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Mechanistic modeling and applications for CHO cell culture development and production

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Chinese hamster ovary (CHO) cell cultures are widely used to produce biologics in the biopharmaceutical industry. The biopharmaceutical industry requires to improve the efficiency of process development as a response to the increasing market competition. The traditional approaches to optimize cell culture are typically empirically-based and generally time-consuming. The use of mechanistic models can be beneficial to these industrially relevant exercises and improve the efficiency of process development. This review introduces stoichiometric and kinetic models, the two commonly used mathematical approaches to describe cell system, and discusses the challenges associated such as parameterization and generalizability with respect to model applications. Examples of applying mechanistic models across the stages of process understanding, optimization and control are reviewed.

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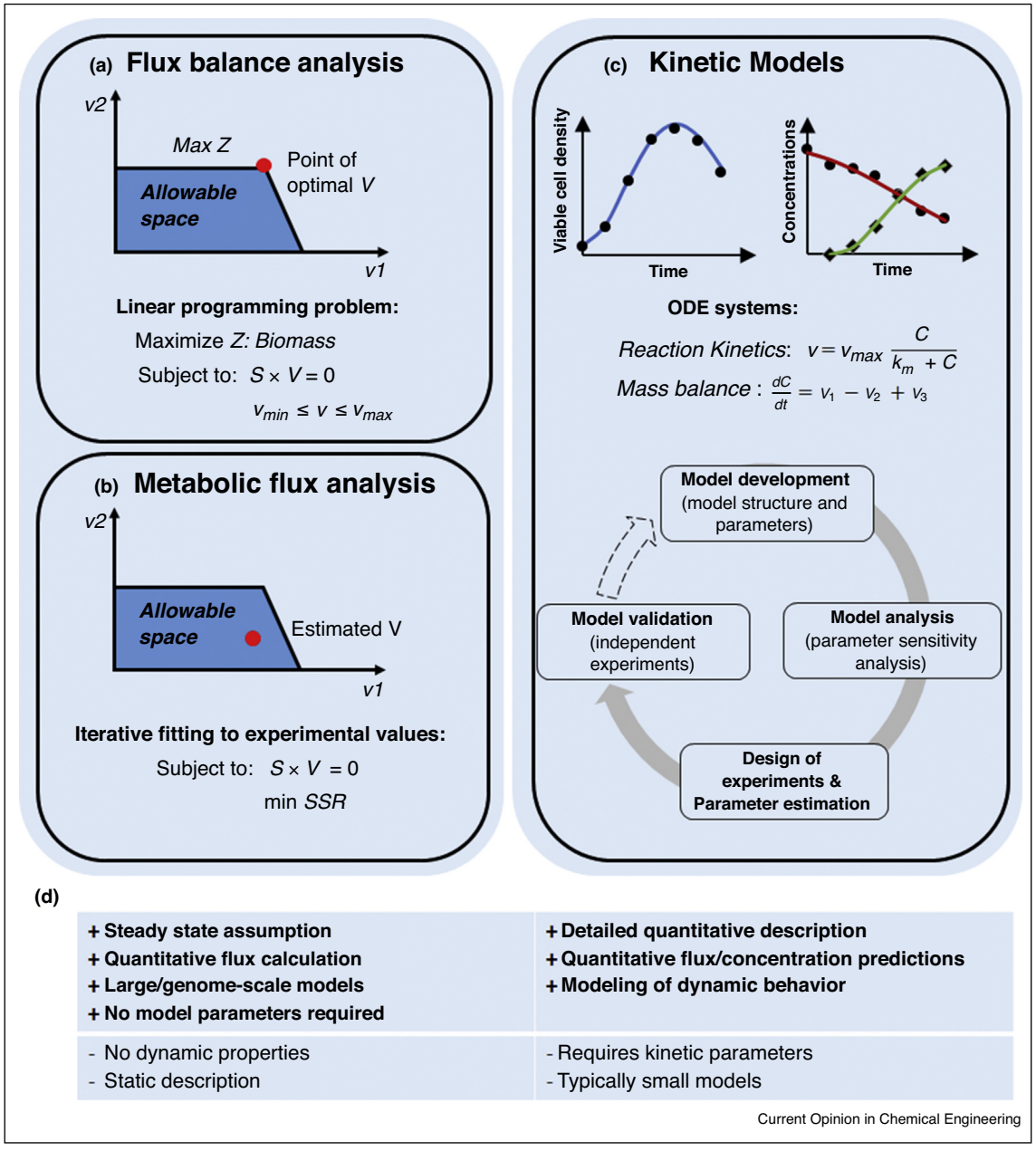
Introduction

