EXPRESSION STREAM

Experience the future of biologics.

Difficult to Express Proteins
Optimizing Protein Expression
Purifying Antibodies

KEYNOTES

Raimund Dutzler, Ph.D., Professor, Biochemistry, University of Zurich

Lorenz M. Mayr, Ph.D., Executive Director, Unit Head Biology, Protease Platform, Novartis

Douglas Cecchini, Ph.D., Director, Technical Development, Biogen Idec, Inc.

RECOMMENDED SHORT COURSES

Biological Mass Spectrometric Applications for Drug Discovery and Product Development

How Size Matters in Therapeutic Antibody Design

Changing Guidelines and Regulatory Expectations for Biologics

Characterization Techniques for Protein Therapeutics – Orthogonal vs. Complementary

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SC1 - MICROFLUIDICS FOR ANTIBODY SELECTIONS AND SCREENING

- Integrated Single-Cell Analysis for Antibody Discovery
- Use of Microfluidics for Ultra-High-Throughput Antibody Screening and Selection
- Comparing Tools for Antibody Screening

J. Christopher Love, Ph.D., Associate Professor, Chemical Engineering, Massachusetts Institute of Technology
David Welz, Ph.D., Gordon McKay Professor, Applied Physics, Harvard University

SC2 - TRANSLATIONAL CONSIDERATIONS FOR DEVELOPMENT OF MONOCLONAL ANTIBODIES: FOCUS ON EARLY DISCOVERY PART 1

- Considerations for target selection, antibody screening and mAb pre-clinical development
- Antibody affinity and biophysical characterization: Biacore, Kineaxa, and FACS
- Pre-clinical Considerations – A science based approach: Design goal, MOA, choice of species, and pre-clinical plans

Mohammad Tabrizi, Ph.D., Vice President, Pre-Clinical Development, AnaptysBio, Inc.
Gadi Bornstein, Ph.D., Principal Scientist, AstraZeneca R&D
Cheryl Funelas, Manager, Bioanalytical Development, Takeda San Francisco
Scott Kikamp, Ph.D., Research Fellow, Biophysical Chemistry and Research Informatics, Takeda San Francisco

SC3 - SCREENING & SELECTING CANDIDATE ANTIBODIES

- Strategies for antibody discovery
- Shortening the timeline for identifying ASCs (antibody secreting cells)
- Integrating technology, such as biosensors and microfluidics

David Lowe, Ph.D., Principal Scientist, Display Technology, MedImmune
XiaoDong Yang, M.D., President & CEO, Apexigen, Inc.
Arnout Gerritsen, Ph.D., Director, Assay & Bioanalytical Science, Genmab

SC4 - TRANSLATIONAL CONSIDERATIONS FOR DEVELOPMENT OF MONOCLONAL ANTIBODIES: FOCUS ON NONCLINICAL DEVELOPMENT TO CLINIC PART 2

- Considerations for immunoassay development in support of pharmacokinetic, immunogenicity & biomarker evaluation
- Antibody safety, species selection, introduction to surrogate approaches in development of monoclonal antibodies
- Translation of exposure – Response data from discovery into the clinic in support of FIH dosing

Mohammad Tabrizi, Ph.D., Vice President, Pre-Clinical Development, AnaptysBio, Inc.
Gadi Bornstein, Ph.D., Principal Scientist, AstraZeneca R&D
Cheryl Funelas, Manager, Bioanalytical Development, Takeda San Francisco
Scott Kikamp, Ph.D., Research Fellow, Biophysical Chemistry and Research Informatics, Takeda San Francisco

SC5 - BIOLOGICAL MASS SPECTROMETRIC APPLICATIONS FOR DRUG DISCOVERY AND PRODUCT DEVELOPMENT

- Geared for individuals supporting biopharmaceutical drug characterization analyses using mass spectrometry in either discovery or product development
- A novel method for assessing disulfide bond networks will be discussed
- Methods for performing glycosylation analyses, PTM analyses, and stress assessments on drug candidates will be covered

Chair: Jennifer Nemeth, Ph.D., Principal Research Scientist, Biologics Mass Spectrometry & Allied Technologies, Centocor R&D, Inc.
Alain Ballard, Ph.D., Scientific Director, Analytical & Formulation Sciences, Amgen Yoshi Hamuro, Director, Analysis, ExSAR
Ron Orlando, Ph.D., Professor, Biochemistry and Molecular Biology, Complex Carbohydrate Research Center, University of Georgia

SC6 - PHAGE AND YEAST DISPLAY LIBRARIES AND THEIR SCREENING

- Phage display and construction of phage-displayed peptide, scFv and Fab libraries
- Yeast display and construction of yeast-displayed scFv and Fab libraries
- Selection and screening technologies that are compatible with phage and yeast-display libraries

Jamie K. Scott, M.D., Ph.D., Professor, Canada Research Chair, Molecular Immunology, Molecular Biology & Biochemistry; Faculty of Health Sciences, Simon Fraser University
Andrew M. Bradbury, M.B. B.S., Ph.D., Staff Scientist, Biosciences, Los Alamos National Laboratory
James D. Marks, M.D., Ph.D., Professor, Anesthesia & Pharmaceutical Chemistry, University of California, San Francisco; Chief of Anesthesia and Vice Chairman, Anesthesia & Perioperative Care, San Francisco General Hospital

