

Holding a tiny Chinese hamster is Dr. George Yerganian, who continues to breed these animals in his laboratory at Cytogen Research and Development (Boston, MA). Photo courtesy of Wei Lian.



## SBE SPECIAL SECTION

### From Chinese Hamsters to Therapeutic Proteins



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# Industry and SBE News

## Averting an Epidemic — Novel Device Detects Avian Flu Virus in Less than Half an Hour

Avian influenza is now deeply entrenched in Asia, with sporadic human infections resulting from either direct contact with infected birds or limited human-to-human transmission. Globalization and seasonal avian migration patterns have resulted in the disease spreading rapidly to other parts of the world. With an early warning detection system in place, a potential avian flu epidemic can be averted.

Researchers at the Institute of Bioengineering and Nanotechnology (IBN), Institute of Molecular and Cell Biology (IMCB) and Genome Institute of Singapore (GIS) have successfully developed a lab-on-a-chip device that can be used to detect the highly pathogenic avian flu (H5N1) virus. According to project leader and lead author of the *Nature Medicine* (DOI: 10.1038/nm1634) publication, IBN research scientist Dr. Juergen Pipper, "With our device, medical or humanitarian aid workers would be able to detect the presence of the H5N1 virus directly from throat swab samples on-site in less than half an hour."

The device comprises a unique platform developed by IBN that uses magnetic force to manipulate individual droplets containing silica-coated magnetic particles. "The novelty of our method lies in the way that the droplet itself becomes a pump, valve, mixer, solid-phase extractor and real-time thermocycler. Complex biochem-

ical tasks can thus be processed in a fashion similar to that of a traditional biological laboratory on a miniature scale," explained Pipper. The all-in-one droplet-based device is superior to commercially available solutions as it integrates the entire workflow of viral RNA isolation, purification, preconcentration, and detection. Tests have shown that IBN's platform is as sensitive as, and around 10 times faster than available tests, yet it could potentially be 40 to 100 times cheaper. IBN has filed five patent applications on this novel device.

## Cell-Free Protein Synthesis Comes of Age

"Can we disassemble a living organism and build a more productive biological system?" This critical question was posed by Dr. James Swartz, the Leland T. Edwards Professor of Engineering in the Dept. of Chemical Engineering and Dept. of Bioengineering at Stanford Univ., at the introduction of his presentation on cell-free protein synthesis (CFPS) at SBE's First International Conference on Accelerating Biopharmaceutical Development held this Spring. Compared with conventional *in vivo* (cellular) expression, CFPS systems offer several advantages, including the potential for higher productivity, parallel production and simplified purification. CFPS can significantly speed up product development time. According to Swartz, it can take from 7–10 months using Chinese hamster ovary (CHO) cells, while with CFPS, it would only take 10 weeks. However, even

## Honoring a Bioengineering Pioneer — Dr. George Georgiou to Receive SBE's James E. Bailey Award

Dr. George Georgiou, the Cockrell Family Regents Chair in Engineering and Professor for the Dept. of Chemical Engineering at the University of Texas, Austin, is the recipient of the SBE's prestigious 2007 James E. Bailey Award (endowed by Cytos Biotechnology). The award is given in tribute of Professor Jay Bailey for his many pioneering contributions to biotechnology. It is presented to an individual who has had an important impact on bioengineering and whose achievements, have advanced this profession in any of its aspects.

Georgiou's research has had a profound impact on protein engineering and therapeutics (*CEP*, July 2007, p. 12). In particular, he has increased the fundamental understanding of protein biogenesis. His contributions to biotechnology include the invention of numerous, commercially important methods for



facilitating protein discovery and manufacturing. He is co-inventor of 34 patents, 19 of which have been licensed to pharmaceutical and biotechnology companies.

A member of the National Academy of Engineering, Georgiou has invented technologies for facilitating protein manufacturing (currently being used for the commercial production of several therapeutic proteins), for high throughput screening of biological molecules and for the efficient engineering of therapeutic antibodies. He is also co-inventor of Anthim (Elusys Therapeutics Inc.), the lead therapeutic antibody for protection against inhalation anthrax.