In the biopharmaceutical industry, Chinese hamster ovary (CHO) cell culture represents an important bioprocess to produce biologics such as monoclonal antibodies. The host cells, culture media and operation parameters in bioprocesses are routinely optimized. Conventionally, these exercises are relied on empirical approaches that can be time- and resource-consuming [1–5]. During bioprocess control, tools are needed in addition for achieving consistent process performance. For all of these needs and from a variety of stages, model-based approaches based on available data and current knowledge of cell culture system can be significantly useful.

Data-driven models are established based on statistical correlations between process variables and outcomes. Partial least squares (PLS) is one of such tools [6]. More powerful predictions of processes are expected by using machine learning technology today to train models from increasing amount of process data [7]. Nevertheless, these models do not count biologically meaningful relationship between inputs and outputs and as such the connections remain as 'black box' mechanistically [8••]. In contrast, mechanistic models take the systematic understanding from bioprocesses into account with a major focus to assess the impacts of culture conditions (such as media composition and process parameters) on culture performance (such as cell growth, production and product quality attributes) by quantitatively describing cellular metabolic activities [9,10•]. One trend in the future could be generation of hybrid models from both mechanistic and data-driven models. This is to provide more practicality to the modeling process, given the challenges seen today in the relative shortage of cell culture understanding at the mechanistic level and the difficulty in parameterizing mechanistic equations in the case of kinetic models [11]. Before that, the principles of mechanistic modeling and the relevant applications of models in bioprocessing need be better reviewed at the moment.

Stoichiometric and kinetic models are the two widely used approaches to describe cellular metabolism [12••,13,14]. These two approaches have different features as summarized in Figure 1. In the next section, these two approaches are introduced in detail and several important considerations regarding model development are discussed. For example, a series of work need be carefully carried out to adapt and validate model parameters before application. Once mechanistic models are established, substantial information can be generated via *in silico* simulation, such as finding rational designs of media component allocation during media optimization [15–17]. Such work can decrease the amount of wet-lab experimentation. A model that is applicable across different cell lines or clones can even more effectively save time and labor. In a later section, examples of applications of models at a variety

Figure 1



Principles of **(a)** FBA, **(b)** MFA, **(c)** kinetic models and **(d)** characteristic comparison between stoichiometric and kinetic model. (+) Advantages; (−) disadvantages.

stages of processes, including process understanding, optimization, process design and control will be demonstrated.

Principles of mechanistic modeling approach

Stoichiometric and kinetic models are both metabolic reaction-based and formulated with a network of equations. Key characteristics are shown in Figure 1a–c and the advantages and disadvantages of each approach are summarized in Figure 1d. A list of common databases and software used for generating these two models for mammalian cells are shown in Table 1.

Features of stoichiometric models

As shown in Figure 1a and b, the stoichiometric models exemplified by flux balance analysis (FBA) and metabolic flux analysis (MFA) are applied to solve intracellular fluxes, using extracellular metabolite consumption or production rates as constraints [18]. Models are structured with small or large networks, stoichiometric coefficients

Table 1

Databases and software used in stoichiometric and kinetic modeling for mammalian cells

