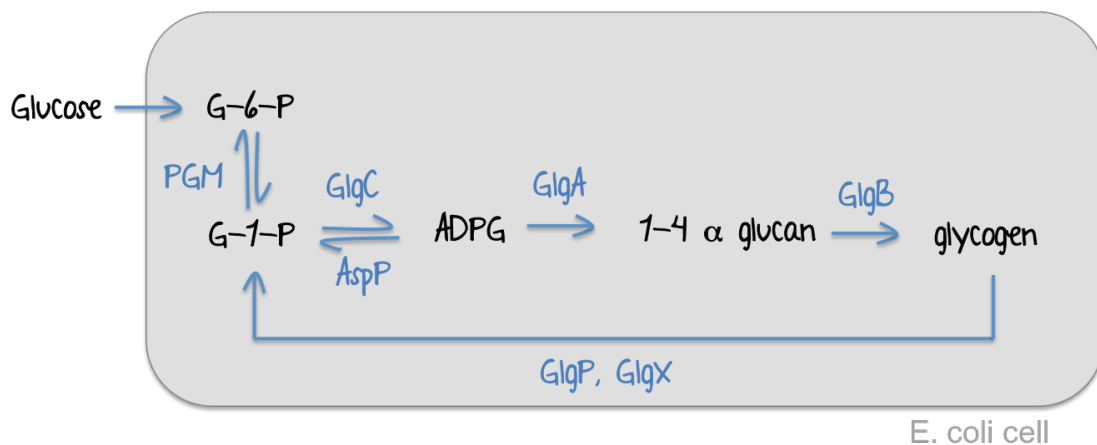


## Kinetic modelling

As a proof of concept, we used glycogen as a model molecule to test the effect of branching and debranching enzymes in *E. coli*.

To analyse the efficiency of the branching (GlgB) and debranching (GlgX) enzymes in glycogen production, we generated a set of differential equations to explain glycogen biosynthesis. We used COPASI (1) (<http://copasi.org/>) to simulate the biochemical reactions involved in the production of glycogen in bacterial cell. We used the the glycogen biosynthesis pathway defined by Wilson WA et al (2) to build our model. The kinetics parameters were found at <http://www.metacyc.org/>

Glycogen Pathway (2)



**Assumptions:** We generated a deterministic model taking into consideration the following assumptions:

- All enzyme concentrations are constant and similar. We fixed the enzyme concentrations to  $1 \times 10^{-5}$  mmol/mL
- Glucose is available in excess.
- Reactions follow simple reversible or irreversible Michaelis-Menten kinetics.
- There is no additional flux of substrates after the beginning of the simulation.
- The intracellular metabolite concentrations (ATP, AMP, ADP and PPi) are constant. They are all set to  $1 \times 10^{-5}$  mmol/mL.
- Only competitive inhibition by the product occurs. For simplicity, other external inhibitors are not considered in the model.

**Enzyme kinetic rates**

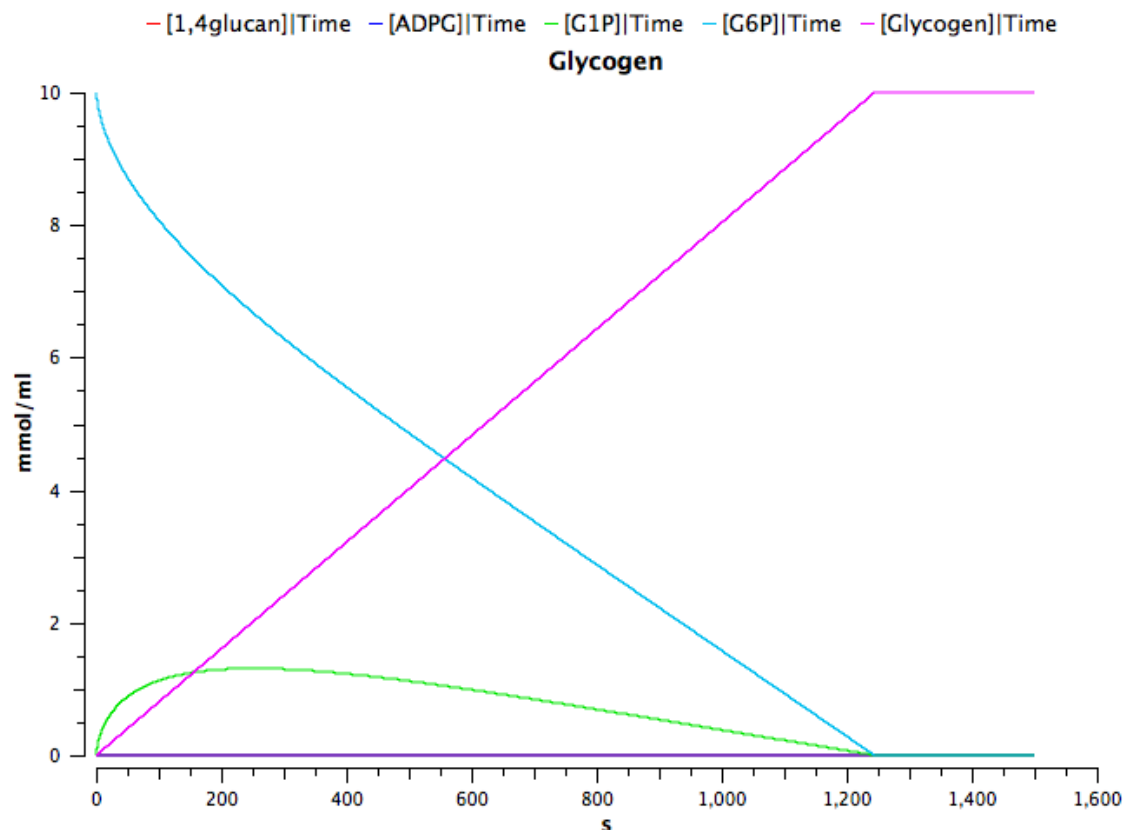
Enzyme	Km (mmol/mL)	Reference
Pgm(G1P)	$2.9 \times 10^{-4}$	(3)
Pgm(G6P)	$5.6 \times 10^{-6}$	(3)
GlgC(ADPG)	$4 \times 10^{-5}$	(4)
GlgC(ATP)	$3.2 \times 10^{-4}$	(4)
GlgA	$3.5 \times 10^{-5}$	(5)
GlgB(Glucan1-4)	$1.42 \times 10^{-5}$	(6)
GlgX (Glycogen)	$1 \times 10^{-6}$	-not found-
GlgP (Glycogen)	$1 \times 10^{-6}$	-not found-

