130. A Fully Reversible, Highly Energy Efficient Glycolysis with Unique Cofactor Utilization in *C. cellulolyticum*

Daniel Amador-Noguez,[1] Junyoung O. Park[1,2]* (jopark@princeton.edu), Zheyun Zhang,[1] Sara A. Rubin,[3] Wenxin Xu,[1] and **Joshua D. Rabinowitz[1,3]**

[1]Lewis-Sigler Institute for Integrative Genomics; [2]Department of Chemical and Biological Engineering; and [3]Department of Chemistry, Princeton University, Princeton, N.J.

Project Goals: The production of biofuels from cellulosic biomass holds promise as a source of renewable clean energy. Members of the genus Clostridium collectively have the ideal set of the metabolic capabilities for biofuel production from cellulosic biomass: *C. acetobutylicum* rapidly ferments glucose to biofuels (butanol, hydrogen) and *C. cellulolyticum* effectively degrades cellulose. Here we aim to integrate metabolomics, genomics and genetic engineering to dramatically advance understanding of metabolism in *C. acetobutylicum* and *C. cellulolyticum*. In so doing, we will lay basic science groundwork for engineering of an organism that cost-effectively converts cellulose into solvents.

C. cellulolyticum is an obligate anaerobe capable of degrading cellulose into simple sugars and converting them into useful biofuels such as ethanol. However, conversion yields and production rates are too low to allow its use for commercial production of biofuels. For example, compared to the typical biofuel producer *C. acetobutylicum*, *C. cellulolyticum* has a very slow sugar catabolism, even when growing on simple sugars such as glucose or cellobiose. To gain a quantitative understanding of the sugar catabolic pathways in this bacterium, we utilize metabolomic tools, in combination with isotope tracers and quantitative flux modeling.

We report that glycolysis in *C. cellulolyticum* is fully reversible, with all of its reactions working near equilibrium. Such reversibility is achieved by replacing the cofactor ATP with pyrophosphate or GTP as the high-energy phosphate donors/acceptors as well as by using canonically anaplerotic and gluconeogenic reactions for glucose catabolism. This results in a highly energy-efficient sugar catabolism that generates more energy (more ATP equivalents) than canonical glycolytic pathways. The unique glycolysis in *C. cellulolyticum* reflects the evolution of the metabolic pathway to cope with the low energy availability, which can be attributed to anaerobiosis on cellulose.

Funding: DOE-DE-SC0006839