SC7 - HOW SIZE MATTERS IN THERAPEUTIC ANTIBODY DESIGN

- Strategies for achieving more powerful and smaller antibody therapeutics
- Designing antibody therapeutics to penetrate tumors and tissue
- Exploiting antibodies’ natural abilities

David Blakey, Ph.D., Chief Scientist, Oncology Discovery, AstraZeneca R&D
K. Dane Wittrup, Ph.D., C.P. Dubbs Professor, Chemical & Biological Engineering, Associate Director, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology
Michael T. Stumpp, Ph.D., CSO, Molecular Partners AG
H. Kaspar Birn, Ph.D., Vice President Technology & Co-Founder, Molecular Partners AG

SC8 - CHANGING GUIDANCES AND REGULATORY EXPECTATIONS FOR BIOLOGICS

- Update on ICH6 pre-clinical guidance document
- Impact on regulatory changes to design and cost of conducting pre-clinical studies

Kevin Mattison, Ph.D., Principal Scientist, Bioanalytics, Malvern Instruments
Ulf Nobbmann, Ph.D., Senior Applications Specialist, Nanometrics, Malvern Instruments
Jason Sanchez, Ph.D., Separations Product Specialist, Malvern Instruments

SC9 - CHARACTERIZATION TECHNIQUES FOR PROTEIN THERAPEUTICS – ORTHOGONAL VS. COMPLEMENTARY

- Introducing concept of First-in-Human (FIH) trials
- Approaches to optimize initial clinical studies, post TeGenero
- Interactive case study exercises on advancing new biologics into Phase I clinical trials

Joy Cavagnano, Ph.D., DABT, RAC, President, Access BIO LC
Julia Barrett, M.D., MPH, Senior Clinical Consultant, Biologics Consulting Group, Inc.

- This workshop covers the theory and general application of both traditional and emerging technologies suitable for protein therapeutic characterization, across the applicable size range of 1 nm to 100 um.
- What’s the difference between 1m measured by DSC, CD, & DLS? Can DLS be used to confirm SEC results? What’s the relevance of protein charge on formulation stability? Can particle counting be achieved in the 100 nm to 1 um size range? What role does protein aggregate “morphology” play?

Kevin Mattison, Ph.D., Principal Scientist, Bioanalytics, Malvern Instruments
Ulf Nobbmann, Ph.D., Senior Applications Specialist, Nanometrics, Malvern Instruments
Jason Sanchez, Ph.D., Separations Product Specialist, Malvern Instruments

* Separate Registration Required
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Michaela Wendeler, Ph.D., Scientist I, Biopharmaceutical Development, complex Biologics
Optimization as the Key for the Development of 11:10 integrated upstream and Downstream with Poster viewing

9:10 The Importance of Characterization in Successful Protein Production
Jeff Culp, Ph.D., Associate Research Fellow, Primary Pharmacology Group, Pfizer Research and Development
It is much easier to express a protein than it is to produce a fully functional protein for use in Drug Discovery. Specific examples will be described of the importance of proper protein characterization to eliminate potential mistakes. Guidelines will be suggested to aid the protein production specialist. Examples will include proteins intended for use in target screens, NMR, protein crystallization and biophysical characterization.

9:40 refolding, Purification, and characterization of the ectodomain complex of the CGRP receptor
Norzehan Abdul-Menan, Ph.D., Researcher I, Gene Expression, Vertex Pharmaceuticals, Inc.
The calcitonin gene-related peptide receptor is a heterodimer of two membrane proteins: calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein 1 (RAMP1). CLR is a class B G-protein-coupled receptor, possessing a large N-terminal extracellular domain (ECD) for ligand recognition and binding. Heterodimerization of CLR with RAMP1 provides specificity for CGRP peptide. The expression, purification, and refolding of the heterodimer of the ectodomain from inclusion bodies will be presented. The refolded complex forms a stable, monodisperse complex and is competent to bind ligands.

10:10 Grand Opening Coffee Break in the Exhibit Hall with Poster Viewing

11:10 Integrated Upstream and Downstream Optimization as the Key for the Development of Complex Biologics
Michaela Wendeler, Ph.D., Scientist I, Biopharmaceutical Development,

Hitting the Ground Running

OPENING KEYNOTE PRESENTATION
8:40 Novel Tools for the Overexpression of Transport Proteins
Raimund Dutzler, Ph.D., Professor, Biochemistry, University of Zurich
The recent success in the structure determination of membrane proteins is tied to the possibility to characterize large numbers of homologues of a particular protein family in different expression systems to identify candidates with superior biochemical properties. I will discuss a novel tool that allows the rapid generation of expression constructs for all common pro- and eukaryotic expression systems and discuss its application to different ion transport families.

11:40 Poster Spotlight Presentation

12:10 pm Advancing Synthetic Gene Design for Optimal Protein Expression
Mark Welch, Ph.D., Director, Gene Design, DNA2.0, Inc.
Advances in gene design and synthesis have enabled greater insight into the workings of the genetic code. This includes how changes in gene sequences can impact the expression of encoded proteins through mechanisms including codon bias, mRNA stability, and translation initiation. Natural gene sequences have been shaped in response to many different evolutionary pressures, but are rarely optimal for “biotechnological fitness.” Here we present how gene design variables such as codon choice and mRNA structure predictably affect the yield of heterologous protein expression, often by up to orders of magnitude increase in expression levels. Host systems validated include mammalian cell lines (CHO/HEK293), yeast (S.cerevisiae/Ppastoris/K.lactis), E.coli and more.

