-417), it was concluded that engineering the ER folding machinery was a prime target and the greatest benefit from a single intervention came from increasing the level of PDI.

This provides encouragement for attempts to engineer comparable improvements in animal cells. However, recent findings have emphasized the significant differences between mammalian cells and yeast in their oxidative folding machinery. In particular, mammalian cells have more complex processes for exploiting the oxidative ability of H2O2 and a considerably greater variety of members of the PDI family than does S. cerevisiae. The paper will review current knowledge of the oxidative folding machinery of mammalian cells and the opportunities for cell engineering to increase oxidative folding capacity.

## T.4.2 Peaceful cell culture processes with real time process monitoring from seed to production

Govind Rao[1], Kurt Brorson[2], Bhargavi Kondragunta[1], Shaunak Uplekar[1], Antonio Moreira[1], Jose Vallejos[1]

[1]Center for Advanced Sensor Technology, [2]Food and Drug Administration, USA

Despite advances in cell and molecular biology, cell culture process development remains as much an art as a science. In particular, early stage cell culture relies on simple,uninstrumented vessels such as T-flasks, petri dishes and spinner flasks generally referred to as process scouting devices (PSD). At the later lab scale bioreactor and above, vessels are generally equipped with pH and oxygen sensors.

In this presentation, we examine cases when PSD equipped with oxygen and pH sensors are monitored and the value of the information obtained. We will propose a cryovial-to-production paradigm for the evolution and scale up of a bioprocess.

## T.4.3 High-end pH-Controlled Delivery of Glucose Effectively Suppresses Lactate Accumulation in CHO Fed-Batch Cultures

Gregory Hiller[1], Matthew Gagnon[1], Yen-Tung Luan[1], Amy Kittredge[2], Denis Drapeau[1]

[1]Pfizer, Inc., Andover, MA , [2]EMD Millipore, Bedford, MA

Lactate accumulation, which can inhibit growth and cellular productivity, has been a significant problem in mammalian cell culture since methods to grow mammalian cells in vitro were first developed. A simple method for control of lactate accumulation in suspension cultures of Chinese Hamster Ovary (CHO) cells based on the culture's pH was developed. When glucose levels in culture reach a low level cells begin to take up lactic acid from the culture medium resulting in a rise in pH. We will describe a nutrient feeding method that has been optimized to deliver a concentrated glucose solution triggered by rising pH. Data will be presented which shows that this high-end pH-controlled delivery of glucose can dramatically reduce or eliminate the accumulation of lactate during the growth phase of a fed-batch CHO cell culture at both bench scale and large scale (2,500-liter). The method has proven applicable to the majority of CHO cell lines producing monoclonal antibodies and other therapeutic proteins. Using this technology in combination with other techniques to enhance a 12-day fed-batch process that already incorporated very high initial cell densities and highly concentrated medium and feeds resulted in an approximate doubling of the final titers for eight cell lines. The increase in titer was due to additional cell growth and higher cell specific productivity. The technology has been used to dramatically increase the productivity for two processes producing two therapeutic proteins for clinical trials. Process performance in terms of increased cell growth, titer increases, and product quality will be presented for the improved processes for the two campaigns at the 2,500-liter scale and compared directly with the original processes previously run at the same scale.

## T.4.4 Pneumatic Mixing Technology for Single-Use Bioreactor Application: A Comparative Analysis of Consistency Across Scale

Brian Lee[1], Manuel Carrondo[2], Matthew Croughan[3], David Fang[4] and Sang-Hoon Paik[5]

[1]PBS Biotech Inc., United States, [2]Instituto de Biologia Experimental e Tecnologica (IBET), [3]KECK Graduate Institute, [4]Systems QbD, [5]Green Cross Central Research Center

Challenges of cell culture process scale up are to maintain the consistency of microenvironment within bioreactors while the vessel sizes increase. Fast mixing and high gas mass transfer rates in bioreactors are often obtained by applying high agitation and gas flow rates, which also increase shear stress levels. A novel pneumatic mixing technology has been developed using an air-wheel mechanism which converts buoyancy of gas bubbles to rotational energy achieving an efficient liquid and gas mixing. The mixing system is scalable from 2L to 5,000L with fast mixing times of 20 to 60 seconds using comparable gas flow rates that are used with stainless steel bioreactors. High mass transfer rate (20 $hr^{-1}$ kLa) was obtained using a micro-sparger. Shear stress ($\tau_{avg}$, in Pa) and turbulent kinetic energy dissipation rate ($\varepsilon$, in $m^2/s^3$) of the system were calculated from computational fluid dynamics (CFD) modeling on Star CCM software and compared to stirred-tank bioreactors at respective working volumes. The average level of shear stress of the pneumatic mixing system (<0.3 Pa) is significantly lower than the conventional stirred bioreactors (1.0–2.4 Pa) and remains constant during scale up from 3L to 2500L. Similarly, constant levels of turbulent kinetic energy dissipation rates ($\varepsilon$, <0.01 $m^2/s^3$) over the same broad range of working volumes indicate more homogeneous environment within the system during scale up. The pneumatic mixing system operates without any external mechanical mixing device, which makes the bioreactor design simple and compact and ideal for single-use bioreactor applications. Cell culture performance of the single-use pneumatic bioreactor system (PBS) was evaluated using CHO cells with the 2L, 50L, and 250 L working volumes and compared to the results with stirred bioreactors. Comparable peak cell densities up to 107cell/mL with > 95% viability and similar monoclonal antibody productivity were achieved in both bioreactor systems. Final purification yield and product quality profiles were also comparable between the materials generated from the bioreactor systems. In addition, baculovirus expansion in Sf9 insect cells expressing enhanced green fluorescent protein (eGFP) and adenovirus expansion in A549 human lung adenocarcinoma cells grown on micro-carrier's have been tested in a 3L PBS and the results will be discussed. In conclusion, PBS offers more homogenous mixing with lower shear stress than the conventional bioreactors and may have potential applications to grow shear-sensitive cells at various scales for viral expansion, cell therapy, and cultivating adherent cells on micro-carriers in addition to the typical suspension cell culture processes.

## T.4.5 Establishing production cell lines for structural biology by targeted integration and cell sorting

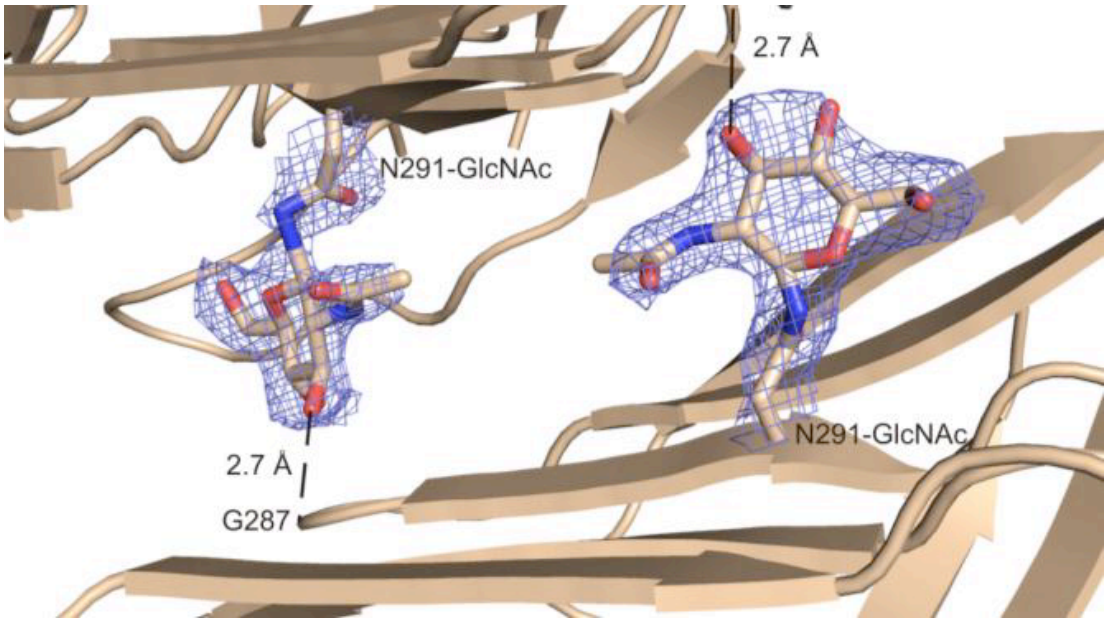
Sonja Wilke, Konrad Büssow[1]

[1]Helmholtz Centre for Infection Research, Braunschweig, Germany

Objectives: Crystal structures of glycoproteins are indispensable for understanding the function of these important molecules and for targeting them with pharmaceuticals. Glycoprotein crystallization benefits from protein production with mutated cell lines that add smaller, more homogeneous glycans to secreted proteins.

Results: We introduce here a new approach to produce glycoproteins with the well-established, glycosylation mutant CHO Lec3.2.8.1 cells. Using preparative cell sorting, stable, high-expressing GFP 'master' cell lines were generated that can be converted fast and reliably by targeted integration via Flp recombinase-mediated cassette exchange (RMCE) to produce any glycoprotein. Small-scale transient transfection of HEK293 cells was used to identify genetically engineered constructs suitable for constructing stable cell lines. Stable cell lines expressing 10 different proteins were established.

Conclusion: The system enabled expression, purification, deglycosylation and crystallization of luminal domains of lysosome-associated membrane proteins (LAMP), leading to the first crystal structure of this protein family.



# ALTERNATIVE EXPRESSION SYSTEMS

## T.5.1 Synthetic Antibodies: New Tools for New Biology

Sachdev Sidhu, The University of Toronto, Toronto, Canada.

Over the last decade, therapeutic monoclonal antibodies represent one of the major breakthroughs for the treatment of cancer and other diseases. To date, most therapeutic antibodies have been obtained by the humanization of rodent-derived antibodies, but in recent years, research in antibody engineering has given rise to a new wave of technologies that promise to transform the field. Phage-displayed libraries of "synthetic antibodies" use entirely man-made antigen-binding sites and thus circumvent the need for natural immune repertoires. Using in vitro selections, highly functional antibodies with fully human frameworks can be generated against virtually any antigen in a matter of weeks. Access to the encoding DNA allows for rapid affinity maturation, fine tuning of specificity and recasting into different molecular formats. We have developed particularly simple synthetic antibodies that use a single human framework and limited chemical diversity in restricted regions of the antigen-binding site. These structural simplifications enhance the performance of the libraries, which have yielded highly functional and stable antibodies against numerous diverse antigens. These libraries are capable of fulfilling all of the roles of natural antibodies, and furthermore, they extend the use of antibody technologies to many challenging problems, such as the recognition of conformational changes, post-translational modifications, structured nucleic acids and integral membrane proteins. Moreover, the recombinant nature of synthetic antibodies makes them ideal reagents that can be used as crystallization chaperones to aid the elucidation of structures for complex antigens.

## T.5.2 LEXSY: Eukaryotic Protein Expression in Leishmania tarentolae

Reinhard Breitling, Jena Bioscience GmbH, Jena, Germany

Leishmania tarentolae is a safe (biosafety group S1), robust and easy-to-culture protozoan organism with the full capacity for eukaryotic protein folding and modification including mammalian-type post-translational glycosylation. We have developed this organism into the flexible and efficient protein expression platform called LEXSY. Recombinant proteins can be expressed intracellularly or be secreted into the cultivation medium. Both, constitutive and inducible expression architectures are available. Protein expression can be monitored online during cultivation by a transcriptionally coupled fluorescence marker. Numerous proteins, including enzymes, surface antigens, toxins, antibodies and membrane proteins have been expressed with LEXSY. Expression yields of up to several hundred mg per liter of culture were obtained, and the purified proteins were successfully employed for diagnostics and R&D including structure determination by NMR and X-ray crystallography. LEXSY has recently been complemented by a version for in vitro translation for cell-free expression of recombinant proteins.

## T.5.3 GSK's cervical cancer vaccine manufactured with the baculovirus expression vector system (BEVS)

Johanne Deman, GSK, Belgium

Cervical cancer is the second most common type of cancer in women worldwide. Persistent infection with an oncogenic human papillomavirus (HPV) type is a necessary cause for the development of cervical cancer. HPV-16 and HPV-18 are oncogenic HPV types that are responsible for ~70% of cervical cancer cases worldwide. To prevent cervical cancer, GlaxoSmithKline Biologicals (GSK) has developed the prophylactic HPV-16/18 vaccine Cervarix®.

Cervarix® vaccine contains the HPV-16/18 L1 proteins that are produced using the baculovirus expression vector system (BEVS), and then purified to form the VLPs. The production of heterologous proteins using the BEVS technology is based on the introduction of a foreign gene into a non-essential region of the baculoviral genome via homologous recombination. The resulting recombinant baculovirus is then used to infect insect cell cultures and produce the protein of interest. This expression system is able to generate complex recombinant proteins with the correct folding since it allows disulfide bond formation, oligomerization and other important post-translational modifications. Consequently, the recombinant protein produced with BEVS should exhibit its expected biological activity and function. Although BEVS-based recombinant subunit vaccines are already available for veterinary applications and proteins produced using BEVS technology have been safely administered in clinical trials investigating other candidate vaccines,GSK's HPV-16/18 cervical cancer vaccine is the first registered human vaccine using this technology.

## T.5.4 Zera® fusions and StorPro®: a versatile expression technology bridging the gap between animal cells and other eukaryotic hosts

Stefan Schmidt, ERA Biotech, Spain

Objectives:
Protein bodies (PBs) are natural endoplasmic reticulum (ER) or vacuole plant derived organelles that stably accumulate large amounts of storage proteins in seeds. The proline rich N-terminal domain derived from the maize storage protein γ zein (Zera) is sufficient to induce PBs in non-seed tissues of Arabidopsis and tobacco. This Zera property opens up new routes for high-level accumulation of recombinant proteins by fusion of Zera with proteins of interest. In this study we extend the advantageous properties of plant seed PBs to recombinant protein production in useful non-plant eukaryotic hosts including cultured fungal, mammalian and insect cells.

Methods:
Protein expression and accumulation is verified by electrophoretic and blotting methods. The density of the PBs is analysed by density gradient centrifugation. Proper functionality of the proteins is confirmed in multiple activity assays.

