

## **Limited Forward Thermodynamic Driving Force in Glycolysis of Slow-Growing Bacteria**

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**Project Goals: Rapid glycolysis during slow growth is a desirable feature for industrial biofuel production. In practice, however, glycolysis tends to slow down together with growth. Here we aim to understand glycolytic thermodynamics and regulation in fast and slow growing bacteria. Specifically, we aim to i) identify Gibbs free energy of glycolytic reactions using a combination of <sup>2</sup>H- and <sup>13</sup>C-labeling, and ii) identify flux control mechanisms by which cells may upregulate glycolysis.**

*C. cellulolyticum* is an obligate anaerobe capable of converting cellulose into biofuels. Using LC-MS metabolomics, we identified its glycolytic pathway that involves unique cofactor (pyrophosphate and GTP) usage, akin to a recent finding in *C. thermocellum*. This results in an energy-efficient sugar catabolism that generates more usable energy in the form of high-energy phosphate bond than canonical glycolytic pathways but at the cost of forward driving force. Using <sup>13</sup>C-tracers, we found that its entire glycolysis is reversible.

The fully reversible glycolysis in *C. cellulolyticum* contrasts the canonical glycolysis as in *E. coli* where phosphofructokinase and pyruvate kinase have classically been assumed to be strongly forward-driven. We have recently quantified  $\Delta G$  in *E. coli* central carbon metabolism by integrating absolute metabolite concentrations and <sup>13</sup>C- tracer data to probe forward and reverse fluxes. We observed relatively even distributions of  $\Delta G$  across glycolysis that reflect sufficient driving force for almost every reaction, such that forward flux is substantially greater than reverse flux and therefore most enzyme is productively utilized. Here we present improved tools for  $\Delta G$  measurement by integrating also <sup>2</sup>H-tracers. Using these tools, we find that glycolysis in *E. coli* growing slowly due to nitrogen limitation is closer to equilibrium than in nutrient-rich conditions. Because net flux through near-equilibrium reactions can change dramatically with small changes in substrate or product, this allows rapid increase in glycolytic flux upon nitrogen upshift with only slight changes in intermediate levels.

Collectively, these observations suggest that slow growing bacteria may engage in glycolysis with limited forward thermodynamic driving force, either to produce more usable energy per carbon in organisms chronically adapted to anaerobic low-sugar environments, or to facilitate seamless adaptation to changing nutrient availability.

## References

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