246b Quantitative Target Identification for Metabolic Engineering of Yeast *Saccharomyces Cerevisiae*: Impacts of Bioreactor Environment

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Designing an effective Metabolic Engineering strategy necessitates systems-level quantification on the linkage between genotypic mutations and phenotypic responses. This linkage is yet to be fully discovered due to the uncertainty in enzyme kinetic parameters and metabolic regulation schemes, plus the complicated interactions between cellular metabolism and its cultivation environments. We have developed a computational framework which employs Monte Carlo method on the (log)linear Metabolic Control Analysis formalism. In this study, we generalize the framework to emphasis the impacts of industrial bioreactor processes on the behavior of intracellular metabolism. It allows a realistic understanding of cellular events by examining them in the context of industrial growth environment. The generalized framework captures the communication between the cellular metabolism and its growth environment via substrate uptake, product excretion, and cell growth.

We apply this framework on the compartmentalized central carbon metabolic network of *S. cerevisiae* cells growing in typical industrial cultivations including batch reactor and chemostat. Large-scale sampling of the parameter space is performed to assess the cellular uncertainty where each sample represents a unique physiological condition of an individual cell. Statistical analysis of the results, in the form of flux control coefficients, reveals considerable impact of growth environment on the distribution of rate-limiting steps in the cellular metabolism. In the batch cultivation, the glycolytic flux is controlled by a few key enzymes including hexose transporters, phosphofructokinase, and pyruvate kinase. However, the glycolytic flux in the chemostat is generally insensitive to most enzyme activity changes. For each growth condition, we have identified potential targets for the optimization of ethanol production and biomass yield. Furthermore, our accurate description of the control scheme in yeast metabolism leads to a fundamental understanding of the operation of cellular metabolism at a systems level.