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Genome-scale metabolic networks in time and space

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Abstract

Constraint-based models (CBMs) are key tools for elucidating the behavior of genome-scale metabolic networks, but the assumption of steady state hinders their application to spatio-temporally varying and multicellular systems. Models that integrate CBMs with kinetics to allow dynamic simulation through dynamic flux balance analysis (DFBA) can circumvent this problem as well as the limitations of purely kinetic models. With many technical barriers for DFBA overcome in recent years, applications traditionally focused on metabolic engineering have expanded to address problems such as evolution of microbial communities, functions of biomedically relevant biofilms, and diet effects on Parkinson's disease. By addressing substantial computational challenges, we expect that such hybrid metabolic models will pave the way towards whole-cell modeling.

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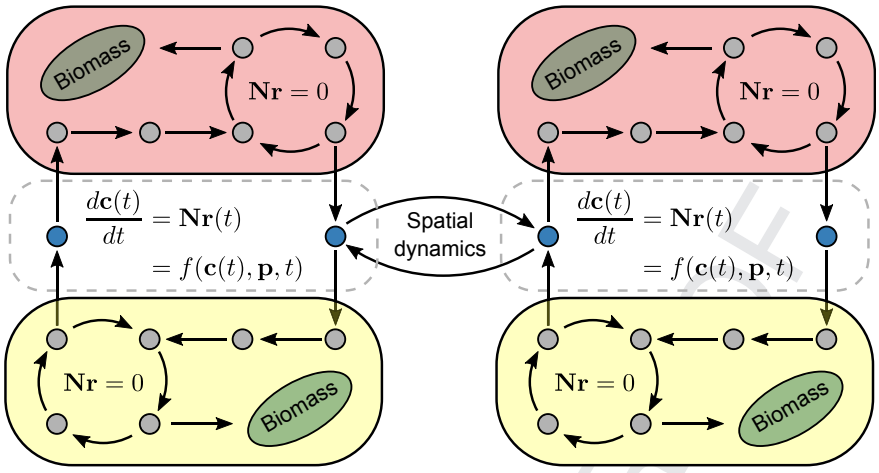
Introduction

Metabolic networks are fundamental in biology, enzymatically converting available substrates into products that include the cellular components needed for survival and growth. Understanding, predicting, and ultimately controlling their behavior is a challenge of crucial importance, not only for basic science but also for manifold applications ranging from metabolic engineering to human health and disease. Experimental technologies, most importantly high-throughput metabolomics, advance rapidly, but system-level mathematical modeling is needed in order to transform data into insight [1].

Constraint-based models (CBMs) are currently the most established and powerful tools for large-scale metabolic network modeling [2]. They are fundamentally based on the quasi-steady-state assumption (QSSA), exploiting that metabolism is fast and therefore approximately invariant on the time-scale of other processes such as gene regulation with which it interacts [3]. This makes CBMs and their analysis linear, parameter-free, and applicable to metabolic networks of virtually any species with a sequenced genome [4,5]. The drawback of relying on the QSSA is that CBMs do not represent metabolite concentrations or their dynamics. Rather, they predict metabolic flux distributions, steady-state reaction rates for all reactions in the network, which have been studied extensively in many organisms, using a plethora of computational methods. The three main computational approaches are (i) flux balance analysis (FBA), which predicts fluxes by assuming cellular objectives such as maximization of growth rate in microorganisms, (ii) pathway analysis, which identifies all possible flux routes through the network, and (iii) random flux sampling, which seeks to determine probability distributions of feasible steady-state fluxes [6].

