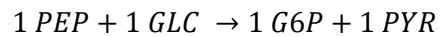


# Transport - PTS

## Reaction equation



phosphoenolpyruvate + glucose = glucose-6-phosphate + pyruvate

BIGG: -1 pep\_c -1 glc\_\_D\_p +1 g6p\_c +1 pyr\_c

## Enzymes and genes

EcoCyc and UniProt

PTS (phosphotransferase system) encoded by

## Regulation

Genetic regulation:

Activators:

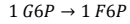
Inhibitors:

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference
Reversible			
k <sub>cat</sub>			
Formation energy X			
K <sub>M</sub>			
K <sub>I</sub>			

# Glycolysis - PGI

## Reaction equation



glucose 6-phosphate = fructose 6-phosphate

BIGG: -1 g6p\_c +1 f6p\_c ([glycolytic direction](#))

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

PGI (glucose 6-phosphate isomerase or phosphoglucose isomerase) is a homodimeric enzyme encoded by the *pgi* gene and located in the cytosol. A  $\Delta pgi$  strain is viable, but grows slower on glucose since the glucose is used via pentose phosphate pathway causing overproduction of NADPH and the Glx shunt is active in this strain.

## Regulation

**Gene regulation:** SoxS (DNA-binding transcription factor) activates transcription initiation of *pgi*.

**Activators:** Activation by CrsA (carbon storage regulator; [Sabnis et al., 1995](#)).

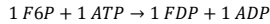
**Inhibitors:** Competitive inhibition by PEP to F6P (phosphoenolpyruvate; [Ogawa et al., 2007](#)), (allosteric?) inhibition by 6PGC which is part of pentose phosphate pathway (6-phosphogluconate; [Schreyer and Böck, 1980](#)) and inhibition by S6P (sorbitol 6-phosphate; [Friedberg, 1972](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	<a href="#">Ishii et al., 2007</a>	-
$k_{\text{cat}}$	1550.0 1/s	0.2 (chosen)	<a href="#">Ishii et al., 2007</a> (calculated from specific activity)	yes
$k_{\text{cat}}$	120.5 1/s	-	<a href="#">Gao et al., 2005</a> (yeast)	no
Formation energy G6P	-1304.7 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy F6P	-1302.1 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ G6P	3.0 mM	0.2 (chosen)	<a href="#">Ishii et al., 2007</a>	yes
$K_M$ G6P	1.018 mM	0.142 mM	<a href="#">Ogawa et al., 2007</a>	no
$K_M$ G6P	0.28 mM	-	<a href="#">Gao et al., 2005</a> (yeast)	no
$K_M$ F6P	0.16 mM	0.2 (chosen)	<a href="#">Ishii et al., 2007</a>	yes
$K_M$ F6P	0.078 mM	0.009 mM	<a href="#">Ogawa et al., 2007</a>	no
$K_M$ F6P	0.2 mM	-	<a href="#">Schreyer and Böck, 1980</a>	no
$K_M$ F6P	0.147 mM	0.006 mM	<a href="#">Gao et al., 2005</a> (yeast)	no
$K_i$ PEP	0.26 mM	0.5 (chosen)	<a href="#">Ogawa et al., 2007</a>	no
$K_i$ 6PGC	0.19 mM	0.5 (chosen)	<a href="#">Schreyer and Böck, 1980</a>	no

# Glycolysis - PFK

## Reaction equation



fructose 6-phosphate + ATP = fructose 1,6-bisphosphate + ADP

BIGG: -1 f6p\_c -1 atp\_c +1 fdp\_c +1 adp\_c (glycolytic direction)

## Enzymes and genes

[EcoCyc PfkA](#), [EcoCyc PfkB](#) and [UniProt PfkA](#), [UniProt PfkB](#)

The PFK (phosphofructokinase) reaction consists of two isozymes: PfkA (PFK I) encoded by the *pfkA* gene and PfkB (PFK II) encoded by the *pfkB* gene. Both isozymes are present in the cytosol, however more than 90% of the total reaction activity in the wild type can be attributed to PfkA ([Kotlarz et al., 1975](#)). PfkA is a homotetrameric enzyme, while PfkB is a homodimeric enzyme. A  $\Delta pfkA$  strain is viable and found to increase the NADPH production via the pentose phosphate pathway, while a  $\Delta pfkB$  strain shows no apparent effect.  $Mg^{2+}$  is a cofactor of both PfkA and PfkB. [Kotlarz and Buc \(1982\)](#) has a nice overview of kinetics and allosteric regulations of both PfkA and pfkB!

## Regulation

**Gene regulation:** F6P (fructose 6-phosphate) binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *pfkA*, thus an increase in F6P increases transcription of *pfkA*. Nac (DNA-binding transcription factor) activates transcription initiation of *pfkB*, while L-arginine binds to ArgR (DNA-binding transcription factor) to enable inhibition and ArgR inhibits transcription initiation of *pfkB*.

**Activators:** Allosteric activation of PfkA by ADP (also GDP; [Blangy and Monod, 1968](#)) and by F6P ([Johnson and Reinhart, 1992](#)), and (product) activation of PfkA by FDP (fructose 1,6-bisphosphate) ([Blangy and Monod, 1968](#)). Allosteric activation of PfkB by HPr (phosphocarrier protein; [Rodionova et al., 2017](#)).

**Inhibitors:** Allosteric inhibition of PfkA by PEP (phosphoenolpyruvate) and by ATP ([Blangy and Monod, 1968](#); [Berger and Evans, 1991](#)). Allosteric inhibition of PfkB by ATP ([Kotlarz et al., 1975](#); [Kotlarz and Buc, 1981](#)), competitive inhibition of PfkB by FDP (fructose 1,6-bisphosphate; [Campos et al., 1984](#)), and (allosteric) inhibition of PfkB by Pi (phosphate; [Parducci et al., 2006](#)).

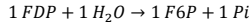
## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
$k_{cat}$ PfkA	110.0 1/s	0.2 (chosen)	<a href="#">Blangy and Monod, 1968</a>	no
$k_{cat}$ PfkA	130.0 1/s	0.2 (chosen)	<a href="#">Berger and Evans, 1991</a>	yes
$k_{cat}$ PfkA	27.4 1/s	-	<a href="#">Ogawa et al., 2007</a> (calculated from specific activity)	no
$k_{cat}$ PfkA	120.0 1/s	-	<a href="#">Auzat et al., 1994</a>	no
$k_{cat}$ PfkA	93.0 1/s	-	<a href="#">Hellinga and Evans, 1987</a>	no
$k_{cat}$ PfkA	88.0 1/s	-	<a href="#">Wang and Kemp, 2001</a>	no
$k_{cat}$ PfkA	167.0 1/s	-	<a href="#">Zheng and Kemp, 1995</a>	no
$k_{cat}$ PfkB	53.0 1/s	0.2 (chosen)	<a href="#">Parducci et al., 2006</a>	yes
$k_{cat}$ PfkB	56.0 1/s	0.2 (chosen)	<a href="#">Villalobos et al., 2016</a>	no
$k_{cat}$ PfkB	61.0 1/s	8 1/s	<a href="#">Baez et al., 2013</a>	no
Formation energy F6P	-1302.1 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy FDP	-2194.8 kJ/mol	2.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy ATP	-2280.7 kJ/mol	2.9 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy ADP	-1405.9 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ F6P of PfkA	0.0125 mM	0.2 (chosen)	<a href="#">Blangy and Monod, 1968</a>	yes
$K_M$ F6P of PfkA	0.030 mM	-	<a href="#">Berger and Evans, 1991</a>	no
$K_M$ F6P of PfkA	0.45 mM	-	<a href="#">Ogawa et al., 2007</a>	no
$K_M$ F6P of PfkA	0.34 mM	-	<a href="#">Auzat et al., 1994</a>	no
$K_M$ F6P of PfkA	0.038 mM	-	<a href="#">Hellinga and Evans, 1987</a>	no
$K_M$ F6P of PfkA	0.16 mM	-	<a href="#">Zheng and Kemp, 1995</a>	no
$K_M$ FDP of PfkA	3.5 mM	0.2 (chosen)	<a href="#">Babul, 1978</a>	yes
$K_M$ FDP of PfkA	15 mM	1.5 (chosen)	Denis' model or unknown	no
$K_M$ ATP of PfkA	0.06 mM	0.2 (chosen)	<a href="#">Blangy and Monod, 1968</a>	yes
$K_M$ ATP of PfkA	0.06 mM	-	<a href="#">Berger and Evans, 1991</a>	no
$K_M$ ATP of PfkA	0.018 mM	-	<a href="#">Ogawa et al., 2007</a>	no
$K_M$ ATP of PfkA	0.057 mM	-	<a href="#">Auzat et al., 1994</a>	no
$K_M$ ATP of PfkA	0.041 mM	-	<a href="#">Hellinga and Evans, 1987</a>	no
$K_M$ ATP of PfkA	0.2 mM	-	<a href="#">Zheng and Kemp, 1995</a>	no
$K_M$ ADP of PfkA	0.025 mM	0.2 (chosen)	<a href="#">Blangy and Monod, 1968</a>	yes
$K_M$ F6P of PfkB	0.052 mM	0.2 (chosen)	<a href="#">Parducci et al., 2006</a>	yes
$K_M$ F6P of PfkB	0.013 mM	-	<a href="#">Babul, 1978</a>	no
$K_M$ F6P of PfkB	0.009 mM	-	<a href="#">Baez et al., 2013</a>	no
$K_M$ F6P of PfkB	0.032 mM	-	<a href="#">Campos et al., 1984</a>	no
$K_M$ F6P of PfkB	0.011 mM	-	<a href="#">Kotlarz and Buc, 1981</a>	no
$K_M$ FDP of PfkB	0.14 mM	0.2 (chosen)	<a href="#">Babul, 1978</a>	yes
$K_M$ FDP of PfkB	15 mM	1.5 (chosen)	Denis' model or unknown	no

$K_M$ ATP of PfkB	0.015 mM	0.5 (chosen)	<a href="#">Parducci et al., 2006</a>	yes
$K_M$ ATP of PfkB	0.008 mM	0.003 mM	<a href="#">Baez et al., 2013</a>	no
$K_M$ ATP of PfkB	0.020 mM	-	<a href="#">Campos et al., 1984</a>	no
$K_M$ ATP of PfkB	0.050 mM	-	<a href="#">Kotlarz and Buc, 1981</a>	no
$K_M$ ADP of PfkB	0.025 mM	0.2 (chosen)	<a href="#">Blangy and Monod, 1968</a>	yes
$K_D$ T PEP of PfkA (and PfkB)	0.75 mM	1.0 (chosen)	<a href="#">Blangy and Monod, 1968</a>	yes
$K_i$ PEP of PfkA	1.953 mM	-	<a href="#">Ogawa et al., 2007</a>	no
$K_i$ PEP of PfkA	0.30 mM	0.01 mM	<a href="#">Paricharttanakul et al., 2005</a>	no
$K_i$ ADP of PfkA	0.048 mM	0.002 mM	<a href="#">Paricharttanakul et al., 2005</a>	no
$K_i$ FDP of PfkB	0.24 mM	-	<a href="#">Campos et al., 1984</a>	no
$K_i$ ATP of PfkB	1.026 mM	0.1 mM	<a href="#">Baez et al., 2013</a>	no

# Glycolysis - FBP

## Reaction equation



fructose 1,6-bisphosphate + H<sub>2</sub>O = fructose 6-phosphate + Pi

BIGG: -1 fdp\_c (-1 h2o\_c) +1 f6p\_c +1 pi\_c ([gluconeogenic direction](#))

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

FBP (fructose bisphosphatase) encoded by the *fbp* gene is part of the gluconeogenesis pathway and is required for growth on acetate, glycerol, or succinate. Fbp (FBP I or FBPA) is a homotetrameric enzyme located in the cytosol. Mg<sup>2+</sup> is a cofactor for both FBP I and II. Research if *Δfbp* mutant is viable, because 77% silencing efficacy resulted in a defect in carbon catabolite repression. Another FBP (FBP II or FBPB) is a homodimeric enzyme encoded by the *glpX* gene with lower specific activity and kinetic properties are available ([EcoCyc](#) and [UniProt](#)). The FBP of *fbp* is a class I and the FBP of *glpX* is a class II and the two enzymes share only 10% amino acid sequence similarity.