12:25 Sponsored Presentation (Opportunity Available)

12:40 Luncheon Presentation I
High-Yield in vitro Protein Expression System for Functional Protein Synthesis Using Immortalized Human Cell Lines
Penny Jensen, Ph.D., Research Scientist, Proteomics Research and Development, Thermo Fisher Scientific
Culturing of mammalian cells for the purpose of protein expression is a time consuming and expensive process. An effective alternative is cell-free expression (i.e., in vitro translation) using extracts prepared from mammalian cells. Several immortalized human cell lines, including HeLa, HuH7, and HEK293 have been used to prepare translationally competent extracts. These results along with information regarding our optimized in vitro expression system based on HeLa extracts for producing several hundred micrograms of recombinant protein per ml of reaction, expression of multiple proteins in a single reaction, and high-throughput compatibility of our system will be discussed.

1:10 Luncheon Presentation II (Sponsorship Opportunity Available) or Lunch on Your Own

1:40 Break

MEMBRANE PROTEINS AND OTHER BEASTS

2:00 Chairperson’s Remarks

2:05 Expression and Purification of Membrane Protein Diacylglycerol Acyltransferase
Heping Cao, Ph.D., Principal Research Scientist, Southern Regional
DGAT knockout mice are resistant to diet-induced obesity and lack milk secretion. DGAT genes have been isolated from many organisms, but progress in characterization of the enzymes has been slow because DGATs are membrane-associated and difficult to express and purify. We developed a procedure for full-length DGAT expression in *E. coli* and yeast. This study represents the first description of a procedure for producing full-length recombinant DGAT protein from any species using an *E. coli* expression system.

### 2:35 Recent Progress in Production of Human Membrane Protein Targets and Use in Drug Discovery

Niek Dekker, Ph.D., Principal Scientist, Discovery Enabling Capabilities & Sciences, AstraZeneca R&D Molndal

Results will be presented on the expression of human ion channels in various eukaryotic expression systems. Total protein expression levels have been analyzed using Western blotting and radio-ligand binding. Target localization has been analyzed using confocal microscopy, and functional properties have been studied using electrophysiology. The combined approaches provided good insight in quality of produced targets in the various expression systems. The successful mg-scale production of a human ligand-gated ion channel will be presented including biophysical verification of ligand-binding properties using circular dichroism and isothermal titration calorimetry. Progress on crystallization of this target and ongoing engineering efforts will be presented. In addition, examples of production of other membrane proteins including GPCRs will be discussed.

### 3:05 Refreshment Break in Exhibit Hall with Poster Viewing

### 3:45 A Sensitive Fluorescent Method for Rapidly Identifying and Characterizing Lead Membrane Protein Constructs

Christopher Koth, Ph.D., Scientist, Structural Biology, Genentech, Inc.

A number of features of membrane proteins render them challenging targets for the structural biologist, among which the most important is the difficulty in obtaining sufficient quantities of properly folded and homogeneous protein. To address this, we have developed a simple, high-throughput procedure to rapidly characterize and optimize membrane protein solubility, homogeneity and aggregation state in various buffers/detergents. This method has aided in the purification of several membrane protein targets including GPCRs and ion channels.

### 4:15 From Clones to Crystals on a Shoestring Budget

Jian Payandeh, Ph.D., Pharmacology, University of Washington

The success of a membrane protein structural biology project may warrant a "try everything" approach, but this is seldom feasible. I will describe practical aspects to achieving high-level expression and sample homogeneity in a standard laboratory setting. Key considerations in devising a streamlined and cost-effective screen will be highlighted, and examples from our current structural biology efforts will be detailed.

### 4:45 Problem Solving Breakout Sessions

Concurrent Problem Solving Breakouts are interactive sessions hosted by a moderator to discuss a topic in depth. They are open to all attendees, sponsors, exhibitors, and speakers and provide a forum for discussing key issues and meeting potential partners. *Please pick a topic of your choice and join in.*

### 5:45 - 6:45 Reception in the Exhibit Hall with Poster Viewing

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**TUESDAY, MAY 10**

8:00 am Morning Coffee

THE TOOLS FOR SUCCESS

8:25 Chairperson’s Remarks

Geoffrey S. Waldo, Ph.D., Bioscience Division, Los Alamos National Laboratory

8:30 The Use of the Improved *E. coli* Cell-Free Protein Synthesis for Structural Biology

Takanori Kigawa, Ph.D., Team Leader, Protein Preparation Team, RIKEN Systems and Structural Biology Center

We have been developing and using *E. coli* cell extract-based cell-free system. Our developments improved the efficiency, productivity, and throughput of our system, enabling us to use the system as one of the standard expression methods. We have determined more than 200 X-ray structures and 1,300 NMR structures of proteins/protein domains using our system as the protein production method.

9:00 Optimization of *in vitro* High-Throughput Chemical Lysis of *Escherichia coli*

Geoffrey S. Waldo, Ph.D., Bioscience Division, Los Alamos National Laboratory

9:30 Production of Multifunctional Chimaeric Enzymes in Plants: A Promising Approach for Degrading Plant Cell Wall from Within

Ling Yuan, Ph.D., Associate Professor, Plant & Soil Sciences, Univ. of Kentucky

Biological processing of biomass requires a large number of lignocellulolytic enzymes that are expensive to produce at industrial scales. We address this hindrance by creating multidomain, multifunctional single enzymes and producing these novel proteins in plants. The advantages of this approach include cost reduction in enzyme production, ease in vector construction and plant transformation, and improved biomass feedstock digestibility.