Georgiou will give his award lecture, "Engineering the Next Generation of Therapeutic Proteins," at AIChE's Annual meeting in Salt Lake City, UT, on Nov. 5. Visit [www.aiche.org/annual](http://www.aiche.org/annual) to find out more.

with all of its advantages, CFPS is still often considered a bench-top novelty. Some of the obstacles that need to be overcome include: limitations in protein folding and assembly, particularly those with disulfide bonds; and a lack of scale-up technologies.

But, these issues are quickly being resolved. Advances made at Stanford and Fundamental Applied Biology, Inc. (FAB), of which Swartz is the chair and founder, are helping CFPS to realize its potential to becoming a viable manufacturing platform. FAB has developed a proprietary biosynthetic Cell-Free Protein Synthesis (Cell-Free) that activates protein expression from combined transcription and translation reactions without the need for living cells.

The company was awarded a \$500,000 Small Business Technology Transfer (STTR) Phase II grant from the National Science Foundation to develop a cell-free process to produce insulin-like growth factor I (IGF-1), which contains three disulfide bonds and can not be readily expressed in a soluble form in *E. coli*. Prior Phase I work showed production of IGF-1 in very high yields (*i.e.*, 800 µg/L) by a careful control of the environmental conditions and the catalysts that were used. These results show that not only the cell-free production of IGF-1 is technically feasible, but also that cell-free technology may be an important method for the production of any disulfide-containing protein that is difficult to produce in bacterial systems. The Phase II project focuses on quality control (*i.e.*, product characterization and optimization), reaction scale-up, and cost reduction.

### Genetically Engineering Microorganisms into Pharmaceutical Factories

Using microorganisms, such as *E. coli*, to efficiently and inexpensively produce novel pharmaceutical compounds, as well as high-value chemicals, is one step closer to becoming a reality thanks to research being conducted by scientists at the University at Buffalo (UB). "Ultimately, we want to be able to take a designed *E. coli* off of the shelf and drop into it the enzymes that constitute a particular biosynthetic pathway in order to make the product we want," says Mattheos Koffas, assistant professor of chemical and biological engineering in the School of Engineering and Applied Sciences and leader of the UB team.

The UB approach to synthetic chemistry addresses some of the challenges in conventional industrial production of specialty chemicals. "Through the use of specially adapted bacteria, specialized enzymes and natural feedstocks, microbial biosynthesis reduces or eliminates the need for petrochemical sources, elevated temperatures,

## Upcoming 2008 SBE Events

### 1<sup>st</sup> International Conference on Stem Cell Engineering, Jan. 20–23, 2008, Coronado Island, CA

This conference emphasizes how basic and applied efforts in stem cell biology and engineering can combine to aid in the development of stem cell therapeutics and bioprocesses. Topics will emphasize how quantitative approaches can yield an increased understanding of the biological mechanisms that underlie these stem cell fate choices, technologies to study stem cell function, and the development of bioprocesses to culture stem cells for commercial applications.

### 4<sup>th</sup> International Conference on Bioengineering and Nanotechnology, July 22–24, 2008, Dublin, Ireland

Leading international bioengineering and nanotechnology experts will share the latest research advancements at the interface of science, engineering, and medicine.

Conference topics include:

- Drug delivery systems and devices
- Protein and gene delivery systems
- Cell and tissue engineering
- Artificial organs and implants
- Medical and biological devices
- Biocatalysis, organocatalysis and nanobiotechnology
- Nanoparticle sequestration in biomolecules

For more information, go to [www.aiche.org/sbe/events](http://www.aiche.org/sbe/events).

toxic heavy metal catalysts, extremes of acidity and dangerous solvents," Koffas said. Also, the natural enzymes can facilitate chemical reactions that are difficult to accomplish through conventional chemistry, such as chiral synthesis, glycosylations and targeted hydroxylations.

Koffas' lab recently achieved the functional expression in *E. coli* of P450 monooxygenases, enzymes that are used widely in nature, but are not readily expressed in industrially important microorganisms. "P450 is important in the synthesis of natural products," said Koffas. "For example, both Taxol, the breast cancer drug that is currently produced from plant cultures, and artemisinin, the anti-malaria drug, have P450 enzymes in their biosynthetic pathways." Koffas' lab has introduced ways to modify both the P450 monooxygenase enzymes and the host cell, thereby improving their yield of flavonoids.

