

160. Improving computational strain design strategies by incorporation of kinetic information

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Project Goals: The goal of this effort is to assess the scope of using a hybrid kinetic and stoichiometric representation of metabolism for computational strain design. In particular, we compared the performance of the k-OptForce protocol with stoichiometry-only procedures under the same condition for which the kinetic model was parameterized as well as a different environmental condition.

A key methodological impediment of existing computational strain-design approaches is the stoichiometry-only representation of metabolism and the on-off representation of regulation. This may lead to a metabolite concentration, enzymatic activity and metabolic regulation-agnostic intervention strategies. Therefore, identified flux redirection predictions (especially up/down flux modulation) are sometimes difficult to translate into actionable genetic interventions. Strain design prediction accuracy has been the focus for many recent efforts through the selective integration of kinetic information into metabolic models. The recently developed k-OptForce procedure [1] extended the previously developed strain-design OptForce algorithm [2] by integrating all available mechanistic detail afforded by kinetic models within a constraint-based optimization framework tractable even for genome-scale models.

Application of k-OptForce in case studies revealed that, in general, the kinetic model prediction quality is determined by the range and scope of genetic and/or environmental perturbations used during its parameterization. In our most recent work [3], we applied the k-OptForce procedure on a kinetic model of *E. coli* core metabolism constructed using the Ensemble Modeling (EM) method and parameterized using seven mutant strains flux data under aerobic respiration with glucose as the carbon source [4]. The kinetic model includes 138 reactions, 93 metabolites and 60 substrate-level regulatory interactions. Minimal interventions are identified that improve succinate yield under both aerobic and anaerobic conditions to test the fidelity of model predictions under both genetic and environmental perturbations. Under aerobic condition, k-OptForce identifies interventions that match existing experimental strategies pointing at a number of unexplored flux redirections such as routing glyoxylate flux through the glycerate metabolism to improve succinate yield. Many of the identified interventions rely on the kinetic descriptions and would not be discoverable by a purely stoichiometric description. In contrast, under fermentative (anaerobic) conditions, k-OptForce fails to identify key interventions including up-regulation of anaerobic reactions and elimination of competitive fermentative products. This is due to the fact that the pathways activated under anaerobic conditions were not properly parameterized as only aerobic flux data were used in the model construction.

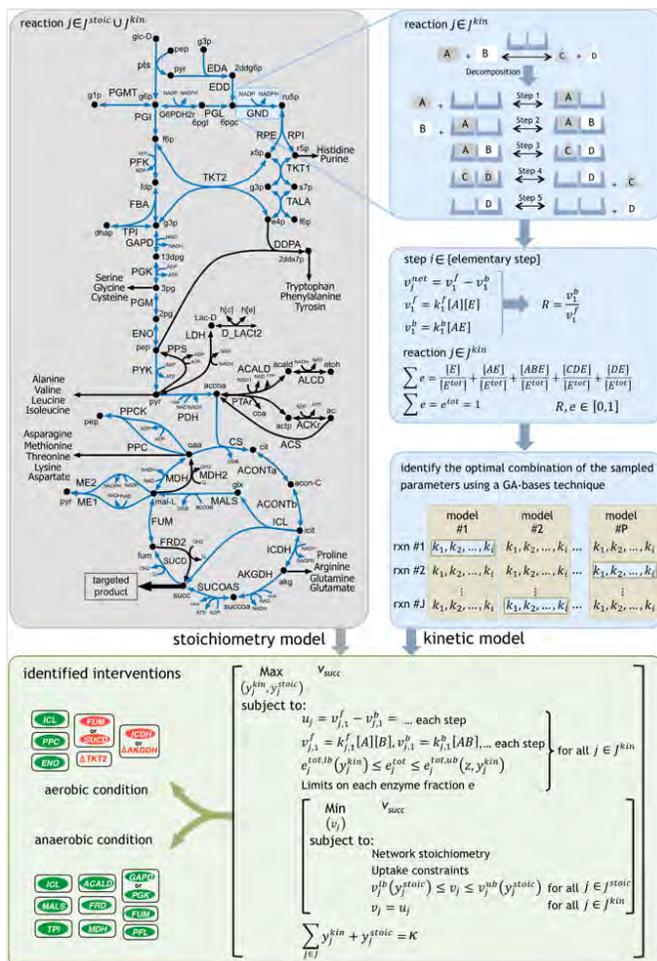
Inspired by these observations, we extend the range and scope of the current kinetic model with inclusion of all relevant reactions from the genome-scale iAF1260 model. Parameterization is carried out using steady-state flux data for 25 mutant strains under both aerobic as well as anaerobic conditions with different carbon sources (glucose, acetate, and pyruvate). The model consists of 457 reactions, 337 metabolites and 295 substrate-level regulatory interactions accounting for glycolysis/gluconeogenesis, Pentose Phosphate (PP) pathway, TCA cycle, anaerobic reactions, amino-acid synthesis/degradation, fatty acid oxidation/synthesis, proline synthesis and a number of reactions in other parts of the metabolism. The developed kinetic description will be ultimately integrated with computational strain

design protocols to improve the accuracy of the identified strategies for overproduction of chemicals of interest.

This study revealed the importance of condition-specific model parameterization and provides guidelines on how to augment kinetic models so as to correctly respond to genetic as well as environmental perturbations.

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A schematic representation of the procedure [3]