10:00 Coffee Break in the Exhibit Hall with Poster Viewing

10:45 Fine Tuning HIV-1 Envelope Glycoprotein Expression for Pre-Clinical Immunogen Testing

George Sellhorn, Ph.D., Staff Scientist, Viral Vaccines, Seattle Biomedical Research Institute

The HIV-1 Envelope (Env) glycoprotein is a heavily glycosylated trimer of two non-covalently associated heterodimeric subunits and is the sole target on anti-HIV-1 for neutralizing antibodies. High-throughput production of soluble, recombinant versions of Env for pre-clinical comparative evaluation faces several challenges including: inherently low expression levels, heterogeneity in glycosylation, weak inter-subunit association and aggregation. Here we discuss recent improvements in Env production and purification.

11:15 Galectin-1 as a Fusion Partner for the Production of Soluble and Folded Human Beta-1,4-glycosyltransferase-T7 in *E. coli*

Pradman K. Qasba, Ph.D., Chief, Structural Glycobiology Section, SAIC-Frederick, Inc., Ctr for Cancer Research Nanobiology Program, NCI-Frederick

The expression of recombinant glycosyltransferases in *E. coli* often produces aggregated proteins known as inclusion bodies. We show that Galectin-1, which binds a beta-galactoside or a hydrophobic protein sequence, can be used as a fusion partner to produce several recombinant glycosyltransferases as soluble folded fusion proteins in *E. coli.*
11:45 Co-Expression of Proteins in Bacteria: Application to the Production of Soluble Core Histone Protein Complexes and Beyond
Robert N. Dutnall, Ph.D., Assistant Professor, Chemistry & Biochemistry, Sect. of Molecular Biology, Div of Biological Sciences, University of San Diego
Co-expression is a viable strategy for producing multiprotein complexes for biochemical and biophysical studies that has several advantages over expressing components individually. We have used co-expression to produce soluble core histone complexes in bacteria that can be purified by simple chromatographic techniques, facilitating studies of chromatin structure and transcription regulatory proteins. We describe strategies to create expression plasmids to produce two or more proteins in various combinations for optimal complex production, solubility or activity.

12:15 pm Expression of an Antibody Fragment utilizing the Pfenex Expression Technology Platform
Georg Klima, Ph.D., Head Process Science Microbial, Boehringer Ingelheim Biopharmaceuticals
This case study will describe how the Pfenex Expression Technology™ platform was applied to rapidly screen and identify an optimal production strain and fermentation process for a proprietary antibody fragment (Fab). Data comparing expression results between Pfenex Expression Technology and other hosts will be presented.

12:45 Luncheon Presentations (Sponsorship Opportunities Available) or Lunch on Your Own

CREATIVE PROBLEM-SOLVING

2:00 Chairperson’s Remarks

2:05 Production of Novel Protein Therapeutics for Cancer Treatment
Sayed Goda, Ph.D., Director, Research and Training, Shafallah Medical Genetics Center
This work shows for the first time the production of an enzyme that is commonly used for cancer treatment that is much more efficient than the wild type one. The work also includes the isolation and molecular characterization of a natural new form of the enzyme. The two forms of the enzyme would solve many of the problems facing ADEPT techniques for cancer treatment.

2:35 Incorporation of Unnatural Amino Acids into Viral Proteins
Sabrina Lusvarghi, Ph.D., Researcher, HIV Drug Resistance Program, National Cancer Institute, NCI-Frederick
Incorporation of unnatural amino acids with unique biophysical properties into proteins has emerged as an important tool in chemical biology. In particular, our group has used different strategies for the insertion of a variety of unnatural amino acids into viral proteins. Methods of incorporation, as well as application of different non-natural analogs, will be described in this presentation.

3:05 Production and Epitope Mapping of Antibodies Targeting Membrane Proteins
Benjamin Doranz, Ph.D., President and CSO, Integral Molecular, Inc.
Lipoparticles containing high concentrations of structurally-intact GPCRs, ion channels, transporters, and oligomeric proteins have been used to generate high titer serum responses (>1:1000) against these conformationally complex proteins. Upon mAb isolation, Shotgun Mutagenesis Epitope Mapping has been used to identify conformational epitopes on structurally diverse proteins, including GPCRs and viral Envelope proteins, by mapping their interactions with mAbs directly within cells in the proteins’ native structures.

3:35 Refreshment Break in Exhibit Hall with Poster Viewing

4:15 Poster Spotlight Presentation

4:45 Co-Expression of Ferrochelatase Allows for Complete Heme Incorporation into Recombinant Proteins Produced in E. coli
Brian R. Crane, Ph.D., Assistant Professor, Chemistry and Chemical Biology, Cornell University
We have determined that recombinant proteins expressed in E. coli often contain less than a full complement of heme because they rather are partially incorporated with free-baseporphyrin. Porphyrin-incorporated proteins have similar spectral characteristics as the desired heme-loaded targets, and thus are difficult to detect, even in purified samples. We present a straightforward and inexpensive solution to this problem that involves the co-expression of native ferrochelatase with the protein of interest. The method is shown to be effective for proteins that contain either Cys- or His-ligated hemes.

