Biotechnology Journal
www.biotechnology-journal.com

Biotechnol. J. 2015, 10, 939–949

DOI 10.1002/biot.201400647

Review

Optimizing eukaryotic cell hosts for protein production through systems biotechnology and genome-scale modeling

Jahir M. Gutierrez[1,2] *and Nathan E. Lewis*[2,3]

[1] Department of Bioengineering, University of California, San Diego, CA, USA
[2] Novo Nordisk Foundation Center for Biosustainability, University of California, San Diego School of Medicine, San Diego, CA, USA
[3] Department of Pediatrics, University of California, San Diego, CA, USA

Eukaryotic cell lines, including Chinese hamster ovary cells, yeast, and insect cells, are invaluable hosts for the production of many recombinant proteins. With the advent of genomic resources, one can now leverage genome-scale computational modeling of cellular pathways to rationally engineer eukaryotic host cells. Genome-scale models of metabolism include all known biochemical reactions occurring in a specific cell. By describing these mathematically and using tools such as flux balance analysis, the models can simulate cell physiology and provide targets for cell engineering that could lead to enhanced cell viability, titer, and productivity. Here we review examples in which metabolic models in eukaryotic cell cultures have been used to rationally select targets for genetic modification, improve cellular metabolic capabilities, design media supplementation, and interpret high-throughput omics data. As more comprehensive models of metabolism and other cellular processes are developed for eukaryotic cell culture, these will enable further exciting developments in cell line engineering, thus accelerating recombinant protein production and biotechnology in the years to come.

Received 26 FEB 2015
Revised 26 APR 2015
Accepted 03 JUN 2015

Keywords: Eukaryotic cell engineering · Genome-scale models · Metabolic networks · Recombinant protein production · Systems Biology

1 Introduction

Eukaryotic cells are the dominant production hosts in the therapeutic protein industry, and contribute substantially to the $140 billion dollars in annual sales [1]. Common hosts, such as Chinese hamster ovary (CHO) cells, are particularly desirable for their capacity to fold and make human-compatible post-translational modifications on recombinant proteins [2]. As the demand for improved quantity, purity, and quality in biotherapeutic products continues to increase, novel strategies for engineering efficient eukaryotic cells become more necessary. Traditional strategies for increasing protein titers and improving cellular performance during culture relied primarily on mutant screens and bioprocess optimizations. For example, culture temperature can be lowered or culture media can be varied to identify conditions resulting in high titers [3–5]. Some initial attempts to utilize metabolic networks on eukaryotic cells for metabolic engineering used dynamic modeling for estimating flux distributions [6–8]. However, with the advent of high throughput "omic" technologies and the application of computational methods in systems biology, it is now possible to elucidate the molecular basis of eukaryotic cell physiology and production capabilities at the genome-scale [9, 10]. Such efforts involve reconstructed and refined genome-scale metabolic network models [11, 12]. These models enable the quantitative analysis of intracellular metabolic fluxes "in silico" (i.e. in a computer simulation) and the prediction of phenotype from genotype [13, 14]. Such predictions are possible since all precursors needed for synthesizing cell biomass and maintaining cell viability are produced

Correspondence: Prof. Nathan E. Lewis, 9500 Gilman Drive Mail Code 0760La Jolla, CA 92093-0760, United States
E-mail: nlewisres@ucsd.edu

Abbreviations: BEV, baculovirus expression vector; **CHO**, Chinese hamster ovary; **COBRA**, constraint-based reconstruction and analysis; **FBA**, flux balance analysis; **GS**, glutamine synthetase; **SOD**, superoxide dismutase

through metabolic pathways. Thus, the metabolic fluxes directly influence cell physiology and their quantification is of great importance to bioprocess engineering [15, 16]. Among the different methodologies, the constraint-based reconstruction and analysis (COBRA) approach has proven quite useful for studying cell metabolism at the genome scale, using algorithms such as flux balance analysis (FBA). Detailed reviews and tutorials on COBRA and FBA are available for the interested reader [17, 18]. In this review we will cover the fundamental goals of systems biotechnology as an emerging field and of COBRA as a modeling framework. Then, we will highlight several research efforts that applied these models to characterize and engineer eukaryotic cell metabolism for bioprocessing.

2 Systems biotechnology and metabolic models

2.1 Introduction to genome-scale reconstructions

Systems biotechnology combines computational and experimental approaches to comprehensively describe the biomolecular mechanisms relevant to bioprocessing [19]. This approach frequently utilizes high-throughput omics data to study and quantify the function of specific pathways (e.g. using pathway maps [20–22], metabolic networks [23], or other interaction databases). In this context, genome-scale metabolic networks contain a comprehensive collection of all known biochemical (i.e. metabolic) information of a specific organism [24, 25]. These networks represent a structured database of the totality of known metabolic processes that take place in the cell, including the metabolites involved, the enzymes catalyzing each of the reactions and the genes that code for the necessary machinery for these processes (Fig. 1). With the proliferation of genome sequencing efforts, many metabolic network reconstructions have been built including eukaryotic genome-scale models that are relevant to industry and medicine [26–28]. These include the filamentous fungi *Saccharomyces cerevisiae* [29] and *Pichia pastoris* [30] (for industrial applications) as well as *Homo sapiens* [31] and *Mus musculus* [32] (which are important models for medicine and drug design).

Biotechnological applications of genome-scale models include metabolic engineering [19], phenotype prediction and characterization [33], identification of genetic targets for cellular engineering [34], and interpretation of high-throughput omics data [35]. Metabolic engineering of production strains has also been facilitated by in silico predictions of gene deletions, alternative metabolic pathways, metabolic coupling of growth rate with secretion of target molecules, and estimations of minimum nutrients in culture media for optimizing growth [36, 37]. Among all the different types of predictions done with metabolic models, one of particular interest to industrial biotechnology is the computation of maximum yield of a target molecule from a given substrate [28].

2.2 Constraint-based reconstruction and analysis of metabolic networks

In order to capture the biologically meaningful pathway usage, or flux distributions, of a metabolic network under a given condition in silico, it is valuable to use approaches that apply known physicochemical constraints, such as mass balance and thermodynamics of each reaction. The constraint-based reconstruction and analysis (COBRA) approach uses such constraints to narrow down the range of feasible flux distributions to recapitulate real pathway usage. COBRA further provides a diverse range of analytical tools for constructing and analyzing genome-scale metabolic networks [38]. The networks are reconstructed by enumerating all biochemical reactions in the organism of interest. Each reaction can be described mathematically using a stoichiometric matrix, which contains the stoichiometric coefficients for each metabolite (rows in the matrix) in each reaction (columns in the matrix, see Fig. 1B). To analyze stoichiometric networks and quantify the metabolic flux distribution of a particular phenotype, COBRA models often assume a steady-state flux and apply fundamental constraints derived from mass conservation and thermodynamics [39]. These constraints can allow for identification of steady-state flux distributions that are thermodynamically feasible and biologically meaningful. Such feasible flux distributions form a solution space, which is a mathematical space containing all possible combinations of steady state reaction fluxes in the metabolic network (Fig. 2, Solution space). Once the solution space is defined, the next step is to choose an objective function, which is a particular reaction whose flux is sought to be maximized or minimized (Fig. 2, Objective and constraints). Finally, by applying linear programming algorithms [40], a particular solution that satisfies both the constraints and the objective function is computed, which provides a prediction of the flux level through each reaction. This optimization technique is commonly called flux balance analysis (FBA) and it is a fundamental COBRA method [41].

