

Toward a Genome Scale Dynamic Model of Cell Free Protein Synthesis in *Escherichia coli*

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Abstract

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Introduction

Mathematical modeling has long contributed to our understanding of metabolism. Decades before the genomics revolution, mechanistically, structured metabolic models arose from the desire to predict microbial phenotypes resulting from changes in intracellular or extracellular states [1]. The single cell *E. coli* models of Shuler and coworkers pioneered the construction of large-scale, dynamic metabolic models that incorporated multiple, regulated catabolic and anabolic pathways constrained by experimentally determined kinetic parameters [2]. Shuler and coworkers generated many single cell kinetic models, including single cell models of eukaryotes [3, 4], minimal cell architectures [5], as well as DNA sequence based whole-cell models of *E. coli* [6]. Conversely, highly abstracted kinetic frameworks, such as the cybernetic framework, represented a paradigm shift, viewing cells as growth-optimizing strategists [7]. Cybernetic models have been highly successful at predicting metabolic choice behavior, e.g., diauxie behavior [8], steady-state multiplicity [9], as well as the cellular response to metabolic engineering modifications [10]. Unfortunately, traditional, fully structured cybernetic models also suffer from an identifiability challenge, as both the kinetic parameters and an abstracted model of cellular objectives must be estimated simultaneously. However, recent cybernetic formulations from Ramkrishna and colleagues have successfully treated this identifiability challenge through elementary mode reduction [11, 12].

In the post genomics world, large-scale stoichiometric reconstructions of microbial metabolism popularized by static, constraint-based modeling techniques such as flux balance analysis (FBA) have become standard tools [13]. Since the first genome-scale stoichiometric model of *E. coli*, developed by Edwards and Palsson [14], well over 100 organisms, including industrially important prokaryotes such as *E. coli* [15] or *B. subtilis* [16], are now available [17]. Stoichiometric models rely on a pseudo-steady-state assumption to reduce unidentifiable genome-scale kinetic models to an underdetermined linear

algebraic system, which can be solved efficiently even for large systems. Traditionally, stoichiometric models have also neglected explicit descriptions of metabolic regulation and control mechanisms, instead opting to describe the choice of pathways by prescribing an objective function on metabolism. Interestingly, similar to early cybernetic models, the most common metabolic objective function has been the optimization of biomass formation [18], although other metabolic objectives have also been estimated [19]. Recent advances in constraint-based modeling have overcome the early shortcomings of the platform, including capturing metabolic regulation and control [20]. Thus, modern constraint-based approaches have proven extremely useful in the discovery of metabolic engineering strategies and represent the state of the art in metabolic modeling [21, 22]. However, genome-scale kinetic models of industrial important organisms such as *E. coli* have yet to be constructed.

Cell-free systems offer many advantages for the study, manipulation and modeling of metabolism compared to *in vivo* processes. Central amongst these advantages is direct access to metabolites and the microbial biosynthetic machinery without the interference of a cell wall. This allows us to control as well as interrogate the chemical environment while the biosynthetic machinery is operating, potentially at a fine time resolution. Second, cell-free systems also allow us to study biological processes without the complications associated with cell growth. Cell-free protein synthesis (CFPS) systems are arguably the most prominent examples of cell-free systems used today [23]. However, CFPS is not new; CFPS in crude *E. coli* extracts has been used since the 1960s to explore fundamentally important biological mechanisms [24, 25]. Today, cell-free systems are used in a variety of applications ranging from therapeutic protein production [26] to synthetic biology [27]. Interestingly, many of the challenges confronting genome-scale kinetic modeling can potentially be overcome in a cell-free system. For example, there is no complex transcriptional regulation to consider, transient metabolic measurements are easier to

obtain, and we no longer have to consider cell growth. Thus, cell-free operation holds several significant advantages for model development, identification and validation. Theoretically, genome-scale cell-free kinetic models may be possible for industrially important organisms, such as *E. coli* or *B. subtilis*, if a simple, tractable framework for integrating allosteric regulation with enzyme kinetics can be formulated.