In work published in *Applied and Environmental Microbiology* in June, Koffas and his colleagues produced about 400 mg of flavonoids per liter of cell culture, far above the next highest yield of about 20 mg/L produced by other microbial synthesis efforts. "We have done this by increasing the amount of precursor available and re-engineering the native microbial metabolism," he explained, adding that they have taken different approaches to identifying the pathways that lead to the biosynthesis of precursors for desired compounds.

# Singapore: An Emerging Leader in Biomedical Sciences

NIKI S. C. WONG  
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**BIOPROCESSING TECHNOLOGY INSTITUTE,  
BIOMEDICAL SCIENCES INSTITUTES**

With a heavy investment in biomedical research — particularly the \$330 million to build Biopolis in 2003 — Singapore is seeing the fruits of its labor blossom.

While New York City is commonly referred to as the financial capital of the world, Singapore is rapidly becoming known as the biomedical sciences capital of the world. Singapore's establishment of Biopolis has put it on the map as the epicenter for the biomedical sciences industry (Figure 1). An integrated complex with 2.4 million ft<sup>2</sup> of space, Biopolis is home to six public research institutes of the nation's Agency for Science, Technology and Research (A\*STAR) — the Bioinformatics Institute (BII); the Bioprocessing Technology Institute (BTI); the Genome Institute of Singapore (GIS); the Institute of Bioengineering & Nanotechnology (IBN); the Institute of Medical Biology (IMB); and the Institute of Molecular and Cell Biology (IMCB) — with a seventh institute (Singapore Institute for Clinical Sciences (SICS)) nearby. Alongside these institutes are several private-sector R&D laboratories, including the Novartis Institute for Tropical Diseases and GSK Cognition and Neurodegeneration Center. Biopolis is equipped with state-of-the-art scientific resources and services, such as flow cytometry, mass spectrometry, bioimaging and specific pathogen free animal facilities.

"Biopolis represents a vision to establish the entire value chain of biomedical sciences activities in Singapore — from research and development to manufacturing and healthcare delivery. In doing so, the biomedical sciences industry will generate economic wealth for Singapore, create jobs for our people and improve human health and quality of life," said Tony Tan Keng Yam, deputy prime minister and coordinating minister for security and defense at the official launch of Biopolis. As anticipated, the impact of Biopolis has been tremendous. Before Biopolis opened,



Figure 1. Opened in 2003, Biopolis, the epicenter of biomedical R&D in Singapore, currently has 2.4 million ft<sup>2</sup> of space.

the manufacturing output for the biomedical sciences industry was \$6.4 billion. The latest data indicate that this figure has more than doubled to \$15.2 billion.

## Bioprocessing activities

The BTI (Figure 2) has dual roles of pursuing cutting-edge bioprocess research leading to technologies that impact biomedical science and biomanufacturing, as well as nurturing talents for the scientific community and industry. BTI partners the universities and polytechnics in Singapore to develop a solid workforce for the biologics industry. True to its mission, over 400 students have completed bioprocess modules, undertaken undergraduate attachment or PhD research at BTI in the past five years.

To attract talented individuals, BTI established the Bioprocess Internship Programme (BIP) in 2005. The program consists of six months of intensive research, coupled with six months of structured training in expression

engineering, cell culture, downstream processes, analytical techniques and GMP. BTI has also been supporting overseas attachment programs (sponsored by the Singapore Economic Development Board), where graduates spend up to 18 months in biologics companies, and return to work in Singapore after their specialized training.

Many R&D achievements have already been attained by BTI. In particular, the institute has developed capabilities in: the production of biotherapeutics from animal and microbial cells; and the expansion of human embryonic stem cells (hESC) for cellular therapeutics. BTI is divided into several groups as discussed below.

**The Expression Engineering group** has been generating novel host cell lines that lead to the production of high quality biotherapeutics at high yield. Chinese hamster ovary (CHO) mutants have been isolated with defects in the apoptosis and glycosylation pathways, which can be used as robust host cell lines for biotherapeutic (recombinant protein) production. Several proteins regulating recombinant protein production at the transcription or translation level have also been identified, which are interesting targets for increasing productivity.

