

## Supporting information

# The pentose phosphate pathway of cellulolytic clostridia relies on 6-phosphofructokinase instead of transaldolase

Jeroen G. Koendjiharie, Shuen Hon, Martin Pabst, Robert Hooftman, David M. Stevenson, Jingxuan Cui, Daniel Amador-Noguez, Lee R. Lynd, Daniel G. Olson, Richard van Kranenburg

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## Negative control *In vitro* phosphofructokinase assays

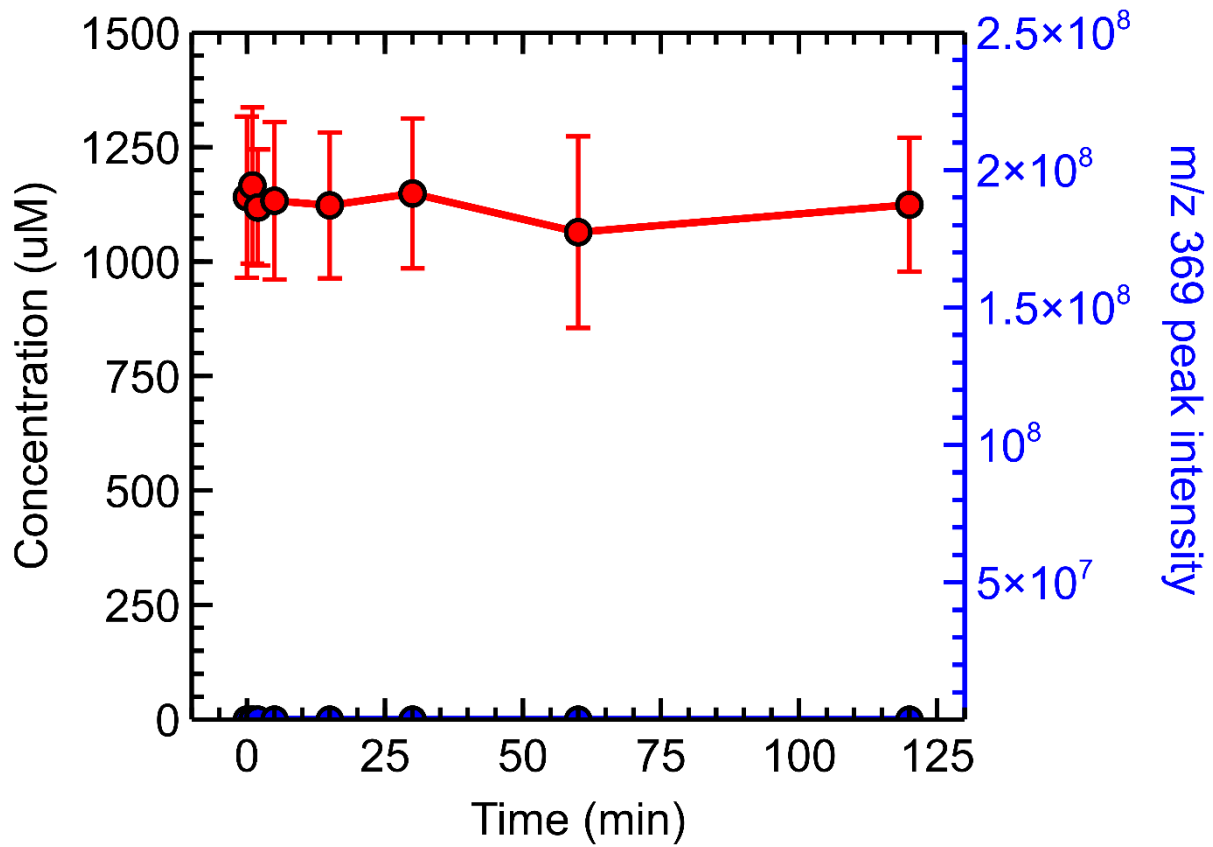
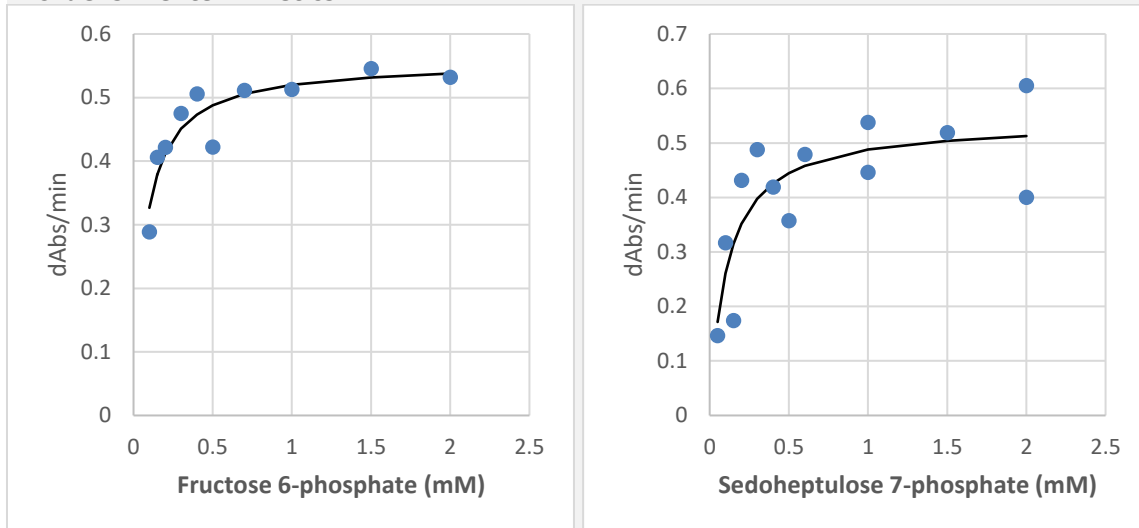


