valve" strategy is the pathway for production of glucaric acid in *E. coli* previously developed in our lab. The initial substrate for the glucaric acid pathway, glucose-6-phosphate, is utilized by the cell in both glycolysis and the pentose phosphate pathway, and static knockout strategies involving these central metabolic pathways can be detrimental to growth and recombinant protein expression.

To develop the metabolite valve concept for this system, we have implemented a strategy based on controlled degradation of a key glycolytic enzyme, phosphofructokinase. Control of enzyme abundance at the post-translational level through degradation allows for rapid response time and can help overcome growth-mediated buffering effects seen with transcriptional control. Combining this with tuning of gene expression levels, we have been able to develop an *E. coli* strain with a "growth mode" very close to wild type and a "production mode" with decreased glycolytic flux. Ongoing work focuses on induction of the system in response to culture conditions to allow for autonomous switching.

15. Flexible and User Friendly Tools for the Incorporation of Fluxomics Data into Metabolic Models

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The measurement of fluxes and the understanding of their control are at the core of Metabolic Engineering (ME). In this context, this work presents two integrated opensource software tools that allow to perform tasks of metabolic flux analysis (MFA). Both are platform independent, written in Java, and interact with the OptFlux framework [1], which also facilitates their communication.

OptFlux is a modular open-source software that incorporates tools for strain optimization, i.e., the identification of ME targets. It also provides tools to use stoichiometric metabolic models for phenotype simulation of both wild-type and mutant organisms, using methods such as the well known Flux Balance Analysis (FBA). Graphical user interfaces are made available for every operation and to check the results that are obtained. Moreover, a network visualization system is offered, where simulation results can be added to overlap the network graph. The developed tools exploit OptFlux's capabilities in terms of model interaction, simulation methods and visualization features.

The first proposed software, named MetaboIIc NEtwork Ratio AnaLysis (MiNeRAl) aims at analyzing labeling experiments to infer flux constraints that for stoichiometric models. From a set of measurements of a $_{13}$C-labelling experiment, mass isotopomer distribution vectors (MDV) are calculated. If aminoacids are measured, the measured fragments, coupled with a carbon transition map provided by the user, are used to determine their precursors, and the corresponding MDVs are calculated. Based on the set of MDVs, the software uses the carbon transitions to determine the flux ratios that produce a given metabolite through the different pathways. These ratios are probabilistic equations that translate how the $_{13}$C-labeling pattern is distributed throughout the metabolic network[2]. Since the calculation of the flux ratios is independent of the flux distribution, this software can be used independently of other flux calculation processes, and the ratios can be further exploited to reduce the degrees of freedom of systems obtained in other MFA approaches [3, 4]. The main differentiating characteristics of this tool are, besides being usr-friendly, the fact that it is generic for any type of metabolite fragmentation originating from GC-MS techniques and metabolic network topology. Furthermore, the software is also able to investigate what flux ratio constraints are possible to be inferred for a certain experiment beforehand.

On the other hand, the second software application here described, jMFA, is focused on using different types of experimental flux data to constrain metabolic models and improve their predictions with a variety of tools. It allows users to define constraints associated with measured fluxes and/ or flux ratios, together with environmental conditions (e.g. media) and reaction/ gene knockouts. The application identifies the set of applicable methods based on the constraints defined from user inputs, allowing to select the desired approach, encompassing algebraic and constraint-based simulation methods (such as Flux Balance Analysis and its variants). Anytime a set of constraints is selected, the software calculates the degrees of freedom of the configured system, and updates the admissible methods depending on whether the system is underdetermined, determined or overdetermined. A method to perform robustness analysis is also implemented. The integration of jMFA within the OptFlux framework allows the use of different model formats and

the integration with complementary methods for phenotype simulation and visualization of the results. Moreover, the flux ratio constraints can be obtained from previous calculations in MiNeRAl, or manually defined by the user. The first option provides a straightforward way to integrate both applications in a ME workflow.

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