Ensemble cell-wide kinetic modeling of anaerobic organisms to support fuels and chemicals production

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Project Goals: The goal of the project is to systematically build dynamic metabolic models of *Clostridium thermocellum* and *Clostridium ljungdahlii* using Ensemble Modeling (EM) paradigm through integrative metabolomic and fluxomic data. These models will be instrumental in exploring genetic interventions for overproduction of biofuel products.

Clostridia possess broad and flexible systems for substrate utilization: *Clostridium thermocellum* can metabolize cellulose, and *Clostridium ljungdahlii* and *Moorella thermoacetica* can fix CO_2 to produce biofuels. However, their metabolism remains poorly characterized. Here we develop kinetic models of clostridia using the Ensemble Modeling paradigm, which require curated genome-scale metabolic (GSM) models as its foundation. For *C. thermocellum*, we constructed a second-generation GSM model (*i*Cth446) with 446 genes, 598 metabolites and 660 reactions, along with gene-protein-reaction associations by updating cofactor dependencies, maintenance (GAM and NGAM) values and resolving elemental and charge imbalances. *i*Cth446 model was subsequently used to develop k-ctherm118, a kinetic model of *C. thermocellum*'s central metabolism containing 118 reactions and 92 metabolites with cellobiose as the carbon source under anaerobic growth condition. k-ctherm118 encompasses the cellobiose degradation pathway, glycolysis/ gluconeogenesis, the pentose phosphate (PP) pathway, the TCA cycle, pyruvate metabolism, anaplerotic reactions, alternative carbon metabolism, nucleotide salvage pathway, along with all biomass precursors and 22 substrate level regulatory interactions extracted from BRENDA. The kinetic model parameters were estimated by simultaneously imposing fermentation yield data in lactate, malate, acetate and hydrogen production pathways for 19 measured metabolites spanning a library of 19 distinct single and multiple gene knockout mutants along with 18 intracellular metabolite concentration data for a *Δgldh* mutant and ten experimentally measured Michaelis-Menten kinetic parameters. k-ctherm118 captures significant metabolic changes including effect of nitrogen limitation on *C. thermocellum*'s metabolism as well as impact of ethanol stress on intracellular metabolite pools due to downregulation of fermentation pathways. Robustness analysis of k-ctherm118 alludes to the presence of a secondary activity of ketol-acid reductoisomerase and possible regulation by valine and/or leucine pool levels.

For the case of *C. ljungdahlii*, we constructed a core metabolic model composed of 79 reactions and 63 metabolites based on a GSM published by Nagarajan *et al*. [1] and including 41 putative substrate-level regulations from BRENDA. The core model accounts for glycolysis, Wood-Ljungdahl pathway, pentose-phosphate pathway and production pathway for major products such as lactate, acetate and ethanol. The TCA cycle is incomplete with missing succinate dehydrogenase, 2-oxoglutarate synthase, and succinyl-CoA synthetase [2]. We have used the wild-type data from the Stephanopoulos group to generate the initial ensemble. The experimental

data included growth rate and the rate of formate, acetate, and ethanol production with CO_2 and/or hexose as carbon source. *C. ljungdahlii* shares its unique metabolic capabilities including CO_2 fixation with another anaerobe of the class clostridia, *M. thermoacetica*. To this end, the Stephanopoulos group performed ^{13}C isotope tracing and metabolic flux analysis to obtain flux ratios between key metabolic pathways, which further constrain the feasible parameter space. The flux analysis revealed that glucose and CO_2 can be consumed simultaneously with CO_2 generating majority of the TCA cycle intermediates. In addition, integrative metabolomic and fluxomic assays under various culture conditions will be performed on *C. ljungdahlii* and *M. thermoacetica* by the Stephanopoulos group and on *C. thermocellum* by the Liao group, which will be used to parametrize and refine the kinetic models. The constructed kinetic models will ultimately aid in exploring the full metabolic capability of clostridial biofuel production.

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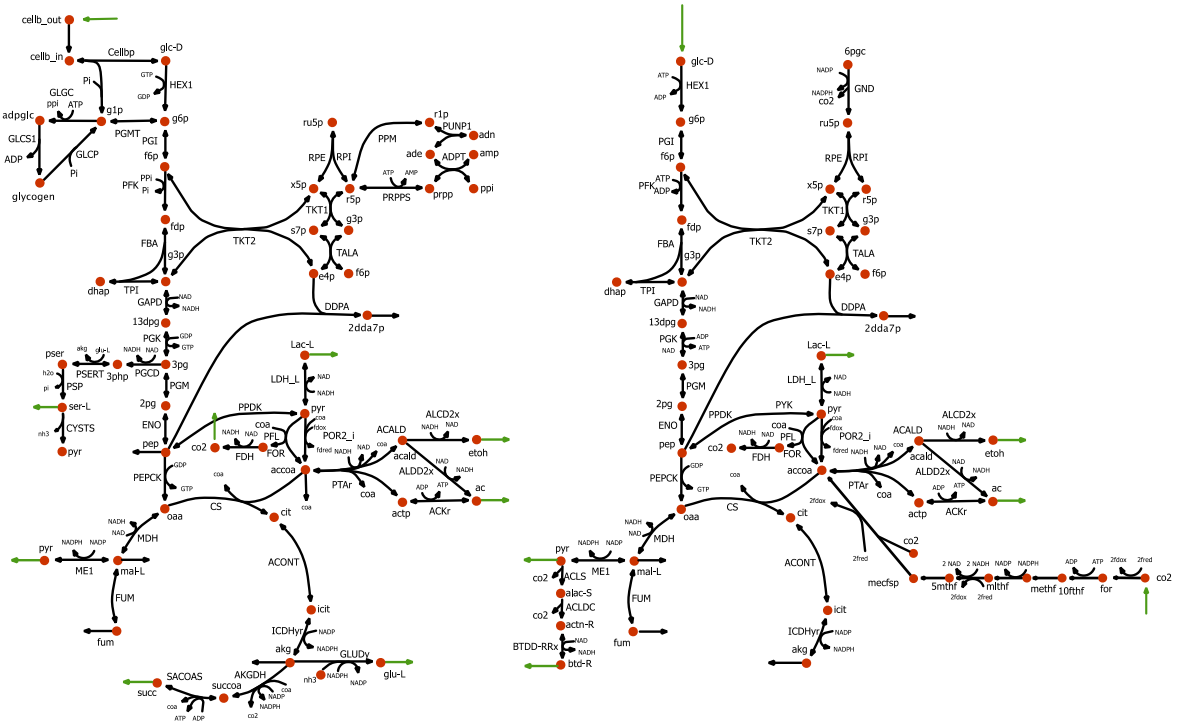

Figure 1: Core metabolic map of *Clostridium thermocellum* and *Clostridium ljungdahlii*. The green arrows represent the metabolite concentrations which are experimentally measured.