In short, FBA consists of a linear programming problem that requires: (i) the set of all biochemical reactions in the system (in the form of a stoichiometric matrix); (ii) an objective function; and (iii) a set of constraints that define the conditions under which the system is allowed to operate. Here we first describe the method conceptually with a simple optimization problem of maximizing the area of a rectangle, and then relate this to modeling metabolism (Fig. 2). When optimizing the area of a rectangle given a constrained perimeter, the rectangle is the system in question and this system can be described with two inde-

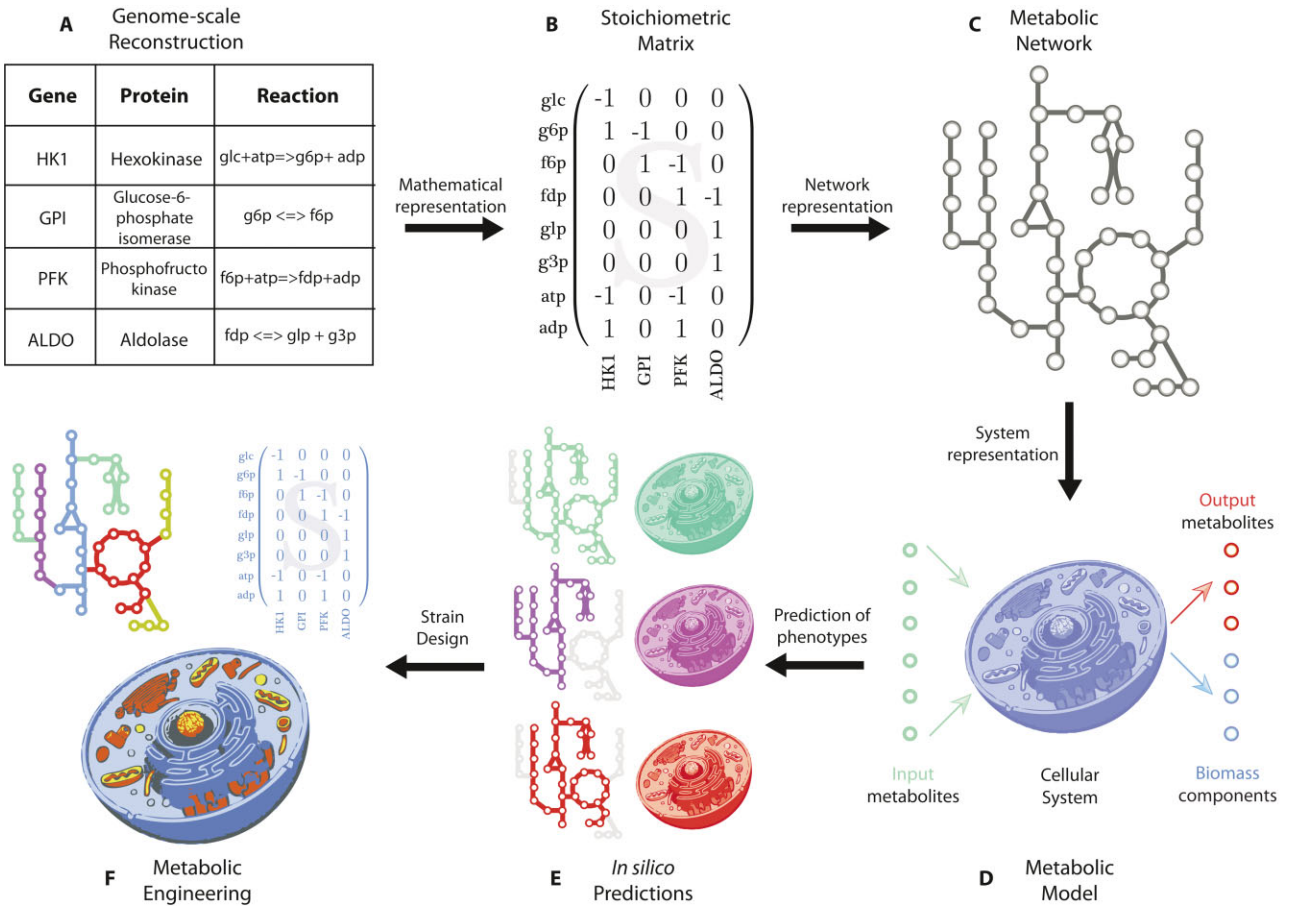


Figure 1. General framework for using genome-scale reconstructions in systems biotechnology. (**A**) First, the reconstruction is assembled from the organism-specific parts list (e.g. genes, proteins, metabolites, and reactions). (**B**) The metabolic reactions in the cell are described mathematically in a stoichiometric matrix, which contains the stoichiometric coefficients for each metabolite (row) in each reaction (column). (**C**) The stoichiometric matrix can be represented graphically as a metabolic network. (**D**) From the metabolic network, a system representation (i.e. metabolic model) of the cell can be obtained by identifying which metabolites are consumed or secreted, as well as the biomass components the cell needs to produce for growing (e.g. ribosomes, proteins, lipids, nucleic acids, etc.). (**E**) By using computational methods such as constraint-based analysis, different phenotypes of interest can be computed by simulating gene knock-outs or nutritional limitations in the media (represented by the different coloring patterns in the networks). (**F**) Finally, the results from these predictions serve as the basis for engineering the metabolism of the host cell towards a desired phenotype.

pendent variables: the width *a* and the length *b*. The area of the rectangle in this case is the objective function, which is computed by taking the product of *a* and *b*. We can construct an infinite number of rectangles by varying the values of *a* and *b*. However, the constraint requiring the perimeter of the rectangle to be the value *L* shortens the range of possible values that both the length and the width can take. Therefore, we have a solution space, and we seek to identify the values of *a* and *b* that maximize the area of the rectangle. This optimal solution is obtained only when *a* and *b* are equal (i.e. when they form a square; Fig. 2, Optimal solution).

For metabolic models, *a* and *b* are reaction fluxes of the metabolic network. The perimeter and the area of the rectangle are also fluxes of the system since their values depend on *a* and *b*. However, we have set a constraint upon the perimeter as it can only take a constant value (*L*). The set of all possible rectangles with perimeter *L* defines the solution space. Finally, the area of the rectangle represents the objective function that we seek to maximize while satisfying the given constraint. In metabolic models, a common objective function is growth (represented by the biomass function, a pseudo reaction in which all metabolites required for the synthesis of cell parts are consumed [42]). The constraints in metabolism include the directionality of the biochemical reactions or the allowed rates of substrate uptake (see bottom panel in Fig. 2).

COBRA methods have been used and implemented to study metabolism for over 30 years now and the universe of possible applications is quite vast [16]. Many applications, including strategies for interpreting high-throughput omics data in the context of metabolic networks, have been previously reviewed [14, 43].

