Title of Project: An open source platform for multi-scale spatially distributed simulations of microbial ecosystems

Institution: Boston University

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Project Results:

The goal of this project was to develop a tool for facilitating simulation, validation and discovery of multiscale dynamical processes in microbial ecosystems. This led to the development of an open-source software platform for Computation Of Microbial Ecosystems in Time and Space (COMETS). COMETS performs spatially distributed time-dependent flux balance based simulations of microbial metabolism. Our plan involved building the software platform itself, calibrating and testing it through comparison with experimental data, and integrating simulations and experiments to address important open questions on the evolution and dynamics of cross-feeding interactions between microbial species.

IN previous related efforts, we had started developing computational tools for synthetic ecology, as a way to understand and engineer microbial ecosystems (Klitgord and Segrè, 2011). Through multi-cellular extensions of genome-scale models of metabolism, we had identified multiple pairs of organisms and environmental conditions that could effectively give rise to artificial communities, based on cross-feeding of relevant nutrients (Klitgord and Segrè, 2010).

The COMETS Software

COMETS uses dynamic flux balance analysis (dFBA) to perform time-dependent metabolic simulations of microbial ecosystems, bridging the gap between stoichiometric and environmental modeling (Harcombe et al., Cell Reports 2014). Simulations occur on a spatially structured lattice of interacting metabolic subsystems (“boxes”), providing at the same time insight on intracellular metabolic fluxes and on ecosystem-level distributions of microbial populations and nutrients. COMETS incorporates two fundamental steps. The first step, cellular growth, is modeled as an increase of biomass at different spatial locations, using a hybrid kinetic-dFBA algorithm. Each box may contain biomass for an arbitrary number of different species. The second step consists of a finite differences approximation of the diffusion of extracellular nutrients and by-products in the environment, and of the expansion of biomass. Simple diffusion simulations in absence of growth behave as expected from classical physics. We have incorporated multiple species into COMETS by importing the corresponding stoichiometric models, either from manually curated reconstructions, or from automated pipelines that construct models from annotated genomes and high-throughput data, such as Model SEED or Kbase. In addition, both spatially and molecularly complex environments can be designed by the user through an interactive toolbox and simulation outcomes can be analyzed through a visualization tool.

Applying COMETS to synthetic microbial communities

In addition to basic testing and calibration (e.g. showing that COMETS recapitulates the growth properties of *E. coli* colonies on different carbon sources), we used COMETS, in tight collaboration with the lab of co-PI Christopher Marx, to

produce predictions relevant for the study of simple artificial microbial communities.

In particular, we next tested the ability of COMETS to predict interactions between members of a previously evolved and studied *E. coli*/*S. enterica* synthetic consortium (Harcombe, Evolution 2010). In lactose medium, *Salmonella enterica* Serovar typhimurium LT2 relies on carbon by-products from an *Escherichia coli* K12 metB mutant. Reciprocally, this auxotrophic *E. coli* requires methionine from its partner in order to grow in minimal medium. Stoichiometric models of each partner were modified to incorporate known genetic constraints. For the *E. coli* strain, the metB mutation was incorporated by constraining to zero the flux through the corresponding reaction (cystathionine γ-synthase). In *S. enterica*, methionine excretion requires gain-of-function mutations in metA (homoserine transsuccinylase). This excretion was modeled as coupled to biomass, so that as cells grew they excreted observed levels of the amino acid. These genetic alterations created an obligate mutualistic interaction *in silico* consistent with that observed in the laboratory; neither species was able to grow in isolation on lactose minimal media, but growth was observed when both species were present. COMETS could additionally accurately recapitulate the abundance of the two species at steady state.

We next challenged COMETS to predict the behavior of a tripartite obligate mutualism. Toward this goal, we used COMETS to reproduce the behavior of an engineered synthetic consortium that incorporates *M. extorquens* AM1 into the previous *E. coli*/*S. enterica* system. This represents a significant advance in complexity relative to obligate consortia that have been previously engineered. *M. extorquens* is the best-studied model system for C1 metabolism and has the ability to obtain energy, carbon, and nitrogen from methylamine. Here, we used a ΔhprA strain that lacks a key enzyme (hydroxypyruvate reductase) for assimilating carbon from methylamine. In media with lactose and methylamine, the ΔhprA *M. extorquens* strain relies on acetate from *E. coli*, while providing the other two species with a source of nitrogen due to dissimilation of methylamine. COMETS again made excellent predictions about the obligate nature of species interactions in the consortium, and the abundance of the three species at steady state.