5:15 End of Conference
MAY 11-12 | EXPRESSION STREAM

OPTIMIZING PROTEIN EXPRESSION

WEDNESDAY, MAY 11

7:00 am Registration and Morning Coffee

CHO & MAMMALIAN EXPRESSION SYSTEMS

8:30 Chairperson’s Opening Remarks

8:40 OPENING KEYNOTE PRESENTATION:

Protein Expression in Drug Discovery – New Challenges, New Solutions

Lorenz M. Mayr, Ph.D., Executive Director, Unit Head Biology, Protease Platform, Novartis Pharma AG

Success in drug discovery relies not only on the appropriate selection of molecular targets, but also on the availability of high-quality recombinant protein and cell lines in sufficient amounts and on short time. Whereas protein expression has long been viewed as a mature science with no need for further improvement, current trends in drug discovery show an increased demand for fast & efficient production systems for recombinant proteins and protein complexes to cope with the demands for protein in sufficient amounts needed for modern hit discovery (HTS, FBS, structure) and lead optimization in discovery research.

9:25 FEATURED PRESENTATION:

High-Level Recombinant Protein Production in CHO Cells Using Lentiviral Vectors and the Cumate Gene-Switch

Bernard Massie, Ph.D., Director, Bioprocess Center, Institute of Research and Biotechnology, Research Council of Canada; President, l’Association de Thérapie Génique du Québec (ATGQ)

Fast and efficient production of recombinant proteins for structural and functional studies is a crucial issue for research and for industry. To this end, we have developed an efficient system to generate, in less than 6 weeks, pools of CHO cells stably expressing high-level of recombinant proteins (>100 mg/L). This system takes advantage of the efficient gene delivery of lentiviral vectors (LVs) in highly active transcription sites, coupled with the powerful cumate-regulated promoter that, not only allows for inducible gene expression, but is also 8-fold stronger in CHO cells than the optimized CMV5 promoter.

9:55 Transient Expression of an IL-23R Extracellular Domain Fc Fusion Protein in CHO vs. HEK Cells Results in Improved Plasma Exposure

John Trauger, Ph.D., Group Leader, Genomics Institute of the Novartis Research Foundation

We found that the plasma exposure in mice of an IL23R extracellular domain Fc fusion protein (IL23R-Fc) was improved about 30-fold when the protein was prepared by transient transfection of CHO vs. HEK cells. Characterization of the CHO- and HEK-expressed IL23R-Fc proteins indicated that the difference in their in vivo plasma exposure is due to differential glycosylation.

10:25 Coffee Break in the Exhibit Hall with Poster Viewing

USING microRNAs TO ENHANCE PROTEIN EXPRESSION IN CHO

11:10 microRNAs: New Tools to Manipulate Protein Expression in CHO Cells

Niall Barron, Ph.D., Program Leader, Mammalian Cell Engineering, National Institute for Cellular Biotechnology, Dublin City University

The ability of miRNAs to influence protein expression is now recognized as a fundamental layer of regulation within the cell. We will provide a brief overview of their biogenesis, genomic organization and mode of action, and then go on to describe some of the approaches we have taken to examine their potential application in the bioprocessing area, with particular emphasis on CHO cell engineering.

11:40 mRNA Stability and Antibody Production in CHO Cells: Improvement through Gene Optimization

Shuangping Shi, Ph.D., Associate Principal Scientist, Bioprocess Development, Merck Research Lab, Merck & Co.

Gene optimization substantially enhances antibody production in Chinese hamster ovary (CHO) cells. When gene optimization was applied to the heavy and light chain genes of a therapeutic antibody, we observed increased antibody production in transient transfection as well as in stable clones. It is also demonstrated that elevated heavy chain mRNA level was associated with the increase of antibody production. Further analysis suggests that the increased antibody expression is attributable to enhanced mRNA stability resulting from gene optimization.

12:10 pm Luncheon Presentation I

Fast, Simple and Efficient Method for Production of Biologics using Stable Extrachromosomal Expression System

Mart Ustav, Ph.D., Founder and CEO, Icosagen Cell Factory Ltd

Icosagen Cell Factory has developed efficient technological platform (QMCF Technology) for production of various recombinant proteins, antibodies and VLPs in mammalian (CHO or 293) cell system. Main goal of QMCF technology is stable maintenance and replication of appropriate expression plasmid, fast and feasible upscale and generation of production cell banks.

12:40 pm Key Aspects of Managing Early Phase Development Programs for Long Term Success

George Koch, CSO, Fujifilm Diosynth Biotechnologies

Highlight several CMC best practices for preclinical activities. From selection of a cell line to release of the first clinical batch, product and process developers make decisions that have timeline, financial, and regulatory consequences.