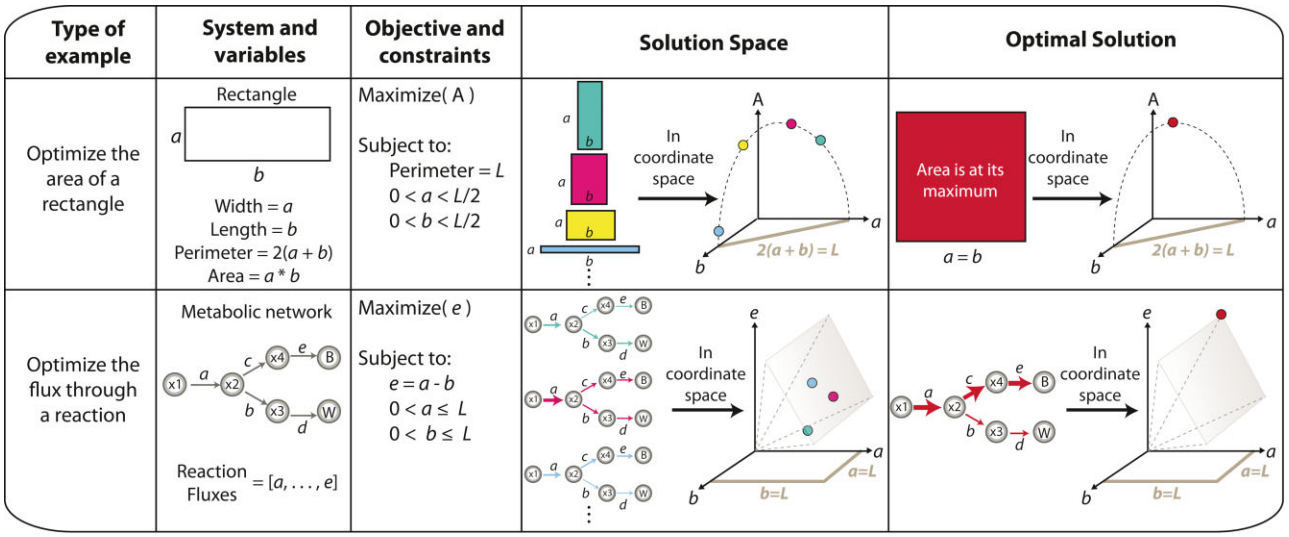


Figure 2. Exemplification of two optimization problems. Two examples of optimization problems are shown here to illustrate flux balance analysis. The first example appears in the context of Euclidean geometry (top row) and the second in the context of metabolic networks (bottom row). In the small metabolic network shown, x1–x4 represent intermediate metabolites, B represents produced biomass and W the secreted waste products. The arrows represent the reactions that connect the metabolites in the network and their width is proportional to the flux.

3 Applications of metabolic models in systems biotechnology for bioprocessing

Metabolic models and stoichiometric equations have been used to gain a systemic understanding of how metabolism dictates the phenotype of various eukaryotic cells in four distinct applications. These include media optimization (section 2.1), characterization of phenotypes under different culture conditions (section 2.2), improvement of cell density (section 2.3), and maximization of protein yield (section 2.4). A summary of these examples is outlined in Table 1.

3.1 Identifying effective cell culture media supplementations

The metabolic phenotype of mammalian cell systems often involves high levels of glucose and glutamine uptake and excessive lactate secretion [44]. Thus, mammalian cell culture media include nutrients that promote both cell growth and the synthesis of the target recombinant protein. Some nutrients, like essential amino acids, vitamins and inorganic salts, cannot be synthesized from the basic carbon (e.g. glucose) and nitrogen (e.g. glutamine) sources. Other nutrients can be synthesized from basic nutrient sources and their supplementation prevents the excessive accumulation of harmful metabolic byproducts (e.g. ammonia in the case of nonessential amino acids). Based on this idea, Xie and Wang formulated a stoichiometric metabolic model to study the nutritional demands for cell growth and protein production in mammalian cell cultures [45]. Using measured cell composition data, the model allowed them to determine the coefficients of a stoichiometric equation governing cell growth. The stoichiometric equation accounts for energy production and synthesis of carbohydrates, lipids, nucleotides and proteins. The researchers subsequently used these results to develop a new medium that allowed for a dramatic improvement in product titers when used in fed-batch cultures of a CRL-1606 hybridoma cell line [37]. Years later, Selvarasu et al. brought this in silico approach for the determination of medium supplementation to the next level by incorporating multivariate statistical analysis and data preprocessing [46]. This allowed for the inference of optimal amino acid concentrations that could be incorporated into the nutrient medium. Furthermore, some negative correlations between non-essential amino acids and cell growth were found, suggesting a way to increase cell viability by reducing the concentrations of some media components [5, 47].

Another common media supplementation in CHO cell cultures includes plant-derived protein hydrolysates from soy, rice or wheat [48]. These supplements support cellular growth and productivity as they serve as raw materials for protein biosynthesis. However, plant-derived protein hydrolysates suffer from high compositional variability which translates into unpredictable culture performance and final product quality. Lee et al. investigated this issue from a systems biology perspective to elucidate the effects of wheat hydrolysates' composition on the metabolic flux distribution of CHO cells [49]. Based on a CHO-320 metabolic network [50], the researchers constructed a constraint-based metabolic model and applied FBA to estimate the metabolic fluxes in cultures with dif-

Table 1. Overview of systems biotechnology applications of stoichiometric equations and metabolic models presented in this review.

Study	Organism/Cell line	Aim(s) of study	Summary of key results
Xie and Wang 1996, [37]	CRL-1606	To construct a simplified stoichiometric network that allows for determination of material balances in animal cell metabolism and potential nutrient supplementations in culture media.	Good agreement between model predictions and experimental data covered in literature. Predictions on media supplementations turned out correct in experiments.
Lee et al. 2014, [49]	GS-CHO and CHO-320	To elucidate the effects of chemical composition from plant-derived supplements on the metabolic flux distribution.	The amino acid and trace element content of wheat hydrolysates induces important variations in central and amino acid metabolism of mammalian cells. Flux distributions with higher cell growth rates were found to have highly active glycine and serine metabolism.
Ivarsson et al. 2015, [55]	CRL-1606	To gain a mechanistic insight into the effect of pH on mammalian cell metabolism.	Significant physiological differences between metabolic flux distributions under two pH conditions were identified by applying FBA to a metabolic model. It was also found that the TCA cycle is regulated by gluconeogenic enzymes at unfavorable pH levels.
Martínez et al. 2013, [58]	CHO-XL99	To use a metabolic network to understand the metabolic fluxes that trigger a metabolic switch in lactate uptake and secretion.	The main differences before and after the metabolic switch were described in terms of ATP usage and redistribution through the core metabolic pathways.
Selvarasu et al. 2012, [59]	in-house IgG-producing CHO cell line and CHO M250-9	To develop a framework for integrating metabolomic data into metabolic networks to gain a mechanistic insight of CHO cell physiology during fed-batch culture and identify the metabolite profile in different growth phases.	Cell-specific biomass composition may lead to erroneous in silico predictions if not properly calculated. Flux distributions of pentose phosphate, amino acid and fatty acid biosynthetic pathways are higher during initial exponential growth phase compared to late exponential growth phase.
Carinhas et al. 2013, [64]	GS-CHO	To contextualize the effects of sodium butyrate on cellular metabolism in a stoichiometric network in the context of low- and high-producing cell lines.	Computational predictions agree very well with experimental data and GS-CHO cell lines' metabolism was found to be characterized by high asparagine uptake and higher metabolic efficiency than other CHO cell lines. Butyrate treatment has a marked effect on increasing biosynthetic activity during stationary phase.
Bernal et al. 2009, [66]	*Spodoptera frugiperda* Sf9 cells	To understand the cell density drop effect observed in high concentration cultures of insect cells infected with a baculovirus expression vector for recombinant protein production.	Redox homeostasis and ATP synthesis, but not byproduct accumulation nor nutrient depletion, have a drastic change after infection, which translates into cell growth arrest and higher conversion of pyruvate to acetyl-CoA.
Carinhas et al. 2010, [69]	*Spodoptera frugiperda* Sf9 cells	To optimize protein production of insect cells and bypass the cell density drop effect by identifying nutrient supplementations from a metabolic network.	It is demonstrated that supplementation of pyruvate and α-ketoglutarate has a six- to seven-fold increase in yield.
González et al. 2003, [70]	*Saccharomyces cerevisiae*	To study the metabolic burden that heterologous protein production imposes on cell growth.	Protein secretion causes a redistribution of the carbon source in the metabolic network of yeast and thus limits growth.
Nocon et al. 2014, [74]	*Pichia pastoris X-33-hSOD*	To engineer central metabolism of *P. pastoris* to enhance protein production by identifying beneficial mutations (i.e. gene knockouts, gene overexpression) via in silico predictions.	The genome scale model used in this study [67] accurately predicts flux changes caused by recombinant protein secretion. About 50% of the single gene mutations significantly improved recombinant protein production.

ferent wheat hydrolysate supplementations. Then, by using principal component analysis (PCA) and partial least squares (PLS), they interpreted the results obtained from FBA and found important characteristics in the central and amino acid metabolic pathways that varied according to the amino acid composition of wheat hydrolysates. These results confirm the usefulness of constraint-based analysis in determining the metabolic regulation in cell cultures under different media supplementations, which have the potential to guide rational design of culture media composition and appropriate supplementations.