COMETS predicts effects of spatial complexity in microbial communities

We used the two-species consortium to investigate the influence of spatial structure on competition in mutualistic systems. As a first step, we tested the growth of each partner as a function of increasing distance between them. Consistent with expectations, both the modeled colonies and the observations of the pair exhibited decreased growth as they were initiated further apart. As growth of communities will rarely be as simple as pairwise interactions between microcolonies, we then asked how additional colonies influence pairwise interactions. When essential metabolites diffuse from a point source one might expect that colonies have an “eclipse” effect, casting a resource shadow that reduces the metabolites available to more distant colonies. Based on this logic, one would expect that the growth rate of a colony would be reduced if a competitor colony is placed between the colony and a mutualistic partner. The extent of negative impact should scale with the rate at which the intermediate colony removes metabolites from the environment. On the other hand, one could argue for an opposite outcome, i.e., that the newly interposed colony, by helping the mutualistic partner, will ultimately benefit the original colony. Intuition alone cannot provide an answer to this conundrum, because its solution depends on the balance among the metabolic rates of the different species, the spatial organization of the colonies, and the diffusion rates.

We used COMETS to simulate the outcome of this gedanken experiment. COMETS predicted that a colony of wild-type *S. enterica* (whose model lacks the imposed methionine excretion of the mutualistic strain) would rapidly remove carbon from its surroundings and diminish the growth of a more distant colony of mutualistic *S. enterica*. However, if the intermediate colony were another mutualistic *S. enterica*, then, based on COMETS, the growth of the distal colony would end up being larger than in the absence of an interfering colony. Though this effect is predicted to be time dependent, it holds over a substantial temporal window. Experimental testing of these predictions confirmed the accuracy of the COMETS simulations. Thus, the intermediate colony increased the growth and excretion of a mutualistic partner, and this amplifying effect outweighed the influence of competition for carbon. Additional insight on the details of this phenomenon would require experimental measurements of metabolite concentrations at different points in space and time, e.g., using imaging mass spectrometry. Interestingly, one can use COMETS to provide some preliminary theoretical insight, by taking advantage of its capacity to record simulated fluxes and metabolites at any given time and location for all organisms.

Additional studies of microbial metabolic dynamics using constraint-based models

As part of our effort to extend existing methods to better characterize the dynamics of intracellular and extracellular metabolites in microbial systems and ecosystems, we performed additional studies that resulted in publications, the most notable of which developed a new Flux Imbalance Analysis approach to study the sensitivity of cellular growth to changes in metabolite pools: Stoichiometric models of metabolism, such as flux balance analysis (FBA), are classically applied to predicting steady state rates - or fluxes - of metabolic reactions in genome-scale metabolic networks. Here we revisit the central assumption of FBA, i.e. that intracellular metabolites are at steady state, and show that deviations from flux balance (i.e. flux imbalances) are informative of some features of in vivo metabolite concentrations. Mathematically, the sensitivity of FBA to these flux imbalances is captured by a native feature of linear optimization, the dual problem, and its corresponding variables, known as shadow prices. First, using recently published data on chemostat growth of *Saccharomyces cerevisae* under different nutrient limitations, we show that shadow prices anticorrelate with experimentally measured degrees of growth limitation of intracellular metabolites. We next hypothesize that metabolites which are limiting for growth (and thus have very negative shadow price) cannot vary dramatically in an uncontrolled way, and must respond rapidly to perturbations. Using a collection of published datasets monitoring the time-dependent metabolomic response of *Escherichia coli* to carbon and nitrogen perturbations, we test this hypothesis and find that metabolites with negative shadow price indeed show lower temporal variation following a perturbation than metabolites with zero shadow price. Finally, we illustrate the broader applicability of flux imbalance analysis to other constraint-based methods. In particular, we explore the biological significance of shadow prices in a constraint-based method for integrating gene expression data with a stoichiometric model. In this case, shadow prices point to metabolites that should rise or drop in concentration in order to increase consistency between flux predictions and gene expression data. In general, these results suggest that the sensitivity of metabolic optima to violations of the steady state constraints carries biologically significant information on the processes that control intracellular metabolites in the cell.

Products Delivered:

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Sara B. Collins*, Ed Reznik*, Daniel Segre': Temporal expression-based analysis of metabolism, PLoS Computational Biology (2012), 8(11): e1002781.

Ed Reznik, Tasso J. Kaper and Daniel Segre': The dynamics of hybrid metabolic-genetic oscillators, Chaos (2013), 23, 013132.

Ed Reznik, Pankaj Mehta and Daniel Segre': Flux imbalance analysis and the sensitivity of cellular growth to changes in metabolite pools, PLOS Computational Biology (2013), 9(8): e1003195.

Ed Reznik, Osman Chaudhary and Daniel Segre': The Average Enzyme Principle, FEBS Letters (2013) 587, 2891–289.

Ed Reznik, Stefan Yohe and Daniel Segre': Invariance and optimality in the regulation of an enzyme, Biology Direct (2013), 8:7.

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