Flexible Nets for the Optimization of IgG Production in CHO cells

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Abstract

Antibody production using CHO cells is often inefficient due to trial-and-error approaches. To improve this, we developed a mathematical model based on Flexible Nets that integrates cell metabolism and bioreactor dynamics. This multi-scale modeling enables systematic, optimized design of antibody manufacturing, enhancing productivity while reducing time and cost.

Introduction

Monoclonal antibodies are vital therapeutic tools used to treat cancers, autoimmune diseases, and more. Their effectiveness depends on low immunogenicity and proper structural processing [1], which is best achieved using mammalian systems like Chinese Hamster Ovary (CHO) cells. CHO cells produce antibodies closely resembling human ones, with reduced contamination risks, making them ideal for large-scale production. Though advances have greatly increased yields, large-scale production remains costly. Traditional optimization methods face limitations in capturing metabolic complexity. Computational modeling offers a more efficient path by simulating cell behavior and guiding experiments. Techniques like Flux Balance Analysis and hybrid models enable dynamic metabolic predictions. Genome-scale models (GEMs) help design experiments but can't fully capture environmental influences. Therefore, new computational frameworks are needed to manage multi-scale, uncertain, and nonlinear biological systems.

This contribution presents the method described in [4].

Materials and methods

Flexible Nets description. Flexible Nets (FNs) consist of two connected subnets, an event net and an intensity net, each of which is a tripartite graph [3]. The three vertices of these nets are:

• Places (): metabolites

• Transitions (): reactions

• Handlers (•): capture the way in which the occurrence of a reaction modifies the concentration of metabolites (event handler) and the way in which the concentration of metabolites modulates the reaction rates (intensity handler).

IgG synthesis modeling in CHO cells. We have used the iCHOv1 model from BiGG Models which contains the metabolic network of Chinese Hamster Ovary (CHO) cells [4]. This model was enriched with the reactions necessary for the synthesis of IgG antibodies:

R_1: 39Ala + 14Arg + ... + 1324GT P + 1326H2O → $IgG_{subunit}$ + ... + 1326H

R_2: 2 $IgG_{subunit}$ → IgG

R_3: IgG → ∅

This is graphically represented in Figure 1.

Step-by-step modeling of the bioreactor. The bioreactor was modeled by:

1. Adding the necessary reactions to the CHO model for producing IgG antibodies.
2. Modeling the nutrients' fluxes from the medium to the tank.
3. Modeling the nutrient uptake from the tank.
4. Modeling the nutrients' fluxes from the bioreactor to the effluent.
5. Modeling the biomass in the bioreactor.
6. Adding the IgG antibody production.

Results

Model validation. To evaluate our model's

predictive capacity, we compared its predicted IgG production with experimentally reported values [4] (Table 1).

Tabla 1. Model validation with two experimental datasets.

	HP	Late Exp phase
Experimental	$2.02 \cdot 10^{-5}$	$2.44 \cdot 10^{-5}$
Model	$2.04 \cdot 10^{-5}$	$3.205 \cdot 10^{-5}$
Relative error	$9.9 \cdot 10^{-3}$	0.3

Optimization of the IgG production. Our model highlighted the significant impact of dilution rate and cell density on IgG production in CHO cell cultures, pinpointing ideal production conditions at specific values of dilution rate and biomass (Figure 2), specifically in the central part of the heatmap.

Medium minimization. We identified nutrients that were supplied in excess and established the lowest concentrations necessary to maintain a targeted level of IgG production. Our optimization efforts focused on: 1) reducing the overall cost of the growth medium, and 2) minimizing the total number of different nutrients included in the medium.

Conclusions

This research introduced Flexible Nets as a modeling framework that integrates intracellular metabolic fluxes with macroscopic bioreactor dynamics under a multi-scale modeling framework, enabling simulation of complex, dynamic, and nonlinear systems. The developed FN model successfully predicted IgG antibody production by incorporating experimental data, media formulation, uptake rates, and continuous culture dynamics.

Our model also identified some amino acids as limiting factors. Adjusting dilution rate and biomass further improved production. By optimizing medium composition and cost, the model guides more efficient IgG production. This highlights the power of combining computational and experimental approaches in bioprocess optimization.

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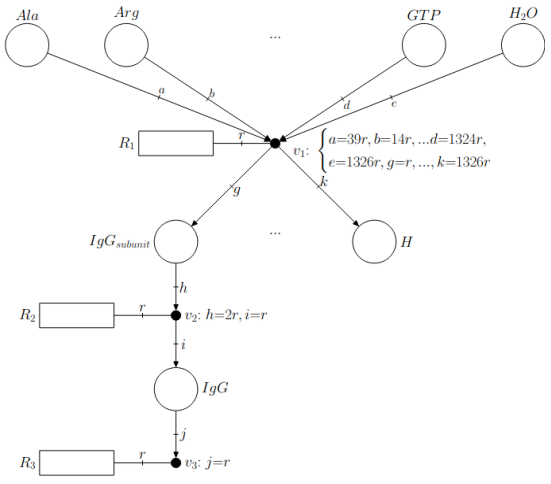

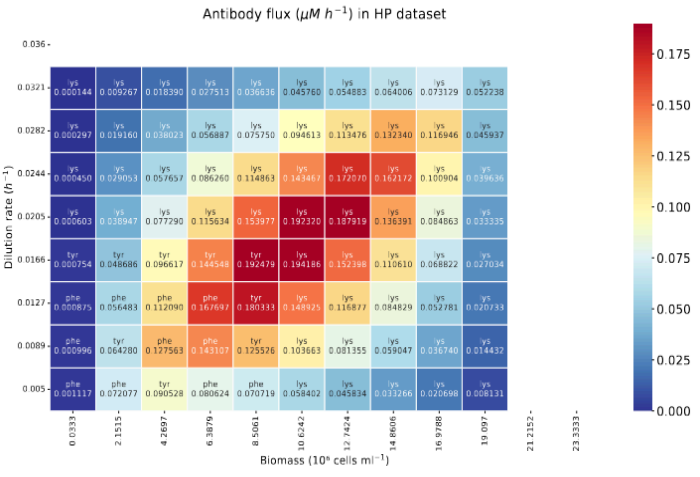

Figure 1. FN modeling the reactions that produce IgG in the iCHOv1 model.
Figure 2. IgG synthesis reaction flux depending on the dilution rate and biomass.