## Regulation

**Gene regulation:** none or unknown for FBP I. cAMP binds to CRP (DNA-binding transcription factor) to enable activation and CRP activates transcription initiation of *glpX*. Glycerol binds to GlpR (DNA-binding transcription factor) to block inhibition, while GlpR inhibits transcription initiation of *glpX*, thus an increase in glycerol increases transcription of *glpX*.

**Activators:** Activation of FBP I by three-carbon carboxylic acids, especially by PEP (phosphoenolpyruvate) and citrate for lower concentrations of up to 5 mM which bind to the active R-state ([Hines et al., 2006](#) and [Hines and Fromm et al., 2007](#), respectively), likely by binding to the allosteric activator site ([Hines et al., 2006](#)). Both PEP (phosphoenolpyruvate) and citrate antagonise/block the inhibition of AMP ([Babel and Guixé, 1983](#) and [Hines and Fromm et al., 2007](#)). (Allosteric?) activation of FBP II by PEP ([Donahue et al., 2000](#)).

**Inhibitors:** Allosteric inhibition of FBP I by AMP ([Babul and Guixé, 1983](#)) and G6P (glucose 6-phosphate; [Hines and Kreusel et al., 2007](#)) at distinct sites of the enzyme in a synergistic manner where the Fbp undergoes a transition into a T-like inactive state. Substrate inhibition of FBP I by FDP (fructose 1,6-bisphosphate) at concentrations above 0.05 mM ([Babel and Guixé, 1983](#)). Competitive (product) inhibition of FBP II by Pi ([Donahue et al., 2000](#) and [Brown et al., 2009](#)) and by F1P. Inhibition of FBP II by ADP ([Donahue et al., 2000](#)) and Li<sup>+</sup> ([Brown et al., 2009](#)). Possible inhibition by fructose 2,6-bisphosphate (which is not present in bacteria; [Marcus et al., 1984](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub> FBP I	14.6 1/s	0.8 1/s	<a href="#">Kelley-Loughnane et al., 2002</a>	no
k <sub>cat</sub> FBP I	24.0 1/s	3 1/s; 0.2 (chosen)	<a href="#">Hines and Fromm et al., 2007</a>	yes
k <sub>cat</sub> FBP I	20.0 1/s	1 1/s	<a href="#">Iancu et al., 2005</a>	no
k <sub>cat</sub> FBP II	2.2 1/s	-	<a href="#">Donahue et al., 2000</a> (calculated from specific activity)	no
k <sub>cat</sub> FBP II	5.7 1/s	0.1 1/s; 0.2 (chosen)	<a href="#">Brown et al., 2009</a>	yes
Formation energy FDP	-2194.8 kJ/mol	2.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy F6P	-1302.1 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy Pi	-1072.6 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	yes
K <sub>M</sub> FDP of FBP I	0.0154 mM	0.002 mM	<a href="#">Kelley-Loughnane et al., 2002</a>	no
K <sub>M</sub> FDP of FBP I	0.0017 mM	0.0001 mM; 0.2 (chosen)	<a href="#">Hines and Fromm et al., 2007</a>	yes
K <sub>M</sub> FDP of FBP I	0.0012 mM	0.00005 mM	<a href="#">Iancu et al., 2005</a>	no
K <sub>M</sub> F6P of FBP I	0.69 mM	1.5 (chosen)	Denis' model or unknown	no
K <sub>M</sub> F6P of FBP I	0.02 mM	0.5 (chosen)	Picked based on previous simulation	yes
K <sub>M</sub> Pi of FBP I	1.0 mM	1.5 (chosen)	Denis' model or unknown	no
K <sub>M</sub> Pi of FBP I	0.02 mM	0.5 (chosen)	Picked based on previous simulation	yes
K <sub>M</sub> FDP of FBP II	0.035 mM	-	<a href="#">Donahue et al., 2000</a>	no
K <sub>M</sub> FDP of FBP II	0.07 mM	0.002 mM; 0.2 (chosen)	<a href="#">Brown et al., 2009</a>	yes
K <sub>M</sub> F6P of FBP II	0.02 mM	0.5 (chosen)	Picked based on previous simulation of FBP I	yes
K <sub>M</sub> Pi of FBP II	0.02 mM	0.5 (chosen)	Picked based on previous simulation of FBP I	yes
K <sub>D</sub> R (A <sub>0.5</sub> ) PEP of FBP I	0.040 mM	-	<a href="#">Hines et al., 2006</a>	no
K <sub>D</sub> R (A <sub>0.5</sub> ) PEP of FBP I	0.027 mM	0.002 mM; 1.0 (chosen)	<a href="#">Hines and Fromm et al., 2007</a>	yes
K <sub>D</sub> R (A <sub>0.5</sub> ) CIT of FBP I	0.21 mM	0.01 mM	<a href="#">Hines and Fromm et al., 2007</a>	no
K <sub>i</sub> (I <sub>0.5</sub> ) AMP of FBP I	0.0181 mM	0.0005 mM	<a href="#">Hines and Fromm et al., 2007</a>	no
K <sub>i</sub> (I <sub>0.5</sub> ) AMP of FBP I	0.008 mM	0.002 mM	<a href="#">Hines and Kreusel et al., 2007</a>	no
K <sub>i</sub> AMP of FBP I	0.0006 mM	0.0001 mM	<a href="#">Iancu et al., 2005</a>	no
K <sub>D</sub> T (I <sub>0.5</sub> ) G6P of FBP I	0.038 mM	0.006 mM; 1.0 (chosen)	<a href="#">Hines and Kreusel et al., 2007</a>	yes

# Glycolysis - FBA

## Reaction equation



fructose 1,6-biphosphate = dihydroxyacetone phosphate + glyceraldehyde 3-phosphate

BIGG: -1 fdp\_c +1 dhap\_c +1 g3p\_c ([glycolytic direction](#))

## Enzymes and genes

[EcoCyc FbaA](#), [EcoCyc FbaB](#) and [UniProt FbaA](#), [UniProt FbaB](#)

The FBA (fructose biphosphate aldolase) reaction consists of two isozymes: FbaA (FBA class II) encoded by the *fbaA* gene and FbaB (FBA class I) encoded by the *fbaB* gene. Both enzymes are present in the cytosol, however only 5% to 10% of the total reaction activity can be attributed to FbaB in general and FbaB is only present when grown on three-carbon substrates such as lactate or glycerol, while FbaA is always present ([Scamuffa and Caprioli, 1980](#)). Thus, FbaA is likely involved in glycolysis and FbaB in gluconeogenesis. FbaA is a homodimeric enzyme, while FbaB is a homodecameric enzyme. A  $\Delta fbaA$  mutant is viable, but has a heat-sensitive defect in rRNA transcription.  $\text{Zn}^{2+}$  is a cofactor of FbaA. Different specific activities of FBA when grown on glucose or glycerol ([Szwergold et al., 1995](#)).

## Regulation

**Gene regulation:** Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *fbaA* and of *fbaB*, thus an increase in fructofuranose 1-phosphate increases transcription of *fbaA* and of *fbaB*. cAMP binds to CRP (DNA-binding transcription factor) to enable activation and CRP activates transcription initiation of *fbaA*. ppGpp activates binding of RNA polymerase to the *fbaB* promotor under the condition of isoleucine starvation.

**Activators:** Activation of FbaA by  $\text{NH}_4$  (ammonium) and  $\text{K}^+$  ([Blom et al., 1996](#)). Activation of FbaB by citrate ([Thomson et al., 1998](#); kinetic values with activation in paper) and PEP (phosphoenolpyruvate; [Baldwin and Perham, 1978](#)).

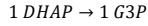
**Inhibitors:** Inhibition of FbaA by  $\text{Ni}^{2+}$  (nickel; [Macomber et al., 2011](#)) and product inhibition of FbaA by DHAP (competitive; dihydroxyacetone phosphate; [Plater et al., 1999](#)) and G3P (uncompetitive). Competitive inhibition of FbaA by 2-phosphoglycolate (2PGly; [Qamar et al., 1996](#)). Non-competitive inhibition of FbaB by PEP ([Ogawa et al., 2007](#)). Irreversible inhibition of FbaB by borohydride ([Thomson et al., 1998](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{\text{cat}}$ FbaA	10.5 1/s	0.07 1/s; 0.2 (chosen)	<a href="#">Zgiby et al., 2002</a> (WT)	no
$k_{\text{cat}}$ FbaA	14.2 1/s	0.6 1/s	<a href="#">Hao and Berry, 2004</a>	no
$k_{\text{cat}}$ FbaA	8.2 1/s	0.4 1/s	<a href="#">Qamar et al., 1996</a>	no
$k_{\text{cat}}$ FbaA	10.3 or 30.3 1/s	0.3 or 0.8 1/s; 0.2 (chosen)	<a href="#">Berry and Marshall, 1993</a>	yes
$k_{\text{app}}^{\text{max}} \times 2 \text{ FBA}$	55.5 $\times 2 = 111.0$ 1/s	-	Calculated from experimental fluxes and protein concentrations ( $\Delta \text{sdhCB}$ )	no
$k_{\text{cat}}$ FbaB	4.14 1/s	0.2 (chosen)	<a href="#">Smallbone et al., 2013</a> (yeast)	no
$k_{\text{cat}}$ FbaB	0.23 1/s	0.007 1/s; 0.2 (chosen)	<a href="#">Thomson et al., 1998</a>	yes
Formation energy FDP	-2194.8 kJ/mol	2.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy DHAP	-1097.2 kJ/mol	1.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy G3P	-1091.5 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_{\text{M}}$ FDP of FbaA	0.17 mM	0.003 mM	<a href="#">Zgiby et al., 2002</a> (WT)	no
$K_{\text{M}}$ FDP of FbaA	0.133 mM	0.5 (chosen)	<a href="#">Babul et al., 1993</a>	no
$K_{\text{M}}$ FDP of FbaA	0.85 mM	-	<a href="#">Stribling and Perham, 1973</a>	no
$K_{\text{M}}$ FDP of FbaA	0.14 mM	0.02 mM	<a href="#">Hao and Berry, 2004</a>	no
$K_{\text{M}}$ FDP of FbaA	0.19 mM	0.03 mM	<a href="#">Qamar et al., 1996</a>	no
$K_{\text{M}}$ FDP of FbaA	(0.24 or) 0.29 mM	(0.03 or) 0.04 mM; 0.2 (chosen)	<a href="#">Berry and Marshall, 1993</a>	yes
$K_{\text{M}}$ DHAP of FbaA	0.03 mM	0.002 mM	<a href="#">Zgiby et al., 2002</a> (WT)	no
$K_{\text{M}}$ DHAP of FbaA	0.088 mM	0.2 (chosen)	<a href="#">Babul et al., 1993</a>	yes
$K_{\text{M}}$ G3P of FbaA	0.088 mM	0.2 (chosen)	<a href="#">Babul et al., 1993</a>	yes
$K_{\text{M}}$ FDP of FbaB	0.451 mM	0.5 (chosen)	<a href="#">Smallbone et al., 2013</a> (yeast)	no
$K_{\text{M}}$ FDP of FbaB	0.055 mM	0.003 mM	<a href="#">Ogawa et al., 2007</a>	no
$K_{\text{M}}$ FDP of FbaB	0.02 mM	0.002 mM; 0.2 (chosen)	<a href="#">Thomson et al., 1998</a>	yes
$K_{\text{M}}$ FDP of FbaB	0.02 mM	-	<a href="#">Stribling and Perham, 1973</a>	no
$K_{\text{M}}$ DHAP of FbaB	2.0 mM	0.5 (chosen)	<a href="#">Smallbone et al., 2013</a> (yeast)	yes
$K_{\text{M}}$ G3P of FbaB	2.4 mM	0.5 (chosen)	<a href="#">Smallbone et al., 2013</a> (yeast)	yes
$K_i$ DHAP of FbaA	0.13 mM	0.011 mM	<a href="#">Plater et al., 1999</a>	no
$K_i$ G3P of FbaA	0.6 mM	-	<a href="#">Babul et al., 1993</a>	no
$K_i$ 2PGly of FbaA	0.009 mM	0.0012 mM	<a href="#">Qamar et al., 1996</a>	no
$K_i$ PEP of FbaB	1.85 mM	-	<a href="#">Ogawa et al., 2007</a>	no

# Glycolysis - TPI

## Reaction equation



dihydroxyacetone phosphate = glyceraldehyde 3-phosphate

BIGG: -1 dhap\_c +1 g3p\_c

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

TPI (triosephosphate isomerase) is a homodimeric enzyme encoded by the *tpiA* gene and located in the cytosol. A  $\Delta tpiA$  mutant is viable, but was unable to grow on glucose, lactate, or other carbon sources that require the activity of both the glycolysis and gluconeogenesis pathways. Expression of *tpiA* is upregulated in a  $\Delta pgi$  mutant and downregulated in a  $\Delta pykF$  mutant.