Figure S1: Negative control of the *In vitro* time-course assays using sedoheptulose-7-phosphate (red), lacking added enzyme. In blue the peak intensity at a m/z of 369, corresponding to sedoheptulose-1,7-bisphosphate. Error bars represent one standard deviation ( $n \geq 2$ ).

## Fitted kinetic models

### *Clostridium thermosuccinogenes* PPI-PFK

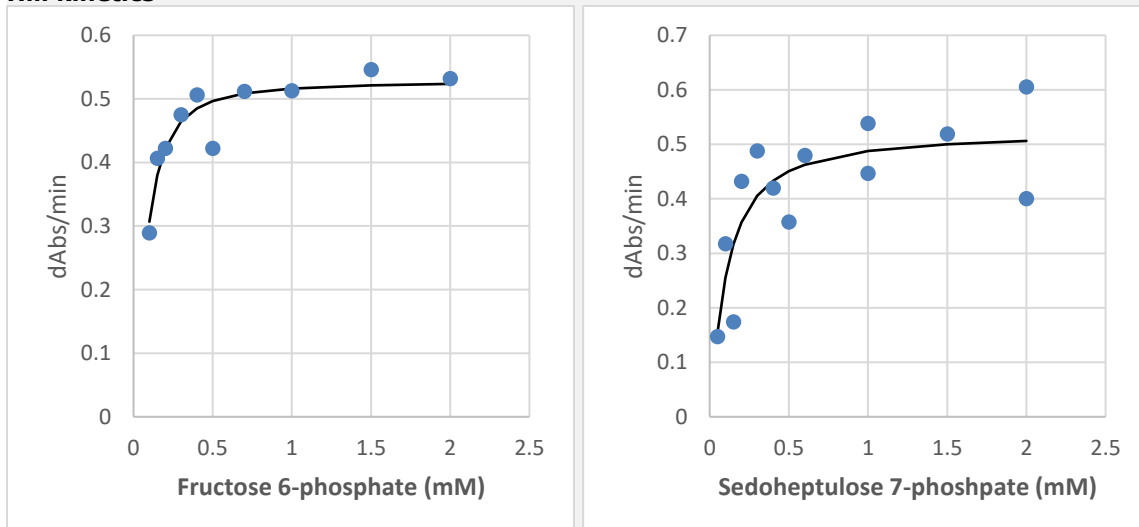
#### Michaelis-Menten kinetics



F6P:  $K_M = 0.07045054$  mM,  $V_{max} = 0.556745262$  dAbs/min

S7P:  $K_M = 0.107440985$  mM,  $V_{max} = 0.540231693$  dAbs/min

#### Hill kinetics



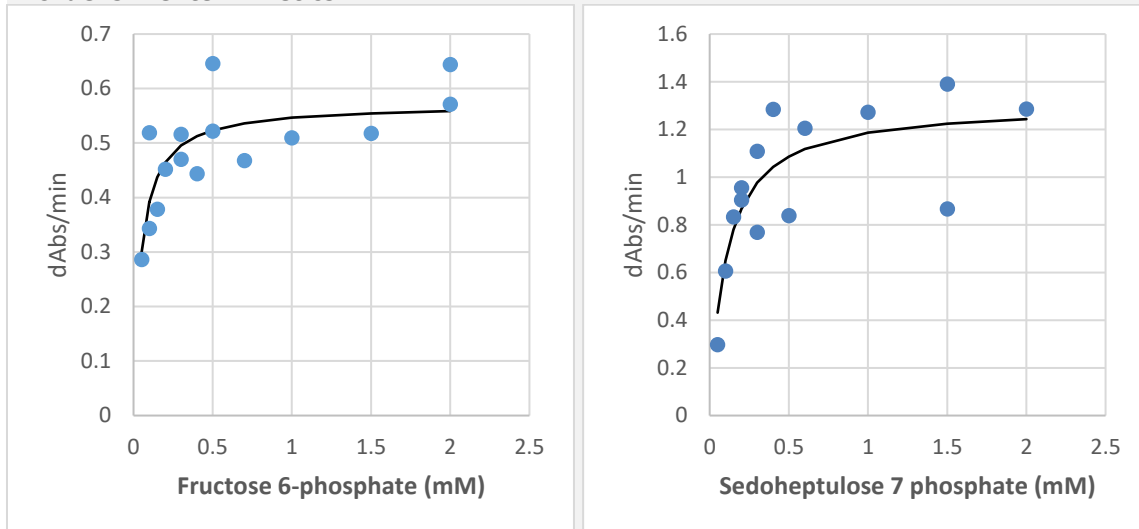
F6P:  $K_{1/2} = 0.080468265$  mM,  $V_{max} = 0.527619413$  dAbs/min,  $n = 1.51508789$

S7P:  $K_{1/2} = 0.103684272$  mM,  $V_{max} = 0.521954034$  dAbs/min,  $n = 1.170962878$

Figure S2: Data of the kinetics assays using *Clostridium thermosuccinogenes* PPI-6-phosphofructokinase (PFK) for the conversion of fructose 6-phosphate (F6P) and sedoheptulose 7-phosphate (S7P). Including fitted models of both Michealis-Menten kinetics and Hill kinetics.