3.2 Characterizing cell physiology under different culture conditions

When cultured mammalian cells grow with excess glucose, lactate dehydrogenase activity increases, leading to a high turnover of intracellular pyruvate and subsequent secretion of lactate into the extracellular medium. As lactate accumulates, both cell growth and cell productivity decrease [51] and certain enzymes in the glycolytic pathway are downregulated [52]. Therefore, an important objective in bioprocess control is to reduce lactate secretion in mammalian cell culture. To achieve this, techniques have been proposed to modulate metabolic pathways via genetic mutations [53] or media optimization [54]. In a recent work [55], however, Ivarsson et al. managed to limit lactate formation and consumption by controlling media pH in CRL-1606 hybridoma cell cultures. The researchers applied FBA to a metabolic network (constructed by Mulukutla and colleagues [56]) in order to see the effect that pH had on lactate metabolism. A reaction for ATP production was chosen as the objective function and thus it was maximized in their constraint-based simulations. The results of this study led to the conclusion that hybridoma cells become more energy-efficient and synthesize more monoclonal antibody at low (6.8) pH levels. The authors were able to identify the consequences of pH on intracellular fluxes, particularly the activation of gluconeogenic enzymes at an unfavorable pH level of 7.8 that regulate the TCA cycle. Importantly, these consequences could not be captured in gene expression analysis under both pH conditions, which highlights the relevance of looking at metabolic fluxes through computational models.

One limitation of mammalian cell cultures is that cells sometimes experience a metabolic switch, leading to an inefficient phenotypic state, e.g. when lactate is secreted while glucose is highly consumed [56]. To understand the mechanism of this phenomenon in the context of metabolic fluxes, Martínez and colleagues derived a CHO XL99 cell metabolic model from a previous mouse genome scale model [57] and performed FBA to yield a detailed analysis of the differences in flux distributions between two phenotypic states: lactate secretion (known to be inefficient) and lactate consumption (which was surprisingly found to be more energy efficient) [58]. For example, by comparing the fluxes in key metabolic pathways (TCA, glycolysis), the researchers found that the lactate-consuming phenotype of CHO cells represents a more efficient state, producing about six times more ATP (80% destined to cell maintenance and 20% to biomass production) compared to the high-lactate-secretion phenotype. The results of this study highlight the power of metabolic models to interpret the consequences of phenotypic changes on cellular metabolism.

In another study, Selvarasu and colleagues presented an integrated framework to characterize the physiology of CHO cells in fed-batch cultures [59]. Their framework consists of combining fed-batch culture data, metabolomics, and in silico metabolic network modeling. This led to an in depth study of three metabolic pathways associated with limitation of CHO cell growth. One surprising finding is reflected in the significant differences in biomass composition (i.e. fraction of lipids, amino acids, and nucleic acids that make up cell biomass) across five different CHO cell lines that the authors were able to analyze. This emphasizes the need for careful quantification of a cell line being studied, since accurate cell biomass composition is important for many modeling uses, such as media optimization [60–62]. Otherwise, models may lead to spurious conclusions if biomass examination is not properly realized.

A common strategy used in CHO cell cultures to stimulate over-expression of the target protein involves treating the cells with sodium butyrate, a histone deacetylase inhibitor that arrests cell growth but sustains recombinant protein productivity [63]. Although this technique increases the specific productivity of CHO cells, it also increases the risk of apoptosis dramatically and can compromise the entire bioprocess. Metabolic models can be used to address pertinent questions on how to optimally culture CHO cells under sodium butyrate treatment. Carinhas et al. [64] realized precisely this in the context of a metabolic network (117 reactions, 24 metabolites) of glutamine synthetase (GS)-CHO cells. By integrating exometabolomic data from different clones at specific growth phases with a metabolic network, the researchers characterized important metabolic trends of GS-CHO cells that influence metabolic transitions in high- and low-producing CHO cell cultures under control and butyrate treatment conditions. Specifically, this study reveals the metabolic efficiency of GS-CHO cells during the transition from exponential to stationary growth, and it also demonstrates a differentiated nitrogen metabolism of GS-CHO cells that is characterized by an increased uptake of asparagine for energy generation.

3.3 Analyzing the energetic basis of cell density to improve cell productivity

Insect cells represent a safe and effective way to produce heterologous proteins and vaccines with protein yields above 500 mg of protein per liter [65]. Here, baculovirus expression vectors (BEVs) are transfected into insect cell hosts and form a production platform of high volumetric productivity [66]. However, a common problem with this system is called the cell density drop effect [67]. This phenomenon refers to a significant reduction of specific productivity (i.e. mass of product produced per cell per unit time) of the insect cells when they have been infected with the BEV at high cell densities [68]. The cell density drop effect thus forces one to perform the BEV transfection at low insect cell concentrations in order to obtain acceptable titers. To understand what happens to insect cells' metabolism before and after BEV infection, Bernal and colleagues embarked on the mission of constructing a core metabolic model of the *Spodoptera frugiperda* Sf9 cell line and performed metabolic flux analysis on the basis of material balances under both conditions [66]. Their core model consisted of 52 internally balanced metabolites and 73 reactions, including reactions from (i) central metabolic pathways such as glycolysis, the pentose phosphate pathway and TCA cycle and (ii) reactions that account for the energetic costs of biomass formation and membrane transport. Interestingly, the results of this study suggest that neither byproduct accumulation nor depletion of nutrients in the culture media are responsible for the cell density drop effect observed in insect cell cultures with high density. Nevertheless, this work sheds light on metabolic regulation occurring in insect cells after infection with BEVs. These include changes in redox homeostasis, augmented ATP synthesis, and enhanced consumption of disaccharides after infection, thus resulting in a higher flux through the conversion of pyruvate into acetyl-CoA. Based upon these results, the same research team subsequently altered Sf9 energy metabolism combining experimental and computational methods, and successfully enhanced protein production [69]. Their strategy involved supplementing the culture media with α-ketoglurate and pyruvate at the time of infection, which resulted in a six-fold increase in yield. These two studies highlight the potential of metabolic models in identifying key culture manipulations for enhancing productivity in a bioprocess, even when the information required to build a genome-scale network is not available.

3.4 Characterizing the energetic trends that favor protein production

Recombinant protein production in yeast is commonly increased using different strategies that range from codon usage to manipulating protein folding processes. However, increasing protein secretion has a draining effect on central metabolic fluxes. In one study of *S. cerevisiae* metabolism, González et al. presented a core stoichiometric model (81 metabolites, 78 reactions) of a human superoxide dismutase (SOD)-producing cell line, and used the model to calculate the metabolic flux distributions in wild type and protein-producing yeast strains [70]. The fundamental differences between both strains were captured in this work; even when glucose consumption and ethanol production remained the same, the key contrasting features lie in the distribution of the carbon source to produce biomass (i.e. growth rate). The synthesis of the recombinant SOD protein was linked to higher fermentation and lower ATP synthesis compared to the wild type strain. This study successfully pin-pointed the energetic trade-off between cell growth and protein synthesis by means of a metabolic model, and thus set the foundations for subsequent research efforts aimed to characterize yeast metabolism via comprehensive stoichiometric networks [71].