## Regulation

**Gene regulation:** Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *tpiA*, thus an increase in fructofuranose 1-phosphate increases transcription of *tpiA*.

**Activators:** Activation by CrsA (carbon storage regulator; [Sabnis et al., 1995](#)).

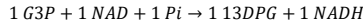
**Inhibitors:** Competitive inhibition by 2-phosphoglycolate ([Mainfroid et al., 1993](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{\text{cat}}$	600.0 1/s	0.2 (chosen)	<a href="#">Hermes et al., 1990</a> (muscle enzyme)	no
$k_{\text{cat}}$	8700.0 1/s	0.2 (chosen)	<a href="#">Mainfroid et al., 1993</a>	yes
Formation energy DHAP	-1097.2 kJ/mol	1.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy G3P	-1091.5 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ DHAP	0.65 mM	0.5 (chosen)	<a href="#">Hermes et al., 1990</a> (muscle enzyme)	no
$K_M$ DHAP	2.8 mM	0.2 (chosen)	<a href="#">Babul et al., 1993</a>	yes
$K_M$ G3P	0.42 mM	0.5 (chosen)	<a href="#">Hermes et al., 1990</a> (muscle enzyme)	no
$K_M$ G3P	1.03 mM	1.0 (chosen)	<a href="#">Mainfroid et al., 1993</a>	no
$K_M$ G3P	0.3 mM	0.2 (chosen)	<a href="#">Babul et al., 1993</a>	yes
$K_i$ 2-PG	0.006 mM	-	<a href="#">Mainfroid et al., 1993</a>	no

# Glycolysis - GAPD

## Reaction equation



glyceraldehyde 3-phosphate + NAD + Pi = 3-phosphoglyceroyl phosphate + NADH

BIGG: -1 g3p\_c -1 nad\_c -1 pi\_c +1 13dpg\_c +1 nadh\_c ([glycolytic direction](#))

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

GAPD (glyceraldehyde 3-phosphate dehydrogenase) is an homotetrameric enzyme encoded by *gapA* gene and is located in the cytosol. A  $\Delta gapA$  mutant with 93% efficacy is viable, but exhibits a severe growth defect. Both GAPD and E4PD (erythrose 4-phosphate dehydrogenase) are able to phosphorylise G3P and E4P, however GAPD is only highly efficient for G3P phosphorylation and E4PD is only highly efficient for E4P phosphorylation.

## Regulation

**Gene regulation:** cAMP binds to CRP (DNA-binding transcription factor) to enable activation and CRP activates transcription initiation of *gapA*. Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *gapA*, thus an increase in fructofuranose 1-phosphate increases transcription of *gapA*.

**Activators:** Activation by arsenate ([Zhao et al., 1995](#)).

**Inhibitors:** Inhibition by iodoacetate ([D'Alessio and Josse, 1971](#)).

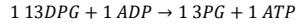
## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
k <sub>cat</sub>	1056.0 1/s	0.2 (chosen)	<a href="#">Soukri et al., 1989</a>	yes
k <sub>cat</sub>	268.0 1/s	6 1/s	<a href="#">Eyschen et al., 1999</a>	no
k <sub>cat</sub>	76.0 1/s	4 1/s	<a href="#">Boschi-Muller et al., 1997</a> ( <i>B. stearrowthermophilus</i> )	no
Formation energy G3P	-1091.5 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy 13DPG	-2212.0 kJ/mol	2.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy Pi	-1072.6 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy NAD <sup>+</sup>	-1146.0 kJ/mol	13.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy NADH	-1079.8 kJ/mol	13.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
K <sub>M</sub> G3P	1.5 mM	0.5 (chosen)	<a href="#">Soukri et al., 1989</a>	no
K <sub>M</sub> G3P	0.89 mM	0.17 mM; 0.5 (chosen)	<a href="#">Eyschen et al., 1999</a>	yes
K <sub>M</sub> G3P	0.90 mM	0.20 mM	<a href="#">Boschi-Muller et al., 1997</a> ( <i>B. stearrowthermophilus</i> )	no
K <sub>M</sub> 13DPG	0.015 mM	0.5 (chosen)	<a href="#">Soukri et al., 1989</a>	yes
K <sub>M</sub> 13DPG	0.005 mM	-	<a href="#">Boschi-Muller et al., 1997</a> ( <i>B. stearrowthermophilus</i> )	no
K <sub>M</sub> Pi	22.0 mM	1.0 (chosen)	<a href="#">Soukri et al., 1989</a>	no
K <sub>M</sub> Pi	0.53 mM	0.11 mM; 0.5 (chosen)	<a href="#">Eyschen et al., 1999</a>	yes
K <sub>M</sub> NAD <sup>+</sup>	0.042 mM	0.5 (chosen)	<a href="#">Soukri et al., 1989</a>	no
K <sub>M</sub> NAD <sup>+</sup>	0.045 mM	0.004 mM; 0.5 (chosen)	<a href="#">Eyschen et al., 1999</a>	yes
K <sub>M</sub> NAD <sup>+</sup>	0.09 mM	0.01 mM	<a href="#">Boschi-Muller et al., 1997</a> ( <i>B. stearrowthermophilus</i> )	no
K <sub>M</sub> NADH	0.011 mM	0.5 (chosen)	<a href="#">Boschi-Muller et al., 1997</a> ( <i>B. stearrowthermophilus</i> )	yes



# Glycolysis - PGK

## Reaction equation



3-phosphoglyceroyl phosphate + ADP = 3-phosphoglycerate + ATP

BIGG: -1 3pg\_c -1 atp\_c +1 13dpg\_c +1 adp\_c ([gluconeogenic direction](#))

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

PGK (phosphoglycerate kinase) is a monomeric enzyme encoded by the *pgk* gene and is located in the cytosol. A  $\Delta pgk$  mutant cannot grow on sugars or gluconeogenic substrates, but a mutant silenced with 79% efficacy did not cause severe growth inhibition.  $Mg^{2+}$  is a cofactor.

## Regulation

**Gene regulation:** cAMP binds to CRP (DNA-binding transcription factor) to enable activation and CRP activates transcription initiation of *pgk*. Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *pgk*, thus an increase in fructofuranose 1-phosphate increases transcription of *pgk*.

**Activators:** Activation by  $Na_2SO_4$  (sodium sulfate; [Fifes and Scopes., 1978](#)).

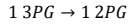
**Inhibitors:** none or unknown.

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{cat}$	349.6 1/s	0.2 (chosen)	<a href="#">Fifes and Scopes., 1978</a> (calculated from specific activity of gluconeogenic reaction)	yes
$k_{cat}$	1480.0 1/s	-	<a href="#">Collinet et al., 2000</a>	no
$k_{cat}$	571.7 1/s	-	<a href="#">Kuntz and Krietsch, 1982</a> (from yeast; calculated from specific activity))	no
$k_{app}^{max} \times 2$	107.4 $\times 2$ = 214.8 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations ( $\Delta sdhCB$ )	no
Formation energy 13DPG	-2212.0 kJ/mol	2.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy 3PG	-1356.7 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy ATP	-2280.7 kJ/mol	2.9 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy ADP	-1405.9 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ 13DPG	0.0018 mM	0.5 (chosen)	<a href="#">Kuntz and Krietsch, 1982</a> (from yeast)	yes
$K_M$ 3PG	0.69 or 1.28 mM	0.5 (chosen)	<a href="#">Kuntz and Krietsch, 1982</a> (from yeast)	yes
$K_M$ ATP	0.48 mM	0.5 (chosen)	<a href="#">Kuntz and Krietsch, 1982</a> (from yeast)	yes
$K_M$ ATP	0.24 mM	-	<a href="#">Fifes and Scopes., 1978</a>	no
$K_M$ ADP	0.2 mM	0.5 (chosen)	<a href="#">Kuntz and Krietsch, 1982</a> (from yeast)	yes

# Glycolysis - PGM

## Reaction equation



3-phosphoglycerate = glycerate 2-phosphate

BIGG: -1 2pg\_c +1 3pg\_c (gluconeogenic direction)

## Enzymes and genes

[EcoCyc dPgm](#), [EcoCyc iPgm](#) and [UniProt dPgm](#), [UniProt iPgm](#)

The PGM (phosphoglycerate mutase) reaction consists of two isozymes: dPgm encoded by *gpmA* gene and iPgm encoded by *gpmM* gene. Both enzymes are present in the cytosol and expressed at high levels, but dPgm has a 10-fold higher specific activity than iPgm ([Fraser et al., 1999](#)). dPgm is a homodimeric enzyme which needs 2,3-bisphosphoglycerate as cofactor (hence the name 2,3-bisphosphoglycerate-dependent Pgm), while iPgm is a monomeric enzyme which needs  $Mn^{2+}$  as cofactor. A *ΔgpmA* mutant is viable, but shows a growth lag in minimal medium. A *ΔgpmM* mutant is viable, while a double mutant, *ΔgpmAΔgpmM*, does not appear to be viable.

## Regulation

**Gene regulation:**  $Fe^{2+}$  binds Fur (DNA-binding transcription factor) to enable inhibition of transcription initiation of *gpmA*. Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *gpmM*, thus an increase in fructofuranose 1-phosphate increases transcription of *gpmM*.

**Activators:** none or unknown.

**Inhibitors:** Inhibition of dPgm by potassium and phosphate ([García-Contreras et al., 2012](#)) and by vanadate ([Fraser et al., 1999](#); includes  $K_i$  values for both competitive and uncompetitive inhibition).

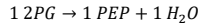
## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{cat}$ dPgm	330.0 1/s	11 1/s	<a href="#">Fraser et al., 1999</a> ; glycolytic	no
$k_{cat}$ dPgm	220.0 1/s	13 1/s, 0.2 (chosen)	<a href="#">Fraser et al., 1999</a> ; gluconeogenic	yes
$k_{cat}$ iPgm	22.0 1/s	1 1/s	<a href="#">Fraser et al., 1999</a> ; glycolytic	no
$k_{cat}$ iPgm	10.0 1/s	0.5 1/s	<a href="#">Fraser et al., 1999</a> ; gluconeogenic	no
Formation energy 3PG	-1356.7 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy 2PG	-1352.2 kJ/mol	1.6 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ 3PG of dPgm	0.20 mM	0.027 mM; 0.5 (chosen)	<a href="#">Fraser et al., 1999</a>	yes
$K_M$ 3PG of iPgm	0.21 mM	0.039 mM	<a href="#">Fraser et al., 1999</a>	no
$K_M$ 2PG of dPgm	0.19 mM	0.035 mM; 0.5 (chosen)	<a href="#">Fraser et al., 1999</a>	yes
$K_M$ 2PG of iPgm	0.097 mM	0.014 mM	<a href="#">Fraser et al., 1999</a>	no

\*Only proteomics data for dPgm.

# Glycolysis - ENO

## Reaction equation



glycerate 2-phosphate = phosphoenolpyruvate + H<sub>2</sub>O

BIGG: -1 2pg\_c +1 pep\_c (+1 h2o\_c) (glycolytic direction)

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

ENO (enolase) is a homodimeric enzyme encoded by the *eno* gene and is located in the cytosol. A  $\Delta eno$  mutant is viable, but glycolytic pathway intermediates accumulated when growing on glucose or glycerol, while growth on glycerate or succinate is undisturbed. Mg<sup>2+</sup> is a cofactor.

## Regulation

**Gene regulation:** L-leucine binds to Lrp (DNA-binding transcription factor) to block activation, while Lrp activates transcription initiation of *eno*, thus an increase in L-leucine decreases transcription of *eno*. Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *eno*, thus an increase in fructofuranose 1-phosphate increases transcription of *eno*.

