Coupling flux predictions to available protein in *Clostridium thermocellum*

Background/Objective

- *Clostridium thermocellum* is a promising candidate for the consolidated bioprocessing of lignocellulosic biomass to ethanol, but inefficient catabolic enzymes burden available protein resources and lead to the generation of undesired end-products.

Approach

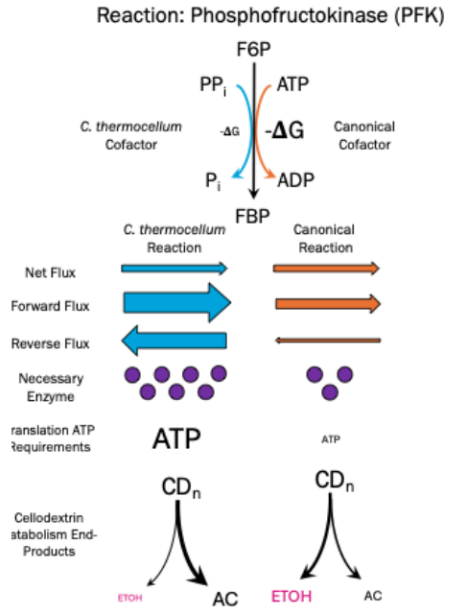
- Resource balance analysis (RBA) models couple predicted reaction fluxes to available enzymes.
- Genome-scale metabolic model *i*CTH669, absolute proteomics, and fluxes from ^{13}C labeling data informed reconstruction of an RBA model of *C. thermocellum*, ctRBA.
- Model predicted impact of altering the cofactor of single enzymes in *C. thermocellum* on maximum theoretical ethanol yields and titers.

Results

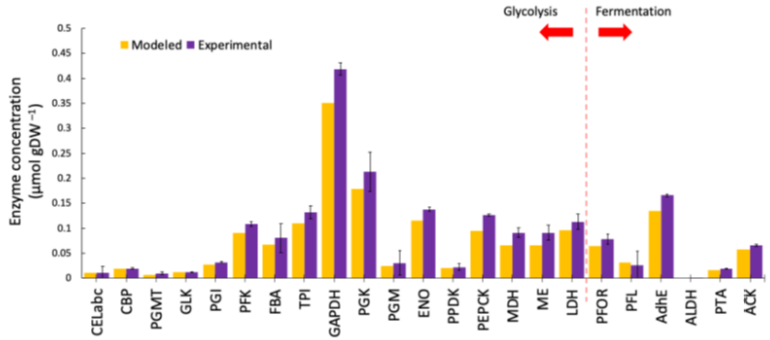
- Glycolytic and fermentation enzyme concentration predictions matched proteomics dataset within 0.1 µmol gDW^{-1}, and computations highlight high-cost glycolytic and fermentation enzymes.
- Simulations of putative strain designs pinpoint altering cofactors in phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, and aldehyde-alcohol dehydrogenase reactions as having meaningful improvements on ethanol production, both via reaction network changes and increases to enzyme efficiency.

Significance/Impact

- Model 'ctRBA' serves as a predictive tool for assessing the effects of genetic perturbations on proteome allocation and metabolic efficiency. The simulation output provides ranking of enzyme-level genetic perturbations showing promise for higher ethanol yields, which can be evaluated *in vivo* in *C. thermocellum* for consolidated bioprocessing applications. The RBA framework could be extended to improve phenotype prediction accuracy by further-detailing analyses of the apparent turnover rates of enzymes and their relationship to cellular conditions.



Cellodextrin catabolism in *C. thermocellum* and the effect of cofactors on fermentation products



Agreement in measured and predicted cellodextrin catabolism enzyme concentrations

Willis et al., *Metab. Eng.* (2025). https://doi.org/10.1016/j.ymben.2025.09.001.