In this study, we present an effective biochemical network modeling framework for building dynamic cell-free metabolic models. The key innovation of our approach is the seamless integration of simple effective rules encoding complex regulation with traditional kinetic pathway modeling. This integration allows the description of complex regulatory interactions, such as time-dependent allosteric regulation of enzyme activity, in the absence of specific mechanistic information. The regulatory rules are easy to understand, easy to formulate and do not rely on overarching theoretical abstractions or restrictive assumptions. We tested our approach by modeling the time evolution of several hypothetical cell-free metabolic networks. In particular, we tested whether our effective modeling approach could describe classically expected enzyme kinetic behavior, and second whether we could simultaneously estimate kinetic parameters and regulatory connectivity, in the absence of specific mechanistic knowledge, from synthetic experimental data. Toward these questions, we explored five hypothetical cell-free networks. Each network shared the same enzymatic connectivity, but had different allosteric regulatory connectivity. We found that simple effective rules, when integrated with traditional enzyme kinetic expressions, captured complex allosteric patterns such as ultrasensitivity or non-competitive inhibition in the absence of mechanistic information. Second, when integrated into network models, these rules captured classical regulatory patterns such as product-induced feedback inhibition. Lastly, we showed, at least for the network architectures considered here, that we could simultaneously estimate kinetic parameters and allosteric connectivity from synthetic data starting from an unbiased collection of possible allosteric structures using

particle swarm optimization. However, when starting with an initial population that was heavily enriched with incorrect structures, our particle swarm approach could converge to an incorrect structure. While only an initial proof-of-concept, the framework presented here could be an important first step toward genome-scale cell-free kinetic modeling of the biosynthetic capacity of industrially important organisms.

The introduction has four paragraphs (introduction no longer than 3 pages). Follow the cell free paper from last year:

1. **First paragraph:** Introduce mathematical modeling, and its role in biochemical engineering.
2. **Second paragraph:** Contrast current static metabolic modeling approaches e.g., FBA with dynamic models.
3. **Third paragraph:** Introduce cell free protein synthesis.
4. **Fourth paragraph:** In this study, [Repeat the abstract with some additional detail].
Taken together, [killer statement].

Results

The results are presented in **past tense**. Each paragraph starts with a statement of the result in that paragraph in active voice. Each results paragraph ends with a Taken together type statement followed by a link statement e.g., Next we considered etc. When referring to figures, state what the figures shows (Fig. ZZ).

1. **First section:**Description of the model biology
2. **Second section:**Estimation of the model parameters, and refinement of the model structure (inclusion of the AA degradation pathways)
3. **Third section:**Analysis of the flux distribution (over the ensemble?), sensitivity results (first parameters, then AA)

Discussion

The discussion has three (sometimes four) paragraphs:

1. **First paragraph:** Present a modified version of the last paragraph of the introduction. In this study, [...]. Taken together, [killer statement]
2. **Second paragraph:** Contrast the key findings of the study with other computational/experimental studies
3. **Third paragraph:** Present future directions. If you had more time, what would like to do? Highlight the key shortcomings of the approach and how will we address them in the future. In this case, we will have a scaling issue if we extend to genome scale. We should extend to dynamic cases, and we need to experimentally validate the findings.

Materials and Methods

Formulation and Solution of the Model Equations We used ordinary differential equations (ODEs) to model the time evolution of metabolite (x_i) and scaled enzyme abundance (ϵ_i) in hypothetical cell-free metabolic networks:

$$\frac{dx_i}{dt} = \sum_{j=1}^{\mathcal{R}} \sigma_{ij} r_j(\mathbf{x}, \epsilon, \mathbf{k}) \quad i = 1, 2, \dots, \mathcal{M} \quad (1)$$

$$\frac{d\epsilon_i}{dt} = -\lambda_i \epsilon_i \quad i = 1, 2, \dots, \mathcal{E} \quad (2)$$

