# Deploying docking calculations and resource balance allocation modeling alongside kinetic model parameterization to elucidate mechanisms controlling metabolism in *Clostridium thermocellum*

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**Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI addresses strategic barriers to the current bioeconomy in the areas of 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition, and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to biofuels using CBP with cotreatment at high rates, titers and yield in combination with catalytic upgrading into drop-in hydrocarbon fuel blendstocks.**

A wide range of natively produced value chemicals and a high native cellulase activity make *Clostridium thermocellum* an attractive bioproduction platform especially for CBP. Despite significant efforts, achieved yields and titers fall below industrially relevant targets. One of the reasons is a limited understanding of enzymatic, regulatory, and/or possible thermodynamic bottlenecks that might be at play in *C. thermocellum's* metabolism. To bridge some of these knowledge gaps and propose engineering strategies for improving yields of desired products, we deploy computational tools to (1) postulate regulatory mechanism active in *C. thermocellum* metabolism, and (2) quantify the enzyme cost of glycolysis and explore the trade-off between more efficient glycolysis and ATP production. We have developed an ensemble docking workflow to compare structural energetics of enzyme-ligand interactions for identifying novel substrate-level regulatory mechanisms with those of experimentally characterized protein structures. By using a kinetic model of *C. thermocellum's* core metabolism as the basis, we hypothesized the presence of several substrate-level enzyme inhibitions based on the improvement in kinetic model's fitness upon their addition. We then used the developed docking workflow to evaluate these enzymatic inhibitions using docking studies of regulatory molecules with enzyme structures involved. We thus flagged a total of 67 substrate-level inhibitions across central carbon metabolism supported by both kinetic formalism and docking analysis. To explore the enzyme cost of metabolism, we constructed a genome-scale resource allocation model of *C. thermocellum* metabolism (i.e., cthRAM), using flux-force efficacy (FFE)[1] constraints to ensure enzyme usage consistent glycolysis operating near thermodynamic equilibrium[2].

References/Publications

1] E. Noor et al., *PLoS Comput. Biol*., 2014, doi: 10.1371/journal.pcbi.1003483.

2] T. Jacobson, et al., *m-Systems*, 2020, doi: 10.1128/mSystems.00736-19.

***The Center for Bioenergy Innovation is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.***