Title: Comparison of Michaelis-Menten and Elementary Decomposition Rate Kinetics for Predicting Enzyme Progress Curves

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Project Goals: *Clostridium thermocellum* is a promising organism for the production of fuels and chemicals from cellulose, but it has an atypical glycolysis that is poorly understood, which limits commercial applications. The overall goal of this project is to develop an improved systems-level understanding of *C. thermocellum* metabolism. We will do this by performing kinetic measurements of purified enzymes and kinetic analysis of enzyme modules in cell lysates. Results from these analyses will be synthesized with a novel multi-scale kinetic modeling approach. This improved understanding will be demonstrated by producing 2,3-butanediol from cellulose *in vivo*.

Abstract Text: Elementary decomposition (ED) provides a way to generate mechanistic kinetic models from reaction stoichiometry, and also a mathematical framework for determining kinetic parameters through non-linear optimization (Gopalakrishnan, Dash, and Maranas 2020) or screening sets of stochastically chosen kinetic parameters (Tran, Rizk, and Liao 2008), both of which attempt to determine enzyme kinetics through global fitting of kinetic parameters (i.e. a top-down approach). These approaches are somewhat limited in that the kinetic parameters estimated in this manner are based on relative enzyme and metabolite concentrations and often make use of lumped reactions and kinetic parameters. For critical metabolic enzymes where more accurate kinetic knowledge is desired, we are interested in using the ED approach for estimating kinetic parameters from progress-curve data for individual enzymes for parameterizing kinetic models of *C. thermocellum* (i.e. a bottom-up approach). To do this, we have created a Nonlinear Programming (NLP) approach. Using spectroscopy data, our approach minimizes error between the measured and calculated concentrations of one reaction participant by solving a system of equations describing the ED kinetics with variable kinetic parameters and participating species concentrations. The process as a whole then performs a bottom-up and absolute estimation of kinetic parameters. To validate our system, we are testing it on formate dehydrogenase (EC 1.2.1.2), a commercially available enzyme that is commonly used for recycling of NAD^+ to NADH in redox reactions. Furthermore, this is one of the few enzymes where a kinetic model has been validated on a complete progress curve, rather than just initial rates (Schmidt et al. 2010). We are interested in addressing the following questions:

1. How does prediction accuracy compare between the two models?
2. For each model, which parameters are well-resolved by time-course kinetic data?
3. How much inaccuracy is introduced by assuming the reaction is irreversible?

4. To what extent can the kinetic mechanism be determined from progress curve data?

References/Publications

Gopalakrishnan, Saratram, Satyakam Dash, and Costas Maranas. 2020. “K-FIT: An Accelerated Kinetic Parameterization Algorithm Using Steady-State Fluxomic Data.” *Metabolic Engineering* 61 (September): 197–205. https://doi.org/10.1016/j.ymben.2020.03.001.

Schmidt, T, C Michalik, M Zavrel, A Spiess, W Marquardt, and M B Ansorge-Schumacher. 2010. “Mechanistic Model for Prediction of Formate Dehydrogenase Kinetics under Industrially Relevant Conditions.” *Biotechnology Progress* 26 (1): 73–78. https://doi.org/10.1002/btpr.282.

Tran, Linh M, Matthew L Rizk, and James C Liao. 2008. “Ensemble Modeling of Metabolic Networks.” *Biophysical Journal* 95 (12): 5606–17. https://doi.org/10.1529/biophysj.108.135442.

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