Resource	Main purposes	URL
Database		
BiGG	Genome-scale models	http://bigg.ucsd.edu/
KEGG	Genes, enzymes, reactions, pathways	https://www.genome.jp/kegg/
MetaCyc	Enzymes, pathways	https://metacyc.org/
BioModels	Published models from literature	https://www.ebi.ac.uk/biomodels-main/
BRENDA	Enzymes (kinetic parameters)	https://www.brenda-enzymes.org/
Software		
OptFlux	FBA, MFA	http://www.optflux.org/
COBRA toolbox	FBA	https://opencobra.github.io/
Sybil	FBA	https://cran.r-project.org/web/packages/sybil/index.html
Metran	MFA	http://www.che.udel.edu/mranton/metran.html
OpenFLUX	MFA	http://openflux.sourceforge.net/index.html
INCA	MFA	http://mfa.vueinnovations.com.
Matlab	FBA, MFA, kinetic model	https://www.mathworks.com/
gPROMS	Kinetic model	https://www.psenterprise.com/

and reaction characteristics (such as reversibility and flux boundary) [19–21,22•]. A small reaction network usually includes glycolysis, TCA cycle and amino acid metabolism, which are the central metabolism crucial for cell growth and protein production and generically applicable to different cell lines. To analyze cell metabolism more comprehensively, CHO genome scale models have been developed given the advance of omics technologies, and are specifically defined for several CHO cell lines (CHO-K1, CHO-S and CHO-DG44) [12••].

MFA and FBA deal with metabolic networks of different degrees of freedom, which are calculated by the difference between the number of unknown intracellular fluxes and that of independent mass balance equations. MFA paired with ^{13}C labeling is used at determined or overdetermined system, typically a small network such as the central metabolism [23–25], while ^{13}C labeling is the empirical approach to track the distribution of carbon source into the metabolic network. At an underdetermined system, objective functions are introduced as equations that quantitatively define how much each reaction contributes to specified objectives (such as cell growth, product formation and ATP generation) [21]. Typically, cell growth is the objective maximized at the cell exponential growth phase and recombinant protein production is the one maximized at the cell stationary phase [26]. A genome-scale model is inherently an underdetermined system with far more reactions than metabolites. Therefore, to solve the system, FBA approach has to be used along with an objective function for an optimum solution to be reached [27].

A quasi-steady state for intracellular metabolites is a critical assumption applied in stoichiometric-based models. Since intracellular fluxes in reality can vary during batch or fed-batch culture, an idea of dynamic flux analysis is used. Such an approach derives a dynamic profile of metabolic rates from the extracellular environment, and inputs these dynamic rates to MFA or FBA to generate a continuous profile of intracellular fluxes. In a sense, this approach generates an infinite number of steady states across a dynamic process and form a continuous view of the fluxes at transient steady states throughout the culture [28].

Features of kinetic models

As shown in Figure 1c, kinetic models are expressed as a series of ordinary differential equations (ODEs) and describe dynamic changes in metabolite concentrations, cell density and product formation over the course of cultivation [29]. Commonly, cell growth and death are linked to critical nutrients and byproducts, while the recombinant protein production is linked to cell growth [17,30], amino acid metabolism [31], mRNA expression [32] and so on. Michaelis-Menten kinetics and Monod kinetics are popularly used in kinetic models to describe enzyme-catalyzed reaction rates [33] and contain kinetic parameters such as enzyme turnover rates and dissociation constants between enzymes and substrates [10•].

Kinetic models are structured with different levels of complexity depending on the details to be covered in culture system and intracellular processes [34]. A model can be as complex as taking into consider the heterogeneity in cell population [35] and different cellular compartments such as endoplasmic reticulum (ER) and Golgi [12••], or can be simplified by lumping reactions to rate-limiting steps, viewing the whole culture as a homogenous population and treating the intracellular environment as one compartment [31,36–41]. Models with substantial details can potentially benefit a need of understanding cell system and finding metabolic engineering targets [42]. However, detailed kinetic models

are inevitably heavy-parameterized and need more experimental data for calibration. There is a risk of over parameterization, where parameter estimation can result in a local solution and poor predictive capability against the models' usability [10•]. A small number of parameters is considered more practical and likely to generate a robust model [43]. Relevant efforts include reducing sophisticated models by omitting unnecessary elements while maintaining the major characteristics of the models [44].