Results:
Various Zera fusions with fluorescent and therapeutic proteins accumulate in induced PBs, the so called StorPro organelles, in all eukaryotic systems tested: tobacco leaves, rice seeds, Trichoderma reesei, several mammalian cultured cells, insect cells, Pichia Pastoris and Microalgae. This accumulation in StorPro organelles insulates both recombinant protein and host from undesirable activities of either. Recombinant protein encapsulation in these PBs facilitates stable accumulation of proteins in a protected subcellular compartment which results in an enhancement of protein production without affecting the viability and development of stably transformed hosts. The induced PBs also retain the high-density properties of native seed PBs which facilitate the recovery and purification of the recombinant proteins they contain.

Conclusion:
The Zera sequence provides an efficient and universal means to produce recombinant proteins by accumulation in ER-derived organelles. The remarkable cross-kingdom conservation of PB formation and their biophysical properties has a broad application in the manufacture of non-secreted recombinant proteins and suggests the existence of universal ER pathways for protein insulation.

# EXPRESSION SYSTEM DEVELOPMENT

## T.6.1 Technology Drivers in the Development of Animal Cell Processes

John Birch, Henley Biotechnology Consultancy, UK

More than half of all recombinant therapeutic proteins are produced in mammalian cell culture systems. The demand for these proteins, and particularly for monoclonal antibodies, has been a major driver for improvements in technology, particularly in the areas of expression technology, fed-batch culture, and process scale-up. This talk will look at some of the key advances that have contributed to modern manufacturing processes, and will consider future trends in products and in process science that will continue to drive innovation in the manufacture of biological products.

## T.6.2 Transient Gene Expression: Gambling with Approaches for Success

Sabine Geisse, Novartis Institutes for BioMedical Research, Basel, Switzerland

Huge efforts are currently invested in the development of therapeutic antibodies in various formats. In order to be successful, these approaches necessitate upfront the production of antigen proteins which should be correctly folded, biologically active and meet high quality standards with respect to biochemical and biophysical parameters. Moreover, these proteins should be readily available in sufficient quantity to initiate immunizations or display technologies as well as the implementation of biological assays.

Transient protein expression in mammalian cell systems has provided a fast route to recombinant protein production, in particular for secreted and membrane bound molecules which are the major targets for intervention by neutralizing antibodies. A plethora of publications describe the transient production of proteins in HEK293 cell lines and, more recently, also in CHO cells on medium to large scale. Yet, novel target proteins are often not well characterized, have not been produced recombinantly or may be of huge size or complex nature, precluding a straightforward strategy of vector design, cloning, expression and subsequent purification.

This presentation covers different approaches to transient protein expression in HEK293 and CHO cell lines with particular focus on difficult-to-express antigens. Modification of expression vector, exchange of leader sequences, and in case of receptors the length of extracellular domain to be incorporated in the expression plasmid will be discussed. Choosing the right boundaries for expression of protein domains for epitope mapping is another important issue, as is the inclusion of a detection/purification tag at the N- versus C-terminus of the gene. Co-expression of chaperones to facilitate secretion can be mandatory in some cases. Likewise, proteolytic instability of some protein candidates may necessitate an elaborate expression process with short run time and close monitoring. Lastly the impact of the individual host cell line on titers, the quality and the biological activity of a candidate protein will be addressed.

In summary, the presentation will cover various critical aspects of antigen production by examples highlighting their crucial importance to successful antibody development.

## T.6.3 Lessons Learned From Platform Antibody Production

Andy Lin, Early Stage Cell Culture, Genentech Inc., USA

Recombinant monoclonal antibodies are well established therapeutics for a wide range of clinical indications. Due to antibodies' generally favorable stability during production, many manufacturers have established platform processes from stable cell line generation to drug product formulation in order to expedite development and save resources. Over the past decades, Genentech has continually improved our version-controlled cell culture platform processes to gain efficiency from early drug development to licensure. The platform processes begin at the antibody candidate selection stage and continues with the use of optimized expression systems that incorporate stringent serum-free selection conditions and chemically-defined medium for production. We will focus in this talk on the integration of antibody candidate selection and the generation of highly productive stable clones. The strategy has evolved over the years and now includes the use of transient transfection generated antibodies for manufacturability assessment, providing valuable information to select for the desired properties in the antibody drug candidates to reduce the chance of delays during clinical development. We will also describe our workflow evolution to demonstrate that shortened stable cell line generation timeline can facilitate acceleration to first-in-human studies.

## T.6.4 Influenza virus production: characteristics of different producer cell lines

Y. Genzel[1], D. Vester[1], B. Isken[1], J. Rödig[1], E. Rapp[1], V. Lohr[1], I. Behrendt[1], K. Scharfenberg[2], A. Kamen[3], G. Schiedner[4], I. Jordan[5], U. Reichl[1,6]

[1]Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany , [2]University of Applied Sciences Emden / Leer. Fachbereich Technik, Emden, [3]Animal Cell Technology, Montreal, Canada, [4]CEVEC Pharmaceuticals GmbH, Köln, Germany , [5]ProBioGen AG, Berlin, Germany, [6]Otto-von-Guericke University Magdeburg, Magdeburg, Germany

With the change from egg-based influenza virus production to cell culture-based production, various cell lines are currently evaluated for virus productivity and process performance. Suspension as well as adherent cell lines are candidates, both having advantages and disadvantages for the respective process. For an adequate comparison, similar process conditions and a systematic evaluation of pros and cons based on validated assays are helpful.

Here, we present a comparison of two adherent cell lines (MDCK, Vero) and five suspension cell lines (MDCK. SUS2, AGE1.CR, AGE.CR.PIX, HEK293, CAP 1D5). The cells were cultivated in different cultivation systems including roller bottles, shaker flasks, small scale stirred tank reactors, and wave bioreactors. Different cultivation strategies were investigated (batch, with medium exchange, without medium exchange, perfusion). Cells were cultivated in their appropriate medium, and infected with several influenza virus strains using different infection conditions (MOI (multiplicity of infection), trypsin concentration). Data on metabolism, cell growth and virus titer but also on infection status, virus-induced apoptosis, glycosylation and proteome will be discussed. These studies clearly indicate that a simple screening of cell lines would not lead to satisfactory results concerning optimum process conditions. In particular, each cell line requires an adaptation to process conditions and can then produce most virus strains in acceptable titers. It remains however unclear, why some virus strains cannot be produced by specific cell lines. Therefore, it seems to be advisable to have several alternatives in case of a pandemic threat.

### T.6.5 Strategies for Expression of Multi-component Protein Complexes in Sf9 Cells

Stephanie Chen, Juan Wang, Linda Myers, Yong Jiang, Ruth Lehr, Elsie Diaz, Leng Nickels, Guofeng Zhang, Benjamin Schwartz, Thomas Sweizter, Sharon Sweitzer

GlaxoSmithKline, USA

The use of baculovirus for expression of recombinant proteins in insect cells has become commonplace. It offers the ability to generate a significant amount of protein with the proper post-translational modifications and folding. While mammalian systems offer these same advantages, generation of mammalian cell lines is labor intensive and expensive when compared to baculovirus expression. Use of baculovirus infected insect cells (BIIC) significantly improves consistency in protein production without the need for a stable system to be generated. Another advantage to using baculovirus is the ability to add two or more viruses encoding different genes to co-express proteins to aid in protein stabilization or form heterogeneous dimers. We have taken co-expression to another level and explored the possibility of using multiple viruses to generate multi-component protein complexes in insect cells. We have investigated the impact of virus ratio and tagging strategies on different subunits on the expression levels, protein quality upon purification, and activity of the resultant complex and have successfully generated up to a 9 component complex with functional activity.

# POSTER PRESENTATIONS

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## P.1 Culture and transient transfection of Sf-9 cells in TubeSpin® bioreactor 50 tubes

Xiao Shen[1], Patrik O. Michel[1], Qiuling Xie[1,2], David L. Hacker[1], Florian M. Wurm[1]

[1]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [2]Jinan University, Guangdong, China

Spodoptera frugiperda Sf-9 insect cells have been widely used for recombinant protein production. Generally, the cells are grown in suspension in either spinner flasks or Erlenmeyer flasks. For gene delivery, infection with a recombinant baculovirus vector or transfection with high-cost liposome-based reagents are commonly used. Here we present TubeSpin® bioreactor 50 tubes (TubeSpins) as a simple, low cost, and highly efficient culture system for the growth and transfection of Sf-9 cells. Sf-9 cells have a considerably better growth performance in TubeSpins than in spinner flasks. Cells were inoculated in the two culture systems at 1 x $10^6$ cells/mL and reached maximal cell densities of 16 x $10^6$ cells/mL in TubeSpins and 6 x $10^6$ cells/mL in spinner flasks. 5-10 mL of culture volume is sufficient in TubeSpins. The cell viability in these batch cultures remained above 90% for 10 days in TubeSpins, whereas a viability drop was observed already after 4 days in spinner flasks. Infection with a recombinant baculovirus coding for green fluorescent protein (GFP) was performed with Sf-9 cells grown in TubeSpins, spinner flasks or Erlenmeyer flasks. All cultures resulted in similar GFP-specific fluorescence levels. Sf-9 cells in TubeSpins were transfected with a plasmid encoding the GFP gene under the control of the baculovirus IE-1 promoter using polyethylenimine (PEI) as the DNA delivery vector. Transfection efficiencies up to 58% were observed. In separate transfections with a plasmid encoding the tumor necrosis factor receptor:Fc fusion protein (TNFR:Fc), volumetric productivity of 90 mg/L was achieved in 5-day cultures. This study highlights the use of TubeSpins for the cultivation and PEI-mediated transfection of Sf-9 cells in suspension. Efforts for further scale-up in orbitally shaken bioreactors are underway.

## P.2 Rapid, high throughput production of human proteases using baculoviruses

Rene Assenberg, Ingrid Bechtold, Kathrin Dresen, Sonia Mechehat, Bruno Martoglio, Lorenz Mayr and Paul Ramage

Novartis Pharma AG, Switzerland

Proteases form an important class of therapeutic targets, yet production of human proteases can be challenging due to the often complex nature of the protease in question. To address this, we have developed a high throughput expression pipeline for the production of human intra- and extracellular proteases for assay development and biophysical studies for drug discovery, using baculoviruses. The methodology is based on multi-well culturing coupled to small scale affinity purification, and can be readily adapted to the localization of the protease (intra- or extracellular, membrane bound or not) and to producing protease complexes. Several examples of successful expression screening, scale-up and purification will be presented of different types of proteases, including complex-forming and secreted (glyco)proteases to illustrate the general applicability. In addition, we are evaluating several novel approaches for improving the production of (secreted) proteases (flashbac bacmids and the pOET3 vector).

**P.3 Extracellular lactate depletion removes metabolic inhibition by rapamycin while sustaining rapamycin-induced over-expression of a recombinant human virus trans-membrane fusion protein in HighFive by BEVS**

Marco Patrone, Nuno Carinhas, Margarida Archer and Paula Alves

Universidade Nova de Lisboa, Oeiras Portugal

Human cytomegalovirus (HCMV), the leading infectious cause of congenital neurodevelopmental defects and a common source of life-threatening complications in transplantation, is in the priority list of U.S. National Institute of Medicine Vaccine Development Strategy since 2000. As being the trigger of viral invasion, HCMV virion fusion factor gB protein is an attractive target for both vaccination and novel anti-viral chemotherapy strategies. A recombinant AcMNPV (rBstII) has been constructed to express in HighFive cells a fusion competent full-length HCMV gB for target development and structure-function studies. Initial attempts to express this trans-membrane protein by BEVS were frustrated by low-level of expression. Rapamycin, a natural toxin inhibiting cap dependent translation in animal cells, was found to increase substantially gB expression. Rapamycin-treated infected cells were atypical as they did not display a size increase. Subsequent analysis revealed that rapamycin strongly inhibits the net consumption of Glutamine (Gln) and Glucose (Glc) from the culture medium. In parallel, a system for extracellular depletion of lactate, based on the enzyme Lactate oxidase, has been developed and proven to increase both Gln and Glc utilization by HighFive cells infected with rBstII. Though modest, lactate depletion itself also improved gB expression. Nevertheless, the combination of rapamycin treatment and lactate depletion had a synergistic effect by restoring nutrient consumption and cell swelling, and supporting gB over-expression.

**P.4 Generation and presentation of membrane protein targets using baculovirus surface display**

Monika Wilde, Miriam Klausberger, Dieter Palmberger, Reingard Grabherr

University of Natural Resources and Life Sciences, Vienna, Austria

Membrane proteins are implicated in essential cellular processes such as signal transduction, transport and cell-to-cell interactions. Approximately 30% of the human genome is devoted to membrane proteins although accounting to their low abundance, hydrophobic nature and heterogeneity are often underrepresented in proteome profiles and hence are difficult to study. Their fundamental role is further emphasized as 70% of all known pharmaceutical targets are represented by this specific protein subset.

The Baculovirus expression system has been widely used for heterologous protein expression and has gained interest as a tool for various surface display applications. The eukaryotic protein processing machinery, well established cloning techniques and possibility to accommodate large insertions of foreign DNA, as well as rapid growth have made the baculovirus-insect cell expression system attractive for display and protein-protein interaction studies. Here, we are suggesting an alternative approach based on this system: the incorporation of human membrane proteins of therapeutic relevance (CD16, CD20 and epidermal growth factor receptor EGFR) into the budded baculovirus surface, based on their native transmembrane. Baculovirus particles display a lipid bilayer membrane spiked with the specific envelope protein gp64 as well as other proteins present in the cellular membrane, e.g. influenza A virus hemagglutinin when being co-expressed. By over-expression of target membrane proteins and down-regulating the abundance of gp64 we are aiming at the generation of virions that are suitable for the display of membrane proteins that serve as a platform for protein-protein interaction studies. Thus, our preliminary goal is to design and produce baculovirus particles displaying decreased amounts of gp64 and to test their suitability for co-displaying foreign membrane bound proteins. Four insect cell lines (Ao38, High Five, Mimic, Sf9) were infected with the baculovirus I-SceI omega lacZ AcMNPV, encoding the ß-galactosidase in order to correlate the absolute amount of gp64 to the number of virus particles. After infection respective supernatants were subjected to SDS-PAGE, Western-Blot, Lectin-Blot and Nano-particle tracking analysis (NanoSight LM20, Amesbury, United Kingdom). Altogether results indicated a smaller proportion of endogenous gp64 envelope protein in Ao38 and High Five cell lines constituting the basis for further studies.

