Systematic Metabolic Flux Modeling Techniques and Applications at the Joint BioEnergy Institute

David Ando[1]* (david.ando@lbl.gov), Amit Ghosh,[1] Weerawat Runguphan,[1] Charles Denby,[1] Chris Shymansky,[1] Yasuo Yoshikuni,[2] Jay D Keasling,[1] and **Hector Garcia Martin**[1]

[1]Joint BioEnergy Institute, LBNL, Emeryville, CA; [2]Joint Genome Institute, LBNL, Walnut Creek, CA

Project Goals: The Joint BioEnergy Institute is focused on developing advanced second generation biofuels which can replace gasoline, diesel and jet fuels while providing significant environmental benefits in the form of reduced global dependence on crude oil and minimizing CO2 production. Our goal is to apply genome scale metabolic flux analysis and other modeling techniques to assist metabolic engineering efforts in fatty acid-derived biofuel production. Our analysis provides the necessary insights into cellular metabolism required for systematic genetic engineering efforts, currently increasing fatty acid production by over 40%. Similarly, our goal is to complete a joint project with JGI to study how substituting the enzymes responsible for most of NADPH production by their equivalents producing NADH in an isopentenol (from the mevalonate pathway) producing strain of *S. cerevisiae* S288c affects targeted production. We find that an optimal set of cofactor swaps is predicted to increase targeted production more than 10x that of the reference strain.

The Joint BioEnergy Institute is a DOE Research Center focused on developing advanced second generation biofuels which can replace gasoline, diesel and jet fuels while providing significant environmental benefits in the form of reduced dependence on crude oil and minimizing CO2 production. In order to produce economically and industrially feasible biofuels it is desirable to be able to systematically determine genetic modifications in biofuel producing organisms which may improve yield. Fluxes describe the flow of molecules through a metabolic pathway, with flux-based metabolic modeling being particularly well suited to providing the insights required in finding genetic modifications which increase biofuel yields since it can provide a global view on how carbon flows from feed to final biofuel product in an organism, and where potential bottlenecks lie. Flux Balance Analysis (FBA) has previously been used successfully for this purpose, which obtains fluxes by using a network of cellular metabolism which includes all reactions coupled with a linear programming assumption that metabolism is tuned, due to evolutionary pressure, to maximize growth rate (or other evolutionary assumptions can be used). Two scale 13C Metabolic Flux Analysis (2S-13C MFA) improves on FBA by retaining the genome scale metabolic network but drops the evolutionary assumption in favor of 13C

constraints from cellular metabolites measured experimentally and applied to the metabolic core of the model[1].

We have applied 2S-13C MFA towards improving production of fatty acids through a biosynthetic pathway developed earlier by Runguphan et al[2]. First we performed 13C tracer experiments and used 2S-13C MFA to measure fluxes both before and after boosting acetyl-CoA production via the addition of the ATP citrate lyase enzyme (ACL). Although acetyl-CoA is the substrate for fatty acid production the introduction of ACL resulted in only a small gain in fatty acid production. 2S-13C MFA identified the most significant sink of acetyl-CoA after the introduction of ACL to be Malate synthesis (MALS). Further downregulating MALS resulted in a more significant increase in fatty acid production of roughly 30%. Finally, as fatty acid production increased as we further engineered for higher fatty acid production 2S-13C showed that the Glycerol-3-phosphate dehydrogenase pathway, which competes for carbon with the acetyl-CoA production pathway, had a carbon flux which similarly increased. We downregulated cytoplasmic Glycerol-3-phosphate dehydrogenase in our engineered strains so more carbon flux would be available for fatty acid production and as expected these strains showed an increase in fatty acid production.

Additionally, we have used Flux Balance Analysis and Minimization of Metabolic Adjustment to determine how different possible combinations of cofactor swaps among a set of NADPH reductases affects isopentenol (from the mevalonate pathway) production in S. cerevisiae strain S288c. In a joint project between JBEI and JGI, we are substituting the enzymes responsible for most of NADPH production by their equivalents producing NADH, with the expectation that the excess NADH will increase isopentenol production due to the reengineered mevalonate pathway's dependence on NADH. Our metabolic modeling simulations support this expectation, with an optimal set of cofactor swaps being predicted to increase targeted production more than 10x that of the reference strain.

References
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