**The Animal Cell Technology (ACT) and Microbial Fermentation groups** develop strategies to enhance recombinant protein yield in mammalian and microbial cell cultures, respectively. The ACT group has developed proprietary protein-free chemically defined media for the cultivation of CHO, 293-HEK and hybridoma cells. A dynamic on-line feeding strategy based on low glutamine has led to 10-fold improvement in recombinant protein yields (1). The advent of '-omic' technologies has created new avenues for more insightful studies of mammalian cells producing recombinant proteins. In one study, novel CHO gene targeted (GT) cell lines were created by manipulating early apoptosis signaling genes which were activated during fed-batch cultures, leading to increased cell densities; as well as improved yield and quality of the recombinant protein produced (2). BTI has co-developed the CHO chip with the University of Minnesota, creating a useful tool to understand the physiology of the cells during recombinant protein production. This has subsequently led to the formation of the Consortium for CHO Cell Genomics in 2006 under the auspices of the Society of Biological Engineering.

In the Microbial Fermentation group, microarray,



Figure 2. In addition to R&D breakthroughs, the Bioprocessing Technology Institute is tasked with the goal of nurturing talented individuals.

proteomic and metabolomic studies have helped to design strategies to direct cellular resources towards secondary metabolite production in *Actinomyces* and plasmid production in *E. coli*. Expertise developed through the handling of these systems is currently being applied to study antibody fragment production in *E. coli*.

**The Stem Cell group** generates technologies for the characterization and large-scale production of hESC as cellular therapeutics. Culture platforms, which have been developed for long term expansion of hESC, include immortal mouse and human feeders (3) and serum-free, feeder-free cultures (4). Antibodies were raised against hESC that can be used to characterize, purify and remove undifferentiated hESC from cultures that have undergone differentia-

tion. One of these proprietary antibodies has specific and quick cytotoxic activity against hESC.

BTI's efforts to support the biologics industry with research and workforce development have become increasingly relevant, with four major biologics investments being committed by Lonza, Genentech and GSK Biologicals in Singapore recently. Maintaining a pipeline of specialized workforce and developing strategic areas of bioprocess research will continue to be important charters for BTI.

CEP

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# Deciphering the Mechanisms of Therapeutic Protein Production

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Demand for therapeutic recombinant proteins will eventually exceed current production capabilities. This is a major driver in further understanding Chinese Hamster Ovary (CHO) cell biology, which is the cell line of choice for producing these proteins. Further R&D into CHO is expected to improve production capacity.

Production of therapeutic recombinant proteins from mammalian systems is one of the fastest growing segments of the pharmaceutical market (1). Several analyses indicate that demand will eventually outpace production of these proteins. This imminent shortage has served to emphasize the need to improve on the yield that can be obtained from the systems currently in place. The majority of these proteins are produced in Chinese Hamster Ovary (CHO) cells, while the remainder are produced in a variety of other cell types including mouse myelomas NS0 and SP2/0, baby hamster kidney (BHK) and a variety of others. CHO cells were first cultured in the late 1950's after their isolation from a Chinese hamster (*Cricetulus griseus*) ovary epithelial tumor (2). Produced in CHO, tPA was the first of these recombinant proteins to receive approval for therapeutic use in 1986 (3). Many changes to the process have occurred since that time, including changes to the cells, the growth medium and the reactor conditions to improve from that first venture.

CHO remains the dominant force in biopharmaceutical production because it represents a cell line that is capable of incorporating the appropriate post-translational modifications, while at the same time maintaining the characteristics ideal for production culture. They are easily maintained in serum-free suspension culture, with high viable cell densities and specific protein productivity. Most commercial procedures can routinely reach titers of 2–5 g/L of recombinant protein using today's methods. This represents a huge leap over the 50–100 mg/L titers achieved from processes over 10 years ago. These incremental changes to the production levels owe

much of their success to increased knowledge of the biology of cells in culture, which led to changes in media formulations and improvements in the processes used to culture the cells (for reviews Refs. 3, 4 and 5).

