176. Opt orce: train design using inetic models and synthetic biology tools

Anupam Chowdhury[1*] (axc1028@psu.edu), Chiam Y. Ng[1], Howard M. Salis[1], and **Costas D. Aranas[1]**

[1]Department of Chemical Engineering, The Pennsylvania State University, University Park, PA.

http://maranas.che.psu.edu

Project Goals: The goals of this effort are to i improve the fidelity of computational strain design procedures by incorporating substrate level regulation in the form of inetic expressions in stoichiometric models using the Opt orce procedure, and ii implement the identified intervention strategies by creating a library of R of different Translation nitiation Rates using the R library calculator to construct synthetic operons that maximi es the yield of the target product.

Existing computational strain-design approaches relying solely on stoichiometry and on- off regulation ignore the effects of metabolite concentrations and substrate-level enzyme regulation while identifying metabolic interventions. In this work, we developed the k- OptForce procedure which integrates the available kinetic descriptions of metabolic reactions with stoichiometric models to sharpen the prediction of intervention strategies for improving the bio-production of a chemical of interest. The interventions suggested by k-OptForce are comprised of both direct enzymatic parameter changes (for reactions with available kinetics) and indirect reaction flux changes (for reactions with only stoichiometric information). Application of k-OptForce to the overproduction of L-serine in *E. coli* and triacetic acid lactone (TAL) in *S. cerevisiae* revealed that the identified interventions tend to cause less dramatic rearrangements of the flux distribution so as not to violate concentration bounds. In some cases, additional modifications are needed to overcome the substrate-level regulations imposed by the representative kinetic model. The mechanism of action of these modifications is often subtle by alleviating substrate inhibition or draining away cofactors from competing pathways. In other cases, kinetic expressions shape flux distributions so as to favor the overproduction of the desired product requiring fewer direct interventions. This work paves the way for the integrated analysis of kinetic and stoichiometric models and enables elucidating system-wide metabolic interventions while capturing regulatory and kinetic effects.

The prioritized set of interventions is subsequently used as a guideline to construct the mutant strain using synthetic biology approaches. Flux through a metabolic reaction is rationally controlled by altering the Translation Initiation Rates (TIR) of the Ribosome Binding Sites (RBSs) using predictive RBS library calculator. Each of this degenerate RBS sequence is able to span a large range of TIRs, allowing us to generate libraries of cells with varying levels of that particular protein. After completing all the computer- aided design, we combine commercial DNA synthesis, Gibson DNA assembly, and multiplex automated genome engineering (MAGE) to construct synthetic bacterial operons. These recombineering techniques are used to insert genetic systems to the *E. coli* chromosome and rationally control the enzyme expression levels via directed genome mutagenesis. We are currently applying this strategy to manipulate the level of proteins of *E. coli* native genes to pinpoint the flux changes consistent with those metabolic interventions predicted by k-OptForce.

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