Engineering *Clostridium thermocellum* glycolysis with ATP-pfk creates a more favorable thermodynamic driving force

Background

- The phosphofructokinase (PFK) reaction in glycolysis represents a critical step in controlling metabolic flux.
- *Clostridium thermocellum*'s PFK reaction is atypical because it uses pyrophosphate (PP_i) instead of ATP as the main cofactor.
- The use of PP_i has a large effect on the overall thermodynamics of glycolysis because the reaction operates closer to thermodynamic equilibrium.
- Replacing PP_i-*pfk* with ATP-*pfk* could be an important engineering step towards achieving higher product titers.

Approach

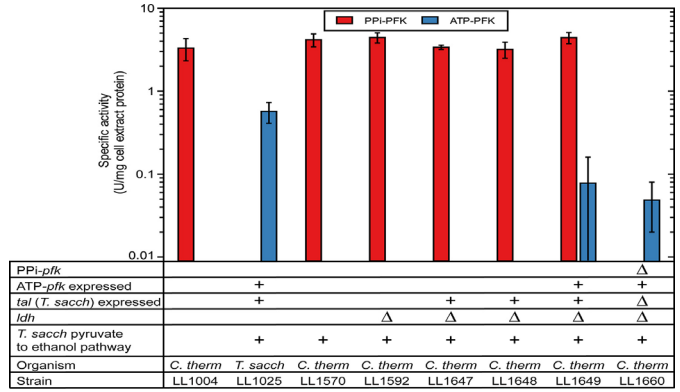
- *C. thermocellum* was engineered to remove native PP_i-*pfk* and express ATP-*pfk* derived from *T. saccharolyticum*.
- Metabolome measurements were performed to assess changes in metabolic flux through the PFK reaction to quantify changes in pathway thermodynamics.

Results

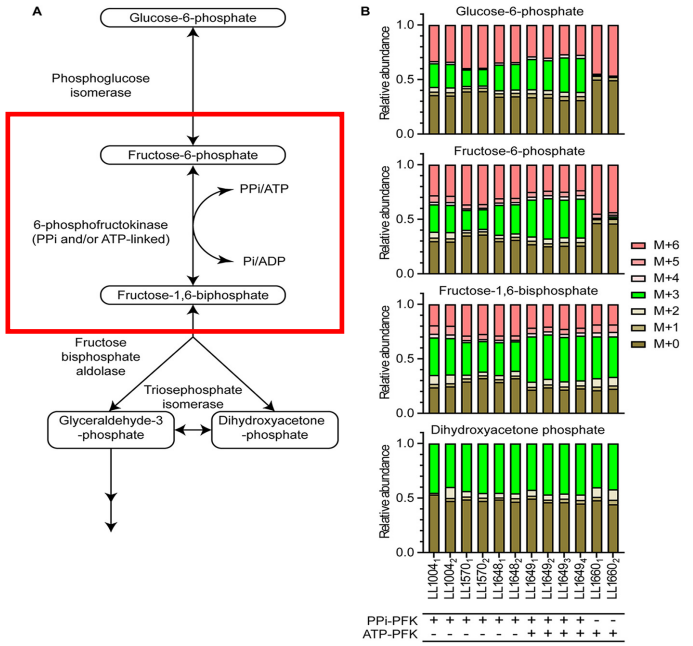
- Deletion of the PP_i-*pfk* gene resulted in loss of PPi-PFK activity, resulting in an ATP-PFK only strain of *C. thermocellum*
- Analysis of metabolic flux data showed that engineered C. *thermocellum* expressing ATP-PFK has a more thermodynamically favorable reaction because the relative amounts of glucose 6-phosphate and fructose 6-phosphate, products of reverse PFK flux, are now significantly reduced.

Significance

- Switching cofactor usage of the PFK reaction from PP_i to ATP significantly reduced the reversibility of the PFK reaction, which indicates greater thermodynamic driving force of the PFK reaction in the forward direction.



Gain of ATP-PFK activity (blue) and loss of PPi-PFK activity (red) in engineered *C. thermocellum* strains



The reversibility of the PFK reaction (in the red box) is effectively eliminated when only ATP-PFK activity is present

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