1:10 Break

ESCHERICHIA COLI & CLONAL CELL PRODUCTION

1:30 Chairperson’s Remarks

1:35 Production of Antibody Mixtures and Bispecifics from Single Clonal Cells

John de Kruif, Ph.D., CSO, Merus Biopharmaceuticals BV

Mixtures of antibodies (mAbs) and bispecific mAbs represent next-generation biopharmaceuticals with improved specificity and efficacy. We have approached the manufacturing complexity of producing and developing these formats by using human mAbs that share the same identical germline-encoded light chain (‘single VL’). We show that native mass spectrometry-based analytical methods allow quantitative measurement of all antibody species in a complex mixture and that cation exchange chromatography can be used to efficiently separate bispecifics from the parental mAbs. This technology facilitates the pharmaceutical production of next generation therapeutic antibodies based on intact IgG molecules.
2:05 Optimization of Protein Expression in E. coli: Best Practices and Unusual Tricks for the Production of Protein Suitable for Structural Studies
Rebecca Page, Ph.D., Assistant Professor, Biology and Principal Investigator, Molecular Biology & Cell Biology & Biochemistry, Brown University
I will be presenting both best practices and unusual (‘last ditch’) methods that are used to successfully express both prokaryotic and eukaryotic proteins in E. coli. Topics to be covered include: solubility tags, purification tags, chaperones and in vivo refolding, soluble expression through protein co-expression, and toxins and eukaryotic kinases.

2:35 Automated Microfluidic Analysis for Enhanced Optimization of Recombinant Protein Expression Platforms
Mark Roskey, Ph.D., Senior Vice President, Applied Biology R&D, Caliper Life Sciences
This talk will focus on the use of high throughput microfluidics based electrophoretic analysis for cell culture optimization and clone selection. Applications discussed will include construct selection, factorial experiment design, analysis of antibody yield and purity, and glycan analysis.

3:05 Refreshment Break in the Exhibit Hall with Poster Viewing

3:50 Strategies for the Use of E. coli as an Expression Host for Challenging Proteins
Bingyuan Wu, Ph.D., Research Scientist, Molecular & Protein Biosciences, Centocor R&D, Inc.
Escherichia coli has been a workhorse for recombinant protein expression due to its well-studied biology, fast growth, and high expression level. However, the expression of mammalian proteins in E. coli often turns out to be challenging. Here a few case studies will be presented on obtaining those difficult proteins using E. coli as an expression host.

4:20 The Challenges and Opportunities for Heterologous Reconstitution of Polyketide and Isoprenoid Natural Product Pathways through E. coli
Blaine Pfeifer, Ph.D., Assistant Professor, Chemical and Biological Engineering, Tufts University
Polyketide and isoprenoid natural products display an impressive therapeutic range that has provided a strong motivation for new technologies to better access this medicinal potential. Equally motivating are the technical challenges associated with production processes reliant on the native host systems responsible for most polyketide and isoprenoid compounds. As a result, heterologous biosynthesis has gained noticeable traction over the last 15 years as a viable route to clinically-relevant natural products. This talk will feature recent successful examples of polyketide and isoprenoid natural products produced heterologously through E. coli. Emphasis will be placed on the technical challenges and strategies associated with functional gene transfer and expression within this alternative host.

4:50 Reception in the Exhibit Hall with Poster Viewing

6:00 End of Day

THURSDAY, MAY 12

8:00 am Morning Coffee

YEAST

8:30 Chairperson’s Remarks

8:35 The Power of Yeast for Protein Expression
Christine Lang, Ph.D., Professor, Institute for Microbiology and Genetics, Institute of Biotechnology, Technical University of Berlin
Both yeasts Saccharomyces cerevisiae and Pichia pastoris are widely recognized and used as robust hosts for recombinant protein expression. These systems are well suited for parallel cloning and expression, and we have used yeast in a comparative expression study of mammalian cDNAs in structural genomics projects. Both yeasts proved to be reliable hosts giving overall expression success rates of 50 – 60% of cDNAs tested. Using different host systems in parallel and varying affinity tags for isolation, bottlenecks in the expression and production of sufficient amounts of soluble proteins can be overcome.

9:05 Yeast-Based Antibody Discovery Platform Enables the Selection of High Expressing Monoclonal Antibodies
Piotr Bobrowicz, Ph.D., Associate Director, Technology & Platform Development, Adimab, Inc.
Adimab has developed a yeast-based antibody discovery platform. The technology inherently isolates antibodies that express well because antibody expression in eukaryotic organism is part of the selection process. Identification of high expressing IgGs at a very early stage ultimately reduces the time and cost to develop antibody therapeutics.

9:35 Production of Recombinant Proteins in the Methylotrophic Yeast Pichia pastoris
James M. Gregg, Ph.D., Research Professor, Keck Graduate Institute of Applied Life Science
As systems for the production of recombinant proteins, yeasts combine the growth and genetic manipulation advantages of bacteria with the ability to perform important post-translational modifications such as proper folding, proteolytic processing, disulfide bridge formation and glycosylation. Pichia pastoris expression strains are easy to scale up from shake-flask cultures to large-volume fermenter cultures growing at cell densities of greater than 100 grams/liter, dry cell weight. The P. pastoris system is particularly valued for its ability to secrete recombinant proteins. Since the organism secretes only low levels of native proteins, the recombinant protein is often the major protein species in the medium.

10:05 Coffee Break in the Exhibit Hall with Poster Viewing

BACULOVIRUS & INSECT CELLS

11:05 The Baculovirus-Insect Cell Expression System: An Overview and Update
Donald L. Jarvis, Ph.D., Professor, Molecular Biology, University of Wyoming
The baculovirus-insect cell system is now well established as a tool for recombinant protein production. This presentation will include a description of this system and its relative strengths and weaknesses. In addition, it will include a discussion of recent developments facilitating the isolation of recombinant baculovirus expression vectors and efforts to humanize protein glycosylation pathways in the lepidopteran insect cell lines that serve as hosts for these vectors.

