

# Enzyme-mechanism models and simulations of central metabolism pathways and amino acid biosynthesis in *Escherichia coli*

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To elucidate the systems biology of the model organism, *Escherichia coli*, we develop mathematical models to extend our simulations of amino acid biosynthesis to the pathways of the TCA cycle and glycolysis. To model the behavior of complex biological systems, we continue to employ a “bottom-up” approach that incorporates detailed enzyme kinetic and pathway-specific regulatory mechanisms from the literature into our model. Here, we present a Random Steady State model to describe catalysis by large enzyme complexes in the TCA cycle. We further use the model along with our previous enzyme models in a simulation of carbon flow through glycolysis and the TCA cycle in *E. coli* and test its behavior under conditions of perturbation and growth on an alternative carbon source. To achieve this goal, we use our kMech and Cellerator packages in Mathematica<sup>TM</sup> to automatically convert our models for enzyme mechanisms and regulatory patterns into association-dissociation reactions, and then into differential equations. These equations are solved by Mathematica<sup>TM</sup> to simulate the model and generate the graphical output. In addition to simplifying the underlining mathematics of writing down differential equations, this approach allows us to examine the biochemical behavior of metabolites and enzyme states in the pathway with greater detail.

The enzyme complexes in the TCA cycle include pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase. These complexes are made up of several copies of three different enzymes, E1, E2 and E3. The E2 components form the core of the complex and are organized in trimers at each corner of a cube while a mixture of E1 and E3 components bind randomly to the core (Figure 1). The model incorporates the rate of the cycle of reactions catalyzed by the three enzymes at one hand and the random nature of the structural organization of the component

enzymes at the other. It also allows for possible sharing of intermediates between neighboring components at corners of the same cube. This sharing might account for the unusual structure, leads to an improvement in the reaction rate and results in a better fit to experimental data.

To verify our simulations and models for enzyme mechanisms for the TCA cycle and glycolysis (Figure 2), we test our models behavior under conditions of metabolic and genetic perturbations, and compare with experimental data from the literature. We further explore a simulation of aerobic growth of *E. coli* on acetate. In preparation for this step, we have performed gene expression profiling experiments of chemostat grown cells comparing growth on glucose to growth on acetate. The results of these experiments (Figure 2) agree completely with a vast literature. When *E. coli* is transferred from glucose to acetate medium, carbon flow is redirected through a glyoxylate bypass to reduce carbon loss and replenish oxaloacetate. We perform a simulation of such growth shift and explore how well this shift correlates with our simulations. We demonstrate in different cases that our simulations closely match our experimental data and data in the literature.

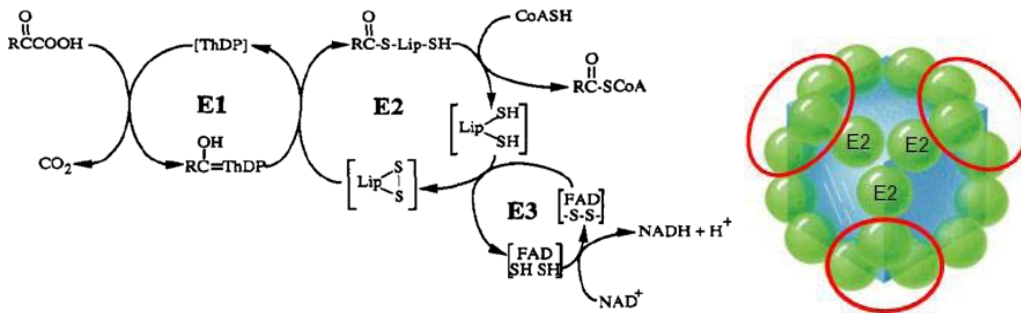


Figure 1: Diagrams showing the cycles of enzyme reactions and the core structural organization of the enzyme complexes, pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase in the TCA cycle. All three enzymes are required for a complete cycle of reactions. The E2 components form the core and each E2 can bind to either an E1 or E3. The rate was derived by solving a structured, 12x12 steady state system symbolically and the random binding of E1 and E3 components to the E2 core was modeled by a binomial distribution of accessible E1 and E3 components at each corner. Sharing of intermediates with 3 neighboring corners (circled in red) was further allowed and described by a sharing factor  $\alpha$  such that the rate is a function of the enzymes on both the same and neighboring corners for values of  $\alpha$  greater than zero.

