Reverse Engineering and Optimization of Metabolic Networks

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Understanding the dynamic behavior of cells and their metabolism is a key goal in systems biology. Reverse engineering metabolic processes from data is crucial for uncovering underlying mechanisms, building predictive models, and optimizing networks for strain engineering and biotechnological applications. Here, we present three representative case studies that highlight different modeling and optimization strategies.

Among the various modeling approaches, S-systems provide a remarkably flexible and interpretable framework. They consist of systems of ordinary differential equations (ODEs) based on a power-law formalism, derived from a first-order Taylor series approximation in logarithmic space. This structure makes them well-suited for approximating reaction dynamics without relying on detailed mechanistic information. We present a case study of glycolysis in *Lactococcus lactis*, modeled using in vivo NMR multivariate time-series of metabolic concentrations [6]. The structural analysis of the proposed model was initially used to assess parameter sensitivity and identifiability, thereby enhancing model interpretability and significantly improving the subsequent reverse engineering step [5].

While classical ODEs offer a powerful framework for modeling continuous nonlinear dynamics, they typically assume that model parameters remain constant over time. However, many biological processes, such as cell growth under nutrient shifts or environmental changes, may exhibit distinct phases and are therefore better modeled using time-varying parameters. Indeed, these transitions can be more effectively captured using hybrid modeling approaches, which combine continuous dynamics with discrete state changes. In this context, we developed SON-EM, a methodology for parameter identification in switched systems [2], which integrates convex optimization, clustering, and a refinement step to detect switching points and estimate local parameters. Applied to multiphasic cell growth, SON-EM successfully captured diauxic transitions and metabolic shifts using a parsimonious structure based on logistic models, with minimal manual intervention [3].

Optimization also plays a crucial role in designing metabolic interventions. We developed OptPipe, a consensus-based pipeline that integrates several algorithms (including OptKnock, RobustKnock, and RobOKoD) to identify promising gene knockouts and rank them based on growth, target production, and adaptability metrics [4]. Complementarily, we explored multi-objective optimization to identify reaction deletions that simultaneously maximize and/or minimize multiple objectives, using multi-objective mixed-integer optimization (MOMO), and applied this approach as a proof of concept to enhance ethanol production in *Saccharomyces cerevisiae* [1].

The presented modeling and optimization methods illustrate how mathematical frameworks, algorithms, and biological insights can be combined to understand, predict, and manipulate metabolic networks.

Keywords: Systems Biology · Strain Design · Multi-Objective Optimization· Kinetic Modeling · Hybrid models

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