**P.5 Influenza virus-like particles as antigen carrier platform for the display of epitopes**

Miriam Klausberger, Monika Wilde, Dieter Palmberger, Florian Krammer, Reingard Grabherr

University of Natural Resources and Life Sciences, Vienna, Austria

Virus-like particles (VLPs) are nanostructures consisting of viral structural proteins arranged in a highly repetitive multivalent format. As VLPs resemble infectious viruses in structure, they are highly immunogenic, inducing vigorous innate and adaptive immune response to antigens exposed on the virus particle surface. Due to their immunogenic properties, VLPs are promising candidates for vaccine strategies in serving as versatile scaffold for the delivery of a wide range of vaccine antigens, especially of epitopes that are normally difficult to invoke an immune response. The Baculovirus/insect cell system (BV/IC) has been proven to be a potent production system for the expression of multiprotein complexes, such as VLPs.

The objective of this research project is the utilisation of influenza VLPs as an antigen-carrier platform for the presentation of antigenic determinants, such as a peptide mimic of the human high molecular weight melanoma-associated antigen (HMW-MAA). Potential insertion sites of the immunodominant antigenic sites A and B in the globular head domain (HA1) on the influenza hemagglutinin (HA, H3 subtype) were tested and compared in terms of insertion capacity, efficiency in epitope display and influence on formation of intact particles. Characterisation was done by transmission electron microscopy, Western Blot, Hemagglutination Assay and ELISA. Randomisation of adjacent residues will serve to fine- tune and optimise the microenvironment of the epitope within the HA framework based on a library approach using the baculovirus insect cell expression system.

Two different insect cell lines, High Five and Ao38, were characterised in respect to VLP yield, shape, and baculovirus background. Purified VLPs were analysed using chemical and biophysical methods such as Western blots, transmission electron microscopy, nanoparticle tracking analysis (NanoSight LM20, Amesbury, United Kingdom) and ELISA. Next steps include immunological testing of purified VLPs in a mouse model to elucidate the specific humoral immune response directed toward the presented epitope on the VLP carrier.

## P.6 Insect cell-based Influenza vaccine bioprocess development

Monteiro, F.[1], Carinhas, N.[1], Peixoto, C.[1], Sousa, M.F.[1], Saelens, X.[2],Carrondo, M.J.T.[1,3]
Bernal, V.[4] and Alves, P.M.[1]

[1]Universidade Nova de Lisboa/Instituto de Biologia Experimental e Tecnológica (ITQB-UNL/IBET), Oeiras, Portugal, [2]Universiteit Gent, Gent, Belgium, [3]Universidade Nova de Lisboa (FCT-UNL), Caparica, Portugal, [4]Universidad de Murcia, Murcia, Spain.

The Insect Cell-Baculovirus Vector Expression System (IC-BEVS) is a powerful protein production platform with high potential in vaccinology, used for the production of a human papilloma virus (HPV) vaccine, recently approved by FDA (CervarixR, GlaxoSmithKline Biologicals) and an Influenza vaccine (FluBlockR, Protein science) that conducted Phase III clinical trials and is under revision for FDA approval.

In this work, IC-BEVS was used for the production of a recombinant tetrameric neuraminidase (rNA) catalytically active and, thus, respecting the correct folding and assembly of all its components. To improve the quantity and quality of the final product, an endogenous secretion signal from Influenza hemagglutinin and a leucine zipper (GCN4) were cloned together with the NA sequence. In a first stage cellular concentration at the moment of infection (CCI), multiplicity of infection (MOI) and time of harvest (TOH) were analysed. Cultures infected with high multiplicity of infection outperformed those infected at low MOI, with specific productivities raging from 20 to 30 Units/106 cells depending on the CCI (1 and 3, respectively). rNA secretion ratios are higher than 90%, when comparing the proportion of enzymatically active protein recovered from the supernatant with the intracellular fraction, being maximum at CCI 3 and MOI 5 attaining 98% of secreted active enzyme. Also, a mammalian based process for the production of rNA was co-evaluated. 293T cells were transfected with different concentrations of a plasmid coding for rNA (3, 5 and 8 μg DNA/$10^6$ cells). The specific productivities between the culture schemes were similar, raging from 0,01 to 0,025 Units/$10^6$ cells. These results highlight the outstanding performance of IC-BEVS system regarding the production of enzymatically active rNA. In parallel, a membrane-based purification process based on anion exchange and affinity is under implementation. Ultimately we hope to establish a high-producing, cost-effective robust and scalable bioprocess for the production of an Influenza vaccine candidate using baculovirus technology.

Acknowledgments: The authors would like to acknowledge Project BACULOME (PTDC/EBBEBI/103359/2008) and Fundação para a Ciência e a Tecnologia (FCT, PhD fellowship SFRH / BD /70139 / 2010 to F. Monteiro and SFRH/BD/36676/2007 to N. Carinhas).

**P.7 Chikungunya virus subunit vaccine candidates produced in insect cells**

Stefan W Metz, Corinne Geertsema, Just M Vlak & Gorben P Pijlman

Wageningen University, Wageningen, The Netherlands

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus (family Togaviridae) that causes large epidemics in Africa, South-East Asia and India. Recently, CHIKV has been transmitted to humans in southern Europe by invading but now established colonies of Asian tiger mosquitoes. To develop a CHIKV subunit vaccine, his-tagged E1 and E2 envelope glycoproteins were produced at high levels in Sf21 insect cells using baculovirus expression vectors. Detailed expression analysis of N-glycosylated E1 and E2 subunits demonstrated partial furin processing of the E3E2 precursor. Deletion of the C-terminal transmembrane domains of E1 and E2 enabled secretion of furin-cleaved, fully processed subunits, which could be efficiently purified from cell culture fluid via metal affinity chromatography. Confocal laser scanning microscopy on living baculovirus-infected cells revealed that full-length E1 and E2 translocated to the plasma membrane, suggesting similar post-translational processing of E1 and E2 as in a natural CHIKV infection. E1 expressed in baculovirus-infected cells retained fusogenic activity as concluded from a pH-dependent syncytium assay, whereas secreted E2 induced a neutralizing antibody response in rabbits. Recombinant CHIKV E1 and E2 can now be tested as candidate vaccines to prevent CHIKV infections.

**P.8 Insect cells for antibody production: evaluation of an efficient alternative**

Dieter Palmberger, Dubravko Rendić, Philipp Tauber, Miriam Klausberger, Iain B. H. Wilson and Reingard Grabherr

University of Natural Resources and Life Sciences, Vienna, Austria

In recent years there has been an increase in both availability and demand for therapeutic monoclonal antibodies. Currently, most of these antibodies are produced by stably transfected mammalian cells. In this study we evaluated the use of different baculoviral insect cell systems as an alternative for commonly used production schemes. We expressed the human anti-gp41 antibody 3D6 in various insect cell lines and compared product yield, the functionality in terms of antigen binding and the ability to elicit effector functions. Especially, the new Ascalapha odorata insect cell line Ao38 has shown to be favourable in terms of yield. Antibodies expressed in all insect cell lines displayed highly specific antigen binding and bound to Fcγ receptors present on effector cells. Due to the importance of glycan residues we further determined the N-linked oligosaccharide structures present on asparagine-297 in IgG1 heavy chains. In general, the insect-produced antibodies carried less complex, fucosylated N-glycans, in some cases including high levels of core α1,3-fucose. The humanized Mimic insect cell line is favourable in terms of glycosylation, but the use for large-scale production is not feasible because of very low yields. By glycoengineering Ao38 cells one can combine the advantage of high yields and mammalian like glycosylation patterns in one cell line, making it an ideal host for fast, easy and cost effective antibody production.

## P.9 Metabolomic studies of Baculovirus infected Insect cells

S. Reid, T. Tran, H. Huynh, L. Nielsen and L. Chan

The University of Queensland, Brisbane, Australia.

The baculovirus-insect cell system has been extensively studied for the production of recombinant proteins, vaccines and vectors for gene therapy, as well as biopesticides to control various insect pests. Metabolomic studies, which allow an analysis of intracellular metabolite levels is a potentially useful approach for understanding host cell and virus interactions as well as the response of cells to their environment. This approach has been employed widely for yeast and bacteria and more recently, for mammalian cells. The application of such metabolomic studies for insect cells has not been well reported to date. The aim of this work was to develop and validate an extraction protocol for infected insect cells which will allow subsequent studies of intracellular metabolite patterns under infection conditions at different time points post infection, using 2 cell lines, each infected with a different baculovirus. An extraction protocol used for mammalian cells, (Dietmair et al, 2010), was trialed and optimized for insect cells, including infected insect cells. A supplement of pluronic, added to a cold sodium chloride quenching solution, was required to protect cells from lysis, especially infected insect cells, during quenching and washing steps. A single wash step was shown to be sufficient for removing medium derived metabolites from cell pellets. The study was conducted with Spodoptera frugiperda, (Sf9), cells infected with a recombinant Autographa californica multiple nucleopolyhedrovirus virus expressing β−Galactosidase, (βGal-AcMNPV), and with Helicoverpa zea, (H.zea), cells infected with a wild type Helicoverpa armigera single nucleopolyhedrovirus, (HaSNPV), virus, suitable for production as a biopesticide. Initial studies indicate that the energy status of infected cells (ATP content/unit biomass), are the same for Sf9 and H.zea cells and remain unaltered for at least 24 hours post infection, when cells are in exponential phase at the time of infection. Further, the data suggest that intracellular amino acid levels increase slightly following infection, but certain amino acids, in particular lysine, are present at very low levels.

Dietmair, S., Timmins, N. E., Gray, P. P., Nielsen, L. K. and Kromer, J.O. (2010).
Towards quantitative metabolomics of mammalian cells: Development of a matabolite extraction protocol. Analytical Biochemistry, 404, 155-164.

**P.10 Vector engineering to enhance protein expression and clone selection into mammalian cells**

Geraldo S. Magalhães[1], Juliana B. Novo[2], Patricia B. Clissa[1], Maisa S Della Casa[1], Diego Butera[1], Ana M. Moura da-Silva[1]

[1]Laboratory of Imunopatology, [2]Center of Biotecnology – Butantan Institute-Brazil

Due to specialized pos-translational machinery, mammalian cells represent an interesting and not fully explored system to express snake toxins. Therefore, in this work we built a new mammalian expression vector that enhances the feasibility to use mammalian cells to express proteins as biomarkers. Among the modifications, an Igκ signal peptide and a 6xHis tag were inserted into the vector to direct the protein to the supernatant and simplify its purification. In addition, to facilitate the selection of high producing clones and tag proteins that may function as a biomarker, the sequence of enhanced green fluorescent protein (EGFP) was added. The efficiency of the resulting vector (pToxEGFP) was tested by cloning and expressing the viper venom disintegrin echistatin (Ech) that due to its affinity to integrin αvβ3 could be used as a molecular marker to characterize angiogenesis. Expression of EGFP-Ech was achieved in CHO-DXB11 cells after transfection resulting in a yield of 22 mg/L. The binding activity of this chimeric protein was successfully achieved in human umbilical vein endothelial cells (HUVECs) which highly express αvβ3. The results indicate that pToxEGFP may constitute an efficient and versatile expression vector to express tagged proteins with potential biomarker activity.

**P.11 Increased understanding of the biochemistry and biosynthesis of the MUC2 gel-forming mucin, a large O-glycoprotein building up the intestinal mucus, through the recombinant expression of different protein sub-domains in CHO cells**

Malin Bäckström[1,2], Daniel Ambort[1], Elisabeth Thomsson[2], Malin E.V. Johansson[1] and Gunnar C. Hansson[1]

[1]Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, University of Gothenburg Box 440, 405 30 Gotehnburg, Sweden, [2]Mammalian Protein Expression Core Facility, Sahlgrenska Academy at the University of Gothenburg, Sweden

The MUC2 mucin, which is a main component of the intestinal mucus, is a large glycoprotein with two long and heavily O-glycosylated mucin domains. The protein also has several von Willebrand D domains in the N-terminal and C-terminal regions flanking the mucin domains, two short cysteine-rich domains (CysD-domains) interspersed within the mucin domains and finally a cysteine knot domain at the C-terminal end. The large size of MUC2 (theoretical mass above 500 kDa excluding the numerous O-glycans) has prohibited its recombinant expression as a full-length protein. In order to study the biosynthesis and biochemistry of MUC2, we have taken the approach of constructing several recombinant proteins consisting of one or several sub-domains of the protein and expressed them in Chinese Hamster Ovary (CHO) cells. With this approach, we could determine that MUC2 forms covalent trimers in the N-terminal domain and also covalent dimers in the C-terminal domain, in this way building up the complex network that constitutes the mucus gel. Furthermore, when one of the CysD domains was expressed in CHO cells as a fusion protein with mouse Ig Fc, it was found that this domain form non-covalent homologous dimers, most likely increasing the cross-linking of the final mucus after secretion.

We have also tried to use the same approach for the expression of different parts of the lung mucin MUC5AC, which has a similar domain structure as MUC2. The MUC5AC N- terminal von Willebrand D domains were fused to green fluorescent protein, just as in the MUC2 N-terminal construct. This protein was however not successfully secreted from CHO cells, illustrating the difficulties in defining the domain borders to get functional proteins.

In conclusion, the combined knowledge obtained from the different MUC2 recombinant proteins has led to an increased understanding of how the intestinal mucus is formed when produced by intestinal goblet cells.