*Clostridium thermocellum* P<sub>Pi</sub>-PFK

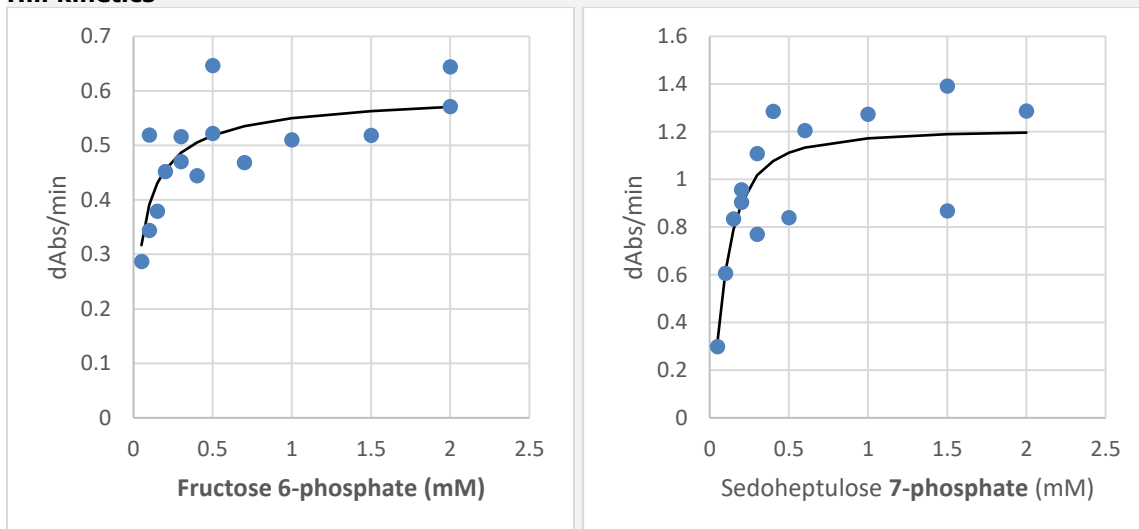
Michaelis-Menten kinetics



F6P:  $K_M = 0.046004018$  mM,  $V_{max} = 0.571547641$  dAbs/min

S7P:  $K_M = 0.10131141$  mM,  $V_{max} = 1.306125685$  dAbs/min

Hill kinetics



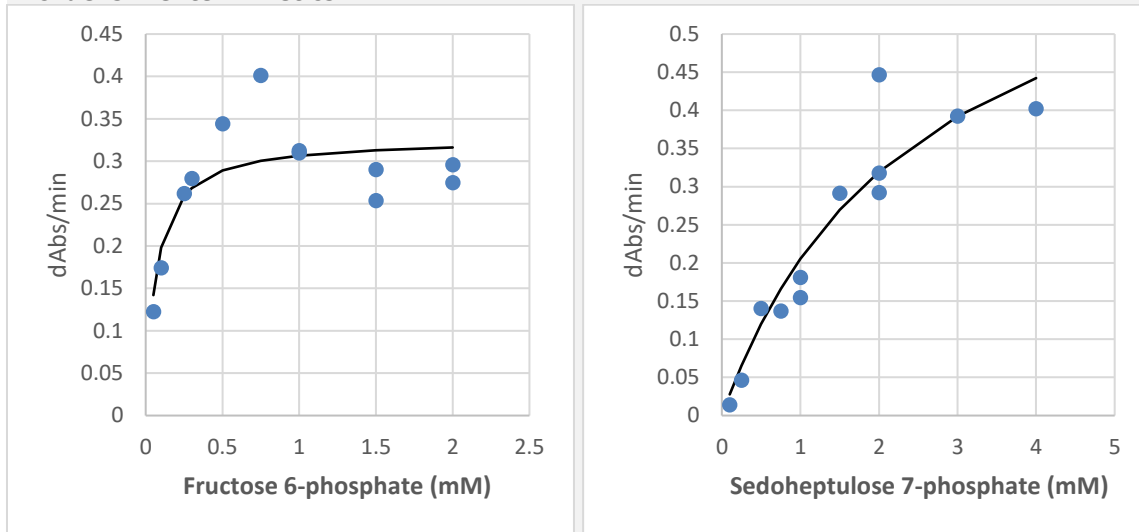
F6P:  $K_{1/2} = 0.04393566$  mM,  $V_{max} = 0.605090926$  dAbs/min,  $n = 0.735169235$

S7P:  $K_{1/2} = 0.098186063$  mM,  $V_{max} = 1.20981373$  dAbs/min,  $n = 1.491188053$

Figure S3: Data of the kinetics assays using *Clostridium thermocellum* P<sub>Pi</sub>-6-phosphofructokinase (PFK) for the conversion of fructose 6-phosphate (F6P) and sedoheptulose 7-phosphate (S7P). Including fitted models of both Michealis-Menten kinetics and Hill kinetics.

