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

Sean R. Hackett, Vito Zanotelli, Jonathan Goya, David H. Perlman, **Joshua D. Rabinowitz** Department of Chemistry and Lewis-Sigler Institute for Integrative Genomics, Princeton University

Project Goals: Metabolic transformation of plant material into biofuels holds promise as a source of renewable clean energy. While the major ongoing focus of our DOE-funded research regards metabolism in the promising biofuel-producing genus Clostridium, we also have a broad interest in methodologies for understanding metabolism and its regulation. The overarching aim of the present work is to develop a quantitative, genome-scale approach to elucidation of metabolic regulation in biofuel-relevant microbes.

We propose a novel, general, and scalable strategy for discovering metabolic regulation through integrative 'omics. 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 notable successes, especially for small networks. Larger dynamical models of nonlinear systems such as metabolism, however, are often intractable. Indeed, even in modeling small networks, we found that the most informative analyses often focused on pre- and post-perturbation pseudo-steady states, rather than dynamics. Specifically, we realized that, at steady-states, regulation can be dissected on an enzyme-by-enzyme basis if one knows the enzyme's concentration, output (flux) and inputs (concentration of all relevant substrates, products, and effectors). Accordingly, we hypothesized that large-scale analysis of different metabolic steady states via integrative 'omics could potentially be a tractable and effective method for revealing metabolic regulation.

To explore this possibility, we elected to use *Saccharomyces cerevisiae* (itself an important biofuel producer) as a test organism. 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 ~ 50 enzymes.

For these ~ 50 enzymes, we assessed whether variation in flux across the 25 experimental conditions could be explained based on the enzyme and metabolite concentrations using an equation of the Michaelis-Menten form. This allowed us to determine Michaelis-Menten parameters, based not on isolated biochemistry but physiological cellular data. For about one-third of enzymes, the concentrations of the enzyme, substrates, and products alone were sufficient to explain the observed fluxes. For another approximately one-third of enzymes, the observed fluxes could be explained if one also included metabolite concentration data for potential effectors (e.g., fructose-1, 6-bisphosphate activation of pyruvate kinase). For the final one-third of enzymes, we have yet to elucidate the missing regulation and/or there is too much noise in the data to obtain a good fit.

Beyond providing proof of concept for integrative 'omic analysis of metabolic regulation, this work also addresses some bigger picture questions, e.g., how much of metabolic flux control resides in enzyme concentrations versus metabolite concentrations? Our results show that across the tested physiological steady states, enzyme and metabolite concentrations make nearly equal flux-control contributions. The general strategy of analyzing many metabolic steady states via integrative 'omics (of fluxes, enzymes, metabolites) thus holds the potential to address long-standing global questions regarding the nature of

metabolic regulation, as well as to identify specific physiologically-relevant instances of regulation both in well-studied organisms like S. cerevisiae and in less studied ones like Clostridia.