**P.12 In silico analysis reveals a putative signature to predict SMAR (Scaffold/Matrix Attachment Regions) genomic elements**

Lucas, SP[1] & Verbisck, NV[2]

[1]Federal University of ABC, Sao Paulo, SP, [2]Health Animal Department, Embrapa Beef Cattle, Campo Grande, MS, Brazil

INTRODUCTION: The eukaryotic chromosomal DNA is attached to the nuclear proteinaceous scaffold/matrix by genomic elements known as SMARs (Scaffold/Matrix Attachment Regions). SMARs have been characterized as non-coding short DNA sequences (100 to 3000 base pairs) able to enhance transcriptional activity and prevent position effect variegation of transgenes (1,2). Even though several structural motifs have been assigned to SMAR elements, no consensus sequences or sequence motifs exclusively associated to them have already been characterized. Here we report an in silico analysis of SMARs and the finding that a combination of motifs has the potential to be used as a new tool to predict SMAR elements.

MATERIALS AND METHODS: Comparative analysis of 34 associated SMAR motifs was performed over 11 well characterized SMAR sequences available at GenBank and SMARt databases. Motif assignment was done with freely available text editor software. CpGPlot at EMBOSS website was used to find CpG islands within the sequences.

RESULTS AND CONCLUSION: We have found that only 9 motifs are common to all SMAR sequences studied. These motifs were used to build maps that have revealed a pattern obtained with only 5 motifs, including SMAR recognition rules formerly described by other groups (3,4). We observed an ordered position distribution for those 5 motifs despite that the distance between them has varied considerably within all the different SMARs studied. The combination of these motifs can be putatively ascribed as a SMAR signature and used to perform future SMAR genomic prediction more successfully.

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## P.13 Targeting of anti-apoptosis genes to organelles in mammalian cells

Abasha Lewis[1], Teuro Hayashi, Tsung-Ping Su, and Michael J. Betenbaugh

[1]Johns Hopkins University, USA

Bcl-2 family proteins are well known for their survival-promoting role in apoptosis and are generally believed to localize at the mitochondria and other cellular organelles to block cytochome c release. Previously, our labs and others have overexpressed heterologous anti-apoptosis proteins to improve survival and provide improved recombinant protein yields. One can speculate that localization of these proteins may in some way have an impact on their function. Thus, we investigated the localization of heterologous Bcl-$x_L$, an anti-apoptotic homologue of Bcl-2, in model mammalian cell lines of commercial significance. We found here by immunostaining and confocal microscopic examinations that heterologous Bcl-$x_L$ exists in CHO cells in three distinct patterns: (1) some Bcl-$x_L$ distributed throughout the mitochondria; (2) about 43% of Bcl-xL clustered at the mitochondria; and (3) interestingly, approximately 20% of Bcl-xL juxtaposed to mitochondria suggesting a Bcl-$x_L$ localization at the ER-mitochondrion membranes termed the MAM (mitochondria-associated ER membranes). Furthermore, a cell fractionation study with standard markers confirms that about 45% of Bcl-$x_L$ localizes to the mitochondria with 15% of Bcl-xL at MAM-enriched membranes whereas only 4% of Bcl-xL resides at the bulk ER. During immunocytochemistry we also found that a portion of Bcl-$x_L$ colocalizes with MAM-specific proteins such as sigma-1 receptor, BiP, and type 3 $IP_3$ receptors; however, these MAM-specific proteins are devoid of mitochondria. When taken together, our results suggest that Bcl-xL resides not only at the mitochondria, but at ER membranes in direct contact with mitochondria. Future efforts will explore the functional role that Bcl-$x_L$ residing at the MAM may play in the altering apoptosis and other cellular events.

## P.14 Modelling Limitations on Recombinant Antibody Production from Mammalian Cell Lines

Emma Mead, Lesley Chiverton, Sarah Spurgeon, C. Mark Smales and Tobias von der Haar

University of Kent, Canterbury, UK

Mammalian cell culture is heavily exploited for the production of therapeutic proteins such as monoclonal antibodies as, despite high associated costs, it has the advantage of correct folding and post-translational modification over other production systems. Potential limiting points on cell line productivity exist throughout the entire gene expression pathway. Design of highly efficient vector systems yielding large amounts of mRNA have not necessarily resulted in increased protein yields, leading us to hypothesise that constraints exist post-transcriptionally. Previously we have shown, using a model rP luciferase, that expression levels are limited at a range of control points in the gene expression pathway from transcriptional to post-translational and have presented some preliminary data from first generation engineering models of recombinant monoclonal antibody production (MAb).

Development of systems biology models is an iterative process and here we present an update on our approach to develop independent biochemical (rate-constant based) (Figure 1) and engineering (nonlinear) models of the entire gene expression pathway of a monoclonal immunoglobulin G4 (IgG4) from four NS0 cell lines with different antibody secretion rates. We demonstrate limitations at a number of points through the gene expression pathway and show that models describing different potential folding pathways of IgG4 can accurately describe antibody expression, be used to identify limitations in the gene expression pathway and suggest engineering strategies to enhance individual cell line specific productivity.

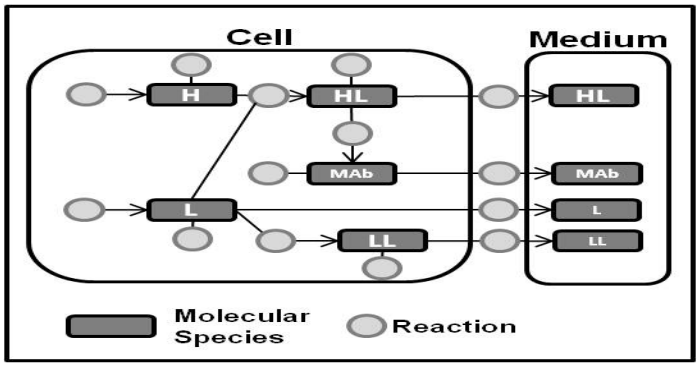


Figure 1. SimBiology model used with MATLAB. Heavy chain (H), Light Chain (L), monoclonal antibody (MAb).

## P.15 Reducing lactate level and increasing antibody production in Chinese Hamster Ovary cells (CHO) by downregulating the expression of lactate dehydrogenase and pyruvate dehydrogenase kinases

Meixia Zhou, Yongping Crawford, Inn Yuk, John Joly, Brad Snedecor and Amy Shen

Genentech Inc, South San Francisco, CA, USA

Large-scale fed-batch cell culture process of CHO cells is the standard platform for the clinical and commercial production of monoclonal antibodies. Lactate is one of the major by-products of CHO fed-batch culture. In pH-controlled bioreactors, accumulation of high levels of lactate is accompanied by high osmolality due to the addition of base to control pH of the cell culture medium, potentially leading to lower cell growth and lower therapeutic protein production during manufacturing. Lactate dehydrogenase (LDH) is an enzyme that catalyzes the conversion of the substrate, pyruvate, into lactate. LDH activity is modulated by many factors including pyruvate concentration. Alternately, pyruvate can be converted to acetyl-CoA by pyruvate dehydrogenases (PDHs), to be metabolized in the TCA cycle. PDH activity is inhibited when phosphorylated by pyruvate dehydrogenase kinases (PDHKs). In this study, we knocked down the gene expression of lactate dehydrogenase A (LDHa) and PDHKs to investigate the effect on lactate metabolism and protein production. We found that LDHa and PDHKs can be successfully downregulated simultaneously using a single targeting vector carrying small inhibitory RNAs (siRNA) for LDHa and PDHKs. Moreover, our fed-batch shake flask evaluation data using siRNA-mediated LDHa/PDHKs knockdown clones showed that downregulating LDHa and PDHKs in CHO cells expressing a therapeutic monoclonal antibody reduced lactate production, increased specific productivity and volumetric antibody production without appreciable impact on cell growth. Similar trends of lower lactate level and higher antibody productivity on average in these siRNA clones were also observed from evaluations performed in bioreactors. Intracellular ATP content analysis showed that siRNA clones have higher intracellular ATP level than the parental line or mock clones, indicating that more pyruvate is channeled into the TCA cycle for each consumed glucose to produce more ATP for cells to use in siRNA clones. In summary, our data demonstrated that simultaneous knockdown of LDHa and PDHK1, 2, and 3 in CHO cells is effective in reducing lactate levels and in increasing antibody titers without noticeable impact on cell growth and product quality.

## P.16 Characterization of PpiB (cyclophilin B) over-expression in CHO-K1 cells and its effect on monoclonal antibody production

Marc Feary[1,2], Robert Young[2], Andy Racher[2], Mark Smales[1]

[1]University of Kent, Canterbury, [2]New Expression Technologies Group, Lonza Biologics plc, Cambridge

The factors contributing to and defining mammalian cell lines that produce large amounts of monoclonal antibody (mAb) are poorly understood. The assembly of mAbs requires the correct folding of two heavy chain (HC) and light chain (LC) polypeptides. Peptidylprolyl cis-trans isomerisation of conserved proline residues in the HC CH1 domain is an essential step in LC CL domain induced folding. In vitro experiments have demonstrated that acceleration of peptidyl cis-trans isomerisation by PpiB, an ER resident peptidylprolyl cis-trans isomerase, can increase the folding rate of the CH1 domain. The aim of this work was to evaluate the effect of over-expression of recombinant human PpiB (rhPpiB) in CHO-K1 cells and in particular the cell specific mAb production rate (qmAb). In order to do this, cell lines permanently over-expressing rhPpiB were generated using the Flp-In CHO system (Invitrogen). The sub-cellular localization of rhPpiB was then determined using differential detergent extraction. The effect of over-expression of rhPpiB on the CHO-K1 proteome was evaluated using 2D-PAGE and its interaction with mAb HC confirmed by immunoprecipitation. Finally, the effect of rhPpiB over-expression on the cell specific production rate of IgG1 and IgG4 mAbs was evaluated in batch cultures. A Flp-In CHO cell line (HP1) over-expressing ~3-fold more PpiB protein than the null cell line (N1) was generated. The majority of rhPpiB was found to be localized to the ER, although some was detected in the cytosol. 2D-PAGE analysis of the proteome following over-expression of rhPpiB identified significant (ANOVA, $p<0.05$) elevated amounts of β-tubulin and decreased amounts of Grp75 and prohibitin. Immunoprecipitation of the IgG4 HC following over-expression of rhPpiB showed an increase in the amount of PpiB protein interacting with the mAb. Cell lines N1 and HP1 had similar specific growth rates (ANOVA, $p>0.05$). However, integral of viable cell concentrations and maximum viable cell concentration were significantly lower (ANOVA, $p<0.05$) in cell line HP1 relative to N1. Further, increased amounts of rhPpiB protein reduced qmAb in IgG super-transfection experiments performed in batch cultures (ANOVA, $p<0.01$). These data therefore suggest that over-expression of rhPpiB protein in CHO-K1 cells has a negative effect on the amount of mAb produced. It is unknown if this relates to PpiBs isomerase or molecular chaperone functions.

## P.17 Targeted transgene integration in a CHO-S hotspot for the fast and reproducible production of high protein titers

Lars Knoch, Karim Mekideche, Emilie Dessez, Jean-Pierre Cabaniols and Christophe Delenda

Cellectis bioresearch, Romainville Cedex, France

The desired characteristics of a producer cell line include the ability to quickly and reproducibly generate stable clones producing high titers of recombinant proteins or monoclonal antibodies (mAbs) with controlled post-translational modifications. Nowadays, these cell lines are however essentially generated by random integration of a gene of interest (GOI) to be expressed with no control over the level and stability of its expression. Several hurdles make this strategy tedious, expensive, and time-consuming, but the real bottleneck of this process is the production, maintenance, and characterization of numerous individual clones. We co-developed a targeted integration system in CHO-S cells, called cellular Genome Positioning System (cGPS® CHO-Sa CEMAX®), based on the stimulation of homologous recombination by meganucleases. Further characterization revealed that the cGPS® CHO-Sa CEMAX® system is more rapid (2-week protocol), more efficient (all selected clones expressed the GOI), reproducible (no or little GOI expression level variation), and stable over time (no change in GOI expression after 15+ weeks of culture) than classical random integration. This fast and robust method opens the door for creating large collections of cell lines expressing high level of recombinant proteins and mAbs.

## P.18 Overexpression of p58 (DnaJ3C) inhibits apoptosis and enhances recombinant antibody output late in culture when stress-induced inhibition of translation initiation occurs

Anne Roobol[1], Jo Roobol[1], Amandine Bastide[2], Anne E.Willis[2] and C. Mark Smales[1]

[1]University of Kent, Canterbury, [2]University of Leicester, UK

We are interested in improving recombinant protein production from mammalian cells. When synthesising large amounts of recombinant protein, cells are likely to encounter a variety of stress conditions that result in the phosphorylation of eukaryotic initiation factor 2alpha (eIF2α) at Ser51 with consequent inhibition of translation initiation. The protein p58, which has a weak endoplasmic reticulum (ER) leader sequence, is an inhibitor of stress-induced eIF2α kinase activation when it is located in the cytoplasm but also has hsp40-type molecular chaperone activity after translocation into the ER lumen, where it acts as a co-chaperone for immunoglobulin binding protein, BiP. p58 is also thought to be central to the mechanism by which B-cells repress eIF2 α phosphorylation during differentiation into plasma cells and subsequent antibody production. We report here on the efficacy of overexpressing p58, both transiently and stably, in Chinese hamster ovary (CHO) cells for overcoming the eIF2α phosphorylation that would normally occur when cells are exposed to amino-acid depletion, ER stress and mild hypothermic stress – these being conditions likely to arise during the later stages of recombinant protein production in mammalian cells. Overexpression of p58 does indeed reduce eIF2α phosphorylation. We further show that overexpression of p58 inhibits apoptosis and enhances recombinant antibody output late in culture when environmental stresses are known to induce phosphorylation of eIF2α.

**P.19 Glucose and glutamine metabolism in a transfected Drosophila melanogaster (S2) cell line**

Aguiar MA[1], Barral MF[2], Léo P[1], Pereira CA[3], Piccoli RAM[1], Augusto EFP[1]

[1]IPT–Instituto de Pesquisas Tecnológicas, São Paulo, SP, Brazil, [2]Engenharia de Materiais, Fundação Santo André, Santo André, SP, Brazil, [3]Laboratório de Imunologia Viral, Instituto Butantan, São Paulo, SP, Brazil.