**Activators:** none or unknown.

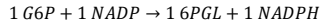
**Inhibitors:** Inhibition by fluoride in the presence of phosphate ([Spring and Wold, 1971](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
k <sub>cat</sub>	138.0 1/s	-	<a href="#">Spring and Wold, 1971</a> (calculated from specific activity)	no
k <sub>cat</sub>	199.3 1/s	10.0 1/s; 0.2 (chosen)	<a href="#">Kühnel and Luisi, 2001</a> (calculated from specific activity)	yes
k <sub>cat</sub>	8.5 1/s	0.2 (chosen)	<a href="#">Krucinska et al., 2019</a>	no
k <sub>app</sub> <sup>max</sup> x2	52.4 x2 = 104.8 1/s	-	Calculated from experimental fluxes and protein concentrations ( $\Delta$ ptsHlcr)	no
Formation energy 2PG	-1352.2 kJ/mol	1.6 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy PEP	-1205.1 kJ/mol	1.6 kJ/mol	<a href="#">eQuilibrator</a>	yes
K <sub>M</sub> 2PG	0.1 mM	1.0 (chosen)	<a href="#">Spring and Wold, 1971</a>	no
K <sub>M</sub> 2PG	0.113 mM	0.5 (chosen)	<a href="#">Krucinska et al., 2019</a>	yes
K <sub>M</sub> PEP	0.1 mM	0.5 (chosen)	<a href="#">Spring and Wold, 1971</a>	yes

## PPP - G6PDH2r

### Reaction equation



glucose 6-phosphate + NADP = 6-phospho-glucono-1,5-lactone + NADPH

BIGG: -1 g6p\_c -1 nadp\_c +1 6pgl\_c +1 nadph\_c

### Enzymes and genes

[EcoCyc](#) and [UniProt](#)

G6PDH2r (glucose 6-phosphate dehydrogenase) is a monomeric enzyme encoded by the *zwf* gene and located in the cytosol. G6PDH2r has a strong preference for NADP<sup>+</sup> over NAD<sup>+</sup> ([Olavarria et al., 2012](#)). A  $\Delta zwf$  mutant grows, but the metabolic flux through CCM is altered which is why the *zwf* gene is a common metabolic engineering target. ([oxidative branch](#))

### Regulation

**Gene regulation:** MarA, Rob, and SoxS (DNA-binding transcription factors) activate transcription initiation of *zwf*. Fe<sup>2+</sup> binds Fur (DNA-binding transcription factor) to enable inhibition of transcription initiation of *zwf* and FNR (DNA-binding transcription factors) inhibits transcription initiation of *zwf*. Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *zwf*, thus an increase in fructofuranose 1-phosphate increases transcription of *zwf*.

**Activators:** none or unknown.

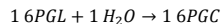
**Inhibitors:** Competitive product inhibition by NADPH to NADP<sup>+</sup> and not to G6P ([Sanwal, 1970](#); [Olavarria et al., 2012](#); [Christodoulou et al., 2018](#)). Allosteric inhibition by NADH ([Sanwal, 1970](#)).

### Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub>	174.0 1/s	2 1/s; 0.2 (chosen)	<a href="#">Olavarria et al., 2012</a>	yes
k <sub>cat</sub>	178.0 1/s	-	<a href="#">Fuentealba et al., 2016</a>	no
k <sub>app</sub> <sup>max</sup> x2	107.5 x2 = 215.0 1/s	-	Calculated from experimental fluxes and protein concentrations ( $\Delta$ ptsHlcr)	no
Formation energy G6P	-1304.7 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy 6PGL	-1377.7 kJ/mol	2.9 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy NADP <sup>+</sup>	-2033.6 kJ/mol	13.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy NADPH	-1967.4 kJ/mol	13.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
K <sub>M</sub> G6P	0.07 mM	-	<a href="#">Sanwal, 1970</a>	no
K <sub>M</sub> G6P	0.145 mM	-	<a href="#">Banerjee and Fraenkel, 1972</a>	no
K <sub>M</sub> G6P	0.2 mM	-	<a href="#">Westwood and Doelle, 1974</a>	no
K <sub>M</sub> G6P	0.174 mM	0.011 mM; 0.5 (chosen)	<a href="#">Olavarria et al., 2012</a>	yes
K <sub>M</sub> 6PGL	0.122 mM	1.0 (chosen)	Estimated from <a href="#">Banerjee and Fraenkel, 1972</a>	yes
K <sub>M</sub> NADP <sup>+</sup>	0.015 mM	-	<a href="#">Sanwal, 1970</a>	no
K <sub>M</sub> NADP <sup>+</sup>	0.015 mM	-	<a href="#">Banerjee and Fraenkel, 1972</a>	no
K <sub>M</sub> NADP <sup>+</sup>	0.02 mM	-	<a href="#">Westwood and Doelle, 1974</a>	no
K <sub>M</sub> NADP <sup>+</sup>	0.0075 mM	0.0008 mM; 0.5 (chosen)	<a href="#">Olavarria et al., 2012</a>	yes
K <sub>M</sub> NADP <sup>+</sup>	0.008 mM	-	<a href="#">Fuentealba et al., 2016</a>	no
K <sub>M</sub> NADPH	0.0168 mM	1.0 (chosen)	Estimated from <a href="#">Banerjee and Fraenkel, 1972</a>	yes
K <sub>i</sub> NADPH or K <sub>o</sub> T NADPH	0.035 mM	0.5 (chosen)	<a href="#">Christodoulou et al., 2018</a>	no
K <sub>i</sub> NADPH	0.014 mM	0.002 mM	<a href="#">Olavarria et al., 2012</a> , but estimated by <a href="#">Christodoulou et al., 2018</a>	no
K <sub>o</sub> T NADH	0.05 mM	1.0 (chosen)	Estimated from Lineweaver-Burk plot in <a href="#">Sanwal, 1970</a>	no
K <sub>o</sub> T 6PGC	0.05 mM	1.5 (chosen)	Realistic estimate of tense dissociation constant with large scale	yes

## PPP - PGL

### Reaction equation



6-phospho-glucono-1,5-lactone + H<sub>2</sub>O = 6-phosphogluconate

BIGG: -1 6pgl\_c (-1 h2o\_c) +1 6pgc\_c

### Enzymes and genes

[EcoCyc](#) and [UniProt](#)

PGL (6-phosphogluconolactonase) is a monomeric enzyme encoded by the *pgl* gene and located in the cytosol. A  $\Delta pgI$  mutant is viable, but has a decreased growth rate ([Kupor and Fraenkel, 1969](#); [Kupor and Fraenkel, 1972](#)). Non-enzymatic hydrolysis is possible, but much slower ( $1.8 \cdot 10^{-4}$  1/s; [Bauer et al., 1983](#)). (oxidative branch)

### Regulation

**Gene regulation:** L-leucine binds to Lrp (DNA-binding transcription factor) to block inhibition of transcription initiation of *pgl*.

**Activators:** none or unknown.

**Inhibitors:** none or unknown. (The *Zymomonas mobilis* enzyme is inhibited by G6P; [Scopes, 1985](#))

### Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub>	2070 1/s	-	<a href="#">Scopes, 1985</a> ( <i>Zymomonas mobilis</i> enzyme)	no
k <sub>cat</sub>	14.3 1/s	-	<a href="#">Bauer et al., 1983</a> (human enzyme)	no
k <sub>cat</sub>	472.0 1/s	0.2 (chosen)	<a href="#">Zimenkov et al., 2005</a> (calculated from specific activity)	yes
k <sub>appt</sub> <sup>max</sup> x2	248.0 x2 = 496.0 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations ( $\Delta sdhCB$ )	no
Formation energy 6PGL	-1377.7 kJ/mol	2.9 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy 6PGC	-1554.0 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
K <sub>M</sub> 6PGL	0.023 mM	0.002 mM; 1.0 (chosen)	<a href="#">Scopes, 1985</a> ( <i>Zymomonas mobilis</i> enzyme)	yes
K <sub>M</sub> 6PGL	0.83 mM	-	<a href="#">Bauer et al., 1983</a> (human enzyme)	no
K <sub>M</sub> 6PGC	10 mM	1.0 (chosen)	<a href="#">Scopes, 1985</a> ( <i>Zymomonas mobilis</i> enzyme)	yes

## PPP - GND

### Reaction equation



6-phospho-gluconate (gluconate 6-phosphate) + NADP = ribulose 5-phosphate + NADPH + CO<sub>2</sub>

BIGG: -1 6pgc\_c -1 nadp\_c +1 ru5p\_D\_c +1 nadph\_c +1 co2\_c

### Enzymes and genes

[EcoCyc](#) and [UniProt](#)

GND (phosphogluconate dehydrogenase) is a homodimeric enzyme encoded by the *gnd* gene and located in the cytosol. A *Δgnd* mutant is viable, but has a decreased growth rate ([Jiao et al., 2003](#); [Zhao et al., 2004](#)). GND cannot use NAD<sup>+</sup> in place of NADP<sup>+</sup> for the reaction. Reported conformational changes upon binding of NADP<sup>+</sup> ([Chen et al., 2010](#)). ([oxidative branch](#))

### Regulation

**Gene regulation:** GadE (DNA-binding transcription factors) activates transcription initiation of *gnd*. Fe<sup>2+</sup> binds Fur (DNA-binding transcription factor) to enable inhibition of transcription initiation of *gnd* and FNR (DNA-binding transcription factors) inhibits transcription initiation of *gnd*. Expression is strongly coupled to the growth rate.

**Activators:** none or unknown.

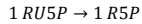
**Inhibitors:** Competitive inhibition by ATP to 6PGC and NADP ([De Silva and Fraenkel, 1979](#)) and competitive product inhibition by NADPH to NADP (Westwood and Doelle, 1974). Product inhibition by RU5P ([De Silva and Fraenkel, 1979](#)) and (allosteric) inhibition by FDP ([Wolf and Shea, 1979](#)). Possible inhibition by PEP ([Peskov et al., 2012](#))

### Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub>	21.1 1/s	0.2 (chosen)	<a href="#">Chen et al., 2010</a>	no
k <sub>cat</sub>	27.5 1/s	-	<a href="#">Veronese et al., 1976</a> (based on specific activity)	no
k <sub>appt</sub> <sup>max</sup>	63.7 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations (Δpgi)	yes
Formation energy 6PGC	-1554.0 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy RU5P	-1223.9 kJ/mol	1.9 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy NADP <sup>+</sup>	-2033.6 kJ/mol	13.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy NADPH	-1967.4 kJ/mol	13.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy CO <sub>2</sub>	-403.1 kJ/mol	5.7 kJ/mol	<a href="#">eQuilibrator</a>	yes
K <sub>M</sub> 6PGC	0.026 mM	-	<a href="#">Wolf and Shea, 1979</a>	no
K <sub>M</sub> 6PGC	0.05 mM	-	<a href="#">Veronese et al., 1976</a>	no
K <sub>M</sub> 6PGC	0.093 mM	0.001 mM; 0.5 (chosen)	<a href="#">Chen et al., 2010</a>	yes
K <sub>M</sub> RU5P	0.044 mM	0.5 (chosen)	Estimated from <a href="#">De Silva and Fraenkel, 1979</a>	yes
K <sub>M</sub> NADP <sup>+</sup>	0.011 mM	-	<a href="#">Wolf and Shea, 1979</a>	no
K <sub>M</sub> NADP <sup>+</sup>	0.033 mM	-	<a href="#">Veronese et al., 1976</a>	no
K <sub>M</sub> NADP <sup>+</sup>	0.049 mM	0.007 mM; 0.5 (chosen)	<a href="#">Chen et al., 2010</a>	yes
K <sub>M</sub> NADPH	0.0034 mM	0.5 (chosen)	Estimated from <a href="#">De Silva and Fraenkel, 1979</a>	yes
K <sub>M</sub> CO <sub>2</sub>	0.1 mM	2.0 (chosen)	unknown	yes
K <sub>i</sub> FDP	0.025 mM	-	<a href="#">De Silva and Fraenkel, 1979</a>	no

# PPP - RPI

## Reaction equation



ribulose 5-phosphate = ribose 5-phosphate

BIGG: -1 r5p\_c +1 ru5p\_D\_c

## Enzymes and genes

[EcoCyc RpiA](#), [EcoCyc RpiB](#) and [UniProt RpiA](#), [UniProt RpiB](#)

The RPI (ribose-5-phosphate isomerase) reaction consists of two isozymes: RpiA encoded by the *rpiA* gene and RpiB encoded by the *rpiB* gene. Both isozymes are present in the cytosol, however more than 99% of the total reaction activity in the wild type can be attributed to RpiA ([Skinner and Cooper, 1971](#)). RpiA is constitutive while RpiB is only present when induced by allose (RpiB is also involved in allose catabolism), but RpiB can substitute RpiA's function ([Skinner and Cooper, 1974](#); [Sørensen and Hove-Jensen, 1996](#)). Both enzymes are homodimeric. A  $\Delta rpiA$  mutant requires ribose for growth, while a  $\Delta rpiB$  mutant cannot catabolise allose. ([non-oxidative branch](#))

## Regulation

**Gene regulation:** L-leucine binds to Lrp (DNA-binding transcription factor) to block inhibition, while Lrp inhibits transcription initiation of *rpiA*, thus an increase in L-leucine increases transcription of *rpiA*. L-leucine binds to Lrp (DNA-binding transcription factor) to block activation, while Lrp activates transcription initiation of *rpiB*, thus an increase in L-leucine decreases transcription of *rpiB*. Glutarate binds to GlrA (DNA-binding transcription factor) to block activation, while GlrA activates transcription initiation of *rpiB*, thus an increase in glutarate decreases transcription of *rpiB*. Allose binds to AlsR (DNA-binding transcription factor) to block inhibition, while AlsR inhibits transcription initiation of *rpiB*, thus an increase in allose increases transcription of *rpiB*.