Initially, these changes were based on the principles of nutritional biochemistry and the formulations were altered to impact the general health and metabolism of the cells in culture. As trends in culture performance were observed upon the addition of specific components, these components were subsequently altered via individual titration to improve media formulations. Gradually, that emphasis shifted to statistical approaches designed to test for synergistic effects among multiple components in a formulation. During that time there was also a paradigm shift in the regulatory domain for biopharmaceuticals. Medium formulations shifted away from the use of animal components in formulations (especially fetal bovine serum) to even more regulatory-friendly, chemically defined components. Other improvements in reactor technology, cell line engineering and clone selection have also served to improve yields.

Together these changes have given us the relatively high levels of productivity that we have today, but in order to take the next steps, it would be prudent to use advanced molecular tools to gain a better understanding of CHO cell biology. With that in mind, there are several potential approaches to gaining this knowledge, including trying to understand what response the cell is having to a stimulus by monitoring the changes in the levels of transcript (genomic/transcriptomic methods) or protein (proteomic methods). It is also possible to monitor the levels of metabolites using various mass

spectrometry based methods, termed metabolomics. In combination, these technologies can give us a much broader understanding of what actually occurs inside the cells in culture. Hopefully this will lead to the development of cells with enhanced characteristics, such as improved viable cell density, specific productivity and epigenetic stability.

### Further investigating CHO biology

The increasing availability of genomic information has fueled the rapid growth of the genomics field by providing research scientists with access to essential sequence information. This has accelerated the development of high-throughput investigational tools, such as microarrays, which have traditionally been used to identify genes that play critical roles in a given phenotype, such as those genes differentially regulated in a diseased versus normal tissue. It is now becoming a common practice to use this genomics/transcriptomics approach to provide a better understanding of mammalian cell cultures, especially those with available genomic sequences. One of the earliest studies in which microarray experiments were performed in this regard was one where researchers hoped to gain a better understanding of the physiological response of human fibroblasts to serum (6). Microarrays have also been used by biopharmaceutical groups to study gene expression profiles of mouse cell lines (NS0) that produce large amounts of therapeutic proteins (7, 8).

As more CHO-specific sequence information becomes available, researchers are utilizing microarrays

to expedite CHO cell line development and bioprocess optimization. Recently, expressed sequence tags (ESTs) from CHO were used to build a CHO-specific DNA microarray that was used to perform a comparative transcriptional analysis between recombinant producing CHO and mouse hybridoma cell lines (9, 10). Interestingly, De-Leon Gatti *et al.* demonstrated that carefully designed mouse probes and subsequent microarrays can be used to characterize the CHO cell transcriptome (9). Other microarray studies in CHO have focused on transcriptional profiles to examine the induction of apoptosis, or to identify key genes associated with recombinant protein productivity (11, 12).

Given the frequency of CHO-production strategies in the market, it is surprising that the biochemical characteristics that define a high-producing CHO cell line remain so poorly understood. To date, the genome of Chinese hamster has not been completely sequenced and the information that is available is not freely accessible. This has served as a substantial barrier to scientists wishing to identify genome or transcriptome changes that are important in high-producing lines, making it difficult to rationally engineer these traits into new lines. We have developed a strategy that utilizes microarrays to elucidate the genetic pathways and identify distinct biomarkers that are characteristic of a high-producing cell line. This information is then translated into an effort to develop media supplements to improve the overall performance of recombinant protein production from an animal cell line and to develop a genetically engineered cell line that has improved performance.

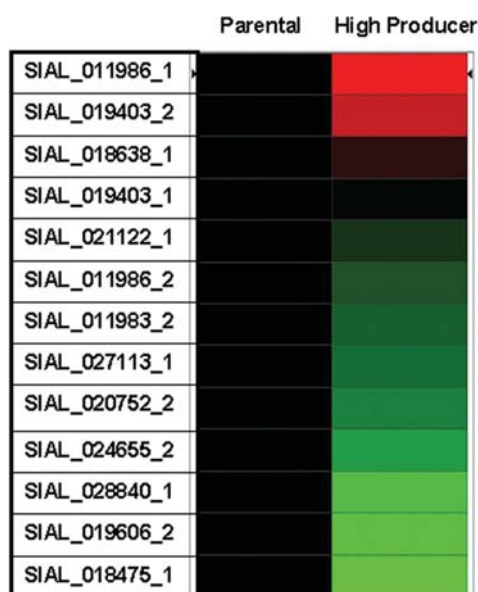
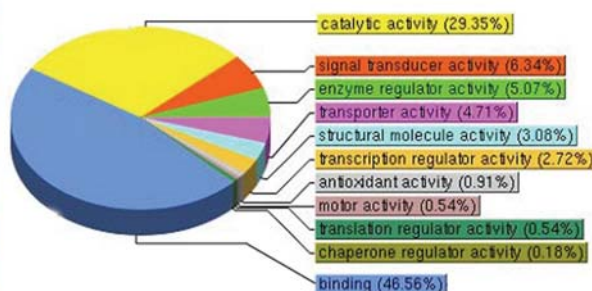