Drosophila melanogaster cell lines have been studied to produce different recombinant proteins, but their metabolism is poorly described in the literature. In this work we discuss the versatility through the evaluation of a transfected S2 strain responses to distinct levels of glucose and glutamine in culture media. Eight runs were carried out at different initial glucose (1<GLC0<15 g/L) and glutamine (0.6<GLN0<7.0 g/L) concentrations, in a 1.5 L bubble free bioreactor, under controlled temperature (28 oC) and dissolved oxygen concentration (30% air saturation) conditions. These variables affected differently maximum specific growth rates (μX,Max), and cell metabolic parameters (yield factors - YX/GLC, YX/GLN, YLAC/GLC, YALA/GLC, YNH4/GLN, YALA/GLN). As a first proof of the versatile metabolism, it was observed that low initial glucose or glutamine concentrations clearly limited cell growth, with well established μX,Max 3 and 2.5 times smaller, respectively, in comparison to intermediate GLC0 and GLN0 conditions. As observed for other animal cell lines, glucose and glutamine metabolism showed a great interdependence. Thus, under glucose excess, cell metabolism was less efficient, with reduction of yield factors YX/GLC (2.3 times) and YX/GLN (4.6 times), and higher generation of by-products, characterized by the augmentation in YALA/GLC (51%), YLAC/GLC (11%) and YNH4/GLN (15%) values. Glutamine excess metabolism showed a reduction in YX/GLN (25 times) and, unexpectedly, also a reduction in by-product release, represented by YNH4/GLN (7 times), and by YALA/GLN (12 times). The effect on glucose metabolism was more intense when glutamine concentration was higher, showing diminution of YX/GLC (3.6 times), and increasing of YALA/GLC (70%), and YLAC/GLC (70%). These results suggest that cells use two pathways to metabolize glutamine: glutaminolysis, in condition of limitation of glucose, or glutamate synthase – NADH-GOGAT, in condition of glucose excess. This cell line showed, also, the ability to synthesize glutamine when its concentrations reaches 50 mg/L, employing ammonium or other amino acids as nitrogen source.

(C. A. Pereira and E.F.P.Augusto are recipients of CNPq fellowship)

## P.20 A plug-and-play disposable insect cell protein production system using the CELL-tainer® and ExpreS2 expression system

W.A. de Jongh[1], N.M.G. Oosterhuis[2], C. Dyring

[1]ExpreS2ion Biotechnologies, Denmark, [2]Cellution Biotech, Assen, The Netherlands

Objective: The aim of the project was to develop an easy to use disposable system for rapid production of proteins at 1.5L to 10L scale.

Methods: Two different proteins were cloned into the pExpreS2-1 expression vector and transfected into a Drosophila S2 insect cell line. Antibiotic selection was used to create stable polyclonal cell lines in three weeks, whereafter the cultures were expanded and inoculated at different concentrations between 2 and 8E6 cells/mL. The cultures were ran using Excell$^{tm}$420 medium in 15L working volume disposable cell-culture bags at a range of volumes. The cells were grown in the single-use CELL-tainer® bioreactor which has a 2-dimensional movement of the bag, with or without dissolved oxygen (DO) based control. Temperature was controlled to 25$^{o}$C, airflow was set to 1.5L-3L/minute and the agitation rate was set to 4 rpm, 6 rpm or controlled through a DO cascade. Furthermore, the data obtained in these experiments were compared to data obtained from shake flask and previous bioreactor experiments with S2 cells.

Results: Independent of starting conditions the S2 insect cell cultures grew to 25-35E6 cells/ml in three to five days in the CELL-tainer®, which is the same as the concentration range attained in shake flask and bioreactor cultures. The viability of the culture remained above 90% throughout the run. The cultures experienced a one day lag phase upon inoculation into the CELL-tainer® using an initial cell concentration between 5 and 8E6 cells/mL, which is similar to the lag-phase observed in bioreactors. It was found that an agitation rate of 6 rpm was sufficient to maintain the required DO for cell growth and protein production for volumes up to 4L with an airflow rate of 3L/min. Furthermore, no pH control was required.

Conclusions: The results demonstrate how the ExpreS2 system in conjunction with the CELL-tainer® can be used to produce proteins without the need for DO or pH control. The process was also robust regarding inoculation cell density in the 5-8E6 cells/mL range. This greatly simplifies the production process and delivers a plug-and-play protein production system for research labs to employ.

**P.21 A practical and fast adaptation of CHO cells expressing human prolactin to grow in suspension and its application to laboratory production**

Arthuso FS, Capone MVN, Suzuki MF, Sousa JM, Oliveira JE, Bartolini P, Soares CRJ.

Cidade Universitária, São Paulo, SP, Brazil.

Human prolactin (hPRL) is a polypeptide with 199 amino acids and a molecular mass of 23 kDa. Previously, a eukaryotic hPRL expression vector based on a selectable dehydrofolate reductase (dhfr) marker was used to transfect anchorage-dependent dhfr- Chinese hamster ovary (CHO) cells. The present work describes a fast and practical laboratory adaptation of these transfected cells, in ~40 days, to grow in suspension in serum-free and protein-free medium. High cell densities of up to $4.0x10^6$ cells/ml were obtained from spinner flask cultures and a stable and continuous production process with a duration of at least 30 days was developed. Two harvesting strategies were set up, 50 or 100% of the total conditioned medium being collected daily and replaced by fresh culture medium. The volumetric productivity was 5-7 µg hPRL/ml, as determined directly in the collected medium via reversed-phase HPLC (RP-HPLC). A two-step process based on a cationic exchanger followed by size exclusion chromatography was applied to obtain purified hPRL from the conditioned medium. Two hPRL isoforms, non-glycosylated (NG-hPRL) and glycosylated (G-hPRL) could also be separated by high-performance size-exclusion chromatography (HPSEC) and, when analysed by RP-HPLC, HPSEC, Western blotting, and bioassay, were found to be comparable to the WHO International Reference Reagents of hPRL. This laboratory scale production was used as a model to compare different culture media and effects of drugs such as cycloheximide and sodium butyrate on hPRL isoforms synthesis. These results provide important subsidies for the practical scale-up to the pilot and industrial scale of a bioprocess based on CHO cell culture.

## P.22 Serum-free Suspension Cultures of MDCK Cells for the Production of Canine Adenovirus Vectors

R. Castro[1], P. Fernandes[1], Y. Genzel[2], K. Scharfenberg[3], E. J. Kremer[4,5], P. M. Alves[1] and A. S. Coroadinha[1]

[1]IBET/ITQB-UNL, Oeiras, Portugal., [2]Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany., [3]University of Applied Sciences Emden/Leer, Emden, Germany., [4]Institut de Génétique Moléculaire de Montpellier, Montpellier, France., [5]Universités de Montpellier I & II, Montpellier, France

The potential of Madine Darby Canine Kidney (MDCK) cells for the production of influenza vaccines have been greatly explored in the past decades. Recently, a new MDCK cell line (MDCKsus) that was able to grow in suspension in a fully defined system was established (Lohr, V., et al., 2010. Vaccine. 28:6256-64). This new cell line is suitable for the development of robust industrial manufacture of viral based products. In this work we studied the production of canine adenoviruses (CAV) for gene therapy. Previously, we have validated the use of MDCK cells for the amplification of CAV-2 vectors in monolayer cultures (Santiago et al., 2010 ASGCT).

In this work, we investigated whether the MDCKsus cell line was suitable for the amplification of CAV-2 vectors in single-cell/small aggregate suspension cultures. We tested four different serum-free media: two formulations of SMIF8, AEM, ExCELL MDCK and OptiPRO SFM. The maximal cell densities achieved varied from 2 x $10^6$ cells/ml (SMIF8 and OptiPRO SFM) to 5 x $10^6$ cells/ml (AEM and ExCELL MDCK). A first screening of CAV 2 production in the four media resulted in low amplification of the vector (below 30) but the reduction of the multiplicity of infection led to an increase of the amplification ratio to values up to 150 (and cell specific productivities up to 450 IP/cell). Although further optimization is in progress, our data show that canine adenovirus vectors can be effectively produced in serum-free suspension cultures of MDCKsus cells. The scalable suspension cultures of MDCK described herein will allow to obtain high titer vector stocks of CAV-2 required in gene therapy trials.

## P.23 Production of Adenovirus Vectors in Human Amniocyte-derived Cells

Silva, A.C.[1,2], Simão, D.[1], Sousa, M. F.Q.[1], Küppers, C.[3], Lucas, T.[3], Cruz, P.E.[1], Carrondo M. J.T.[1,4], Kochanek, S.[3], Alves, P.M.[1,2]

[1]IBET, Animal Cell Technology Unit, Oeiras, Portugal, [2]ITQB-UNL, Animal Cell Technology Unit, Oeiras, Portugal, [3]University of Ulm, Ulm, Germany, [4]FCT/UNL, Laboratório de Engenharia Bioquímica, Monte da Caparica, Portugal

Adenovirus vectors (AdV) have been extensively used as vectors for vaccines, protein production and gene therapy. Strategies that will effectively respond to the increasing demands of such vectors are thus urgently required. Primary human amniocytes can be efficiently transformed by adenoviral E1 functions allowing the production of AdV with potentially reduced generation of replication competent adenovirus (RCA), as compared to production in HEK293 cells, thus opening up a new host alternative.

The aim of this work was to evaluate the growth behavior of a newly developed human amniocyte-derived cell line and to investigate its ability to produce AdV. Transformed human amniocyte cells were adapted to grow in suspension in serum-free media. The $pO_2$ content and pH of the medium were monitored accurately and non-invasively during cell growth and AdV production in shake flasks, allowing identifying critical points in a small scale. The effects of multiplicity of infection, harvesting time, and medium formulation on AdV production were evaluated in shake flasks. The effect of cell concentration at infection was also evaluated in shake flasks and a bioreactor. The production yields were determined by Flow Cytometry and PCR; 293 cells were used as control. Data on the metabolic characterization during cell growth and infection will be presented.

The cells in a bioreactor with serum-free medium reached cell densities up to $9x10^6$ cells/ml. Using these cells it was possible to perform infections at concentrations higher than $3x10^6$ cells/mL without a critical loss in the cell specific productivity (>1000 infectious particles (ip) per cell) thus increasing the volumetric productivity to more than $3x10^9$ ip/ml. Our data indicate that this cell line suffered less from the cell density effect than normally occurs in 293 cells leading to AdV volumetric productivities values below $1x10^9$ ip/mL.

These results constitute valuable information for the development of cell culture process for AdV production using a potentially safer human cell line ensuring simultaneously the product quality, potency and safety necessary for clinical and research applications.

This research was supported by the EU Network of Excellence, the project CLINIGENE.

**P.25 Production and purification of an anti-CD163 antibody from a 500L-Single Use Bioreactor using hybridoma cells.**

Devender Sandhu, Houssam Alosta, Kathryn Matthews, Steven Brookes, Gord Adamson, Katherine Lu, Nishi Singh, Mukesh Mayani, Jin Seog, Sri Wanduragala Amber Li, Rob Fantaske, Neetha Murthy, John Shi and David Bell

Therapure Biopharma, Canada

Recombinant antibody and protein-drug conjugates are increasingly being used as therapeutics for the targeted delivery and improved safety of therapeutic drug molecules. Therapure has generated an antibody to human CD163, a cysteine-rich scavenger receptor expressed on peripheral blood monocytes, some tissue macrophages and on a small percentage of CD34+ hematopoietic progenitor cells (Matthews et al, 2006). We have demonstrated that incubation of the antibody (TBI 304) with CD34+ cells and subsequent plating on colony-forming assays allows for the generation of larger, more hemoglobinized erythroid colonies (BFUe) indicating an increase in erythroid proliferation and differentiation.

A 250-L upstream process for the production of TBI 304 was started from a single cryovial, and pre-culture was divided into two stages which included 3 L spinner flasks in the first stage and expansion in bioreactors (20 L Applikon bioreactor and a 50 L single-use Hyclone bioreactor) in the second stage. Both bioreactors were operated in batch mode. The culture from seed stage two was used to inoculate the 500 L single-use bioreactor from Hyclone operating at 250 L. The bioreactor system uses a DeltaV platform running in a PC-based software controller. A two-stage harvest (clarification and sterile filtration) by filtration process was used to collect conditioned medium which was stored at 4°C for downstream processing. The mAb was isolated using a non protein A mixed mode capture resin (MEP HyperCel) and further purified using anion exchange membrane chromatography. The purity was >95% and included a low pH hold viral inactivation step and a viral filtration step As per an established analytical control plan, different purity, identity, sterility and impurities tests were completed at pre-defined upstream and downstream steps. Upstream samples collected were analyzed for 16 different parameters which included cell density, viability, and metabolites evaluation using the BioProfile Flex analyzer (Nova Biomedical).

## P.26 Semliki Forest Virus vector for recombinant rabies virus glycoprotein expression and for rabies immunization

Astray RM[1], Wagner R[2], Boldorini VL[1], Puglia ALP[1], Jorge SAC[1] Pereira CA[1]

[1]Laboratório de Imunologia Viral, Instituto Butantan, Brazil, [2]Université de Strasbourg, France.

