

## Development of a genome-scale *Escherichia coli* kinetic metabolic model satisfying flux data for multiple mutant strains

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**Project Goals: The goal of this effort is to construct a genome-scale kinetic model of *Escherichia coli* metabolism by making use of Ensemble Modeling (EM) concepts. Model parameterization is carried out using multiple flux datasets for different substrates and growth (aerobic vs. anaerobic) conditions.**

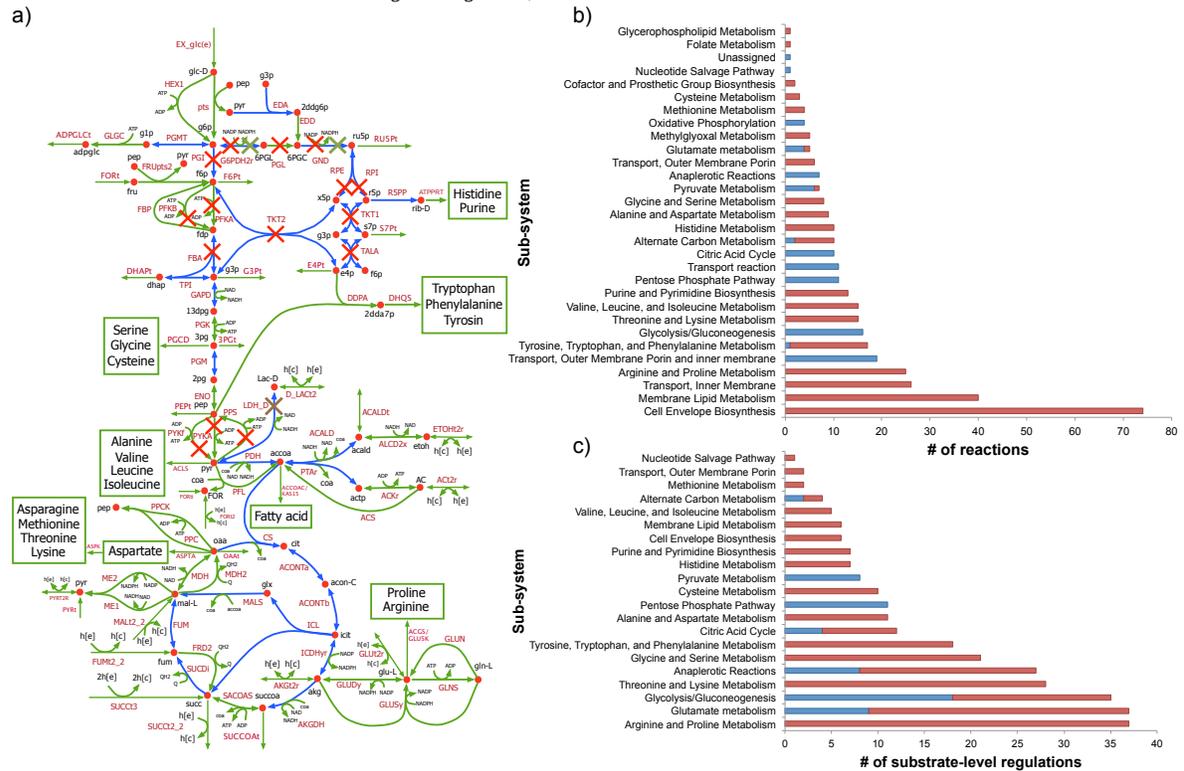
Kinetic modeling paradigm provides a promising platform to broaden our knowledge of cellular capacity and cell physiology beyond stoichiometric descriptions. However, developing kinetic models of metabolism at a genome-scale that faithfully recapitulate the effect of multiple genetic interventions is still an ongoing challenge. To this end, we introduce k-ecoli457, a genome-scale kinetic model of *Escherichia coli* metabolism that satisfies fluxomic data for a wild-type and 25 mutant strains for different substrates and growth (aerobic vs. anaerobic) conditions. The k-ecoli457 model contains 457 reactions and 337 metabolites accounting for all relevant reactions from the genome-scale iAF1260 model that carry flux under the experimental conditions of the flux measurements. These include reactions in glycolysis/gluconeogenesis, the Pentose Phosphate (PP) pathway, the TriCarboxylic Acid (TCA) cycle, anaplerotic reactions, amino acid synthesis/degradation, fatty acid oxidation/synthesis and a number of reactions in other parts of the metabolism, such as folate metabolism, cofactor and prosthetic group synthesis, alternative carbon, membrane lipid, cell envelope, nucleotide salvage and oxidative phosphorylation pathways. In addition, 295 regulatory interactions were extracted from BRENDA and EcoCyc and included in k-ecoli457. The model was also supplemented with a simplified version of the biomass equation including all the constituent precursors. Model predictions were tested against multiple experimentally measured datasets that were not used during model parameterization. These included (i) 898 steady-state metabolite concentrations for twenty of the mutant strains [1-4], (ii) 319 Michaelis-Menten constants (211  $K_m$  and 108  $k_{cat}$  values) from BRENDA and EcoCyc, and (iii) 320 experimentally reported product yields for designed strains spanning 24 different bioproducts. Comparisons revealed that 63% of the predicted metabolite concentrations as well as 60% and 64% of the estimated  $K_m$  and  $k_{cat}$  values, respectively, are within the experimentally reported ranges. These levels of agreement, in overall, exceed the previous effort [5], despite the significantly increased scope of the model and coverage of less studied pathways. The average relative error of k-ecoli457 predictions for the yield of 16 bioproducts in 140 designed strains is 0.1, while stoichiometric model based techniques such as flux balance analysis (FBA) or minimization of metabolic adjustment (MOMA) yield corresponding relative errors of 1.05 and 1.19, respectively.

This modeling effort describes a stepwise procedure for construction of genome-scale kinetic models with robust parameterization consistent with multiple sets of omics information for *E. coli* and provides guidelines for developing genome-scale kinetic models for other well-studied organisms.

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**Figure 1** (a) A pictorial representation of the constructed kinetic model of *E. coli* metabolism. Red, brown and green marks denote the knockout mutants grown aerobically on glucose, anaerobically on glucose and aerobically on pyruvate, respectively, with flux data for the reactions shown in blue. (b) Sub-system classification of reactions in the constructed kinetic model. (c) Sub-system classification of the integrated regulatory interactions. Blue bars denote the content of the core model [5] while red denotes the additional reactions/regulations included in k-ecoli457.