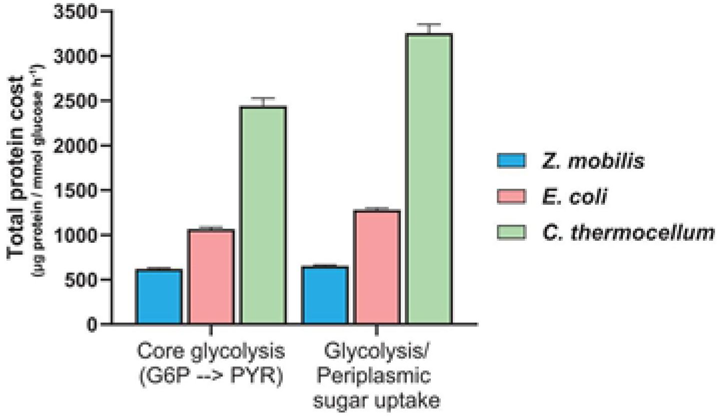
In vivo evidence reveals how thermodynamics shape enzyme investment in glycolysis

Background/Objective

- Computational studies predict that thermodynamically constrained reactions and pathways impose greater protein demands on cells, requiring a larger amount of enzyme to sustain a given flux compared to those with stronger thermodynamics. This study provides *in vivo* evidence that thermodynamic driving forces are a key parameter influencing the enzyme burden of metabolic pathways.



Protein cost of glycolytic processes in three bacterial species

Approach

- Scientists quantified absolute concentrations of glycolytic enzymes in three bacterial species (*Zymomonas mobils*, *Escherichia coli*, and *Clostridium thermocellum*) that employ distinct glycolytic pathways with varying thermodynamic driving forces and integrated enzyme data with corresponding *in vivo* metabolic fluxes and ΔG measurements.

Results

- Results showed the more thermodynamically favorable Entner-Doudoroff pathway in *Z. mobilis* requires one fourth as much enzymatic protein to sustain the same flux as the less favorable pryophosphate-dependent glycolytic pathway in *C. thermocellum*; the Embden-Meyerhof-Parnas pathway in *E. coli* showed intermediate thermodynamic favorability and enzyme demand. The highly reversible fermentation pathway in *C. thermocellum* requires 10x more protein than the irreversible pathways in *Z. mobilis*.

Significance/Impacts

- This study provides *in vivo* evidence that strongly thermodynamically favorable metabolic pathways require significantly lower enzyme concentrations to sustain a given flux than thermodynamically constrained pathways. The insights and quantitative proteomic data generated will serve as a valuable resource for developing constraint-based genome-scale metabolic models.

Khana, D. et al. Thermodynamics shapes the in vivo enzyme burden of glycolytic pathways. mBio, **0**, e01837-25. (2025). [DOI:10.1128/mbio.01837-25]