where \mathcal{R} denotes the number of reactions, \mathcal{M} denotes the number of metabolites and \mathcal{E} denotes the number of enzymes in the model. The quantity $r_j(\mathbf{x}, \epsilon, \mathbf{k})$ denotes the rate of reaction j . Typically, reaction j is a non-linear function of metabolite and enzyme abundance, as well as unknown kinetic parameters \mathbf{k} ($\mathcal{K} \times 1$). The quantity σ_{ij} denotes the stoichiometric coefficient for species i in reaction j . If $\sigma_{ij} > 0$, metabolite i is produced by reaction j . Conversely, if $\sigma_{ij} < 0$, metabolite i is consumed by reaction j , while $\sigma_{ij} = 0$ indicates metabolite i is not connected with reaction j . Lastly, λ_i denotes the scaled enzyme degradation constant. The system material balances were subject to the initial conditions $\mathbf{x}(t_o) = \mathbf{x}_o$ and $\epsilon(t_o) = 1$ (initially we have 100% cell-free enzyme abundance).

The reaction rate was written as the product of a kinetic term (\bar{r}_j) and a control term (v_j), $r_j(\mathbf{x}, \mathbf{k}) = \bar{r}_j v_j$. In this study, we used either saturation or mass action kinetics. The control term $0 \leq v_j \leq 1$ depended upon the combination of factors which influenced rate process j . For each rate, we used a rule-based approach to select from competing control factors. If rate j was influenced by $1, \dots, m$ factors, we modeled this relationship as $v_j = \mathcal{I}_j(f_{1j}(\cdot), \dots, f_{mj}(\cdot))$ where $0 \leq f_{ij}(\cdot) \leq 1$ denotes a regulatory transfer function quantifying the influence of factor i on rate j . The function $\mathcal{I}_j(\cdot)$ is an integration rule which maps the output of regulatory transfer functions into a control variable. Each regulatory

transfer function took the form:

$$f_{ij}(\mathcal{Z}_i, k_{ij}, \eta_{ij}) = k_{ij}^{\eta_{ij}} \mathcal{Z}_i^{\eta_{ij}} / (1 + k_{ij}^{\eta_{ij}} \mathcal{Z}_i^{\eta_{ij}}) \quad (3)$$

where \mathcal{Z}_i denotes the abundance factor i , k_{ij} denotes a gain parameter, and η_{ij} denotes a cooperativity parameter. In this study, we used $\mathcal{I}_j \in \{\max, \min\}$ [?]. If a process has no modifying factors, $v_j = 1$. We used multiple saturation kinetics to model the reaction term \bar{r}_j :

$$\bar{r}_j = k_j^{\max} \epsilon_i \left(\prod_{s \in m_j^-} \frac{x_s}{K_{js} + x_s} \right) \quad (4)$$

where k_j^{\max} denotes the maximum rate for reaction j , ϵ_i denotes the scaled enzyme activity which catalyzes reaction j , and K_{js} denotes the saturation constant for species s in reaction j . The product in Equation (4) was carried out over the set of *reactants* for reaction j (denoted as m_j^-).

Generation of model ensemble Fill me in.

Global and local sensitivity analysis We conducted a global sensitivity analysis, using the variance-based method of Sobol, to estimate which parameters controlled the performance of the reduced order model [28]. We computed the total sensitivity index of each parameter relative to two performance objectives, the peak thrombin time and the area under the thrombin curve (thrombin exposure). We established the sampling bounds for each parameter from the minimum and maximum value of that parameter in the parameter set ensemble. We used the sampling method of Saltelli *et al.* [29] to compute a family of $N(2d + 2)$ parameter sets which obeyed our parameter ranges, where N was the number of trials, and d was the number of parameters in the model. In our case, $N = 10,000$ and $d = 22$, so the total sensitivity indices were computed from 460,000 model evaluations. The variance-based sensitivity analysis was conducted using the SALib module encoded

in the Python programming language [30].

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