11:35 Insect Cells for Cytokine Production and Stem Cell Mediated Gene Therapy Applications
Satya Prakash, Ph.D., Professor, Biomedical Engineering, McGill University
Recombinant proteins produced in insect cell systems are useful in molecular biology research and in production of specialized proteins and other applications. Insect cells have been used to produce recombinant protein productions using the BEVS (baculovirus expression vector system) and other systems. Recently we have shown their prospects in excellent expression of therapeutic proteins. Here we discuss the application of this system in producing human interleukin-7, a cytokine protein that may become a valuable supplementary agent for immunotherapeutic treatments in patients with HIV infection, immunodeficiency and other diseases. In addition, we will introduce a new concept of using insect cells in stem cell-mediated gene therapy applications using polymeric membrane microcapsules.

12:05 pm End of Conference
MAY 12-13 | EXPRESSION STREAM

PURIFYING ANTIBODIES

THURSDAY, MAY 12

12:00 pm Registration

1:30 Chairperson’s Opening Remarks

1:40 OPENING KEYNOTE PRESENTATION:
Can the Antibody Purification Platform be Improved?
Douglas Cecchini, Ph.D., Director, Technical Development, Biogen Idec, Inc.

In recent years, technological development for the purification of monoclonal antibodies and Fc fusion proteins has, to some extent, reached a plateau. However, new challenges posed by highly productive cell culture processes, the increasing demands on multi-product manufacturing facilities, and the Quality by Design initiative would benefit from further improvements to purification technologies. A number of innovative approaches that address these challenges will be presented.

2:10 FEATURED PRESENTATION:
Purification of Antibody Fragments and Alternative Protein Scaffolds using the Strep-Tag, the His-Tag and Other Affinity Tags
Arne Skerra, Ph.D., Professor, Biological Chemistry, Technical University of Munich

The quick purification of recombinant proteins under standardized conditions is crucial for their functional optimization, in particular during biological drug discovery and development. While many affinity tags have been proposed over the years, only few offer the beneficial features of high purification efficiency, re-use of the affinity matrix, native elution and minimal interference with protein structure and function. A survey of established affinity tags and some case studies of Fab fragments, Anticalins and PASylated biologics will be presented.

2:40 Continuous mAb Purification using Simulated Moving Bed: Taking the Chromatography Platform to the Next Level
Alla Zilberman, Ph.D., Director, Applications, Semba Biosciences, Inc.

Chromatography as a bioprocessing platform has not kept pace with the demand for higher flexibility, higher productivity and lower costs in the purification of protein-based pharmaceuticals. Continuous multicoloumn protein separations through simulated moving bed chromatography elevate the chromatographic platform by conversion of the conventional batch process to a continuous process. Several purification methods including the “industry-standard” Protein A and size exclusion chromatography can be performed continuously on an automated bench top Octave™ Chromatography System. The results demonstrate more efficient use of chromatography media, reduced resin and buffer consumption, and higher throughput, making continuous chromatography an improved alternative to single column methods.

4:00 Options for the Production and Purification of Fab Antibody Fragments
Gavin Wild, Ph.D., Senior Scientist, Antibody Biology, UCB New Medicines

Antibody fragments such as Fab offer a structurally simple and physically robust format for therapeutic candidates. The purification and subsequent conjugation of antibody fragments at scale bring several challenges which must be overcome. A number of options for robust, efficient, and scalable processes have been developed by exploitation of the target molecule properties, combined with a range of chromatography matrices. Downstream purification case studies covering the harvest/extraction, primary capture and subsequent polishing steps for Fab expressed in both mammalian and E.coli systems will be presented.

4:30 mAb Downstream Filtration Development: A Platform Approach for Variable Feed Streams
Bruno Marques, Ph.D., Investigator, Biopharmaceutical Development, GlaxoSmithKline

In order to de-bottleneck high-producing monoclonal antibody (mAb) processes at the commercial scale, highly efficient, cost-effective, and predictable platform downstream unit operations are required. This paper presents the development of robust downstream filtration steps – employing depth, nano-, ultra-, and membrane filter technology – capable of scaling up to 20,000 L bioreactors with MAb titers of 3 g/L and above. By utilizing a statistical approach to experimental design, various filter fouling models, as well as high-throughput systems to alter buffer conditions, we were able to identify a combination of operating parameters and raw materials capable of processing MAbS with different biophysical properties, impurity profiles, and formulation requirements.

5:00 Close of Day

FRIDAY, MAY 13

7:45 am Continental Breakfast in the Exhibit Hall with Poster Viewing

INNOVATING ANTIBODY PURIFICATION

8:30 Chairperson’s Remarks

8:35 Innovation in Antibody Purification: Are Old Ways Better than New?
William W. Ward, Ph.D., Associate Professor, Biochemistry & Microbiology; Director, CREBB, School of Environmental and Biological Sciences, Rutgers University

As we develop new technologies for protein purification, we sometimes overlook old methods that can be greatly improved. We have modified one of the earliest methods for purifying antibodies, ammonium sulfate precipitation, improving this method to the extent that it now out-performs affinity chromatography on Protein-A. Used to purify therapeutic monoclonal antibodies, our substitute method eliminates the need to validate total removal of Protein-A, a potentially harmful ligand that always leaches from these columns.