*E. coli* PfkA

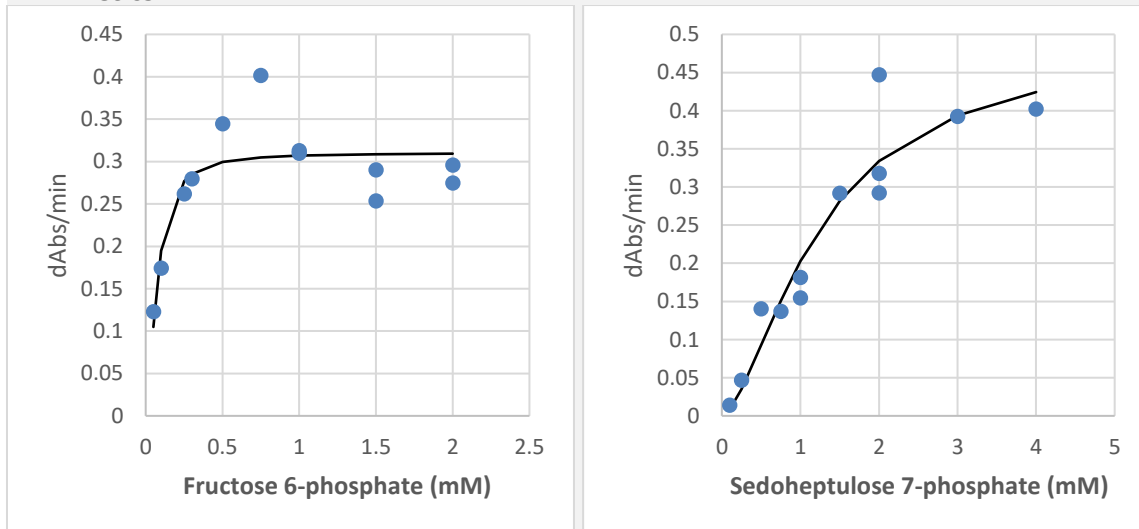
Michaelis-Menten kinetics



F6P:  $K_M = 0.064895717$  mM,  $V_{max} = 0.326490009$  dAbs/min

S7P:  $K_M = 2.489181822$  mM,  $V_{max} = 0.717467989$  dAbs/min

Hill kinetics



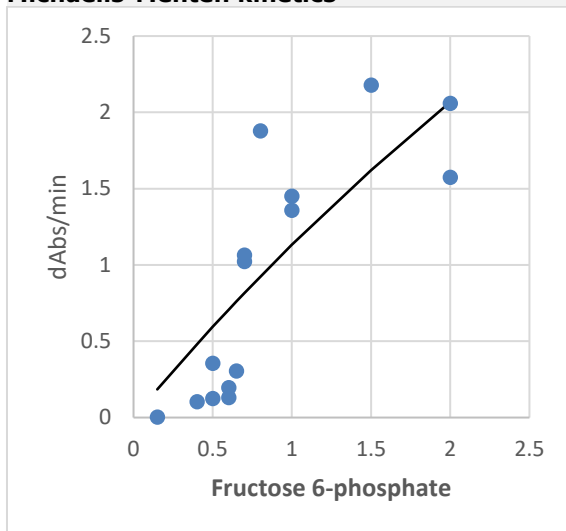
F6P:  $K_{1/2} = 0.073763326$  mM,  $V_{max} = 0.310312305$  dAbs/min,  $n = 1.729659661$

S7P:  $K_{1/2} = 1.24179394$  mM,  $V_{max} = 0.48943044$  dAbs/min,  $n = 1.603138135$

Figure S4: Data of the kinetics assays using *E. coli* 6-phosphofructokinase (PfkA) for the conversion of fructose 6-phosphate (F6P) and sedoheptulose 7-phosphate (S7P). Including fitted models of both Michealis-Menten kinetics and Hill kinetics.

*P. thermosuccinogenes* ATP/GTP-PFK

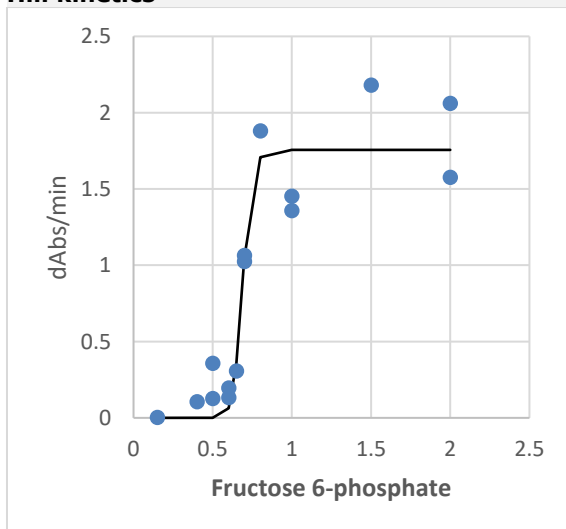
**Michaelis-Menten kinetics**



F6P:  $K_M = 9.550905907$  mM,  $V_{max} = 11.94361907$  dAbs/min

S7P: No activity

**Hill kinetics**



F6P:  $K_{1/2} = 0.68836161$  mM,  $V_{max} = 1.756115449$  dAbs/min,  $n = 23.87224309$

S7P: No activity

**Figure S5:** Data of the kinetics assays using *Clostridium thermosuccinogenes* ATP/GTP-6-phosphofructokinase (PFK) for the conversion of fructose 6-phosphate (F6P) and sedoheptulose 7-phosphate (S7P). Including fitted models of both Michealis-Menten kinetics and Hill kinetics.