**Activators:** none or unknown.

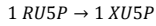
**Inhibitors:** Competitive inhibition of RpiA by arabinose 5-phosphate (A5P), E4P (erythrose 4-phosphate), erythronic acid (EA), and 4-phosphoerythronic acid (PEA) ([Zhang et al., 2003](#)). Inhibition of RpiA by AMP ([Essenberg and Cooper, 1975](#)). Inhibition of RpiB by iodoacetate ([Essenberg and Cooper, 1975](#)) and non-competitive inhibition by G6P (glucose 6-phosphate; [David and Wiesmeyer, 1970](#), not found in [Essenberg and Cooper, 1975](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{cat}$ RpiA	2100.0 1/s	300.0 1/s	<a href="#">Zhang et al., 2003</a>	yes
$k_{cat}$ RpiB	52.0 1/s	2 1/s	<a href="#">Roos et al., 2008</a>	no
$k_{app}^{max} \times 2$	114.6 x2 = 229.2 1/s	-	Calculated from experimental fluxes and protein concentrations ( $\Delta p_{tsHlcr}$ )	no
Formation energy RU5P	-1223.9 kJ/mol	1.9 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy R5P	-1226.0 kJ/mol	2.0 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ RU5P of RpiA	0.0035 mM	0.5 (chosen)	Estimated from <a href="#">Zhang et al., 2003</a>	yes
$K_M$ R5P of RpiA	6.2 mM	-	<a href="#">Skinner and Cooper, 1971</a>	no
$K_M$ R5P of RpiA	1.85 - 2.59 mM	-	<a href="#">David and Wiesmeyer, 1970</a>	no
$K_M$ R5P of RpiA	4.4 mM	0.5 mM	<a href="#">Essenberg and Cooper, 1975</a>	no
$K_M$ R5P of RpiA	3.1 mM	0.2 mM; 0.5 (chosen)	<a href="#">Zhang et al., 2003</a>	yes
$K_M$ RU5P of RpiB	0.0035 mM	-	Estimated from <a href="#">Zhang et al., 2003</a>	no
$K_M$ R5P of RpiB	0.95 mM	-	<a href="#">Skinner and Cooper, 1971</a>	no
$K_M$ R5P of RpiB	0.13 - 0.25 mM	-	<a href="#">David and Wiesmeyer, 1970</a>	no
$K_M$ R5P of RpiB	0.83 mM	0.13 mM	<a href="#">Essenberg and Cooper, 1975</a>	no
$K_M$ R5P of RpiB	1.1 mM	0.2 mM	<a href="#">Roos et al., 2008</a>	no
$K_i$ A5P of RpiA	2.1 mM	0.9 mM	<a href="#">Zhang et al., 2003</a>	no
$K_i$ E4P of RpiA	0.67 mM	-	<a href="#">Zhang et al., 2003</a>	no
$K_i$ EA of RpiA	0.32 mM	-	<a href="#">Zhang et al., 2003</a>	no
$K_i$ PEA of RpiA	0.004 mM	-	<a href="#">Zhang et al., 2003</a>	no
$K_i$ G6P of RpiB	0.060 - 0.085 mM	-	<a href="#">David and Wiesmeyer, 1970</a>	no

## PPP - RPE

### Reaction equation



ribulose 5-phosphate = xylulose 5-phosphate

BIGG: -1 ru5p\_D\_c +1 xu5p\_D\_c

### Enzymes and genes

[EcoCyc](#) and [UniProt](#)

RPE (ribulose 5-phosphate 3-epimerase) is a monomeric enzyme encoded by the *rpe* gene and located in the cytosol.  $\text{Fe}^{2+}$  is a cofactor and can be replaced by  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Zn}^{2+}$ . When  $\text{Fe}^{2+}$  is used as cofactor, the RPE enzyme is vulnerable to damage by  $\text{H}_2\text{O}_2$  (hydrogen peroxide; [Sobota and Imlay, 2011](#)). A *Δrpe* mutant only grows on minimal medium, when ribose and xylose are provided and has a strongly impaired growth rate on glucogenic substrates ([Lyngstadaas et al., 1998](#)). [\(non-oxidative branch\)](#)

### Regulation

Gene regulation: none or unknown.

Activators: none or unknown.

Inhibitors: none or unknown.

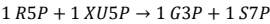
### Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{\text{cat}}$	67000 1/s	7700 1/s	<a href="#">Sobota and Imlay, 2011</a>	no
$k_{\text{cat}}$	3800.0 1/s	160 1/s	<a href="#">Chan et al., 2008</a> : with $\text{Co}^{2+}$	yes
$k_{\text{app}}^{\text{max}} \times 2$	289.1 $\times 2 = 578.2$ 1/s	-	Calculated from experimental fluxes and protein concentrations ( $\Delta \text{pgi}$ )	no
Formation energy RU5P	-1223.9 kJ/mol	1.9 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy XU5P	-1227.3 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_{\text{M}}$ Ru5P	1.6 mM	0.2 mM	<a href="#">Sobota and Imlay, 2011</a>	no
$K_{\text{M}}$ Ru5P	1.2 mM	0.1 mM	<a href="#">Chan et al., 2008</a> : with $\text{Co}^{2+}$	yes
$K_{\text{M}}$ Ru5P	1 mM	-	<a href="#">Hurwitz and Horecker, 1956</a> ; <i>Lactobacillus pentosus</i>	no
$K_{\text{M}}$ Ru5P	0.872 mM	-	Estimated from <a href="#">Hurwitz and Horecker, 1956</a>	no
$K_{\text{M}}$ XU5P	0.5 mM	0.5 (chosen)	<a href="#">Hurwitz and Horecker, 1956</a> ; <i>Lactobacillus pentosus</i>	yes
$K_{\text{M}}$ XU5P	0.893 mM	-	Estimated from <a href="#">Hurwitz and Horecker, 1956</a>	no



# PPP - TKT1

## Reaction equation



ribose 5-phosphate + xylulose 5-phosphate = glyceraldehyde 3-phosphate + sedoheptulose 7-phosphate

BIGG: -1 r5p\_c -1 xu5p\_D\_c +1 g3p\_c +1 s7p\_c

## Enzymes and genes

[EcoCyc TktA](#), [EcoCyc TktB](#) and [UniProt TktA](#), [UniProt TktB](#)

The TKT1 (transketolase 1) reaction consists of two isozymes: TktA (TKT I) encoded by the *tktA* gene and TktB (TKT II) encoded by the *tktB* gene. Both isozymes are present in the cytosol, however TktA is responsible for the major TKT activity ([Iida et al., 1993](#)). Overexpression of *tktB* and thus overproduction of TktB suppresses the *tktA* phenotype. TktA is a homodimeric enzyme, while TktB is a monomeric enzyme. TKT mutants cannot grow on pentoses and a  $\Delta tktA\Delta tktB$  double mutant requires pyridoxine, aromatic amino acids, and vitamins for growth. The TKT reaction is a common metabolic engineering target. Expression of *tktA* and *tktB* is complementary, resulting in approximately constant levels of transketolase expression throughout growth. TktA and TktB have high sequence similarity. Thiamine diphosphate (ThDP) and  $Mn^{2+}$  (or  $Mg^{2+}$  or  $Ca^{2+}$  or  $Co^{2+}$ ) are cofactors of TktA; one per subunit. TktA can use other substrates than in TKT1 and TKT2 (review: [Schenk et al., 1998](#)). (non-oxidative branch)

## Regulation

**Gene regulation:** none or unknown for TktA. Phosphate binds to PhoB (DNA-binding transcription factor) to enable activation of transcription initiation of *tktB*. DksA and ppGpp (under conditions of isoleucine starvation) activate binding of RNA polymerase to the promotor.

**Activators:** none or unknown.

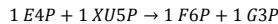
**Inhibitors:** Competitive inhibition of TktA by arabinose 5-phosphate (ASP; [Sprenger et al., 1995](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{cat}$ TktA	61.3 1/s	0.2 (chosen)	<a href="#">Sprenger et al., 1995</a> (calculated from specific activity)	yes
$k_{cat}$ TktA	84.6 1/s	-	<a href="#">Gyamerah and Willetts, 1997</a> (other substrates)	no
$k_{cat}$ TktB	61.3 1/s	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA; calculated from specific activity)	no
$k_{app}^{max} \times 2$	21.8 $\times 2$ = 43.6 1/s	-	Calculated from experimental fluxes and protein concentrations ( $\Delta pgi$ )	no
Formation energy R5P	-1226.0 kJ/mol	2.0 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy XU5P	-1227.3 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy G3P	-1091.5 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy S7P	-1365.6 kJ/mol	3.7 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ R5P of TktA	1.4 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ XU5P of TktA	0.16 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ G3P of TktA	2.1 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ S7P of TktA	4.0 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ R5P of TktB	1.4 mM	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA)	no
$K_M$ XU5P of TktB	0.16 mM	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA)	no
$K_M$ G3P of TktB	2.1 mM	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA)	no
$K_M$ S7P of TktB	4.0 mM	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA)	no
$K_i$ ASP of TktA	6.0 mM	-	<a href="#">Sprenger et al., 1995</a>	no

## PPP - TKT2

### Reaction equation



erythrose 4-phosphate + xylulose 5-phosphate = fructose 6-phosphate + glyceraldehyde 3-phosphate

BIGG: -1 e4p\_c -1 xu5p\_D\_c +1 f6p\_c +1 g3p\_c

### Enzymes and genes

[EcoCyc TktA](#), [EcoCyc TktB](#) and [UniProt TktA](#), [UniProt TktB](#)

The TKT1 (transketolase 2) reaction consists of two isozymes: TktA (TKT I) encoded by the *tktA* gene and TktB (TKT II) encoded by the *tktB* gene. Both isozymes are present in the cytosol, however TktA is responsible for the major TKT activity ([Iida et al., 1993](#)). Overexpression of *tktB* and thus overproduction of TktB suppresses the *tktA* phenotype. TktA is a homodimeric enzyme, while TktB is a monomeric enzyme. TKT mutants cannot grow on pentoses and a  $\Delta tktA \Delta tktB$  double mutant requires pyridoxine, aromatic amino acids, and vitamins for growth. The TKT reaction is a common metabolic engineering target. Expression of *tktA* and *tktB* is complementary, resulting in approximately constant levels of transketolase expression throughout growth. TktA and TktB have high sequence similarity. Thiamine diphosphate (ThDP) and  $\text{Mn}^{2+}$  (or  $\text{Mg}^{2+}$  or  $\text{Ca}^{2+}$  or  $\text{Co}^{2+}$ ) are cofactors of TktA; one per subunit. TktA can use other substrates than in TKT1 and TKT2 (review: [Schenk et al., 1998](#)). (non-oxidative branch)

### Regulation

**Gene regulation:** none or unknown for TktA. Phosphate binds to PhoB (DNA-binding transcription factor) to enable activation of transcription initiation of *tktB*. DksA and ppGpp (under conditions of isoleucine starvation) activate binding of RNA polymerase to the promoter.