P. pastoris is a methylotrophic yeast that has drawn the attention of many systems biologists, since it is an effective host for heterologous protein production. Several fully compartmentalized genome-scale metabolic reconstructions of this organism are now available [30, 72, 73]. Using a genome-scale reconstruction (built by Sohn and colleagues [72]), Nocon et al. [74] demonstrated significant changes in flux distributions of a *P. pastoris* strain when forced to produce recombinant protein. They utilized the algorithms "minimization of metabolic adjustment" (MOMA, [75]) and "flux scanning based on enforced objective function" (FSEOF, [76]) to predict appropriate genetic modifications (i.e. knockout or overexpression) that would translate into increased recombinant protein production. From there, the researchers were able to highlight the most important features of the regulatory flexibility of *P. pastoris* metabolic network to redirect resources for protein production thanks to the predicted genetic manipulations (see Table 1). This study goes to show that metabolic models not only provide powerful descriptions of yeast metabolism to enhance secretion of small molecules (e.g. succinate, sesquiterpenes [77]) but also secretion of macromolecules and polymers.

4 Challenges and future perspectives

The use of genome-scale metabolic models for enhancing recombinant protein production is still in its infancy. As can be inferred from the studies reviewed here, the discovery of more sophisticated and novel biotechnological strategies for enhancing recombinant protein production will rely on the refinement and analysis of these models. Some immediate areas of research that will have the greatest impact on model-based improvements of protein secretion are as follow. First, advances that

address the higher complexity and compartmentalization of metabolic processes in eukaryotes will be highly valuable. Second, the physiology of only a few eukaryotes (e.g. yeast) has been studied in depth, and continued efforts in characterizing the more complex metabolism in higher order organisms (e.g. mouse, hamster) [71] will enable more detailed and accurate predictions with genome-scale metabolic models for these protein secretion hosts. Third, technological advances in regard to the generation of complex high-throughput datasets (beyond the genome or the proteome) will further benefit future work with eukaryotes. In particular, the areas of glycobiology and phosphoproteomics, when mapped to metabolic and genetic networks, will help us understand how to control post-translational modification of products and better account for key regulatory events in the cell. Fortunately, at least for CHO cells, there have been several efforts to generate these types of datasets for the N-glycoproteome [78], O-glycoproteome [79] and the transcriptome [80].

Major successes in the use of genome-scale models for metabolic engineering have been achieved in the development of production hosts for small molecules [81–83]. Recent expansions of these models have given place to the next generation of genome-scale models of bacteria, also known as ME-models (metabolic and gene expression models). These models incorporate non-enzymatic events such as transcription, translation [84, 85] as well as translocation [86], and allow for the estimation of the optimal functional proteome required by the prokaryotic cell under particular conditions [87, 88]. Although the task would be enormous, the ME model framework could be used to expand and refine eukaryotic cell models. Beyond transcription, translation and signaling, the coupling of additional process such as protein secretion and associated post-translational modifications would also greatly benefit the development of eukaryotic protein production hosts. For example, protein folding in the endoplasmic reticulum via chaperone activity imposes an additional energetic cost (e.g. consuming ATP, sugar nucleotides, etc.) that is not explicitly accounted in metabolic models simply because this process cannot be stoichiometrically described. The same applies to redox balancing when creating disulfide bonds in proteins or to the impact of amino acid composition on metabolic flux distributions [89, 90]. Recent studies are now addressing these issues, such as Feizi and colleagues [91], who have reconstructed the first genome-scale model of the yeast protein secretory pathway. Furthermore, significant progress in modeling the eukaryotic glycosylation pathways has been made. These research efforts (reviewed previously [92, 93]) aim to gain a systemic insight of the glycosylation capabilities of cell hosts. The computational tools derived from these efforts could be easily incorporated into the systems biotechnology toolbox for practical applications in the near future. As these models continue to be deployed, it is anticipated that they will prove exceptionally valuable for engineering the next generation of protein-producing eukaryotic cell factories. Specifically, they will help identify targets for genetic modification, improve cellular metabolic capabilities, optimize media, and interpret high-throughput omics data to elucidate the biomolecular mechanisms controlling recombinant protein production yield and quality.

Nathan E. Lewis is an assistant professor in the Division of Pediatric Pharmacology & Drug Discovery at the University of California, San Diego School of Medicine. He was trained in biochemistry and proteomics at Brigham Young University, bioengineering and systems biology at the University of California, San Diego, and genome editing technologies at Harvard Medical School. His lab now integrates these techniques, leveraging genome editing technologies and systems biology modeling of cell processes relevant to protein secretion, metabolism, and gene regulation. With these technologies, they aim to develop platforms and techniques that enable the rational engineering of protein producing host cells.

Jahir M. Gutierrez is a doctoral student in the Department of Bioengineering at the University of California, San Diego. Gutierrez earned his B.Sc. degree in Biomedical Engineering from the University of Guadalajara Mexico and his M.S. in Bioengineering from the University of California, San Diego. His doctoral research focuses on two main areas: (i) Phenotypic meta-analysis of CHO cells and (ii) development of constraint-based models of eukaryotic cell systems.

This work was completed with generous support from the Novo Nordisk Foundation provided to the Center for Biosustainability at the Technical University of Denmark and a graduate fellowship from CONACYT and the University of California Institute for Mexico and the United States.

All authors contributed equally to this work and declare no financial or commercial conflict of interest.