Figure 1. Microarray data yields differences in transcript level between two phenotypes. Shown is a list of genes with different levels of expression in a parental CHO cell line vs. a corresponding recombinant clone expressing high levels of IgG. These types of lists can be used to generate pathways and activities that are changed in highly productive clones based on gene ontology designations or other criteria.



In order to build a functional microarray platform, we first established a CHO sequence database. Through our collaboration with the Consortium for Chinese Hamster Ovary Cell Genomics, as well as our own sequencing efforts, we have constructed a CHO database containing approximately 21,000 unique CHO sequences, about 9,000 of which encode known proteins. We have currently assembled a combined 64,162 CHO ESTs (10) and continue to update this database with new sequence additions.

Using this database, we have designed a CHO microarray to identify changes to the transcriptome in various CHO clones. Our current CHO microarray design contains 30,784 probes designed from our CHO sequence database, which consists of both genomic and mitochondrial derived sequences, 10,505 orthologous sequences from known protein encoding genes that were absent in our current CHO database, 2,488 probes that represent our housekeeping and selection marker control genes, and 1,417 non-CHO control genes. Based on the mouse transcript information available from the FANTOM3 annotation and sequence comparison studies performed between CHO and mouse, we predict that our array covers a large portion of the predicted CHO transcriptome (10, 13). We are currently using the Agilent 4 x 44 k custom array platform to perform all of our microarray experimentation. This platform fits our needs for flexibility, as we are able to easily add or redesign our array with the new addition of CHO specific probes as our CHO sequence database continues to expand.

In brief, our experimental strategy examines gene expression profiles from multiple variables including measurements over a time course, different producing phenotypes, and many different cell lines to obtain a common gene expression profile synonymous to all conditions. These gene expression profiles are then mined for biological significance through detailed pathway analysis and molecular network analysis utilizing GeneSifter and Ingenuity software applications (Figure 1). We believe that as we continue to build out this dataset with more phenotypes we will be able to pinpoint the commonalities between them that lead to the identification of key genes associated with the most important pathways for highly productive CHO clones.

### CHO biology makes an impact

Gene targets specific to high producing CHO cell lines identified through microarray analysis or other analytical techniques provide a diverse set of potential biomarkers that need to be validated for their effects on productivity. One of the first key steps in validation and application of the identified gene targets is comprehensive pathway analysis to classify individual targets into functional cate-

gories or key signaling cascades based on the gene annotations. These categories and/or cascades can then be evaluated for their potential effects on protein production. Once the key gene targets and biological pathways have been identified, several different approaches can be taken to modify the intracellular activity of these genes and/or pathways, with the ultimate result being increased therapeutic protein production or the production of therapeutic proteins with increased efficacy. The expression levels of key proteins can be altered through gene knockdown mechanisms or transgene over-expression. Alternatively, based upon a recent agreement between Sigma-Aldrich and Sangamo Biosciences, the genetic alterations could be made using Zinc Finger Nucleases (ZFNs) (14). Also, proteins and/or small molecules can be added to the cell culture medium, thereby regulating the activity of key intracellular signaling cascades. In the following paragraphs, each one of these applications will be described in more detail, and examples will be provided.