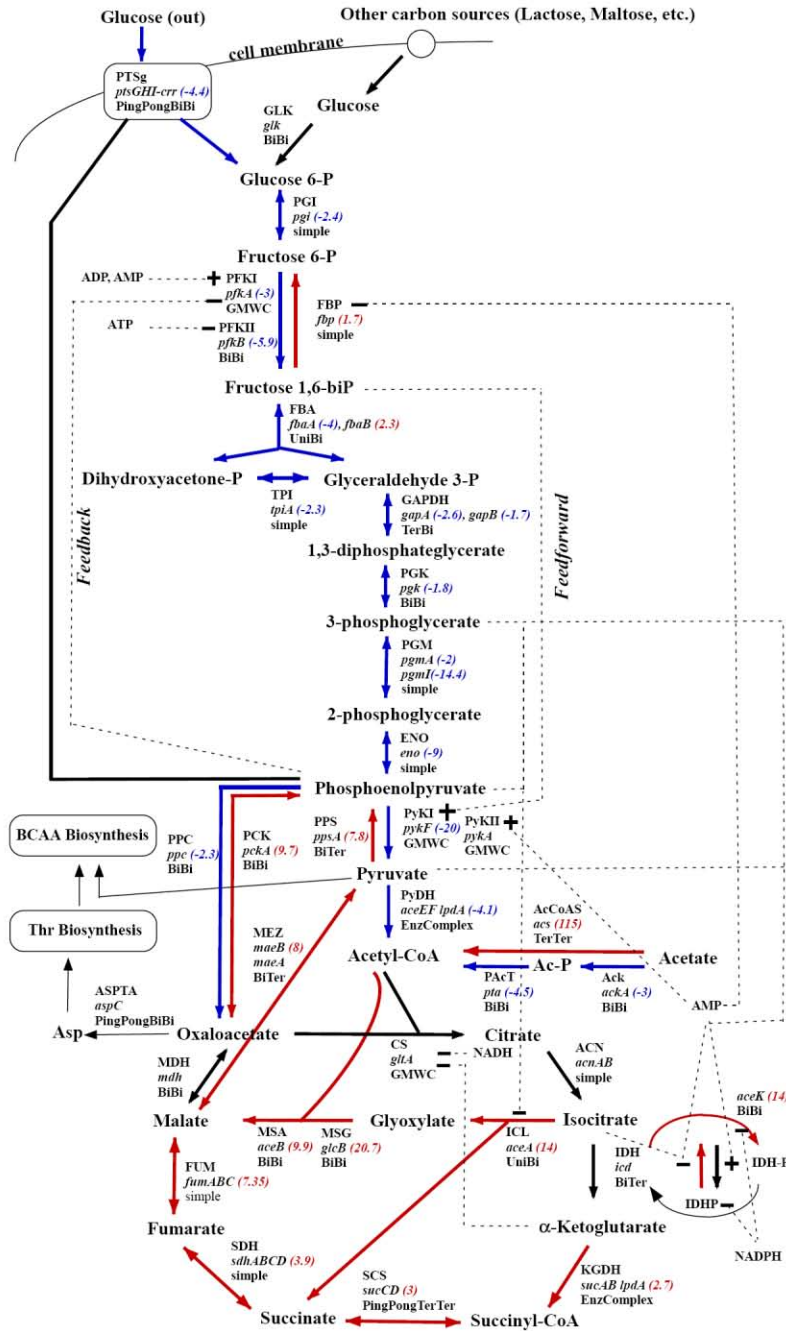


Figure 2: Metabolic pathways of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass in *E. coli*. Double arrows represent reversible reactions; Solid black arrows indicate no change in gene expression when cells are grown on acetate; Solid Blue arrows indicate a down-regulation in gene expression when cells are grown on acetate; Solid red arrows indicate an up-regulation in gene expression when cells are grown on acetate. Dashed lines represent regulatory circuits. Plus signs indicate activation; negative signs indicate repression. “(out)” indicates metabolites outside of the cell membrane. Each reaction step is labeled with an abbreviated enzyme name (1st row), gene name (italic, 2nd row), fold change in gene expression from microarray data and the kMech enzyme model name (3rd row).