479d Metabolic Engineering of Escherichia Coli through in Silico Design and Experimental Evolution

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Metabolic engineering efforts using microbes have recently grown both in scope and success. However, one of the challenges remaining in metabolic engineering is to utilize system-level approaches to facilitate and expedite experimental strain design. The work described here represents one approach that has been developed to incorporate system-level analyses to computationally design, experimentally construct, and iteratively improve strain designs for metabolite overproduction.

In implementing this approach, a genome-scale metabolic reconstruction of *E. coli* was used in conjunction with the OptKnock bi-level optimization algorithm to computationally predict gene deletion strategies to couple secretion of lactic acid to biomass production. Three different strain designs resulting from the computational predictions were constructed and experimentally evolved for 60 days with results demonstrating direct coupling of lactic acid secretion to cellular growth as designed and predicted computationally. In addition, gene expression analysis was conducted for all evolved strains and used to reconcile differences between experimental and computational phenotypes, thus providing a means of directing an iterative step of strain improvement. In summary, a multi-stage process for metabolic engineering will be presented in tandem with results demonstrating the utility and limitations of this approach.