**Activators:** none or unknown.

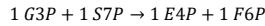
**Inhibitors:** Irreversible inhibition/inactivation of TktA by superoxide ([Benov and Fridovich, 1999](#)).

### Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{\text{cat}}$ TktA	133.8 1/s	0.2 (chosen)	<a href="#">Sprenger et al., 1995</a> (calculated from specific activity)	yes
$k_{\text{cat}}$ TktA	84.6 1/s	-	<a href="#">Gyamerah and Willetts, 1997</a> (other substrates)	no
$k_{\text{cat}}$ TktB	133.8 1/s	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA; calculated from specific activity)	no
$k_{\text{app}}^{\text{max}} \times 2$	18.8 $\times 2$ = 37.6 1/s	-	Calculated from experimental fluxes and protein concentrations ( $\Delta pgi$ )	no
Formation energy E4P	-1156.0 kJ/mol	3.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy XU5P	-1227.3 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy F6P	-1302.1 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy G3P	-1091.5 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ E4P of TktA	0.09 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ XU5P of TktA	0.16 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ F6P of TktA	1.1 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ G3P of TktA	2.1 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ E4P of TktB	0.09 mM	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA)	no
$K_M$ XU5P of TktB	0.16 mM	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA)	no
$K_M$ F6P of TktB	1.1 mM	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA)	no
$K_M$ G3P of TktB	2.1 mM	-	<a href="#">Sprenger et al., 1995</a> (TktB is similar to TktA)	no

# PPP - TALA

## Reaction equation



glyceraldehyde 3-phosphate + sedoheptulose 7-phosphate = erythrose 4-phosphate + fructose 6-phosphate

BIGG: -1 g3p\_c -1 s7p\_c +1 e4p\_c +1 f6p\_c

## Enzymes and genes

[EcoCyc TalaA](#), [EcoCyc TalaB](#) and [UniProt TalaA](#), [UniProt TalaB](#)

The TALA (transaldolase) reaction consists of two isozymes: TalaA encoded by the *talA* gene and TalaB encoded by the *talB* gene. Both isozymes are located in the cytosol, however only TalaB has been biochemically characterised and is likely the dominant isozyme. Both a  $\Delta talA$  or  $\Delta talB$  do not cause a growth defect. TalaB is a homodimeric enzyme, while TalaA is likely a monomeric enzyme. *talA* expression increases in early stationary phase just as expression of *talB* decreases. (non-oxidative branch)

## Regulation

**Gene regulation:** Phosphate binds to PhoB (DNA-binding transcription factor) to enable activation of transcription initiation of *talA*. DksA and ppGpp (under conditions of isoleucine starvation) activate binding of RNA polymerase to the promotor. None or unknown for *talB*.

**Activators:** none or unknown.

**Inhibitors:** Competitive inhibition of TalaA by FDP to F6P and uncompetitive inhibition by FDP to E4P ([Ogawa et al., 2016](#)). Competitive inhibition of TalaB by arabinose 5-phosphate (ASP), glyceraldehyde, and phosphate ([Sprenger et al., 1995](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{cat}$ TalaA	38.0 1/s	-	<a href="#">Ogawa et al., 2016</a> (calculated from specific activity)	no
$k_{cat}$ TalaB	46.7 1/s	0.2 (chosen)	<a href="#">Sprenger et al., 1995</a> (calculated from specific activity)	yes
$k_{cat}$ TalaB	72.0 1/s	-	<a href="#">Schörken et al., 1998</a>	no
$k_{cat}$ TalaB	79.4 1/s	-	<a href="#">Schörken et al., 2001</a>	no
$k_{cat}$ TalaB	53.0 1/s	6 1/s	<a href="#">Schneider et al., 2008</a>	no
$k_{cat}$ TalaB	46.7 1/s	-	<a href="#">Ogawa et al., 2016</a> (calculated from specific activity)	no
$k_{app}^{max} \times 2$	25.6 x2 = 51.2 1/s	-	Calculated from experimental fluxes and protein concentrations (WT)	no
Formation energy G3P	-1091.5 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy S7P	-1365.6 kJ/mol	3.7 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy E4P	-1156.0 kJ/mol	3.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy F6P	-1302.1 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ G3P of TalaA	0.038 mM	-	<a href="#">Sprenger et al., 1995</a> (TalaA is likely similar to TalaB)	no
$K_M$ S7P of TalaA	0.285 mM	-	<a href="#">Sprenger et al., 1995</a> (TalaA is likely similar to TalaB)	no
$K_M$ E4P of TalaA	0.14 mM	0.04 mM	<a href="#">Ogawa et al., 2016</a>	no
$K_M$ F6P of TalaA	1.5 mM	0.22 mM	<a href="#">Ogawa et al., 2016</a>	no
$K_M$ G3P of TalaB	0.038 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ S7P of TalaB	0.285 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ E4P of TalaB	0.09 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ E4P of TalaB	0.295 mM	-	<a href="#">Schörken et al., 1998</a>	no
$K_M$ E4P of TalaB	0.1 mM	-	<a href="#">Schörken et al., 2001</a>	no
$K_M$ E4P of TalaB	0.084 mM	0.01 mM	<a href="#">Ogawa et al., 2016</a>	no
$K_M$ F6P of TalaB	1.2 mM	0.5 (chosen)	<a href="#">Sprenger et al., 1995</a>	yes
$K_M$ F6P of TalaB	0.94 mM	-	<a href="#">Schörken et al., 1998</a>	no
$K_M$ F6P of TalaB	1.2 mM	-	<a href="#">Schörken et al., 2001</a>	no
$K_M$ F6P of TalaB	3.0 mM	0.2 mM	<a href="#">Schneider et al., 2008</a>	no
$K_M$ F6P of TalaB	0.9 mM	0.12 mM	<a href="#">Ogawa et al., 2016</a>	no
$K_i$ FDP of TalaA	3.2 mM	-	<a href="#">Ogawa et al., 2016</a>	no
$K_i$ FDP of TalaB	2.85 mM	-	<a href="#">Ogawa et al., 2016</a>	no
$K_i$ ASP of TalaB	0.05 mM	-	<a href="#">Sprenger et al., 1995</a>	no

# PPP - EDD

## Reaction equation



6-phosphogluconate = 2-dehydro-3-deoxygluconate 6-phosphate + H<sub>2</sub>O

BIGG: -1 6pgc\_c +1 2ddg6p\_c (+1 h2o\_c)

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

EDD (6-phosphogluconate dehydratase) is a monomeric enzyme encoded by the *edd* gene and is located in the cytosol. EDD is induced by gluconate and enables growth on gluconate. EDD requires and iron-sulfur cluster (4Fe-4S specifically) as a cofactor and is thus sensitive to superoxide and hydrogen peroxide. Reduced expression levels of the GroEL-GroES molecular chaperonin complex during steady-state growth increases the synthesis of EDD and EDA, suggesting a regulatory function. A *Δedd* mutant grows more slowly on gluconate. Mutations in *iscS* involved in iron-sulfur cluster biosynthesis result in decreased activity of EDD and a *ΔsufABCDSE* deletion mutant shows reduced EDD activity during iron starvation. The *edd* gene is a common metabolic engineering target. The enzyme EDD has not been biochemically well characterised. ([Entner-Doudoroff shunt](#))

## Regulation

**Gene regulation:** Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *edd*, thus an increase in fructofuranose 1-phosphate increases transcription of *edd*. Gluconate binds to GntR (DNA-binding transcription factor) to block inhibition, while GntR inhibits transcription initiation of *edd*, thus an increase in gluconate increases transcription of *edd*. KdGR (DNA-binding transcription factor) inhibits transcription initiation of *edd*.

**Activators:** none or unknown.

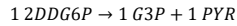
**Inhibitors:** Inhibition by superoxide (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and stabilised by fluoride ([Gardner and Fridovich, 1991](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub>	0.4 1/s	-	<a href="#">Wang and Dykhuizen, 2001</a> (calculated from specific activity)	no
k <sub>appt</sub> <sup>max</sup> x2	212.6 x2 = 425.2 1/s	-	Calculated from experimental fluxes and protein concentrations (Δtpi)	yes
Formation energy 6PGC	-1554.0 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy 2DDG6P	-1445.9 kJ/mol	4.6 kJ/mol	<a href="#">eQuilibrator</a>	yes
K <sub>M</sub> 6PGC	1.356 mM	0.1 mM	<a href="#">Wang and Dykhuizen, 2001</a>	no
K <sub>M</sub> 6PGC	0.6 mM	0.5 (chosen)	<a href="#">Kovachevich and Wood, 1955</a> ; <i>Pseudomonas putida</i>	yes
K <sub>M</sub> 2DDG6P	10 mM	1.5 (chosen)	Picked because of irreversible reaction	yes

## PPP - EDA

### Reaction equation



2-dehydro-3-deoxy-gluconate 6-phosphate = glyceraldehyde 3-phosphate + pyruvate

BIGG: -1 2ddg6p\_c +1 g3p\_c +1 pyr\_c

### Enzymes and genes

[EcoCyc](#) and [UniProt](#)

EDA (2-dehydro-3-deoxy-phosphogluconate or KDPG aldolase) is a homotrimeric enzyme encoded by the *eda* gene and is located in the cytosol. EDA has two additional functionalities, however at lower activity levels. EDA is induced by gluconate and enables growth on gluconate, but is constitutively expressed. A *Δeda* mutant cannot grow on gluconate. The *eda* gene is a common metabolic engineering target. KDPG is a toxic compound and stops growth. ([Entner-Doudoroff shunt](#))

### Regulation

**Gene regulation:** Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *eda*, thus an increase in fructofuranose 1-phosphate increases transcription of *eda*. Gluconate binds to GntR (DNA-binding transcription factor) to block inhibition, while GntR inhibits transcription initiation of *eda*, thus an increase in gluconate increases transcription of *eda*. KdgR (DNA-binding transcription factor) inhibits transcription initiation of *eda*. Phosphate binds to PhoB (DNA-binding transcription factor) to enable inhibition of transcription initiation of *eda*.

**Activators:** none or unknown.

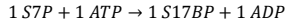
**Inhibitors:** Competitive inhibition by 6PGC (6-phosphogluconate) and product inhibition by G3P ([Pouysségur and Stoeber, 1971](#)).

### Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub>	283.8 1/s	3.4 1/s	<a href="#">Fong et al., 2000</a>	no
k <sub>cat</sub>	80.0 1/s	2 1/s	<a href="#">Walters et al., 2008</a>	no
k <sub>cat</sub>	83.0 1/s	2 1/s; 0.2 (chosen)	<a href="#">Cheriyen et al., 2007</a>	yes
k <sub>app</sub> <sup>max</sup> x2	37.4 x2 = 74.8 1/s	-	Calculated from experimental fluxes and protein concentrations (Δtpi)	no
Formation energy 2DDG6P	-1445.9 kJ/mol	4.6 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy G3P	-1091.5 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy PYR	-355.2 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	yes
K <sub>M</sub> 2DDG6P	0.2 mM	-	<a href="#">Pouysségur and Stoeber, 1971</a>	no
K <sub>M</sub> 2DDG6P	0.35 mM	0.01 mM	<a href="#">Fong et al., 2000</a>	no
K <sub>M</sub> 2DDG6P	0.1 mM	0.02 mM	<a href="#">Walters et al., 2008</a>	no
K <sub>M</sub> 2DDG6P	0.1 mM	0.01 mM; 0.5 (chosen)	<a href="#">Cheriyen et al., 2007</a>	yes
K <sub>M</sub> G3P	10 mM	1.5 (chosen)	<a href="#">Wymer et al., 2001</a> (similar to PYR, both products)	yes
K <sub>M</sub> PYR	10 mM	0.5 (chosen)	<a href="#">Wymer et al., 2001</a>	yes
K <sub>i</sub> 6PGC	0.8 mM	-	<a href="#">Pouysségur and Stoeber, 1971</a>	no
K <sub>i</sub> G3P	0.4 mM	-	<a href="#">Pouysségur and Stoeber, 1971</a>	no

## PPP - PFK\_3

### Reaction equation



sedoheptulose 7-phosphate + ATP = sedoheptulose 1,7-bisphosphate + ADP

BIGG: -1 s7p\_c -1 atp\_c +1 s17bp\_c +1 adp\_c

### Enzymes and genes

[EcoCyc](#) and [UniProt](#) same as PfkA

PFK\_3 (phosphofructokinase s7p) is a promiscuous activity of PfkA. This activity was discovered when the TALA reaction was blocked by deletion of talA and talB, and by feeding either xylose or gluconate as substrate ([Nakahigashi et al., 2009](#)). The activity only occurs when S7P accumulates in the cell (e.g. blocking of the TALA reaction) and would thus not likely occur in the wild type and is better to leave out of the model ([Riemer et al., 2013](#)). This promiscuous activity is slower than the native activity, so lower affinity for S7P as substrate compared to F6P. PfkA is a homotetrameric enzyme. A  $\Delta pfkA$  strain is viable and found to increase the NADPH production via the pentose phosphate pathway.  $Mg^{2+}$  is a cofactor of both PfkA. ([sedoheptulose biphosphate bypass](#))

### Regulation

**Gene regulation:** F6P (fructose 6-phosphate) binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *pfkA*, thus an increase in F6P increases transcription of *pfkA*.