5 References

[1] Walsh G., Biopharmaceutical benchmarks 2014. *Nat. Biotechnol.* 2014, *32*, 992–1000.
[2] Harcum S. W., How to reconnect US academic and industrial researchers to better utilize Chinese hamster ovary 'omics in bioprocessing. *Pharm. Bioprocess.* 2014, *2*, 383–388.
[3] Kaufmann H., Mazur X., Fussenegger M., Bailey J. E., Influence of low temperature on productivity, proteome and protein phosphorylation of CHO cells. *Biotechnol. Bioeng.* 1999, *63*, 573–582.
[4] Yoon S. K., Song J. Y., Lee G. M., Effect of low culture temperature on specific productivity, transcription level, and heterogeneity of erythropoietin in Chinese hamster ovary cells. *Biotechnol. Bioeng.* 2003, *82*, 289–298.
[5] Chen P., Harcum S. W., Effects of amino acid additions on ammonium stressed CHO cells. *J. Biotechnol.* 2005, *117*, 277–286.
[6] Provost A., Bastin G., Dynamic metabolic modelling under the balanced growth condition. *J. Process Control* 2004, *14*, 717–728.
[7] Llaneras F., Picó J., A procedure for the estimation over time of metabolic fluxes in scenarios where measurements are uncertain and/or insufficient. *BMC Bioinformatics* 2007, *8*, 421.
[8] Teixeira A. P., Alves C., Alves P. M., Carrondo M. J. T. et al., Hybrid elementary flux analysis/nonparametric modeling: Application for bioprocess control. *BMC Bioinformatics* 2007, *8*, 30.
[9] Kildegaard H. F., Baycin-Hizal D., Lewis N. E., Betenbaugh M. J., The emerging CHO systems biology era: Harnessing the 'omics revolution for biotechnology. *Curr. Opin. Biotechnol.* 2013, *24*, 1102–1107.
[10] Borth N., Opening the Black Box?: Chinese hamster ovary research goes genome scale. *Pharm. Bioprocess.* 2014, *2*, 367–369.
[11] Smallbone K., Mendes P., Large-scale metabolic models: From reconstruction to differential equations. *Ind. Biotechnol.* 2013, *9*, 179–184.
[12] Kaas CS., Fan Y., Weilguny D., Kristensen C., Kildegaard H. F., Andersen M. R., Toward genome-scale models of the Chinese hamster ovary cells: Incentives, status and perspectives. *Pharm. Bioprocess.* 2014, *2*, 437–448.
[13] Hefzi H., Lewis N. E., From random mutagenesis to systems biology in metabolic engineering of mammalian cells. *Pharm. Bioprocess.* 2014, *2*, 355–358.
[14] Bordbar A., Monk J. M., King Z. A., Palsson BØ., Constraint-based models predict metabolic and associated cellular functions. *Nat. Rev. Genet.* 2014, *15*, 107–120.
[15] Stephanopoulos GN., Aristidou AA., Nielsen J., *Metabolic Engineering*. Elsevier, 1998.
[16] Lewis N. E., Nagarajan H., Palsson B. Ø., Constraining the metabolic genotype-phenotype relationship using a phylogeny of in silico Methods. *Nat. Rev. Microbiol.* 2012, *10*, 291–305.
[17] Schellenberger J., Que R., Fleming R. M. T., Thiele I. et al., Quantitative prediction of cellular metabolism with constraint-based models: The COBRA Toolbox v2.0. *Nat. Protoc.* 2011, *6*, 1290–1307.
[18] Orth J. D., Thiele I., Palsson B. Ø., What is flux balance analysis? *Nat. Biotechnol.* 2010, *28*, 245–248.
[19] Lee S. Y., Lee D-Y., Kim T. Y., Systems biotechnology for strain improvement. *Trends Biotechnol.* 2005, *23*, 349–358.
[20] Kanehisa M., Goto S., Sato Y., Kawashima M. et al., Data, information, knowledge and principle: Back to metabolism in KEGG. *Nucleic Acids Res.* 2014, *42*, 199–205.
[21] Cerami E. G., Gross B. E., Demir E., Rodchenkov I. et al., Pathway commons, a web resource for biological pathway data. *Nucleic Acids Res.* 2011, *39*, 685–690.
[22] Croft D., Mundo A. F., Haw R., Milacic M. et al., The reactome pathway knowledgebase. *Nucleic Acids Res.* 2014, *42*, 472–477.
[23] Kim T. Y., Sohn S. B., Kim H. U., Lee S. Y., Strategies for systems-level metabolic engineering. *Biotechnol. J.* 2008, *3*, 612–623.
[24] Carinhas N., Oliveira R., Alves P. M., Carrondo M. J. T. et al., Systems biotechnology of animal cells: The road to prediction. *Trends Biotechnol.* 2012, *30*, 377–385.
[25] Price N. D., Reed J. L., Palsson B. Ø., Genome-scale models of microbial cells: Evaluating the consequences of constraints. *Nat. Rev. Microbiol.* 2004, *2*, 886–897.
[26] Kim T. Y., Sohn S. B., Kim Y. B., Kim W. J. et al., Recent advances in reconstruction and applications of genome-scale metabolic models. *Curr. Opin. Biotechnol.* 2012, *23*, 617–623.
[27] Mardinoglu A., Nielsen J., Systems medicine and metabolic modelling. *J. Intern. Med.* 2012, *271*, 142–154.
[28] King Z. A., Lloyd C. J., Feist A. M., Palsson B. Ø., Next-generation genome-scale models for metabolic engineering. *Curr. Opin. Biotechnol.* 2015, *35*, 23–29.
[29] Herrgard M. J., Swainston N., Dobson P., Dunn W. B., et al., A consensus yeast metabolic network reconstruction obtained from a community approach to systems biology. *Nat. Biotechnol.* 2008, *26*, 1155–1160.
[30] Chung B. K., Selvarasu S., Andrea C., Ryu J. et al., Genome-scale metabolic reconstruction and in silico analysis of methylotrophic yeast *Pichia pastoris* for strain improvement. *Microb. Cell Fact.* 2010, *9*, 50.
[31] Thiele I., Swainston N., Fleming R. M. T., Hoppe A. et al., A community-driven global reconstruction of human metabolism. *Nat. Biotechnol.* 2013, *31*, 419–425.
[32] Selvarasu S., Karimi I. A., Ghim G. H., Lee D. Y., Genome-scale modeling and in silico analysis of mouse cell metabolic network. *Mol. Biosyst.* 2010, *6*, 152–161.
[33] Templeton N., Lewis A., Dorai H., Qian E. A. et al., The impact of anti-apoptotic gene Bcl-2Δ expression on CHO central metabolism. *Metab. Eng.* 2014, *25*, 92–102.
[34] Naderi S., Meshram M., Wei C., McConkey B. et al., Development of a mathematical model for evaluating the dynamics of normal and apoptotic Chinese hamster ovary cells. *Biotechnol. Prog.* 2011, *27*, 1197–1205.
[35] Oberhardt M., Palsson B. Ø., Papin J., Applications of genome-scale metabolic reconstructions. *Mol. Syst. Biol.* 2009, *5*, 661.
[36] Zhou M., Crawford Y., Ng D., Tung J. et al., Decreasing lactate level and increasing antibody production in Chinese hamster ovary cells (CHO) by reducing the expression of lactate dehydrogenase and pyruvate dehydrogenase kinases. *J. Biotechnol.* 2011, *153*, 27–34.
[37] Xie L., Wang D. I., Material balance studies on animal cell metabolism using a stoichiometrically based reaction network. *Biotechnol. Bioeng.* 1996, *52*, 579–590.
[38] Lewis N. E., Jamshidi N., Thiele I., Palsson B. Ø., Metabolic systems biology: A constraint-based approach, in: *Encyclopedia of Complexity and Systems Science*, Springer, 2008, pp. 5535–5552.
[39] Palsson, B. Ø., *Systems Biology: Constraint-Based Reconstruction and Analysis*, Cambridge University Press, 2015.
[40] Vanderbei R. J., *Linear Programming: Foundations and Extensions*. Springer, 2014.
[41] Schilling C. H., Letscher D., Palsson B. Ø., Theory for the systemic definition of metabolic pathways and their use in interpreting metabolic function from a pathway-oriented perspective. *J. Theor. Biol.* 2000, *203*, 229–248.
[42] Feist A. M., Palsson B. Ø., The biomass objective function. *Curr. Opin. Microbiol.* 2010, *13*, 344–349.
[43] Hyduke D. R., Lewis N. E., Palsson B. Ø., Analysis of omics data with genome-scale models of metabolism. *Mol. Biosyst.* 2013, *9*, 167–174.
[44] Quek L. E., Dietmair S., Kromer J. O., Nielsen L. K., Metabolic flux analysis in mammalian cell culture. *Metab. Eng.* 2010, *12*, 161–171.
[45] Xie L., Wang D. I., Stoichiometric analysis of animal cell growth and its application in medium design. *Biotechnol. Bioeng.* 1994, *43*, 1164–1174.