RNA interference (RNAi) is a sequence-specific, post-transcriptional gene-silencing mechanism that is mediated by double stranded RNA molecules (15, 16). One strand of the short RNA duplexes functions as a template leading to the degradation of the complementary mRNA (17). RNAi can be induced in mammalian cells by transfecting in either synthetic short-interfering RNAs (siRNAs) or expression vectors encoding for short-hairpin RNAs (shRNAs) that are then processed into siRNAs inside the cells. RNAi is now widely being used as a gene silencing strategy in mammalian therapeutic protein production systems. Lim *et al.* used RNAi to knockdown the expression of the pro-apoptotic genes Bax and Bak in a recombinant CHO K1 cell line producing IFN- $\gamma$  (18). These cells displayed an extended lifespan and increased viable cell densities in fed-batch culture, thereby leading to increased production of recombinant therapeutic protein. In another study, Mori *et al.* used RNAi to knockdown the gene expression of  $\alpha$ 1,6 fucosyltransferase (Fut8) in a CHO-DG44 cell line that produces a recombinant monoclonal antibody (19). The effect of this was the production of defucosylated antibody with increased antibody-dependent cellular cytotoxicity (ADCC). Ngantung *et al.* used another RNAi approach to help improve the production of functional recombinant protein from CHO cells by knocking down sialidase in a recombinant CHO cell line expressing IFN- $\gamma$  (20). Sialidase is an enzyme that cleaves terminal sialic acids on recombinant proteins. Desialylated glycoproteins have significantly lower circulatory half-lives, reducing overall product quality.

We developed a high-throughput assay using RNAi and the Cell Xpress technology driven by Laser Enabled Analysis and Processing (LEAP) to evaluate the effects of targeted gene knockdown on therapeutic protein pro-