The Semliki Forest Virus (SFV) system is based on a positive single-strand RNA virus carrying the gene of interest under the control of a viral promoter. After infection, a great amount of recombinant mRNA is produced leading to high expression of the recombinant protein. Provided that recombinant SFV performs a single round of infection and does not integrate into the host cell genome, SFV has also been studied as a vector for immunization. Objectives: To evaluate the expression of the immunogenic recombinant rabies virus glycoprotein (RVGP) in animal cells infected with recombinant SFV (SFV-RVGP), and to evaluate the utilization of SFV-RVGP for immunization against rabies. Methods: The SFV-RVGP was utilized for the infection of mammalian cells and the amount of RVGP mRNA as well as RVGP produced were determined by quantitative PCR and ELISA, respectively. The amount of infected cells was determined by flow cytometry and viability was analysed. Immunization studies were performed in mice, which were inoculated with SFV-RVGP and were analysed for anti-RVGP antibody titres in serum by ELISA. Results: Immediately after infection cells showed an exponential accumulation of RVGP mRNA, which reached maximum values in 12 h. RVGP was detected at levels of 0.03 μg/mL after 6 h of infection and accumulated over 30 h of culture, reaching 0.3 μg/mL (2.5 μg/$10^6$ cells). Around 80% of cells were infected and viable cell concentration declined during all post-infection times. The immunization of mice with SFV-RVGP induced a humoral immune response similar to the immunization with commercial rabies vaccine. Anti-RVGP antibodies reached levels of 2.0 to 4.0 UI/mL. The immune response was also characterized by the predominance of IgG2a-biased subtype antibody, which are potent inducers of anti-viral effector functions. Conclusion: The development of a third generation rabies vaccine with SFV-RVGP can be achieved by the production of RVGP in cell cultures or by direct immunization with SFV-RVGP generatng in vivo expression of RVGP.
Supported by FAPESP (10/08742-8) (10/52255-4) and CNRS (114298)

## P.27 Recombinase Mediated Cassette Exchange (RMCE) as an alternative method for establishment of CHO producer cell lines

Nathalie Veith1, Dagmar Wirth[2] , Thorsten von Hoff[1], Barbara Beuerle[1], Sevim Duvar[1], Holger Ziehr[1]

[1]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Braunschweig, Germany
[2]Helmholtz Centre for Infection Research, Braunschweig, Germany

Objectives

The pharmaceutical market today is under intense cost and time-to-market pressure. The adherence to pre-determined cost and time lines often decides about failure or success of a project. Conventional procedures for establishment of a production cell line rely on random integration of the gene of interest (GOI) into the genome of the host cell line. However, this approach is very time-consuming and takes up to 18 months. To overcome the long generation time, alternative strategies for cell line development are needed. Using recombinase mediated cassette exchange (RMCE) for establishment of a CHO production cell line the generation can be reduced to 6 weeks.

Methods

The basic principle of RMCE involves a tagging and screening step, in which suitable chromosomal loci are identified and marked by the integration of specific recognition sequences for a recombinase enzyme. These sites can hence later be used for specific integration.

Results

First, RMCE compatible tagging plasmids and lentiviral constructs, either carrying a marker gene or the GOI or both, were tested. With suitable tagging vectors high and stable expression loci in CHO genome were identified in suspension as well as in adherent CHO host cells. Several single copy tagged cell clones were monitored for over three months for productivity and stability. Finally, these tagged cell clones were targeted with various expression cassettes including constructs coding for an IgG2a and IgG1a.

Conclusion

RMCE can provide stable, high-expressing CHO cell lines for production of recombinant antibodies and other therapeutic proteins. If a stable and well- expressing locus is found, this site can be re-used routinely and efficiently to reduce generation time for a new CHO production cell line.

## P.28 CAP Technology: Production of Biopharmaceuticals in Human Amniocytes

Schiedner, G.

CEVEC Pharmaceuticals GmbH, Köln, Germany

Addressing the needs of biopharmaceutical manufacturing, CEVEC has developed a human cell expression system for the production of safe and effective biopharmaceuticals. Although the current animal cell-based expression technologies for proteins are commercially proven platform with a widely recognized robustness and productivity, a human cell-derived expression technology offers significant advantages in quality, serum-half life and safety. Whereas the expression of very complex proteins demand authentic human glycosylation patterns, also antibody expression technologies rise to the challenge for high titers and for avoiding non-human glycan structures.

Based on primary human amniocytes, the CAP technology has been generated in order to reach competitive yields and superior posttranslational modification patterns. The stable producing CAP and the new transiently producing CAP-T cells meet all regulatory guidelines, they are of non-tumor origin and from ethically accepted source.

CEVEC is routinely using its CAP technology for expression of biotherapeutics and we will present results exemplarily from a very complex, highly glycosylated protein but also from antibodies. We have optimized expression plasmids in order to achieve high levels and ensure consistent performance. We have developed defined protocols for cell line development in order to simplify and shorten the development time lines. Applying these improvements, we have produced very high titers for both antibodies and very complex proteins, with authentic human glycosylation patterns, very short development time lines and thus optimized cost efficiency.

The combined use of the CAP-T cell, a new version of CAP for transient protein expression for very early discovery or preclinical evaluation, with the CAP cells for stable expression up to clinical supply offer the advantage of a one stop protein expression solution with identical genetic origin, high titers, authentic human glycosylation and shortened time lines for production of safe and effective therapeutic proteins.

**P.29 Rabies virus glycoprotein (RVGP) and messenger RNA (mRNA) expression in transfected Drosophila melanogaster S2 cells**

Puglia ALP, Ventini DC, Boldorini VL, Pereira CA, Astray RM

Laboratório de Imunologia Viral, São Paulo, Brazil

S2 cells have been successfully utilized for RVGP expression. The aim of this study was to evaluate the influence of cell culture temperature on expression of RVGP and its mRNA (RVGPmRNA) in recombinant S2 (rS2) cells. rS2 cells were kept in T flasks with SF900II culture medium for 15 days at 28ºC or 25ºC. jCells were then inoculated into shake flasks with 20 mL medium at 100 rpm. Cultures were then inoculated in triplicates with 5x105 cells/ml. Another protocol used a sharp decrease in temperature from 28ºC to 25ºC after 48 h of cultivation. rS2 cells were also cultivated in a bioreactor (90 rpm, 50% DO, 28°C and sub optimal temperatures). The cellular concentrations were determined by hemocytometer counting, the RVGP concentrations by ELISA, RVGPmRNA by qRT-PCR, glucose, lactate and glutamine concentrations by biochemical methods. The decrease of culture temperature of rS2 cells resulted in decreased cell growth. The cell cultures at 28ºC reached the end of the exponential phase at 60 to 72 h while the cultures at lower temperatures reached it at 72 to 96 h. The metabolic analysis showed no differences in glucose and glutamine consumption or in lactate production. We observed a reduction or no difference in the cellular and volumetric RVGP concentrations in cultures at sub-optimal temperatures. The RVGPmRNA levels (shake flasks) showed increasing values between 72h to 120 h, but did not present direct correlation with RVGP concentration or volumetric productivity. The RVGPmRNA levels (shake flasks) were most likely related to the culture phase. When cells showed maximum cell growth rate, a lower RVGPmRNA relative concentration was found. At stationary growth phase the relative RVGPmRNA concentration increased. This profile can be a consequence of a decrease in the levels of other mRNAs linked to the cell cycle. The increased amounts of RVGPmRNA in relation to total mRNA, did not generate an increase in RVGP levels because cells usually have less translational activity when entering in stationary culture phase.

Financial support: FAPESP and PAP Program

**P.31 Production of Ebola and Marburg Virus-Like Particles for the Development of Differential Immuno-Assays**

L. Hudson, K. S. Richards, H. Love, E. N. C. Newman, L. Easterbrook, J. Plank, H. Tolley, and C. B. Bruce.

Health Protection Agency, Porton Down, UK

With the ever increasing levels of international travel, trade and threat of bio-terrorism, the need to quickly and efficiently identify filovirus infections is critical. Furthermore, due to their varying degrees of case fatality, confirming the virus sub-type involved is crucial in the efficient containment and management of outbreaks.

Specialised high-containment laboratories are required to work with hazard group-4 (HG) agents (e.g. Ebola and Marburg viruses); such facilities can restrict experimental procedures, and are costly to run/maintain. Alternate work strategies are therefore required to facilitate the swift and efficient development of new assays required to support diagnostic and vaccine development programmes.

Our work focuses on the development of differential immuno-assays for identification and characterization of filovirus sub-types. Here we describe a strategy for producing monoclonal antibodies (mAb) to HG-4 agents in Biological Safety Level-2 (BSL) facilities. One of the key aspects to this work was the use of baculovirus and mammalian expression systems to produce antigenic, non-infectious, species-specific virus-like particles (VLP). Verification of VLP morphology and detection of antigenic sub-units (Major matrix protein [VP40] & Glycoprotein [GP]) were performed by Transmission Electron Microscopy and biochemical analysis, respectively.

MAbs to GP of specific species and strains of Ebola and Marburg viruses were produced by immunising BALB/c mice with VLPs containing the relevant GP; resulting mAbs were screened by Enzyme-Linked Immuno-Sorbent Assay for species-specificity and cross-reactivity; positive clones will be screened for virus neutralization activity using an enzymatic-VLP assay. Monoclonal antibodies identified with neutralizing properties will be tested on live virus in our BSL-4 facility.

## P.32 Generation of a Chinese Hamster Ovary cell line producing recombinant human glucocerebrosidase using the dihydrofolate reductase selection system

Juliana Branco Novo[1,2], Ligia Morganti[3], Ana Maria Moro[4], Isaías Raw[1] and Paulo Lee Ho[1,2]

[1]Instituto Butantan, SP, Brazil, [2]Instituto de Ciências Biomédicas, USP, SP, Brazil, [3]Centro de Biotecnologia, IPEN, SP, Brazil, [4]Laboratório de Biofármacos em Células Animais, Instituto Butantan, SP, Brazil

Impaired activity of the lysosomal enzyme glucocerebrosidase (GCR) results in the inherited metabolic disorder known as Gaucher's disease. Current treatment consists of enzyme replacement therapy by administration of exogenous GCR. Although effective, it is exceptionally expensive and patients worldwide have a limited access to this medicine. In Brazil, the public healthcare system provides the drug free of charge for all Gaucher's patients, which reaches the order of $ 84 million per year. However, the production of GCR by public institutions in Brazil would reduce significantly the therapy costs and the near expiration of the GCR patent prompted us to generate a Chinese Hamster Ovary (CHO) cell line for future production of this enzyme. Recombinant GCR was expressed in CHO-DXB11 (dihydrofolate reductase-deficient) cells after stable transfection and gene amplification with methotrexate, using traditional methods to screen high producer clones. As expected, glycosylated GCR was detected by immunoblotting assay both as cell-associated (~ 64 kDa) and in secreted (63-69 kDa) form. Analysis of subclones allowed the selection of stable CHO cells producing a secreted functional enzyme, with a calculated productivity of 5.14 pg/cell/day for the highest producer. To the best of our knowledge, this is the first report on a robust protocol for the generation of a cell line producing recombinant human GCR. Although being laborious, traditional methods of screening high-producing recombinant cells may represent a valuable alternative to generate otherwise expensive biopharmaceuticals in countries with limited resources.

Financial Support: FAPESP, CNPq and Fundação Butantan.
Keywords: glucocerebrosidase; Gaucher's disease; enzyme replacement therapy; CHO cells; dihydrofolate reductase.

**P.33 Viral pseudoparticles expressing viral proteins in mammalian cells**

Ribeiro LC[1], Rezende, AG[1], Lemos MAN[1], Silva, FG[1], Astray, RM[1], Wagner, R2 Pereira CA[1], Jorge SAC[1]

[1]Laboratório de Imunologia Viral, Instituto Butantan, São Paulo - SP, Brasil. , [2]Département Récepteurs et Protéines Membranaires, CNRS Strasbourg, France

Introduction: The rabies virus glycoprotein (RVGP) has been recognized as an antigen able to induce neutralizing antibodies, conferring protective immunity against rabies. Gene expression in cells has been a powerful tool in biotechnology. Some viral particles (viral like particles-VLP) have been produced in cell cultures to express different viral proteins. Chronic hepatitis caused by Hepatites C Virus (HCV) is a world health problem. The estimative of infected people by HCV is 170 millions, almost 3% of the world population. Approximately 70% of the chronically infected patients reach a cirrhosis state. In this work we have constructed pseudoviral particles to express RVGP and non structural protein 3 (NS3) of HCV in mammalian cells.

Objectives: To construct plasmids to produce virus pseudoparticles carrying an mRNA to express an NS3 protein of HCV or RVGP. We used two systems, a Semliki Forest Virus and another with HCV structural proteins associated with MLV Gag/Pol.

Methods: The vectors were constructed by digestion of cDNA fragments (NS3 or RVGP) and by ligation with respective expression vectors.

Results: We obtained expression vectors and produced pseudoparticles containing RVGPmRNA and NS3mRNA by co-transfection procedures. Samples of pseudoparticles were used to infect hepatocarcinoma (Huh7.0) or baby hamster kidney cells (BHK-21). The RVGP produced in infected cells was measured by ELISA. We obtained 1,4µg of RVGP/1E7cells

Conclusion: Preliminary results with RVGP expression showed the efficacy of the system, but better results are needed.

Suported by FAPESP, FUNDAP (Instituto Butantan), CNPq

## P.34 Production of Human recombinant IgE receptor (FcεRIα) -a case study

N Rahman[1,2], JE Nettleship[1,2], LE Bird[1,2], Y Zhao[2] and RJ Owens[1,2]

[1]R92 Rutherford Appleton Laboratories, Harwell Oxford, Didcot, UK, [2]Universtity of Oxford, Oxford, UK

The OPPF-UK has implemented a pipeline for the production of glycoproteins for structural studies, using human embryonic kidney (HEK) 293 cells. A number of vectors have been developed which are based on the pTT backbone and InFusion cloning (Takara Bio). The vector of choice for secreted expression is pOPINTTGneo, containing an N-terminal signal sequence and a C-terminal His6-tag, along with the neo gene for selection during the formation of stable cell lines. Two further vectors based on pOPINTTGneo containing cleavable C-terminal fusion tags of CD4 or HaloTag7 (Promega) are also available to increase the yield of difficult-to-express proteins.

Using the human IgE receptor, FcεRIα, as an example, we have compared scale-up technologies in terms of yield of secreted protein, timeline and resources. These comparisons include the use of transient transfection vs. stable cell line development; automated vs. manual transfection; and attached vs. suspension cultures. These experiments have allowed us to develop a robust pipeline for production of secreted products in mammalian cells for structural studies.

## P.36 Development of an insect cell factory for production of complex biopharmaceuticals using flipase-mediated cassette exchange

Fabiana Fernandes[1,2], João Vidigal[1,2], Hélio Tomás[2], Ana Teixeira[1,2], Ana Coroadinha[1,2], Paula Alves[1,2]

[1]ITQB-UNL, Oeiras, Portugal, [2]IBET, Oeiras, Portugal

The baculovirus expression vector system (BEVS) has been widely used as a production platform for recombinant proteins and vaccines in insect cells [1,2], mainly because of the high cell densities reached in serum-free suspension cultures and the relative short timelines required for new production process establishment. However, inherent drawbacks of this system include the effort to maintain the virus stock, the lytic infection cycle which can compromise the quantity and quality of the final product, and the recognized difficulties to separate recombinant from viral proteins during purification [2]. Stable expression in insect cells using recombinase-mediated cassette exchange (RMCE) systems potentially represents a valuable alternative to the BEVS. This new technology enables the repeated use of pre-characterized genomic sites with good expression rates to produce multiple recombinant proteins, and has been widely applied in mammalian cells [e.g. 3].