[46] Selvarasu S., Kim D. Y., Karimi I. A., Lee D. Y., Combined data preprocessing and multivariate statistical analysis characterizes fed-batch culture of mouse hybridoma cells for rational medium design. *J. Biotechnol.* 2010, *150*, 94–100.
[47] Broadhurst E. R., Butler M., The inhibitory effect of glutamate on the growth of a murine hybridoma is caused by competitive inhibition of the x(-) (C) transport system required for cystine utilization. *Cytotechnology* 2000, *32*, 31–43.
[48] Ballez J. S., Mols J., Burteau C., Agathos S. N. et al., Plant protein hydrolysates support CHO-320 cells proliferation and recombinant IFN-gamma production in suspension and inside microcarriers in protein-free media. *Cytotechnology* 2004, *44*, 103–114.
[49] Lee H. W., Christie A., Starkey J. A., Read E. K. et al., Intracellular metabolic flux analysis of CHO cells supplemented with wheat hydrolysates for improved mAb production and cell-growth. *J. Chem. Technol. Biotechnol.* 2015, *90*, 291–302.
[50] Zamorano F., Vande Wouwer A., Jungers R. M., Bastin G., Dynamic metabolic models of CHO cell cultures through minimal sets of elementary flux modes. *J. Biotechnol.* 2013, *164*, 409–422.
[51] Ahn W. S., Antoniewicz M. R., Metabolic flux analysis of CHO cells at growth and non-growth phases using isotopic tracers and mass spectrometry. *Metab. Eng.* 2011, *13*, 598–609.
[52] Leite T. C., Coelho R. G., Da Silva D., Coelho W. S. et al., Lactate downregulates the glycolytic enzymes hexokinase and phosphofructokinase in diverse tissues from mice. *FEBS Lett.* 2011, *585*, 92–98.
[53] Paredes C., Prats E., Cairó J. J., Azorín F. et al., Modification of glucose and glutamine metabolism in hybridoma cells through metabolic engineering. *Cytotechnology* 1999, *30*, 85–93.
[54] Altamirano C., Illanes A., Becerra S., Cairó J. J. et al., Considerations on the lactate consumption by CHO cells in the presence of galactose. *J. Biotechnol.* 2006, *125*, 547–556.
[55] Ivarsson M., Noh H., Morbidelli M., Soos M., Insights into pH-induced metabolic switch by flux balance analysis. *Biotechnol. Prog.* 2015, *31*, 347–357.
[56] Mulukutla B. C., Gramer M., Hu W-S., On metabolic shift to lactate consumption in fed-batch culture of mammalian cells. *Metab. Eng.* 2012, *14*, 138–149.
[57] Quek L. E., Nielsen L. K., On the reconstruction of the *Mus musculus* genome-scale metabolic network model. *Genome Inform.* 2008, *21*, 89–100.
[58] Martínez V. S., Dietmair S., Quek L. E., Hodson M. P. et al., Flux balance analysis of CHO cells before and after a metabolic switch from lactate production to consumption. *Biotechnol. Bioeng.* 2013, *110*, 660–666.
[59] Selvarasu S., Ho Y. S., Chong W. P. K., Wong N. S. C. et al., Combined in silico modeling and metabolomics analysis to characterize fed-batch CHO cell culture. *Biotechnol. Bioeng.* 2012, *109*, 1415–1429.
[60] Szappanos B., Kovács K., Szamecz B., Honti F. et al., An integrated approach to characterize genetic interaction networks in yeast metabolism. *Nat. Genet.* 2011, *43*, 656–662.
[61] Carnicer M., Baumann K., Toplitz I., Sanchez-Ferrando F. et al., Macromolecular and elemental composition analysis and extracellular metabolite balances of *Pichia pastoris* growing at different oxygen levels. *Microb. Cell Fact.* 2009, *8*, 65.
[62] Raghunathan A. U., Pérez-Correa J. R., Bieger L. T., Data reconciliation and parameter estimation in flux-balance analysis. *Biotechnol. Bioeng.* 2003, *84*, 700–709.
[63] Jiang Z., Sharfstein S. T., Sodium Butyrate Stimulates monoclonal antibody over-expression in CHO cells by improving gene accessibility. *Biotechnol. Bioeng.* 2008, *100*, 189–194.
[64] Carinhas N., Duarte T. M., Barreiro L. C., Carrondo M. J. T. et al., Metabolic signatures of GS-CHO cell clones associated with butyrate treatment and culture phase transition. *Biotechnol. Bioeng.* 2013, *110*, 3244–3257.
[65] Ozturk, S., Hu, W. (Ed.), *Cell Culture Technology for Pharmaceutical and Cell Based Therapies*, Taylor & Francis, 2006.
[66] Bernal V., Carinhas N., Yokomizo A. Y., Carrondo M. J. T. et al., Cell density effect in the baculovirus-insect cells system: A quantitative analysis of energetic metabolism. *Biotechnol. Bioeng.* 2009, *104*, 162–180.
[67] Doverskog M., Ljunggren J., Ohman L., Haggstrom L., Physiology of cultured animal cells. *J. Biotechnol.* 1997, *59*, 103–115.
[68] Taticek R. A., Shuler M. L., Effect of Elevated oxygen and glutamine levels on foreign protein production at high cell densities using the insect cell-baculovirus expression system. *Biotechnol. Bioeng.* 1997, *54*, 142–152.
[69] Carinhas N., Bernal V., Monteiro F., Carrondo M. J. T. et al., Improving baculovirus production at high cell density through manipulation of energy metabolism. *Metab. Eng.* 2010, *12*, 39–52.
[70] Gonzalez R., Andrews B. A., Molitor J., Asenjo J. A., Metabolic analysis of the synthesis of high levels of intracellular human SOD in *Saccharomyces cerevisiae* rhSOD 2060 411 SGA122. *Biotechnol. Bioeng.* 2003, *82*, 152–169.
[71] Graf A., Dragosits M., Gasser B., Mattanovich D., Yeast systems biotechnology for the production of heterologous proteins. *FEMS Yeast Res.* 2009, *9*, 335–348.
[72] Sohn S. B., Graf A. B., Kim T. Y., Gasser B. et al., Genome-scale metabolic model of methylotrophic yeast *Pichia pastoris* and its use for in silico analysis of heterologous protein production. *Biotechnol. J.* 2010, *5*, 705–715.
[73] Caspeta L., Shoaie S., Agren R., Nookaew I. et al., Genome-scale metabolic reconstructions of *Pichia stipitis* and *Pichia pastoris* and in silico evaluation of their potentials. *BMC Syst. Biol.* 2012, *6*, 24.
[74] Nocon J., Steiger M. G., Pfeffer M., Sohn S. B. et al., Model based engineering of *Pichia pastoris* central metabolism enhances recombinant protein production. *Metab. Eng.* 2014, *24*, 129–138.
[75] Segre D., Vitkup D., Church G. M., Analysis of optimality in natural and perturbed metabolic networks. *Proc. Natl. Acad. Sci. USA* 2002, *99*, 15112–15117.
[76] Choi H. S., Lee S. Y., Kim T. Y., Woo H. M., In silico identification of gene amplification targets for improvement of lycopene production. *Appl. Environ. Microbiol.* 2010, *76*, 3097–3105.
[77] Asadollahi M. A., Maury J., Patil K. R., Schalk M. et al., Enhancing sesquiterpene production in *Saccharomyces cerevisiae* through in silico driven metabolic engineering. *Metab. Eng.* 2009, *11*, 328–334.
[78] Baycin-Hizal D., Tabb D. L., Chaerkady R., Chen L. et al., Proteomic analysis of Chinese hamster ovary cells. *J. Proteome Res.* 2012, *11*, 5265–5276.
[79] Yang Z., Halim A., Narimatsu Y., Joshi H. J. et al., The GalNAc-type O-glycoproteome of CHO cells. *Mol. Cell. Proteomics* 2014, *13*, 3224–3235.
[80] Vishwanathan N., Yongky A., Johnson K. C., Fu H. Y. et al., Global insights into the Chinese hamster and CHO cell transcriptomes. *Biotechnol. Bioeng.* 2015, *112*, 965–976.
[81] Yim H., Haselbeck R., Niu W., Pujol-Baxley C. et al., Metabolic engineering of *Escherichia coli* for direct production of 1,4-butanediol. *Nat. Chem. Biol.* 2011, *7*, 445–452.
[82] Lee J. W., Na D., Park J. M., Lee J. et al., Systems metabolic engineering of microorganisms for natural and non-natural chemicals. *Nat. Chem. Biol.* 2012, *8*, 536–546.
[83] Lee S. K., Chou H., Ham T. S., Lee T. S. et al., Metabolic engineering of microorganisms for biofuels production: From bugs to synthetic biology to fuels. *Curr. Opin. Biotechnol.* 2008, *19*, 556–563.
[84] Thiele I., Jamshidi N., Fleming R. M. T., Palsson B. Ø., Genome-scale reconstruction of *Escherichia coli*'s transcriptional and translational

machinery: A knowledge base, its mathematical formulation, and its functional characterization. *PLoS Comput. Biol.* 2009, *5*, e1000312.