**Activators:** Allosteric activation of PfkA by ADP (also GDP; [Blangy and Monod, 1968](#)) and by F6P ([Johnson and Reinhart, 1992](#)), and (product) activation of PfkA by FDP (fructose 1,6-bisphosphate) ([Blangy and Monod, 1968](#)).

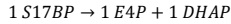
**Inhibitors:** Allosteric inhibition of PfkA by PEP (phosphoenolpyruvate) and by ATP ([Blangy and Monod, 1968](#); [Berger and Evans, 1991](#)).

### Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
$k_{app}^{max} \times 2$	67.6 x2 = 135.2 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations (WT)	yes
Formation energy S7P	-1365.6 kJ/mol	3.7 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy S17BP	-2257.1 kJ/mol	4.0 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy ATP	-2280.7 kJ/mol	2.9 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy ADP	-1405.9 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ S7P	0.16 mM	-	<a href="#">Karadsheh et al., 1973</a> ; rabbit liver	yes
$K_M$ S7P	0.6 mM	-	<a href="#">Karadsheh et al., 1973</a> ; rabbit muscle	no
$K_M$ S17BP	10 mM	1.5 (chosen)	Picked because of irreversible reaction	yes
$K_M$ ATP of PfkA	0.06 mM	0.5 (chosen)	<a href="#">Blangy and Monod, 1968</a>	yes
$K_M$ ATP of PfkA	0.06 mM	-	<a href="#">Berger and Evans, 1991</a>	no
$K_M$ ATP of PfkA	0.018 mM	-	<a href="#">Ogawa et al., 2007</a>	no
$K_M$ ATP of PfkA	0.057 mM	-	<a href="#">Auzat et al., 1994</a>	no
$K_M$ ATP of PfkA	0.041 mM	-	<a href="#">Hellinga and Evans, 1987</a>	no
$K_M$ ADP of PfkA	0.025 mM	0.5 (chosen)	<a href="#">Blangy and Monod, 1968</a>	yes
$K_D$ T PEP of PfkA	0.75 mM	1.0 (chosen)	<a href="#">Blangy and Monod, 1968</a>	no
$K_i$ PEP of PfkA	1.953 mM	-	<a href="#">Ogawa et al., 2007</a>	no
$K_i$ PEP of PfkA	0.30 mM	0.01 mM	<a href="#">Paricharttanakul et al., 2005</a>	no
$K_i$ ADP of PfkA	0.048 mM	0.002 mM	<a href="#">Paricharttanakul et al., 2005</a>	no

## PPP - FBA3

### Reaction equation



sedoheptulose 1,7-bisphosphate = erythrose 4-phosphate + dihydroxyacetone phosphate

BIGG: -1 s17bp\_c +1 e4p\_c +1 dhap\_c

### Enzymes and genes

[EcoCyc](#) and [UniProt](#) are the same as FbaA

FBA3 (sedoheptulose 1,7-bisphosphate D-glyceraldehyde-3-phosphate-lyase) is a promiscuous activity of FbaA. This activity was discovered when the TALA reaction was blocked by deletion of talA and talB, and by feeding either xylose or gluconate as substrate ([Nakahigashi et al., 2009](#)). The activity only occurs when S7P accumulates in the cell (e.g. blocking of the TALA reaction) and would thus not likely occur in the wild type and is better to leave out of the model ([Riemer et al., 2013](#)). This promiscuous activity is slower than the native activity, so lower affinity for S17BP as substrate compared to FDP. FbaA is a homodimeric enzyme. A  $\Delta fbaA$  mutant is viable, but has a heat-sensitive defect in rRNA transcription.  $\text{Zn}^{2+}$  is a cofactor of FbaA. ([sedoheptulose biphosphate bypass](#))

### Regulation

**Gene regulation:** Fructofuranose 1-phosphate binds to Cra (DNA-binding transcription factor) to block inhibition, while Cra inhibits transcription initiation of *fbaA*, thus an increase in fructofuranose 1-phosphate increases transcription of *fbaA*. cAMP binds to CRP (DNA-binding transcription factor) to enable activation and CRP activates transcription initiation of *fbaA*.

**Activators:** Activation of FbaA by  $\text{NH}_4$  (ammonium) and  $\text{K}^+$  ([Blom et al., 1996](#)).

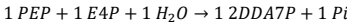
**Inhibitors:** Inhibition of FbaA by  $\text{Ni}^{2+}$  (nickel; [Macomber et al., 2011](#)) and product inhibition of FbaA by DHAP (competitive; dihydroxyacetone phosphate; [Plater et al., 1999](#)) and G3P (uncompetitive). Competitive inhibition of FbaA by 2-phosphoglycolate (2PGly; [Qamar et al., 1996](#)).

### Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{\text{cat}}$	0.052 1/s	-	<a href="#">Nakahara et al., 2003</a> ; yeast (calculated from specific activity)	no
$k_{\text{app}}^{\text{max}} \times 2$	17.9 $\times 2$ = 35.8 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations (WT)	yes
Formation energy S17BP	-2257.1 kJ/mol	4.0 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy E4P	-1156.0 kJ/mol	3.4 kJ/mol	<a href="#">eQuilibrator</a>	yes
Formation energy DHAP	-1097.2 kJ/mol	1.1 kJ/mol	<a href="#">eQuilibrator</a>	yes
$K_M$ S17BP	0.23 mM	0.5 (chosen)	<a href="#">Flechner et al., 1999</a> ; <i>Cyanophora paradoxa</i>	yes
$K_M$ S17BP	0.625 mM	-	<a href="#">Nakahara et al., 2003</a> ; yeast	no
$K_M$ E4P	0.088 mM	1.5 (chosen)	<a href="#">Babul et al., 1993</a> (maybe the same as DHAP, the other product)	yes
$K_M$ DHAP of FbaA	0.03 mM	0.002 mM	<a href="#">Zgiby et al., 2002</a> (WT)	no
$K_M$ DHAP of FbaA	0.088 mM	0.5 (chosen)	<a href="#">Babul et al., 1993</a>	yes
$K_i$ DHAP of FbaA	0.13 mM	0.011 mM	<a href="#">Plater et al., 1999</a>	no
$K_i$ G3P of FbaA	0.6 mM	-	<a href="#">Babul et al., 1993</a>	no
$K_i$ 2PGly of FbaA	0.009 mM	0.0012 mM	<a href="#">Qamar et al., 1996</a>	no

# Shikimate - DDPA

## Reaction equation



phosphoenolpyruvate + erythrose 4-phosphate + H<sub>2</sub>O = 2-dehydro-3-deoxyarabino-heptulosonate 7-phosphate + phosphate

BIGG: -1 pep\_c -1 e4p\_c (-1 h2o\_c) +1 2dda7p\_c +1 pi\_c

## Enzymes and genes

[EcoCyc Tyr](#), [EcoCyc Phe](#), [EcoCyc Trp](#) and [UniProt Tyr](#), [UniProt Phe](#), [UniProt Trp](#)

The DDPA (3-deoxy-7-phosphoheptulonate synthase) reaction consists of three isozymes: DDPA Tyr-sensitive encoded by the *aroF* gene, DDPA Phe-sensitive encoded by the *aroG* gene, and DDPA Trp-sensitive encoded by the *aroH* gene. DDPA Tyr is an homodimeric enzyme, DDPA Phe is an homotetrameric enzyme, DDPA Trp is an homodimeric enzyme, and all isozymes are located in the cytosol. There is a high degree of sequence identity (41%) between the three isozymes and the polypeptides are nearly identical in size. DDPA Phe makes up about 80% of the total DDPA activity, DDPA Tyr makes up about 20%, and DDPA Trp makes up about 1% ([Tribe et al., 1976](#)). All three isozymes are metalloenzymes and require a divalent metal for catalysis and/or structural integrity: DDPA Tyr can use several metal ions, DDPA Phe uses Fe<sup>2+</sup> mostly, and DDPA Trp is activated by Fe<sup>2+</sup>. Certain mutations in all three isozymes lead to insensitivity towards the corresponding aromatic amino acid. The role of metal ion in DDPA is to position the amino acids with the appropriate geometry required to coordinate and activate the water molecule; the rate constant varies with the bound metal ion ([Furdui et al., 2004](#)).

## Regulation

**Gene regulation:** SoxR (DNA-binding transcription factor) activates transcription initiation of *aroF*, Nac (DNA-binding transcription factor) inhibits transcription initiation of *aroF*, and L-tyrosine binds to TyrR (DNA-binding transcription factor) to enable inhibition and TyrR inhibits transcription initiation of *aroF*. L-leucine binds to Lrp (DNA-binding transcription factor) to block either activation or inhibition of transcription initiation of *aroG* by Lrp. Phosphate binds to CpxR (DNA-binding transcription factor) to enable activation and CpxR activates transcription initiations of *aroG*, while L-phenylalanine binds to TyrR (DNA-binding transcription factor) to enable inhibition and TyrR inhibits transcription initiation of *aroG*. Competitive inhibition of L-tyrosine and L-phenylalanine binding to TyrR. L-tryptophan binds to TrpR (DNA-binding transcription factor) to enable inhibition and TrpR inhibits transcription initiation of *aroH*.

**Activators:** none or unknown.

**Inhibitors:** Allosteric (potential) inhibition of DDPA Tyr by L-tyrosine, noncompetitive inhibition of DDPA Tyr by phosphate and competitive for PEP (product) inhibition of DDPA Tyr by 2DDA7P (2-dehydro-3-deoxyarabino-heptulosonate 7-phosphate) ([Schoner and Herrmann, 1976](#)). Allosteric inhibition of DDPA Phe by L-phenylalanine ([McCandliss et al., 1978](#)) and inhibition of DDPA Phe by L-alanine and L-dihydrophenylalanine ([McCandliss et al., 1978](#)). Competitive inhibition of DDPA Phe by 2,3-bisphosphoglycerate, 2-phosphoglycerate (2PG; glycerate 2-phosphate), 3-methylphosphoenolpyruvate, and 3-propylphosphoenolpyruvate ([Simpson and Davidson, 1976](#)). (Noncompetitive, possibly allosteric) inhibition of DDPA Trp by L-tryptophan ([Ray and Bauerle, 1991](#); [Camakaris and Pittard, 1974](#)) and DDPA Trp follows non-Michaelis-Menten kinetics ([Akowski and Bauerle, 1997](#)).

