

# Gene regulation of metabolic pathways: a continuous model for *Escherichia coli* carbon sources uptake

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## Abstract

A “realistic” dynamical model concerning carbon source uptake and consumption was assembled from related metabolic and regulatory networks of *E. coli* using an ODE formalism. Parameter values and kinetics used for metabolic reactions are mostly based on published experimental measurements and/or estimations. This kind of integration is rarely seen in literature and challenges the integrated models out of their boundary conditions into a more complex dynamic scenario. This scenario is particularly interesting when the integrated models comprise both metabolic and gene regulatory networks, providing sometimes new insights into microorganism function that might be exploitable for several purposes, such as growth-optimization, increasing by-product formation or bioreactor design. The assembled model was used for simulations of known biological events in order to evaluate its capacity to reflect real biological phenomena. Glucose uptake rate, which in the literature shows an enormous variance, was first underestimated by the model in nearly an order of magnitude because of an undocumented accumulation of Sed-7-phosphate in the Pentose Phosphate Pathway, as shown by the simulation in Figure 1.

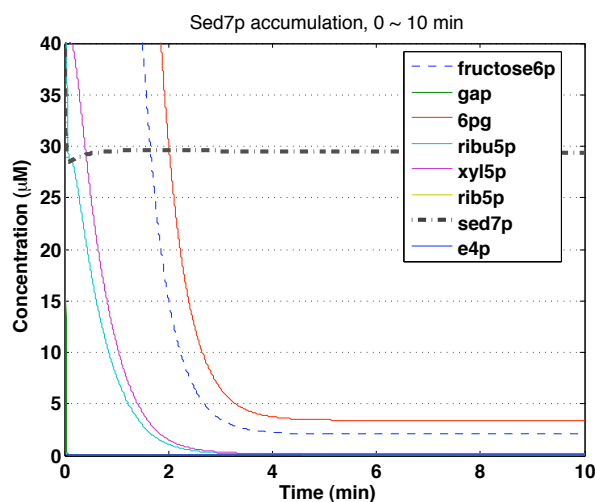


Figure 1: The accumulation of *Sedoheptulose-7-phosphate* is shown in comparison to the other intermediate products of the *Pentose-Phosphate Pathway*. Although not shown here, at time  $T = 3\text{min}$ , the flux of *Glucose* through the *PTS* systems was already stable at the low rate of  $69\ \mu\text{M}/\text{min}$ .

This result questions the presumed reaction kinetics (or its parameters) of the reactions involving this metabolite, shown in Figure 2. Because of this feature, the glycolysis and pentose phosphate pathways cannot catch-up with the amount of glucose entered by the PTS system. The system is thus unable to replenish the necessary Pep-Pyr relation that drives the PTS engine. However, modifying particular enzymes concentrations or reactions kinetics related to the accumulation points does not produce a significant difference on the low glucose consumption, pointing to a structural problem of the glycolysis and pentose phosphate model.

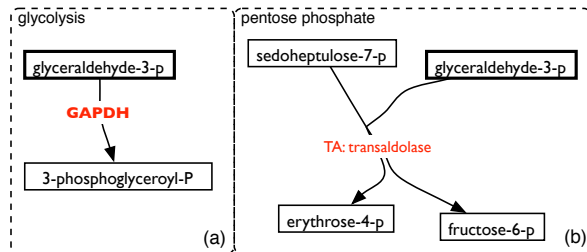


Figure 2: The consumption of *glyceraldehyde-3-p* (*gap*), occurs both in glycolysis (a) and in the pentose-phosphate pathway (b). The reaction catalysed by the enzyme *GAPDH* has a dramatically faster kinetics than that catalysed by the enzyme *TA*. This results in the lack of *gap* to co-react with *sedoheptulose-7-p*, producing the accumulation of the latter and the loss of a great deal of carbon source that otherwise could be used by glycolysis in the process of regeneration of *PEP*.

The model could effectively reflect the known preference of *Escherichia coli* for glucose over glycerol. This was accomplished by the interaction of cAMP-CRP, Mlc and GlpR regulators with the operons related to Glycerol uptake. Once activated by lack of glucose, Glycerol was transported into the cell at a similar rate as was Glucose before depletion, suggesting that the Glycerol uptake speed could be controlled by consumption kinetics in Glycolysis rather than by kinetics of Glycerol uptake enzymes.

## Model description

The integrated metabolic pathways include the uptake systems of Glucose (PTS), Glycerol, Mannose and two components of the central carbon metabolism, Glycolysis and the Pentose Phosphate Pathway. Additionally, gene regulation of the enzymes participating in the uptake pathways was built from scratch, based on the experimentally described behaviour of essential regulators CRP-cAMP, Mlc and GlpR when regulating both the operons of the uptake systems and themselves. Degradation and synthesis kinetics were estimated from published experimental data for Proteins and mRNA. The model is essentially centred on the glucose uptake system, PTS, and the regulation it exerts on other systems. The whole model, including the mentioned gene regulation and metabolic pathways, includes 234 kinetic parameters, 130 macromolecules and 130 reactions that relate them. Hence, a system of 130 differential equations was built.