

167. An integrated 'omics approach to large-scale quantitative analysis of cellular metabolic regulation

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Project Goals: Quantitative and comprehensive understanding of natural metabolic regulatory mechanisms should facilitate metabolic engineering efforts. The overarching goal of this project is to quantitatively integrate systems-level data on metabolite concentrations, enzyme concentrations, and metabolic flux to elucidate physiologically relevant metabolic regulation.

We present a strategy for discovering metabolic regulation through analysis of steady-state integrative 'omics data. Previously, we and others have attempted to elucidate regulation through measurement of time-dependent changes in metabolite concentrations (e.g., in response to acute nutrient perturbation) and dynamical modeling of the resulting data. This approach has had successes, especially for small networks. Larger dynamical models of nonlinear systems such as metabolism, however, are often intractable. To avoid complications associated with dynamic interplay between reactions, the present approach examines steady-state flux control on a reaction-by-reaction basis. Building from systems-level data on metabolite concentrations, enzyme concentrations, and fluxes across many different steady-state conditions, we assess whether the observed fluxes can be accounted for by a Michaelis-Menten relationship between enzyme, substrate and product concentrations or whether further regulation is necessary to explain flux.

As an initial test case, we used *Saccharomyces cerevisiae*, itself an important biofuel producer. Cells were grown in chemostats at 25 different steady states. Concentrations of metabolites were measured by LC-MS-based metabolomics and of metabolic enzymes by LC-MS/MS-based proteomics. To infer fluxes, uptake and excretion rates of the diversity of metabolites were measured, as was detailed biomass composition; together these measurements were sufficient to constrain a genome-scale flux-balanced metabolic model, resulting in reliable determination of many core metabolic fluxes. Full information (flux, enzyme concentration, and all relevant metabolite concentrations) was obtained across all 25 conditions for 55 enzymes. For about 25 of these 55 enzymes, the concentrations of the enzyme, substrates, and products alone suffice to explain the observed fluxes. For another 20 enzymes, the observed fluxes can be explained by including a single allosteric effector (e.g., fructose-1, 6- biphosphate activation of pyruvate kinase). The approach was also able to identify an example of unexpected enzyme regulation that was biochemically verified.

A key output of the method is a quantitative assessment of which factors, overall, most strongly control metabolic flux under physiological circumstances. For reversible reactions, we find that most flux control occurs via changes in substrate and product concentrations. In contrast, irreversible reactions involve important contributions also from enzyme concentrations and allostery. Overall, the fraction of flux control residing in enzyme concentrations was modest, hinting at the difficulty of re-wiring core metabolism solely through controlling enzyme levels. Due to its reaction-by-reaction approach, the present method does not require extensive prior knowledge and thus appears well-suited to deciphering regulation also in less well studied biofuel producers.

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