[85] Lerman J. A., Hyduke D. R., Latif H., Portnoy V. A. et al., In silico method for modelling metabolism and gene product expression at genome scale. *Nat. Commun.* 2012, *3*, 929.

[86] Liu J. K., O'Brien E. J., Lerman J. A., Zengler K. et al., Reconstruction and modeling protein translocation and compartmentalization in *Escherichia coli* at the genome-scale. *BMC Syst. Biol.* 2014, *8*, 110.

[87] O'Brien E. J., Lerman J. A., Chang R. L., Hyduke D. R. et al., Genome-scale models of metabolism and gene expression extend and refine growth phenotype prediction. *Mol. Syst. Biol.* 2013, *9*, 693.

[88] O'Brien E. J., Palsson B. Ø., Computing the functional proteome: Recent progress and future prospects for genome-scale models. *Curr. Opin. Biotechnol.* 2015, *34*, 125–134.

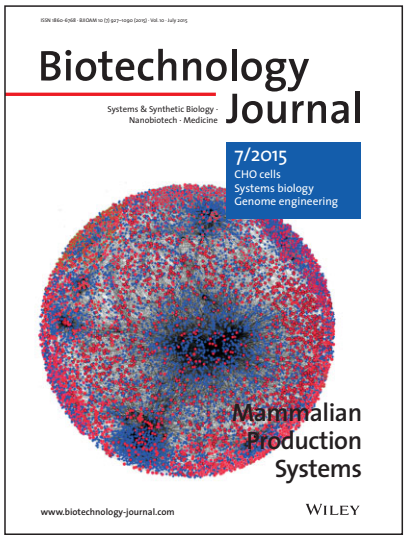
[89] Oddone G. M., Mills D. A., Block D. E., A dynamic, genome-scale flux model of *Lactococcus lactis* to increase specific recombinant protein expression. *Metab. Eng.* 2009, *11*, 367–381.

[90] Margittai E., Sitia R., Oxidative protein folding in the secretory pathway and redox signaling across compartments and cells. *Traffic* 2011, *12*, 1–8.

[91] Feizi A., Osterlund T., Petranovic D., Bordel S. et al., Genome-scale modeling of the protein secretory machinery in yeast. *PLoS One* 2013, *8*, e63284.

[92] Spahn P. N., Lewis N. E., Systems glycobiology for glycoengineering. *Curr. Opin. Biotechnol.* 2014, *30*, 218–224.

[93] Neelamegham S., Liu G., Systems Glycobiology: Biochemical reaction networks regulating glycan structure and function. *Glycobiology* 2011, *21*, 1541–1553.



Cover illustration

Special issue: Mammalian Production Systems. This special issue is edited by Nicole Borth and Lars Nielsen. It focuses on the use of CHO cells and their improvement to produce recombinant proteins. The articles cover the application of CRISPR technology, systems biology and modeling approaches as well as bioinformatics methods. The cover visualizes the highly interconnected metabolic pathway map of CHO cells based on a genome-scale metabolic reconstruction (blue: reactions, red: molecular species). Image by Michael Hanscho.

Biotechnology Journal – list of articles published in the July 2015 issue.

Editorial: On the cusp of rational CHO cell engineering
Lars Nielsen and Nicole Borth
http://dx.doi.org/10.1002/biot.201500375

Review
CHOgenome.org 2.0: Genome resources and website updates
Benjamin G. Kremkow, Jong Youn Baik, Madolyn L. MacDonald and Kelvin H. Lee
http://dx.doi.org/10.1002/biot.201400646

Review
Optimizing eukaryotic cell hosts for protein production through systems biotechnology and genome-scale modeling
Jahir M. Gutierrez and Nathan E. Lewis
http://dx.doi.org/10.1002/biot.201400647

Review
Towards next generation CHO cell biology: Bioinformatics methods for RNA-Seq-based expression profiling
Craig Monger,Paul S. Kelly, Clair Gallagher, Martin Clynes, Niall Barron and Colin Clarke
http://dx.doi.org/10.1002/biot.201500107

Review
Epigenetic regulatory elements: Recent advances in understanding their mode of action and use for recombinant protein production in mammalian cells
Niamh Harraghy, David Calabrese, Igor Fisch, Pierre-Alain Girod, Valérie LeFourn, Alexandre Regamey and Nicolas Mermod
http://dx.doi.org/10.1002/biot.201400649

Review
CRISPR/Cas9-mediated genome engineering of CHO cell factories: Application and perspectives
Jae Seong Lee, Lise Marie Grav, Nathan E. Lewis and Helene Faustrup Kildegaard
http://dx.doi.org/10.1002/biot.201500082

Review
Industrial production of clotting factors: Challenges of expression, and choice of host cells
Sampath R. Kumar
http://dx.doi.org/10.1002/biot.201400666

Review
Mammalian designer cells: Engineering principles and biomedical applications
Mingqi Xie and Martin Fussenegger
http://dx.doi.org/10.1002/biot.201400642

Research Article
NF-κB, CRE and YY1 elements are key functional regulators of CMV promoter-driven transient gene expression in CHO cells
Adam J. Brown, Bernie Sweeney, David O. Mainwaring, David C. James
http://dx.doi.org/10.1002/biot.201400744

Research Article
Re-programming CHO cell metabolism using miR-23 tips t he balance towards a highly productive phenotype
Paul S. Kelly, Laura Breen, Clair Gallagher, Shane Kelly, Michael Henry, Nga T. Lao, Paula Meleady, Donal O'Gorman, Martin Clynes and Niall Barron
http://dx.doi.org/10.1002/biot.201500101

Research Article
Chemical manipulation of the mTORC1 pathway in industrially relevant CHOK1 cells enhances production of therapeutic proteins
Nazanin Dadehbeigi and Alan J. Dickson
http://dx.doi.org/10.1002/biot.201500075

Research Article

Low glucose depletes glycan precursors, reduces site occupancy and galactosylation of a monoclonal antibody in CHO cell culture

Carina Villacrés, Venkata S. Tayi, Erika Lattová, Hélène Perreault and Michael Butler

http://dx.doi.org/10.1002/biot.201400662

Research Article

Optimization of bioprocess conditions improves production of a CHO cell-derived, bioengineered heparin

Jong Youn Baik, Hussain Dahodwala, Eziafa Oduah, Lee Talman, Trent R. Gemmill, Leyla Gasimli, Payel Datta, Bo Yang, Guoyun Li, Fuming Zhang, Lingyun Li, Robert J. Linhardt, Andrew M. Campbell, Stephen F. Gorfien and Susan T. Sharfstein

http://dx.doi.org/10.1002/biot.201400665

Research Article

Deep sequencing reveals different compositions of mRNA transcribed from the *F8* gene in a panel of FVIII-producing CHO cell lines

Christian S. Kaas, Gert Bolt, Jens J. Hansen, Mikael R. Andersen and Claus Kristensen

http://dx.doi.org/10.1002/biot.201400667