9:05 Purifying Antibodies from Complex Biological Milieu with Nanoparticles: Exquisite Specificity, Maximum Yield
David O’Connell, Ph.D., Senior Scientist, School of Medicine, University
conditions allows us to identify operating conditions for antibodies. The simultaneous screening of a large number of plates to evaluate the fit of antibodies to our chromatography development of purification processes for therapeutic antibodies. High-throughput screening can be used to accelerate the development of platforms for new classes of antibodies. Case studies will be presented where high-throughput screening has accelerated the development of antibodies that represent both a good and poor fit in the purification platform.

2:00 Production and Purification of Soluble VH Domains Derived from a Naïve Human VH Library

Csaba Pazmany, Senior Scientist, Head, Throughput Production and Purification, X-Body BioSciences

We have established a robust high-throughput E. coli expression platform to produce soluble VH domains identified from a naïve human VH library for binding and affinity screening. A single-step purification process yields highly pure, monomeric and stable proteins for rapid lead characterization and selection. Extrinsic and intrinsic factors that may contribute to the solubility of these VH antibody fragments will be discussed.

2:30 Refreshment Break

PURIFYING ANTIBODIES FROM TRANSGENIC SOURCES

3:00 Purification of Human Antibodies Expressed in Plants using Liquid/Liquid Extraction

Daniel Forciniti, Ph.D., Professor, Chemical and Biological Engineering, Department, Missouri University of Science and Technology

The use of liquid/liquid extraction for the purification of transgenic human antibodies will be discussed. The method will be illustrated by the purification of a de-glycosylated antibody expressed in corn. The effects of glycosylation on the purification strategy will also be discussed.

3:30 GMP-Compliant Production of a Full-Sized mAb in Tobacco Plants

Stephan Hellwig, Ph.D., Head, Manufacturing, Integrated Production Platforms, Fraunhofer IME

A process was developed for the production of an HIV-binding humanized full-sized monoclonal antibody in greenhouse-grown tobacco plants. The process was scaled up to 200 kg leaf biomass and clinical-grade API for topical administration. IME was one of the first players when using plants as an expression system for biopharmaceuticals was in its infancy, and one of the first to produce an API under GMP. IME holds a manufacturing license for antibodies produced in plants. The talk will highlight the special features of downstream processing from this unusual source and quality control of the purified bulk.

4:00 Strategies for Purification of Human Polyclonal Antibodies from Transgenic Bovine Plasma

Jin-an Jiao, Ph.D., Executive Vice President, Product Development and Manufacturing, Hematech, Inc.

Hematech is developing a novel technology platform to produce human polyclonal antibodies in transgenic cattle for a wide variety of therapeutic applications, including immune deficiency, infectious diseases, cancers, and biodefense. Due to the complicated nature of the feedstock material (transgenic bovine plasma) and the product (human polyclonal antibodies), specific purification methods are required to achieve high yields and purity of polyclonal human antibodies with minimal levels of bovine impurities. We have developed unique purification methods using caprylic acid fractionation and llama VH ligand as affinity ligands.

4:30 End of Conference
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PEGS – the essential protein engineering summit, is coming off a very successful 2010 event. This year’s summit will prove to be even better. PEGS will assemble international innovation leaders who are striving to learn the newest approaches and technologies in the field of life science that will enable the next generation of biologics.

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Discounted Room Rate Cut-off Date: April 8, 2011
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Pricing and Registration Information

PRE-CONFERENCE SHORT COURSES
SUNDAY, MAY 8
Morning Courses 10:00 am-1:00 pm
- SC1 Microfluidics for Antibody Selection and Screening
- SC2 Translational Considerations for Development of Monoclonal Antibodies: Focus on Early Discovery (Pt. 1)
Afternoon Courses 2:00 - 5:00 pm
- SC3 Screening and Selecting Candidate Antibodies
- SC4 Translational Considerations for Development of Monoclonal Antibodies: Focus on Nonclinical Development to Clinic (Pt. 2)
- SC5 Biological Mass Spectrometric Applications for Drug Discovery and Product Development
- SC6 Phage and Yeast Display Libraries and their Screening
Dinner Short Courses
TUESDAY, MAY 10 | 6:00 - 9:00 pm
- SC7 How Size Matters in Therapeutic Antibody Design
THURSDAY, MAY 12 | 5:30 - 8:30 pm
- SC8 Changing Guidances and Regulatory Expectations for Biologics
- SC9 Characterization Techniques for Protein Therapeutics: Orthogonal vs Complementary

CONFERENCE PRICING

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<th>Regular Rate</th>
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<tr>
<td>PREMIUM: (Includes access to conference options I, II, III)</td>
<td>after April 8, 2011</td>
<td>$2,825</td>
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CONFERENCE OPTIONS
Based on your pricing package, please select the conferences you will most likely attend
NOTE: Choose one program per option

I. May 9 - 10
- Phage & Yeast Display
- Difficult to Express Proteins
- Characterization of Biotherapeutics
- Antibodies for Cancer Therapy
II. May 11 -12
- Engineering Antibodies
- Optimizing Protein Expression
- Protein Aggregation and Stability
- Bispecific Antibodies
III. May 12 -13
- Antibody Optimization
- Purifying Antibodies
- Immunogenicity
- Antibody-Drug Conjugates

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