Herein, we report the establishment of an Sf9 insect cell line using RMCE. Two complementary vectors (the tagging and target vectors) were constructed, flanked by two recognition sites to enable flipase-mediated recombination. In both vectors, the OpIE2 promoter drives the expression of one reporter protein (dsRed in the tagging and eGFP in the target). To explore the full potential of this technology, only one copy of the tagging cassette can be integrated in the cell genome. Two transfection methods were used to evaluate the probability of obtaining single copy integration: a cellfectin-based method and an electroporation-based method. The transfection efficiency was significantly higher for the cellfectin-based transfection and clone screening is ongoing for each population. Preliminary cassette exchange studies were already performed in a few clones by co-transfecting cells with the flipase and target constructs. Exchange of the tagging with the target triggers the activation of the neomycin resistance gene; as cells resistant to neomycin could be selected, the exchange was successfully accomplished. The possibility of re-using a well characterized locus for easy gene integration by-passes the need for clone screening each time a new protein needs to be produced. This new cell line will have additional advantages compared to commercial Sf9 cells for protein production as well as fundamental physiological studies.

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Acknowledgments: Funding by Fundação para a Ciência e Tecnologia through the project (PTDC/EBBEBI/102266/2008) and PhD fellowship (SFRH/BD/43830/2008)

## P.37 Impact of Gene Vector Design on the Control of Recombinant Monoclonal Antibody Production by CHO Cells

Sarah L. Davies[1], Peter M. O'Callaghan[1], Jane McLeod[1], Leon P. Pybus[1], Yun Hee Sung[2], James Rance[3], Stephen J. Wilkinson[1], Andrew J. Racher[3], Robert J. Young[3], David C. James[1]

[1]University of Sheffield, Sheffield, U.K.,[2]LG Life Sciences, Biopharmaceutical R&D, Daejeon, South Korea, [3]Lonza Biologics, Slough, U.K.

In this study we systematically compare two vector design strategies for recombinant monoclonal antibody (Mab) synthesis by Chinese Hamster Ovary (CHO) cells; a dual opening reading frame (ORF) expression vector utilizing separate CMV promoters to drive heavy chain (HC) and light chain (LC) expression independently, and a single ORF vector design employing a single CMV promoter to drive HC and LC polypeptide expression joined by a foot and mouth disease virus F2A polypeptide self-cleaving linker sequence. Initial analysis of stable transfectants showed that transfectants utilizing the single ORF vector designs exhibited significantly reduced Mab production. We employed an empirical modeling strategy to quantitatively describe the cellular constraints on recombinant Mab synthesis in all stable transfectants. In all transfectants an intracellular molar excess of LC polypeptide over HC polypeptide was observed. For CHO cells transfected with the single ORF vectors, model-predicted and empirical intracellular intermediate levels could only be reconciled by inclusion of nascent HC polypeptide degradation. Whilst a local sensitivity analysis showed that qMab of all transfectants was primarily constrained by recombinant mRNA translation rate, our data indicated that all single ORF transfectants exhibited a reduced level of recombinant gene transcription and that Mab folding and assembly reactions generically exerted greater control over qMab. We infer that the productivity of single ORF transfectants is limited by ER processing/degradation "capacity" which sets a limit on transcriptional input. We conclude that gene vector design for oligomeric recombinant proteins should be based on an understanding of protein-specific synthetic kinetics rather than polypeptide stoichiometry.

## P.38 A Mechanistic Understanding of Production Instability in CHO Cell Lines Expressing Recombinant Monoclonal Antibodies

Minsoo Kim[1], Peter M. O'Callaghan[1], Kurt A. Droms[2], David C. James[1]

[1]University of Sheffield, Sheffield, U.K., [2]Pfizer Corporation, Chesterfield, MO

One of the most significant problems in industrial bioprocessing of recombinant proteins using engineered mammalian cells is the phenomenon of cell line instability, where a production cell line suffers a loss of specific productivity (qP). This phenomenon occurs with unpredictable kinetics and has been widely observed in Chinese hamster ovary (CHO) cell lines and with all commonly used gene expression systems. The underlying causes (both genetic and physiological) and the precise molecular mechanisms underpinning cell line instability have yet to be fully elucidated, although recombinant gene silencing and loss of recombinant gene copies have been shown to cause qP loss. In this work we have investigated the molecular mechanisms underpinning qP instability over long-term sub-culture in CHO cell lines producing recombinant IgG1 and IgG2 monoclonal antibodies (Mab's). We demonstrate that production instability derives from two primary mechanisms: (i) epigenetic - methylation-induced transcriptional silencing of the CMV promoter driving Mab gene transcription and (ii) genetic - progressive loss of recombinant Mab gene copies in a proliferating CHO cell population. We suggest that qP decline resulting from loss of recombinant genes is a consequence of the inherent genetic instability of recombinant CHO cell lines.

## P.39 Transient expression of antibodies in CHO and HEK293 cells using the cost-effective transfection reagents LPEI-MAX and PEI, respectively

Christina Rottbøll Andersen, Lars S. Nielsen and Anne B. Tolstrup

Symphogen, Lyngby, Denmark

During preclinical development of recombinant antibodies large amounts of potential antibody drug candidate material is often desired for in-vivo studies, frequently at times where the time-consuming generation of stable cell lines has not been initiated. Instead, transient expression platforms may be used for the production of preclinical material.

Multiple transient expression platforms exist, utilizing different cell types and transfection reagents, many of which are rather expensive. To reduce the costs of transient expression, we have investigated the use of the cheaper reagents PEI and LPEI-MAX for transfection of HEK293and CHO cells, respectively. Both systems were in-licensed from Dr. Yves Durocher, University of Montreal, Canada.

Using these transient expression platforms we have expressed several antibodies covering a wide range of production levels, including antibodies with low, intermediate and high expression capacity. Transient expression in CHO cells generally resulted in lower antibody titers than in HEK293 cells as expected from literature. However, we observed that some antibodies gave higher antibody titers when transiently expressed in CHO cells compared to HEK293 cells. Notably, the antibodies showing increased titers in CHO cells were those least efficiently expressed in HEK293 cells. This indicates that cell lines are differently equipped for expression of different antibodies, suggesting that different cell lines might be evaluated for optimal expression of a specific antibody.

To produce larger amounts of antibody in a short and cost-effective timeframe we tested the scalability of PEI mediated transfections of an antibody efficiently expressed in HEK293 cells. The antibody was expressed in different scales and vessels ranging from 200 ml in a shaker to 1 L in a disposable wave bag. Highly similar antibody titers above 200 mg/L were observed across the scales. Together these data demonstrate that the cost-effective PEI transfection reagent for transient expression in HEK293 cells can be used for large scale expression of antibodies, with transient expression in CHO cells being an alternative platform for efficient expression of antibodies with low expression capacity in HEK293 cells.

## P.40 An Efficient, Integrated Platform for Protein Expression in Both Spodoptera and Mammalian Cell Systems

Robert Kirkpatrick, Wangfang Hou and Thau Ho

GlaxoSmithKline, USA

The baculovirus (BV) expression system is one of the most versatile methods used for the production of diverse protein classes. Although recombinant production in baculovirus-insect cells provides a robust, cost effective alternative to mammalian cell expression, insect cell production is limited in its ability to produce more complex post-translational modifications which may be needed to confer full biological activity. Consequently, separate expression in both BV and mammalian systems may be needed in order to compare relative activities of proteins produced prior to committing to large scale production. Here we describe the application of a recombinant baculovirus engineered with a dual CMV polyhydrin promoter capable of driving high level expression in both Spodoptera and mammalian cell lines. This hybrid-promoter virus has been successfully used to express GFP, along with other mammalian proteins in both baculovirus-infected Spodoptera frugiperda insect cells and bacmam virus transduced HEK293F and U2OS cells. The ability to use the same virus to express proteins for expression in both BV and mammalian expression systems provides an efficient, simplified method for the direct comparison of mammalian and insect systems in the identification of a suitable production method.

**P.41 Generation of mammalian cell lines overproducing clotting factors VIII and IX and PDGF**

Marcos A. A. Demasi, Theri L. Degaki Ana Claudia O. Carreira & Mari C. Sogayar

University of São Paulo, SP, Brazil

Objective: Generation of mammalian cell lines over-producing recombinant proteins (human Factor VIII and IX and PDGF-Platelet-derived growth factor) to be used as biopharmaceuticals in treatment of hemophilia A and B, respectively, and in cell therapy of orthopedic and odonthological lesions and wound healing.

Methods and Results: Expression of active human clotting factor VIII (FVIII) was achieved using two alternatives strategies, namely: 1) CHO-DG44 cell clones highly producing FVIII (as determined by the chromogenic assay) were obtained through stable co-transfection of FVIII light (Lch) and heavy (Hch) chains sequences cloned into a dhfr bicistronic expression vector, followed by co-amplification of the dhfr gene, upon selection with methotrexate and isolation of highly producing clones; and 2) CHO-DG44 cell populations highly producing the FVIII Hch were firstly established through stable transfection of the FVIII Hch dhfr bicistronic expression vector, followed by co-amplification of dhfr. These cell populations were then stably transfected with the FVIII Lch expression vector containing the G418-resistance sequence with active FVIII highly producing clones being isolated. Higher levels of the FVIII Hch and a more balanced expression of the two chains were obtained using the second strategy, as determined by ELISA. FIX and PDGF-B cDNAs were amplified from a cDNA bank, constructed by our lab, and sequenced. FIX was cloned into the same dhfr bicistronic expression vector used to express FVIII aiming at the isolation of highly producing cell clones. These clones produce medium levels (determined by ELISA) of active FIX (determined by a chromogenic assay). PDGF-B was transferred into a lentiviral expression vector, and after lentivirus production, CHO-dhfr$^{-/-}$ infected cells over-expressing PDGF-B were obtained. Characterization of PDGF-overproducing mammalian cell clones is underway.

Conclusion: This NUCEL initiative to produce recombinant proteins in mammalian cells is not only generating biopharmaceuticals of major social and economical importance for Public Health in Brazil, but, also, promoting entrepreneurship and greater interaction between the University and the private biotech and pharmaceutical sectors, in addition to enhancement of personnel qualification to operate in cell and molecular therapy translational research. Support: FAPESP, CNPq, CAPES, BNDES, FINEP, MS-DECIT, MCT.

## P.42 Cell line specific control of PEI-mediated transient transfection optimised with 'Design of Experiments' methodology

Ben C. Thompson[1], Camille R. J. Segarra[1], Olivia L. Mozley[1], Olalekan Daramola[2], Ray Field[2], Peter R. Levison[3], David C. James[1]

[1]University of Sheffield, Sheffield, U.K., [2]MedImmune, Cambridge, U.K., [3]Pall Europe Ltd, Portsmouth, U.K.

We describe a Design of Experiments (DoE) response surface modeling strategy to optimize the concentration of basal variables underpinning polyethylenimine (PEI) mediated transfection of different CHO-K1 derived parental cell populations in a chemically defined medium, specifically the relative concentration of linear 25 kD PEI, host CHO cells and plasmid DNA. Utilizing recombinant secreted alkaline phosphatase (SEAP) reporter activity as the modeled response, a discrete simple maximum was predicted for each CHO host cell population. Differences between the modeled optima derived from host cell specific differences in PEI cytotoxicity, such that the PEI : cell interaction effectively limited PEI-DNA polyplex load at a relatively constant PEI : DNA ratio. However, across the three CHO host cell populations, SEAP reporter production was not proportional to plasmid DNA input at the host cell specific predicted basal variable optima. A ten-fold variation in SEAP reporter output per mass of plasmid DNA delivered was observed. To determine the cellular basis of this difference in transient productivity, host CHO cells were transfected with fluorescently labeled polyplexes followed by flow cytometric analysis. Each CHO host cell population exhibited a distinct functional phenotype – varying in the extent of PEI-DNA polyplex binding to the cell surface and degree of polyplex internalization. SEAP production was directly proportional to the level of polyplex internalization and heparan sulphate proteoglycan level. Taken together, these data show that choice of host CHO cell line is a critical parameter which should rationally precede cell line specific transient production platform design using DoE methodology.

## P.43 VARIANTS OF ANTI-DIGOXIN MONOCLONAL ANTIBODY OBTAINED BY PHAGE DISPLAY

Murata, Viviane M[1].; Tsuruta, Lilian R[1]; Schmidt, Mariana C.B[1]. & Moro, Ana Maria[1,2]

[1]Instituto Butantan, Sao Paulo, Brasil, [2]INCT-iii, Science and Technology Ministry, Brasil

Introduction: Digoxin is a pharmaceutical used in the control of cardiac dysfunction. Its therapeutic window is narrow, with the therapeutic dosage very close to the toxic dosage. To counteract the toxic effect, a polyclonal anti-digoxin Fab fragment (anti- Dig Fab) is available commercially. The work present herein is based on monoclonal anti-digoxin antibodies, which would account for a product with a specific potency and more precise dosage for the detoxification of patients under digoxin treatment. The phage display technology allows the selection of high affinity and specific antibody sequences to a determined antigen and their production in unlimited amounts. This technology makes use of filamentous phages able to incorporate fragments of exogenous DNA and expose the synthesized protein on its surface, like antibody fragments, that can be selected by the appropriate antigen.

Objective: To obtain variants by phage display and characterize anti-digoxin Fab fragments by its affinity.
Methodology: An anti-digoxin mAb was generated at the Heart Institute (Sao Paulo, Brasil). This work started with the total RNA extraction for the cDNA synthesis. Specific primers were used for the light chain and Fd amplifications, then cloned sequencially in a phagemid vector (pComb3XTT) for the combinatorial Fab library construction. Clones displayed on the surface of phages were selected by the binding to the antigen (digoxin-BSA conjugate). Random selected clones were evaluated for the presence of light and heavy chains and the positive clones were sequenced. The clones displaying variation in the variable regions were induced to produce soluble Fabs.