GlgB catalyzes 2 consecutive reactions. First, it cleaves an alpha 1,4 glycosidic linkage in a 1,4-alpha-D-glucan to form a non-reducing-end oligosaccharide chain that is transferred to a C-6 hydroxyl group of the same or another alpha-1,4-D-glucan. (Preiss, J. 2009. Glycogen Biosynthesis.) GlgX and GlgP were considered together as the action of both enzymes is required to get debranching of glycogen.

2 substrate irreversible reaction:  $V_{max} \frac{substrateA \cdot substrateB}{(K_mB \cdot substrateA + K_mA \cdot substrateB + substrateA \cdot substrateB)}$

All the reactions of the pathway are highly efficient, as all the initial glucose-6-P converted to glucose-1-P and ADP-glucose is used in the production of glycogen. Part of the glucose-1-P is recovered due to the GlgX debranching activity.

We run our model for 2500 seconds and collect the data in intervals of 0.05 seconds starting with a concentration of 10 mmol/mL of Glucose-6-P (G6P). After the time course, the majority of the starting glucose has been used to produce glycogen and only a small part of the glucose stays unbranched.



## Differential equations

$$\frac{d([G6P])}{dt} = - \left( \frac{\frac{V_f(P_{gm}) \cdot [G6P]}{K_{ms}(P_{gm})} - \frac{V_r(P_{gm}) \cdot [G1P]}{K_{mp}(P_{gm})}}{1 + \frac{[G6P]}{K_{ms}(P_{gm})} + \frac{[G1P]}{K_{mp}(P_{gm})}} \right)$$

$$\begin{aligned} \frac{d([G1P])}{dt} = & + \left( \frac{\frac{V_f(P_{gm}) \cdot [G6P]}{K_{ms}(P_{gm})} - \frac{V_r(P_{gm}) \cdot [G1P]}{K_{mp}(P_{gm})}}{1 + \frac{[G6P]}{K_{ms}(P_{gm})} + \frac{[G1P]}{K_{mp}(P_{gm})}} \right) \\ & - \left( \frac{V_{max}(GlgC) \cdot [G1P] \cdot [ATP]}{K_{mB}(GlgC) \cdot K_{mA}(GlgC) + K_{mB}(GlgC) \cdot [G1P] + K_{mA}(GlgC) \cdot [ATP] + [G1P] \cdot [ATP]} \right) \\ & + \left( \frac{V_{(GlgX-GlgP)} [Glycogen]}{K_{m(GlgX-GlgP)} + [Glycogen]} \right) \\ & + \left( \frac{V_{(AspP)} \cdot [ADPG]}{K_{m(AspP)} + [ADPG]} \right) \end{aligned}$$

$$\begin{aligned} \frac{d([ADPG])}{dt} = & - \left( \frac{V_{(GlgA)} \cdot [ADPG]}{K_{m(GlgA)} + [ADPG]} \right) \\ & + \left( \frac{V_{max}(GlgC) \cdot [G1P] \cdot [ATP]}{K_{mB}(GlgC) \cdot K_{mA}(GlgC) + K_{mB}(GlgC) \cdot [G1P] + K_{mA}(GlgC) \cdot [ATP] + [G1P] \cdot [ATP]} \right) \\ & - \left( \frac{V_{(AspP)} \cdot [ADPG]}{K_{m(AspP)} + [ADPG]} \right) \end{aligned}$$

$$\begin{aligned} \frac{d([1,4glucan])}{dt} = & + \left( \frac{V_{(GlgA)} \cdot [ADPG]}{K_{m(GlgA)} + [ADPG]} \right) \\ & - \left( \frac{V_{(GlgB)} \cdot [1,4glucan]}{K_{m(GlgB)} + [1,4glucan]} \right) \end{aligned}$$

$$\begin{aligned} \frac{d([Glycogen])}{dt} = & + \left( \frac{V_{(GlgB)} \cdot [1,4glucan]}{K_{m(GlgB)} + [1,4glucan]} \right) \\ & - \left( \frac{V_{(GlgX-GlgP)} \cdot [Glycogen]}{K_{m(GlgX-GlgP)} + [Glycogen]} \right) \end{aligned}$$

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