Kinetic Modeling of Metabolic Pathways in *Zymomonas mobilis* to Optimize Pentose Fermentation

Dhinakar S. Kompala* (kompala@colorado.edu)

University of Colorado, Boulder, Colorado

Project Goals: To investigate the complex network of metabolic pathways in engineered bacterium *Zymomonas mobilis*. To develop a quantitative understanding of the metabolic fluxes along the newly engineered pathways for pentose fermentation.

Zymomonas mobilis has been engineered with four new enzymes to ferment xylose along with glucose. A network of pentose pathway enzymatic reactions interacting with the native glycolytic Entncr Doudoroff pathway has been hypothesized. We have analyzed the complex interactions between the pentose phosphate and glycolytic pathways in this network by developing a large-scale kinetic model for all the enzymatic reactions. Based on the experimental literature on *in vitro* characterization of each of the 20 enzymatic reactions, the large-scale kinetic model is numerically simulated to predict the dynamics of all the intracellular metabolites along the network of interacting metabolic pathways. This kinetic model takes into account all the feedback and allosteric regulations on the enzymatic reaction rates and is better suited to the systems level analysis of interacting metabolic pathways compared to the standard linearized methods of metabolic flux analysis and metabolic control theory.

This nonlinear kinetic model is simulated to perform numerous *in silico* experiments by varying different enzyme concentrations and predicting their effects on all the intercellular metabolic concentrations and the ethanol production rates in continuous fermentors. Among the five enzymes whose concentrations were varied and given as input to the model, the ethanol concentration in the continuous fermentor was optimized with xylosc isomerase was needed at the highest level, followed by the transaldolase. Predictions of the model, that interconnecting enzyme phosphoglucose isomerase, does not need to be overexpressed, were recently confirmed through experimental investigations. Through this kinetic modeling approach, we can develop efficient ways of maximizing the fermentation of both glucose and xylose,

while minimizing the expression of the heterologous enzymes.

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MEWG

Foundational Advances in RNA Engineering Applied to Control Biosynthesis

Christina D. Smolke* (smolke@cheme.caltech.edu)

California Institute of Technology, Pasadena, California

Project Goals: The goals of this project are to: (i) generate RNA-based molecular sensors to key metabolites along the BIA pathway; (ii) engineer *Saccharomyces cerevisiae* to synthesize early BIAs; (iii) integrate the molecular sensors into the transgenic yeast strain for the noninvasive, real-time detection of key metabolite levels along this heterologous pathway; (iv) expand the utility of the engineered molecular sensors to the dynamic regulation of enzyme levels in response to metabolite accumulation; and (v) solidify a strong base in biomolecular and metabolic engineering and encourage its advancement by training and educating scientists through cutting-edge, integrated research and educational plans.

Recent progress in developing frameworks for the construction of RNA devices is enabling rapid advances in cellular engineering applications. These devices provide scalable platforms for the construction of molecular communication and control systems for reporting on, responding to, and controlling intracellular components in living systems. Research that has demonstrated the modularity, portability, and specificity inherent in these molecules for cellular control will be highlighted and its implications for synthetic and systems biology research will be discussed. In addition, tools that translate sequence information to device function to enable the forward design and optimization of new devices will be discussed. The flexibility of the specified framework enables these molecules to be integrated as systems that perform higher-level signal processing based on molecular computation strategies. The application of these molecular devices to studying cellular systems through non-invasive *in vivo* monitoring of biomolecule levels and to regulating cellular behavior will be discussed, in particular in the control and optimization of the biosynthesis of alkaloids in *Saccharomyces cerevisiae*.