Results and Discussion: The constructed library was analyzed for anti-digoxin expression. Out of 10 clones randomly chosen, 6 were positive. The sequence showed 2 identical clones and one presented a pseudogene in the light chain. Four clones presenting variations in the framework 1 were induced to express soluble Fabs, which were positive for anti-digoxin binding in ELISA assays. The clones will be further analyzed by their production capacity and kinetic analyses by BIAcore technology to evaluate affinity association and dissociation constants.

Financial support: CNPq

## P.44 Production of Recombinant Human Growth Factors in Mammalian Expression Systems for Angiogenesis and Wound Healing

Ana Claudia O. Carreira, Gustavo G. Belchior, Fernanda M.C. Sodré, Theri L. Degaki, Raquel S. Cruz and Mari C. Sogayar

University of São Paulo, SP, Brazil

Wound healing is a complex process involving four different phases: haemostatic, inflammatory, proliferative and remodeling. By manipulating the composition of a set of growth factors, it is possible to accelerate and modify this process. VEGF is a key factor for the proliferative phase through angiogenesis, which is a process involved in several diseases and of great importance for establishing a scaffold over which tissue remodeling takes place. Therefore, the use of recombinant human VEGF (rhVEGF) and other specific factors is of great therapeutical interest. In order to achieve a robust production of proteins, lentiviral vectors were the system of choice for their ability to efficiently transduce a wide diversity of dividing and non-dividing cells and to stably integrate the transgene into the host genome, generating long-term protein expression. This system is suitable for robust expression of recombinant proteins in mammalian cells bearing the proper post-translational machinery for correct production, folding and biological activity of glycoproteins. PDGF-B, VEGF121 and VEGF165 cDNAs were amplified and cloned into a lentiviral vector containing a GFP reporter gene. For other growth factors involved in the process (G-CSF, GM-CSF, TGF-β1, TGF-β3) a plasmid vector was chosen. All cDNAs were amplified from our human full-length cDNA Bank and subcloned into the pGEM®-T-Easy vector. E. coli transformants were screened by colony PCR. Upon DNA sequencing, the inserts were transferred to the mammalian expression vectors and cells were co-transfected with CSFs, PDGF-B and VEGFs constructs along with a Hygr vector for clone selection. CSFs and PDGF-B were successfully expressed in different mammalian cell lines, which were assessed by Western blot. Biological activity of recombinant human CSFs was confirmed in vitro. Preliminary results show that mammalian cells were efficiently transfected with the VEGF121 transgene via GFP fluorescence. Characterization of the mammalian cell clones for the other growth factors is underway. Ex-vivo aortic ring and fibrin gel assays were established to measure the VEGFs biological activity. The production of these biopharmaceuticals in our mammalian expression system provides a promising alternative treatment for diseases which have angiogenesis and wound healing as their central issue.
Support: FAPESP, CNPq, CAPES, BNDES, FINEP, MS-DECIT, MCT. Keywords: Wound Healing, VEGF, recombinant growth factors/biopharmaceuticals, angiogenesis.

**P.45 Development of a cell-based enzyme fragment complementation assay for NRF2 activators using the BacMam delivery technology**

Wensheng Xie, Christina Pao, Taylor Graham, Ed Dul, Quinn Lu, Thomas D. Sweitzer, Robert S. Ames, and Hu Li

GlaxoSmithKline, Collegeville, PA

The nuclear factor-E2 related factor 2 (Nrf2) plays an important role in regulating Phase II genes, a panel of protective proteins and enzymes. Under basal conditions, Nrf2 binds to KEAP1 which targets Nrf2 for ubiquitin mediated degradation. Under stress conditions, the Keap1-dependent ubiquitination of Nrf2 is disrupted and Nrf2 is accumulated in the cell, and translocated into nucleus to interact with its binding partners (such as MafK and RunX2) which leads to induction of Phase II genes. Nrf2 pathway is directly involved in many types of diseases, including COPD, cancer and inflammatory disease, and thus, regulation of NRF2 activity represents an attractive opportunity for drug discovery.

We have developed a cell-based luciferase enzyme fragment complementation (EFC) assay to monitor NRF2 cellular activity using BacMam delivery technology. We used this assay to detect the interaction of Nrf2 and its binding protein MafK or RunX2, both of which were fused with fragments of luciferase. Luciferase activity would reflect the stabilization and activation of Nrf2 protein when the cells are stressed. This assay was validated with known tool compounds and enabled a linear, sensitive, and robust assay that is amenable for screening large compound collection.

## P.46 Different but the same? Enzymatic and structural analysis of JAK2/3-Inhibition

Adrian Schomburg[1], Martin A. Augustin[1], Holger Steuber[1,2], Lars Neumann[1], Jark Böttcher[1], Sabine Höppner[1] and Dirk Ullmann[1]

[1]Proteros biostructures, Germany [2]Universität zu Lübeck, Germany

The Janus kinase family in mammals (JAK1, JAK2, JAK3 and Tyk2) comprises four non-receptor protein tyrosine kinases (PTK) that are involved in the signalling cascades (e.g. JAK/STAT pathway) of a wide range of type I and type II cytokine receptors. The kinase activity of JAK proteins resides in the C-terminal JAK homology 1 domain (JH1) adjacent to the regulatory pseudokinase domain (JH2). The N-terminal domains (JH3-JH7) are responsible for receptor interaction and protein stabilization (Williams et al., 2009).

Mutations in JAK proteins are associated with – for example – leukemia, cardiovascular diseases or rheumatoid arthritis and therefore the JAK family represents an important drug target. The family has been extensively characterized, including crystallographic analysis of the kinase domains (KD) of JAK1, 2 and 3 (Boggon et al., 2005; Lucet et al., 2006; Williams et al., 2009). In the paper by Williams et al., the structure of a pan-JAK inhibitor (CP690,550) has been determined in complex with the kinase domains of both, JAK1 and JAK2. The binding mode of CP690,550 is highly similar in both proteins and the authors suggest the same binding mode also for JAK3. Here we present structural and biochemical data on the interaction of CP690,550 with JAK2 and JAK3 kinase domain.

## AUTHORS INDEX

# List of participants

| | |
|---|---|
| Airenne, Kari | University of Eastern Finland |
| Alves, Paula | IBET and ITQB-UNL |
| Ames, Robert | GlaxoSmithKline |
| Andersen, Christina Rottbøll | Symphogen |
| Assenberg, Rene | Novartis AG |
| Astray, Renato Mancini | Instituto Butantan |
| Augusto, Elisabeth | IPT - Technological Research Institute |
| Bäckström, Malin | University of Gothenburg |
| Balaji Chandra Sekhar, Sinhadri | Free University Berlin |
| Bandeiras, Tiago | IBET |
| Barron, Niall | National Institute for Cellular Biotechnology |
| Belchior, Gustavo | University of São Paulo |
| Birch, John | Henley Biotechnology |
| Blissard, Gary | Cornell University |
| Boehnlein, Ernst | TET Systems GmbH & Co. KG |
| Böhm, Ernst | Baxter BioScience |
| Bosques, Carlos | Momenta Pharmaceuticals |
| Bosteels, Hella | GlaxoSmithKline |
| Branco Novo, Juliana | Instituto Butantan |
| Bredl, Simon | Redbiotec AG |
| Breitling, Reinhard | Jena Bioscience GmbH |
| Büssow, Konrad | Helmholtz Centre for Infection Research |
| Butler, Michael | University of Manitoba |
| Capdevila, Cécile | Sanofi-Aventis |
| Chen, Stephanie | GlaxoSmithKline |
| Chiou, Henry | Life Technologies |
| De Jongh, Wian | ExpreS2ion Biotechnologies |
| Degaki Theri Leica | Sao Paulo University |
| Deman, Johanne | GSK Biologicals |
| Demasi, Marcos | University of Sao Paulo |
| Eckardt, Nicole Susann | Miltenyi Biotec |
| Ernst, Wolfgang | University of Natural Resources and Life Sciences |
| Faust, Nicole | Cevec Pharmaceuticals GmbH |
| Feary, Marc | Lonza Biologics |
| Freedman, Robert | University of Warwick |
| Geisse, Sabine | Novartis |
| Genzel, Yvonne | Max Planck Institute |
| Grabherr, Reingard | University of Natural Resources and Life Sciences |
| Hargreaves, Emma | University of Kent |

| | |
|---|---|
| Hayes, Ph.D, Scott | Mirus Bio LLC |
| Heine, Peer | MaxCyte, Inc. |
| Hiller, Gregory | Pfizer, Inc. |
| Ho, Ying Swan | Bioprocessing Technology Institute |
| Hou, Wangfang | GlaxoSmithKline |
| Hudson, Laura | Health Protection Agency |
| Izeddin, Ignacio | Ecole normale supérieure |
| James, David | University of Sheffield |
| Jans, Erik | MaxCyte, Inc. |
| Jarvis, Donald | University of Wyoming |
| Johnson, Matt | Abcam plc |
| Jones, Meredith | Life Technologies |
| Jorge, Soraia | Instituto Butantan |
| Kadwell, Sue | GlaxoSmithKline |
| Kamen, Amine | National Research Council Canada |
| Kaseko, Galina | The Stephen Sanig Research Institute |
| Kaufman, Randal | Sanford-Burnham Medical Research Institute |
| Kayser, Kevin | SAFC |
| Kemp, Christopher | Kempbio, Inc. |
| Klausberger, Miriam | University of Natural Resources and Life Sciences |
| Knoch, Lars | Cellectis bioresearch |
| Koch, Stefan | Roche Diagnostics GmbH |
| Kost, Tom | GlaxoSmithKline |
| Kwang, Jimmy | National University of Singapore |
| Lee, Brian | PBS Biotech |
| Lee, Gyun Min | KAIST |
| Lee, Jae Seong | KAIST |
| Lee, So Jeong | KAIST |
| Lee, Yih Yean | CSL Limited |
| Lewis, Abasha | DHHS/NIH/NIDA |
| Lièvremont, Jean-Philippe | GlaxoSmithKline Biologicals |
| Lin, Andy | Genentech |
| Liu, Jimmy | BioMarin Pharmaceuticals |
| López Almagro , Rosa | Almirall, S.A. |
| Lu, Quinn | GlaxoSmithKline |
| Lundstrom, Kenneth | PanTherapeutics |
| Magalhães, Geraldo | Instituto Butantan |
| Mahaworasilpa, Tohsak | The Stephen Sanig Research Institute |
| Mann, Kimberly | EMD Millipore |
| McCall, Eileen | AstraZeneca Pharmaceuticals |

| | |
|---|---|
| Michel, Patrik | Ecole Polytechnique Federale de Lausanne |
| Monteiro, Francisca | IBET ITQB-UNL |
| Moro, Ana | Instituto Butantan |
| Mummery, Christine | Leiden University Medical Centre |
| Nakane, Aya | Chugai Pharmaceutical Co., Ltd. |
| Nettleship, Joanne | OPPF-UK, Oxford University |
| Nielsen, Lena | BioInvent International AB |
| Palmberger, Dieter | University of Natural Resources and Life Sciences |
| Patrone, Marco | ITQB_UNL/ IBET |
| Pereira, Carlos | Instituto Butantan Laboratorio de Imunologia Viral |
| Pijlman, Gorben | Wageningen University, Laboratory of Virology |
| Quinsey, Noelene | Monash University |
| Rafty, Louise | DNA2.0 Inc |
| Rahman-Huq, Nahid | OPPF-UK |
| Rao, Govind | University of Maryland, Baltimore County |
| Real, Gonçalo | IBET, ITQB-UNL |
| Rebordosa, Xavier | HIPRA |
| Reid, Steven | The University of Queensland |
| Reinl, Tobias | GlycoThera GmbH |
| Rendeiro, Dennis | Merck-Bioprocess Development |
| Rysiok, Thomas | Merck KGaA |
| Sadhukhan, Ramkrishna | Abbott Labs |
| Sandhu, Devender | Therapure Biopharma |
| Sanford, James | Life Technologies |
| Saunders, Fay | UCB |
| Schiedner, Gudrun | Cevec Pharmaceuticals GmbH |
| Schleef, Martin | PlasmidFactory |
| Schmid, Georg | F. Hoffmann-La Roche |
| Schmidt, Stefan | ERA Biotech |
| Schnapp, Gisela | Boehringer Ingelheim Pharma |
| Sebastià, Eduard | HIPRA |
| Shah, Girish | GlaxoSmithKline |
| Shea, Kenneth J. | University of California |
| Shiloach, Joseph | NIDDK/NIH |
| Shimizu, Noriaki | Hiroshima University |
| Shrestha, Binesh | Novartis Pharma |
| Sidhu, Sachdev | University of Toronto |
| Smales, Mark | University of Kent |
| Soares, Carlos | IPEN-CNEN/SP |

| | |
|---|---|
| Sodoyer, Regis | Sanofi Pasteur |
| Sramon, Ashwajit | MCU |
| Sterner, Jane | Eli Lilly & Company |
| Steven, John | Pfizer UK |
| Stevenson, Jessica | MedImmune |
| Stutzman-Engwall, Kim | Pfizer, Inc |
| Szewczyk, Boguslaw | University of Gdansk |
| Szymanik, Michal | Adamed Sp. z o.o. |
| Tang, Yvette H. | BioMarin Pharmaceuticals |
| Teixeira, Ana | IBET, ITQB-UNL |
| Tenenbaum, Scott | University at Albany-SUNY |
| Tornberg, Ulla-Carin | BioInvent International AB |
| Trill, John | GlaxoSmithKline |
| Van Dongen, Frank | Cellution Biotech |
| Veith, Nathalie | Fraunhofer ITEM Pharmaceutical Biotechnology |
| Verbisck, Newton | EMBRAPA Gado de Corte |
| Villalobos, Alan | DNA2.0 |
| Volkman, Loy | University of California |
| Von Strandmann, Ralph | TAP Biosystems |
| Wilcox, Andrew | Abcam plc |
| Wilde, Monika | University of Natural Resources and Life Sciences |
| Williams, Steven | AvidBiotics Corp. |
| Zhou, Meixia | Genentech Inc |
| Zoro, Barney | TAP Biosystems |

**NOTES**