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Predicting metabolic interactions between multiple species from first principles

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Predictive genome-scale metabolic models (GSMs) have become standard tools for systems-level analysis of metabolism in contexts ranging from biotechnology to cancer 3. They rely on incorporating mass balances and potentially other constraints on the operation of metabolic networks at steady-state. In biased methods such as flux balance analysis (FBA), assumptions on biological objectives (e.g., bacteria maximizing their growth rate) allow to predict specific flux distributions by optimization (e.g., by linear programming in FBA). Unbiased methods for the analysis of all feasible flux distributions are subsumed under metabolic pathway analysis; they typically involve solving large combinatorial problems. However, many interactions in microbial consortia or tissues of multicellular organisms rely on networks of metabolite exchanges, where multiple species interact metabolically via a shared environment. In the multi-species setting, biased methods either require assumptions on community objectives that are biologically questionable, or model augmentations by often unknown kinetics. Unbiased methods are limited by the scalability of current metabolic pathway concepts to multi-species networks 4.

For unbiased methods, we aim to increase predictive power by relying on first principles and by systematically quantifying uncertainties. This involves integrating thermodynamic constraints via probabilistic thermodynamic analysis (PTA) 2, kinetics via ENKIE, a predictor of enzyme kinetic parameters 1, and optimization over enzyme costs. The resulting method termed global enzyme cost minimization (GECM) combines MCMC sampling with biconvex optimization to allow predictions such as alternative metabolic strategies for *E. coli* growth that are consistent with experimental observations. GECM thereby provides predictions of single-species behaviors and their uncertainty—albeit at high computational cost.

As a potentially complementary unbiased method, we define minimal pathways (MPs) that yield compact representations of metabolic network capabilities 5. They generalize existing pathway concepts by allowing inhomogeneous constraints as well as the targeted analysis of subnetworks. Computationally, enumeration and sampling of MPs is efficient via iterative minimization and pathway graphs. This enables applications such as assessing quantitative gene essentiality in *E. coli* central metabolism, predicting metabolite exchanges associated with homeostasis and health in a host-microbe model of the human gut, and designing butyrate-producing microbial communities.

In combination, we envisage MPs and GECM to predict metabolic interaction networks between multiple species as follows: MPs restrict the set of potential metabolite exchange patterns between individual species and the environment

in the community context. Application of GECMs to the reduced problem(s) could then follow to predict actual interactions and their uncertainties. However, this requires improvements to computational efficiency and numerical stability of GECM. In perspective, this integration could enable predictions of emergent community functions and of experimentally testable interactions for applications such as the human gut microbiome.

Keywords: Genome-scale metabolic models · Metabolic interactions · Thermodynamics · Kinetics · Metabolic pathways · Microbiome.

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