As shown in Figure 1c, once a model is structured, initial values of the parameters can be obtained from literature and databases (Table 1) [10•,45,46]. Based on preliminarily parameterized models, a sensitivity analysis is used to define the parameters that must require estimation from experiments. Subsequently, design of experiments (DoE) are conducted and parameters are estimated. Independent experimental data is used afterward to validate the predictive capability of models [47•]. In an iterative way, models can be further modified to improve its predictive capability. Moreover, model parameters that are known to change during a process can be updated with online estimation instead of being constants throughout the course of cultivation, especially when the models are used in online monitoring or control [48••].

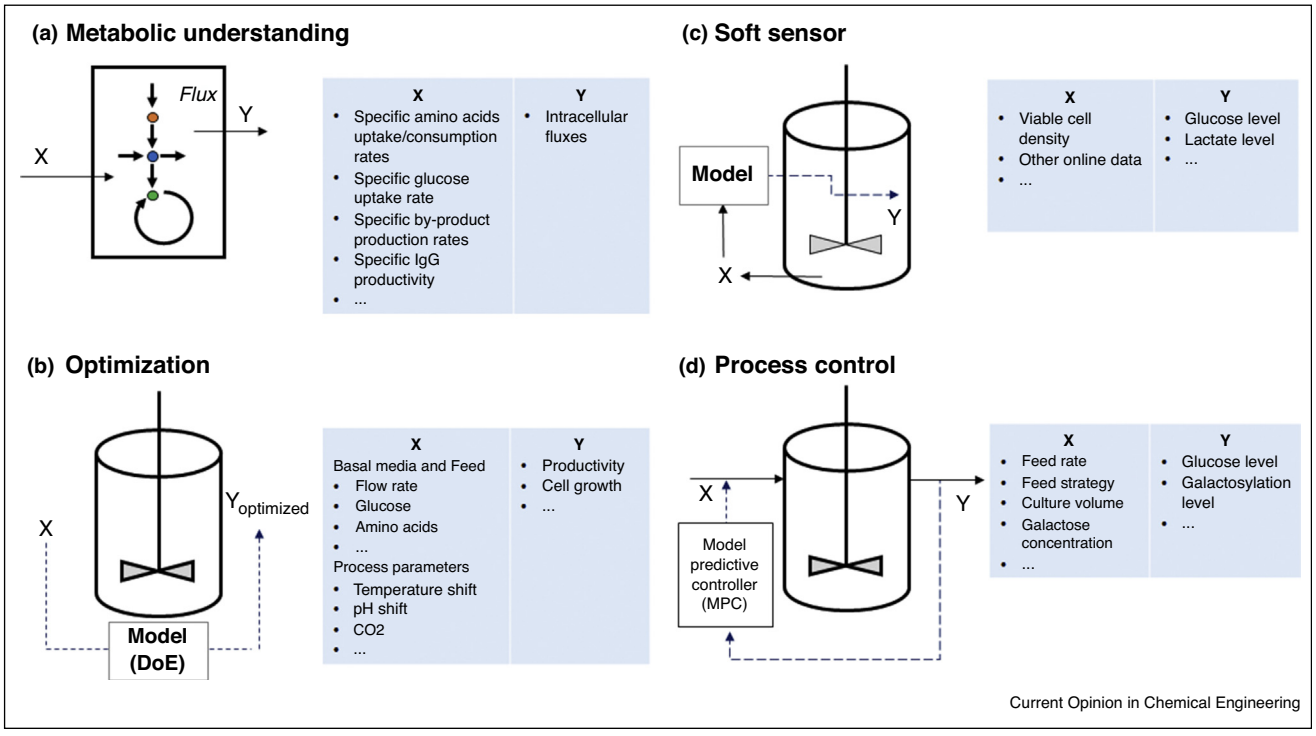
To apply kinetic models across different cell lines and clones or other processes, parameters generally need to be re-estimated [49]. Otherwise, efforts have been made to develop generic model structures that would be applicable broadly across cell lines and processes. These efforts are made by firstly excluding cell line or process specific details to minimize the parameters that need adaption and secondly incorporating mathematical correlations between process variables (e.g. cell lines) and model parameters (e.g. enzyme turnover rates) so models can perform self-adjustment of parameter values giving different process platforms.