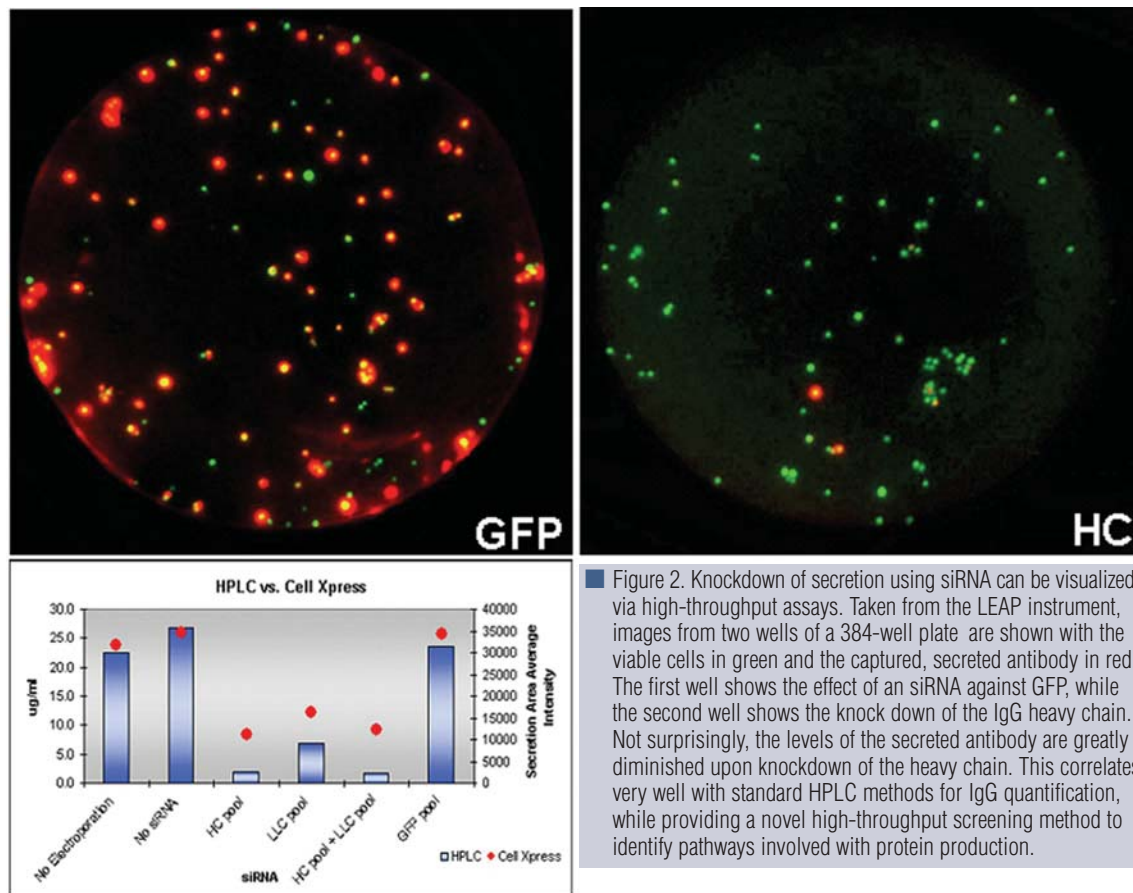


Figure 2. Knockdown of secretion using siRNA can be visualized via high-throughput assays. Taken from the LEAP instrument, images from two wells of a 384-well plate are shown with the viable cells in green and the captured, secreted antibody in red. The first well shows the effect of an siRNA against GFP, while the second well shows the knock down of the IgG heavy chain. Not surprisingly, the levels of the secreted antibody are greatly diminished upon knockdown of the heavy chain. This correlates very well with standard HPLC methods for IgG quantification, while providing a novel high-throughput screening method to identify pathways involved with protein production.

duction. The LEAP instrument, manufactured by Cytintellect, is a unique laser-based cell processing system that combines cell imaging and laser-mediated cell manipulation in an automated and high-throughput manner via large field of view optics and galvanometer steering. The Cell Xpress platform is an automated protocol that runs the imaging and laser-mediated purification functions of the LEAP instrument. We utilized the imaging capabilities of Cell Xpress to rapidly quantify IgG secretion from CHO cells (Figure 2). This system provides us with a phenotypic assay that we can use to test the effects of siRNAs designed against our microarray targets. Once the transient targeted gene knockdowns have been validated for their desired effects on productivity, we can engineer stable CHO lines with enhanced recombinant protein production capabilities using the shRNA technology of Sigma-Aldrich's RNAi Consortium.

Target gene over-expression is another common approach researchers are using to alter the intracellular activity of key proteins and/or pathways with the intent of having positive effects on therapeutic protein produc-

tion from mammalian systems. For example, Ferrara *et al.* used a HEK293 transient expression system to co-express monoclonal antibody heavy and light chain genes as well as a gene encoding recombinant  $\beta$ 1,4-N-acetyl-glucosaminyltransferase III (GnT-III) (21). They showed that over-expression of GnT-III leads to antibodies enriched in bisected oligosaccharides as well as non-fucosylated and hybrid oligosaccharides, thereby increasing antibody dependent cellular cytotoxicity (ADCC). Sauerwald *et al.* generated stable CHO lines over-expressing multiple inhibitors of apoptosis (22). They showed that simultaneous over-expression of both upstream and downstream apoptosis inhibitors is beneficial in extending CHO cell culture lifetimes. Multigene engineering is a rather recent approach in which targeted gene knock down and over-expression technologies are now being used simultaneously. With this, one can alter the endogenous expression levels of several different genes in the same recombinant cell line. For instance, Greber and Fussenegger engineered a system in which target genes can be both over-expressed and knocked-down (23). They developed multicistronic vectors that

are capable of coordinated expression of up to three transgenes and three siRNAs off of a single genetic platform. As our genetic knowledge of CHO cell production systems increases, technologies like this will prove to be invaluable in cell line engineering efforts.

### Moving forward

With the onset of the big push to make all mammalian therapeutic production system serum-free, or even chemically defined, one of the most common approaches to optimizing therapeutic protein production from CHO cells has been through the development of enhanced media formulations. The addition of many media supplements, including growth factors, hormones, transport proteins, carbohydrates, and other small molecules, is based on the composition of serum, and the overall effects of these additives on CHO cell biology have not been well defined. With the data gathered from our CHO genomics/transcriptomics studies and pathway analysis, we will be able to take a more rational approach to media design and identify key proteins and/or small molecules that can be added to the cell culture medium to regulate the activity of key intracellular signaling cascades that are involved in therapeutic protein production.

We believe that by taking a systems-biology discovery approach to increase our understanding of CHO cellular

and molecular biology, we will be able to identify key gene targets and cellular signaling pathways that can be modulated to have significant effects on recombinant therapeutic protein production. As described previously, we have currently established and validated an in-house RNAi screening assay as well as transgene over-expression systems that can be used to engineer better cell lines for therapeutic protein production. Also, we believe that the information obtained from our genomics/transcriptomics studies will enable us to develop enhanced cell culture media formulations.

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