CBMs have been remarkably successful in areas that range from systematically representing biological knowledge and data [7] to devising metabolic engineering strategies [8]. However, the QSSA limits their applicability beyond the obvious: they do not capture the dynamic behavior of cells in changing environments. Even for a constant environment, CBMs alone cannot predict fluxes from metabolite concentrations (and vice versa). Bridging this gap between fluxes and concentrations is a fundamental challenge of particular importance for multicellular systems such as microbial communities or human tissues because the metabolite concentrations in the environment couple the cells' behaviors to each other (Figure 1). In such cases, methods established for unicellular CBMs are not directly applicable because of many challenges that range from model construction, to assumed cellular objectives, to context-dependent interactions between cells [9]. For example, modified cellular objectives for FBA of microbial communities [10,11] are hard to justify biologically (e.g., why should an individual strain's objective be to maximize the overall growth rate of the community?). For unbiased methods such as pathway analysis and random sampling, the size of multiple interacting metabolic networks exacerbates the computational challenges and makes flux prediction for multicellular systems practically infeasible [12,13].

Figure 1



Metabolic models for multicellular systems. Cells of potentially different types (indicated by colors) contain intracellular metabolic networks composed of chemical species (grey nodes) and reactions (arrows) that are formally represented by a stoichiometric matrix **N**. CBMs for individual cells rely on the QSSA, assuming that the intracellular metabolite concentrations $\mathbf{c}(t)$ do not change over time, such that the mass balances lead to a linear problem in which the flux distribution (set of reaction rates) **r** is the only unknown. For extracellular metabolites (blue nodes) accessible to several cells, kinetics (time-dependent exchange rates $\mathbf{r}(t)$ that are functions of extracellular concentrations $\mathbf{c}(t)$ and kinetic parameters **p**) need to be considered even in a constant environment to capture how resources are distributed between cells. Spatial extensions follow the same logic, where kinetics (for example, describing the diffusion of metabolites) connect different compartments (left and right).

Without fundamental progress in the computational methods, flux predictions for multicellular systems require an integration of CBMs with extracellular concentrations through experimental data, metabolite uptake kinetics, or both. We argue that models that combine CBMs with kinetics are the most promising approach to connect fluxes to concentrations because they rely less on dynamic metabolite data than state-of-the-art methods for integrating metabolomics with CBMs [14,15]. They also need fewer kinetic rate laws and parameters than detailed kinetic models that are often hard to identify and computationally expensive to simulate [16]. Hybrid models can be simulated through dynamic flux balance analysis (DFBA) approaches [17], which connect kinetics to flux predictions from FBA without needing multicellular objectives. Alternative approaches to simplify dynamics, cybernetic models, consider more detailed, optimal pathway-level resource allocation; they are computationally expensive and currently not applicable to genome-scale [18]. Here, we therefore discuss recent advances in DFBA models, and their applications to diverse uni- and multicellular systems with temporal and spatiotemporal resolution.

Metabolic models and dynamic FBA

Mass balances for metabolites that constrain the static or dynamic fluxes are the basis for all metabolic models. DFBA models contain two distinct sets of mass balances, one dynamic and one static (Figure 1). The QSSA virtually always applies to the balances of all intracellular metabolites; the metabolic network is considered static on the extracellular time-scale. Dynamics are modeled by a sequence of instantaneous steady-state responses to environmental changes, mediated by the kinetics of metabolite uptake and secretion. In this case, the dynamic components are biomass and metabolites that can be exchanged between the cell and the environment. Multiple CBMs can share the same pool of dynamic metabolites, which in turn can connect to pools at other points in space.

Because the dynamic and static mass balances are interdependent, one has to solve them together (Figure 2). This amounts to jointly solving ordinary differential equations (ODEs) for dynamic balances and linear algebraic equations for static balances, and integration of the former requires flux predictions from the latter. Any realistic metabolic network contains more reactions than metabolites, such that the static system is generally underdetermined. Together with flux capacity constraints, this defines a CBM whose solution space contains an infinite number of feasible steady-state flux distributions. FBA uses linear programming to identify a specific solution that optimizes an objective function, typically growth rate as represented by the flux of a biomass reaction, and DFBA uses FBA to get the static fluxes needed for integration of the ODE system. The static fluxes, in turn, are constrained by the state of the dynamic system—the environment—through simple binary rules or kinetic equations. In multicellular settings, environmental resources are allocated to individual cells at each time step, and competition and cooperation can emerge over time through dynamic interactions. Importantly, it is not necessary to define