Applying mechanistic models in bioprocessing

Gaining understanding of cell culture processes

The mechanistic understanding of cellular metabolism by intracellular fluxes (as shown in Figure 2a) plays an important role in planning and designing processes. Using MFA or FBA, researchers once characterized the presence of high oxidative metabolism in high-producing cells [23] and recognized the higher activity of energy metabolism in antibody-producing CHO cell lines compared to their parental cell lines [27]. These results indicate necessary cellular machineries in high-production processes. It is generally understood that perfusion culture can achieve higher viable cell density and therefore higher titer, but Templeton *et al.* found that the specific productivity from perfusion culture was actually lower than the stationary phase of fed-batch. By using ^{13}C MFA, a higher portion of energy was used to support gross growth rather

Figure 2



The applications of models in bioprocess. **(a)** Metabolic understanding; **(b)** process optimization; **(c)** soft sensor; **(d)** process control.

than IgG production in perfusion cell culture [24]. This study uncovered a limitation of cellular behavior in perfusion culture and thus potentially a chance to overcome it. The study of Pereira *et al.* using ^{13}C MFA examined the response of central carbon metabolism after re-formulating media via amino acid composition manipulation. The study successfully decreased the ammonium production rate by 40% (from approximately 0.65 to 0.4 μmol/million cell/day) while maintaining the same cell growth and production after media formulation. Underneath this phenotype, ^{13}C flux analysis provided an intracellular fluxes' view that the central carbon metabolism was not affected [25].

An understanding of the effects of temperature shift on bioprocesses was gained via both FBA and kinetic models in the studies by Sou *et al.* The researchers found an increase of production but a decrease of highly processed glycoforms on products, once culture temperature shifts from 36.5 °C to 32 °C. Using FBA approach, a root cause was found at a reduction of the carbon species fluxes into nucleotide and nucleotide sugar synthesis. A kinetic model was next used to describe glycosylation and the kinetic parameters were specifically estimated under 36.5 °C and 32 °C. The difference of the estimated parameters unveiled two main factors that were affected by temperature: the production of nucleotide sugars and the expression of galactosyltransferase. These findings were further verified by experimental profiles of nucleotide sugar and gene expression of galactosyltransferase [26,50]. This understanding may enhance a future strategy to remedy the glycosylation change from temperature shift strategy in cell culture.

Optimizing culture and feed media

Some studies have optimized culture or feed media based on the understanding from MFA or FBA. As one example, the study conducted by Xing *et al.* identified excess and limiting amino acids in cell culture by using MFA. According to this result the researchers adjusted batch and feed media aimed for a better balance of amino acids. The optimized cell culture achieved increased peak cell density and protein titer [15]. Another approach to optimize media is based on kinetic models and model-based *in silico* DoE, as shown in Figure 2b. One example is the study conducted by Möllerl *et al.* to determine the glutamine concentration and feed rates for optimal cell growth. The model was first parameterized using a couple of cultivation runs and achieved an R-Square of 0.74 when compared to the verification experiments [16]. Similarly, Kiparissides *et al.* used a Monod kinetics model to identify optimal feed strategies for improving mAb titer. Their fed-batch experiment following the model-based optimization led to an increase of final titer by 30% [17]. Other models reported for media optimization include [31,51–53].

Optimizing process parameters

Process parameters such as oxygen, pH, temperature and agitation may be considered in models because of their impact on cell culture performance. Optimization of these process parameters can be made via models for maximal cell growth and production (as shown in Figure 2b). For example, Hogiri *et al.* developed a pH-dependent dynamic model where IgG production is a function of viable and apoptotic cells. The researchers applied cubic functions to describe the relationship between model parameters and pH value. A pH shift schedule was optimized by the model and increases mAb production by 40% compared to the control condition [30]. Fox *et al.* optimized the time points for shifting culture temperature from 37 °C to 32 °C to achieve maximum production. Temperature-specific parameters were estimated from culture respectively at 37 °C and 32 °C. Following that, the authors simulated the culture with starting temperature shift at different days and determined the optimal day for maximizing production. The results were validated by experiments to show an expected enhancement on the volumetric productivity [54].

Cell line engineering targets

Using mechanistic models, cell line engineering targets that can potentially improve cellular phenotypes are identified. For example, McDonald *et al.* constructed a kinetic model for glycosylation process. Via model simulation, the researchers found that different isoforms of galactosyltransferase 4 could control the metabolic flux through glycosylation pathways and determine the antennarity of *N*-glycan branching on nascent proteins. The authors also noticed that the glycoform branching ultimately affect biological activity of proteins [42]. In another study, Nolan and Lee used their models to assess the possibility to improve antibody production by changing process variables and enzyme expression. A candidate knockdown enzyme was identified and in the meantime the outcomes of modifying this enzyme under different process conditions were evaluated [55].

MFA and FBA, especially genome-scale models can be used to identify engineering targets by simulating manipulations of the presence or absence of gene expression. However, this application has not yet been very common in CHO cells. Examples to find target knock-out genes to increase production have been demonstrated with microbial species [56]. It is promising that a combination of genome-scale FBA and CRISPR technology can provide a range of customized editing to improve biopharmaceutical cell lines.

Process monitoring and control

The technology of soft sensors is interested by manufacturers for its role to estimate/calculate key bioprocess variables that are not readily measurable online by

available techniques [57,58]. Raman spectroscopy combined with statistical models is one example that uses online data (spectrum) to derive process parameters (biomass, amino acid concentration, etc. [6]). Like statistical models, mechanistic models can also generate a linkage between online data and unmeasurable parameters, as shown in Figure 2c. One example is that Kornecki and Strube adopted a model from Xing *et al.* to enable the prediction of glucose and lactate concentrations from online measured viable cell density [37,59].

Model predictive control (MPC) is a technique that uses models to predict future trajectory in processes and takes a feedback or feedforward action to maintain process parameters at a fixed set-point [60]. The concept is shown in Figure 2d. Craven *et al.* established a non-linear MPC based on a model built by Monod kinetics to describe a fed-batch CHO cell culture. The strategy was applied to control glucose concentration at a fixed set point [38]. In the work of Downey *et al.*, a model was developed to estimate the culture products' galactosylation level based on the galactose level in the media. A CHO cell perfusion culture applied this model in MPC to control the galactosylation level on products [61].

Conclusions and future prospective

Mechanistic modeling is a valuable effort to meet the demand of bioprocess optimization and control. Even though developing mechanistic models requires a dedicating process, the investment in establishing frameworks that can be adaptable to different processes with a reasonable amount of model parameterization and/or input customization is worthwhile [48••]. The efforts from academia can play an important role in advancing the systematic understanding in culture processes. CHO omics (including genomic, transcriptomic, proteomic and metabolomic) currently employed to obtain better mechanistic understandings is promising to derive either detailed or generalized models, that can fit to different goals of applications [62,63]. Data from industrial processes will be an important resource to help the development of models with general structure and that are broadly applicable to various cell lines and processes. This article has demonstrated the appearing directions of mechanistic models in bioprocessing, however, the practice of applying models in industrial processes is still little. Acceleration of the adoption of currently existing models to industrial practice need collaboration between academia and industry. Moving forward, it is necessary to advance software and user-friendly tool package to promote the acceptance and usability in the industry [64]. These improvements require interdisciplinary efforts, such as computer science, bioprocess engineering and systems biology.

Conflict of interest statement

Nothing declared.

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