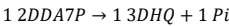
## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub> DDPA Tyr	60.3 1/s	-	<a href="#">Schoner and Herrmann, 1976</a> (based on specific activity)	no
k <sub>cat</sub> DDPA Tyr	29.5 1/s	0.2 (chosen)	<a href="#">Ramilo and Evans, 1997</a>	yes
k <sub>cat</sub> DDPA Phe	32.0 1/s	-	<a href="#">Sundaram et al., 1998</a>	no
k <sub>cat</sub> DDPA Phe	62.3 1/s	0.5 1/s; 0.2 (chosen)	<a href="#">Xu et al., 2004</a>	yes
k <sub>cat</sub> DDPA Phe	71.0 1/s	-	<a href="#">Williamson et al., 2005</a>	no
k <sub>cat</sub> DDPA Trp	20.6 1/s	1.1 1/s; 0.2 (chosen)	<a href="#">Akowski and Bauerle, 1997</a>	yes
Formation energy PEP	-1205.1 kJ/mol	1.6 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy E4P	-1156.0 kJ/mol	3.4 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy 2DDA7P	-1513.8 kJ/mol	2.7 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy Pi	-1072.6 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> PEP of DDPA Tyr	0.00585 mM	-	<a href="#">Schoner and Herrmann, 1976</a>	no
K <sub>M</sub> PEP of DDPA Tyr	0.013 mM	0.2 (chosen)	<a href="#">Ramilo and Evans, 1997</a>	yes
K <sub>M</sub> E4P of DDPA Tyr	0.0965 mM	-	<a href="#">Schoner and Herrmann, 1976</a>	no
K <sub>M</sub> E4P of DDPA Tyr	0.0814 mM	0.2 (chosen)	<a href="#">Ramilo and Evans, 1997</a>	yes
K <sub>M</sub> PEP of DDPA Phe	0.08 mM	0.04 mM	<a href="#">Simpson and Davidson, 1976</a>	no
K <sub>M</sub> PEP of DDPA Phe	0.009 mM	-	<a href="#">Sundaram et al., 1998</a>	no
K <sub>M</sub> PEP of DDPA Phe	0.035 mM	0.004 mM; 0.5 (chosen)	<a href="#">Xu et al., 2004</a>	yes
K <sub>M</sub> E4P of DDPA Phe	0.9 mM	0.3 mM	<a href="#">Simpson and Davidson, 1976</a>	no
K <sub>M</sub> E4P of DDPA Phe	0.086 mM	-	<a href="#">Sundaram et al., 1998</a>	no
K <sub>M</sub> E4P of DDPA Phe	0.25 mM	0.052 mM; 0.2 (chosen)	<a href="#">Xu et al., 2004</a>	yes
K <sub>M</sub> E4P of DDPA Phe	0.021 mM	-	<a href="#">Williamson et al., 2005</a>	no
K <sub>M</sub> (S <sub>0.5</sub> ) PEP of DDPA Trp	0.0053 mM	0.2 (chosen)	<a href="#">Akowski and Bauerle, 1997</a>	yes
K <sub>M</sub> E4P of DDPA Trp	0.076 mM	0.005 mM	<a href="#">Camakaris and Pittard, 1974</a>	no
K <sub>M</sub> (S <sub>0.5</sub> ) E4P of DDPA Trp	0.035 mM	0.2 (chosen)	<a href="#">Akowski and Bauerle, 1997</a>	yes
K <sub>M</sub> 2DDA7P	0.01 mM	2.0 (chosen)	Picked based on previous simulation	no
K <sub>M</sub> Pi	1.0 mM	1.5 (chosen)	Picked (weakly informative)	no
K <sub>i</sub> (K <sub>D</sub> T) Tyr of DDPA Tyr	0.009 mM	0.2 (chosen)	<a href="#">Ramilo and Evans, 1997</a>	yes
K <sub>i</sub> Tyr of DDPA Tyr	0.082 mM	-	<a href="#">McCandliss et al., 1978</a>	no
K <sub>i</sub> (K <sub>D</sub> T) PHE of DDPA Phe	0.013 mM	0.2 (chosen)	<a href="#">McCandliss et al., 1978</a>	yes
K <sub>i</sub> 2PG of DDPA Phe	1.0 mM	0.1 mM	<a href="#">Simpson and Davidson, 1976</a>	no
K <sub>i</sub> (K <sub>D</sub> T) Trp of DDPA Trp	0.0014 mM	0.2 (chosen)	<a href="#">Akowski and Bauerle, 1997</a>	yes



# Shikimate - DHQS

## Reaction equation



2-dehydro-3-deoxyarabino-heptulosonate 7-phosphate = 3-dehydroquininate + phosphate

BIGG: -1 2dda7p\_c +1 3dhq\_c +1 pi\_c

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

DHQS (3-dehydroquininate synthase) is a monomeric enzyme encoded by the *aroB* gene and is located in the cytosol. DHQS requires multiple cofactors: NAD<sup>+</sup> and Zn<sup>2+</sup> or Co<sup>2+</sup>. Co<sup>2+</sup> as cofactor results in higher specific activity, Zn<sup>2+</sup> is more readily available in nature.

## Regulation

**Gene regulation:** Glutarate binds to GlaR (DNA-binding transcription factor) to block inhibition, while GlaR inhibits transcription initiation of *aroB*, thus an increase in glutarate increases transcription of *aroB*. It is noteworthy that *aroB* is less regulated than the other genes in the shikimate pathway and the *aroB* expression is not repressed by chorismate, any of the aromatic amino acids or by the transcription factors TrpR and TyrR ([Tribe et al., 1976](#)).

**Activators:** none or unknown (apart from the cofactors; [Bender et al., 1989](#)).

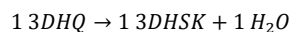
**Inhibitors:** Competitive inhibition by 2DDA7P (2-dehydro-3-deoxyarabino-heptulosonate 7-phosphate; substrate inhibition) and variants of 2DDA7P ([Myrvold et al., 1989](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub>	72.9 or 137.5 1/s	0.2 (chosen)	<a href="#">Bender et al., 1989</a> (calculated for Zn <sup>2+</sup> )	yes
k <sub>cat</sub>	16.0 1/s	0.2 1/s; 0.2 (chosen)	<a href="#">Negron and Parker, 2011</a>	no
Formation energy 2DDA7P	-1513.8 kJ/mol	2.7 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy 3DHQ	-608.7 kJ/mol	12.1 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy Pi	-1072.6 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> 2DDA7P	0.018 mM	-	<a href="#">Myrvold et al., 1989</a>	no
K <sub>M</sub> 2DDA7P	0.033 mM	-	<a href="#">Maitra and Sprinson, 1978</a>	no
K <sub>M</sub> 2DDA7P	0.0055 mM	0.2 (chosen)	<a href="#">Bender et al., 1989</a>	yes
K <sub>M</sub> 2DDA7P	0.0057 mM	0.5 (chosen)	<a href="#">Negron and Parker, 2011</a>	no
K <sub>M</sub> 3DHQ	0.002 mM	2.0 (chosen)	Picked based on previous simulation	no
K <sub>M</sub> Pi	1.0 mM	1.5 (chosen)	Picked (weakly informative)	no
K <sub>M</sub> NAD	80 nM	-	<a href="#">Bender et al., 1989</a>	no

# Shikimate - DHQTI

## Reaction equation



3-dehydroquinate = 3-dehydroshikimate + H<sub>2</sub>O

BIGG: -1 3dhq\_c +1 3dhsk\_c (+1 h2o\_c)

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

DQDH (3-dehydroquinate dehydratase) is an homodimeric enzyme encoded by the *aroD* gene and is located in the cytosol.

## Regulation

**Gene regulation:** Nac (DNA-binding transcription factor) inhibits transcription initiation of *aroD*.

**Activators:** none or unknown.

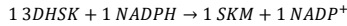
**Inhibitors:** Competitive inhibition by acetate, succinate, tartrate, and chloride; inhibition by diethylpyrocarbonate, and sodium borohydride ([Chaudhuri et al., 1986](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
k <sub>cat</sub>	135.0 1/s	-	<a href="#">Kleanthous et al., 1992</a>	no
k <sub>cat</sub>	142.0 1/s	2 1/s; 0.2 (chosen)	<a href="#">Leech et al., 1995</a>	yes
k <sub>cat</sub>	29.53 1/s	-	<a href="#">Liu et al., 2015</a>	no
Formation energy 3DHQ	-608.7 kJ/mol	12.1 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy 3DHSK	-463.0 kJ/mol	12.2 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> 3DHQ	0.018 mM	-	<a href="#">Chaudhuri et al., 1986</a>	no
K <sub>M</sub> 3DHQ	0.073 mM	-	<a href="#">Chaudhuri et al., 1986</a> (different buffer)	no
K <sub>M</sub> 3DHQ	0.44 mM	-	<a href="#">Mitsuhashi and Davis, 1954</a>	no
K <sub>M</sub> 3DHQ	0.016 mM	-	<a href="#">Kleanthous et al., 1992</a>	no
K <sub>M</sub> 3DHQ	0.017 mM	0.0009 mM; 0.2 (chosen)	<a href="#">Leech et al., 1995</a>	yes
K <sub>M</sub> 3DHQ	0.188 mM	-	<a href="#">Liu et al., 2015</a>	no
K <sub>M</sub> 3DHSK	0.004 mM	0.2 (chosen)	Picked based on previous simulation	yes
K <sub>i</sub> acetate	102 mM	-	<a href="#">Chaudhuri et al., 1986</a>	no
K <sub>i</sub> succinate	74 mM	-	<a href="#">Chaudhuri et al., 1986</a>	no
K <sub>i</sub> chloride	17 mM	-	<a href="#">Chaudhuri et al., 1986</a>	no
K <sub>i</sub> tartrate	21 mM	-	<a href="#">Chaudhuri et al., 1986</a>	no

# Shikimate - SHK3Dr

## Reaction equation



BIGG: -1 3dhsk\_c -1 nadph\_c +1 skm\_c +1 nadp\_c

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

SHK3Dr (shikimate dehydrogenase) is a monomeric enzyme encoded by the *aroE* gene and is located in the cytosol. Another SHK3Dr homodimeric enzyme is encoded by the *ydiB* gene and kinetic properties are available ([EcoCyc](#) and [UniProt](#)). The SHK3Dr from *aroE* is NADP<sup>+</sup>-specific and has much higher catalytic efficiency than the SHK3Dr from *ydiB*, which has broader substrate specificity and can use either NADP<sup>+</sup> or NAD<sup>+</sup> as a co-substrate ([Michel et al., 2003](#)). Mutant data and the results from metabolic engineering experiments strongly suggest that the SHK3Dr from *ydiB* is unable to replace the SHK3Dr from *aroE* under normal physiological conditions. An *ΔaroE* mutant is viable, but accumulates 3-dehydroshikimate in minimal medium and is able to grow on media supplemented with shikimate, phenylalanine, and tyrosine. In the "reverse" direction, AroE appears to be able to further dehydrogenate 3-dehydroshikimate to 3,5-dehydroshikimate, which can spontaneously convert to gallic acid ([Muir et al., 2011](#)). The physiological relevance is unclear.

## Regulation

**Gene regulation:** Phosphate binds to ZraR (DNA-binding transcription factor) to enable activation and ZraR activates transcription initiation of *aroE*. Nac (DNA-binding transcription factor) inhibits transcription initiation of *aroE*.

**Activators:** none or unknown.

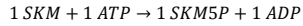
**Inhibitors:** (Linear) mixed inhibition by shikimate ([Dell and Frost, 1993](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub>	236.7 1/s	0.2 (chosen)	<a href="#">Michel et al., 2003</a>	no
k <sub>cat</sub>	8750.0 1/s	-	<a href="#">Muir et al., 2011</a>	no
k <sub>cat</sub>	190.0 1/s	5.0 1/s; 0.2 (chosen)	<a href="#">García-Guevara et al., 2017</a>	no
k <sub>cat</sub>	178.0 1/s	7.0 1/s; 0.2 (chosen)	<a href="#">Noble et al., 2006</a>	yes
Formation energy 3DHSK	-463.0 kJ/mol	12.2 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy SKM	-403.4 kJ/mol	12.3 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy NADP <sup>+</sup>	-2033.6 kJ/mol	13.4 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy NADPH	-1967.4 kJ/mol	13.4 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> 3DHSK	0.11 mM	0.5 (chosen)	<a href="#">Draths et al., 1999</a>	no
K <sub>M</sub> 3DHSK	0.11 mM	0.017 mM; 0.2 (chosen)	<a href="#">Noble et al., 2006</a>	yes
K <sub>M</sub> SKM	0.065 mM	0.5 (chosen)	<a href="#">Michel et al., 2003</a>	no
K <sub>M</sub> SKM	0.102 mM	-	<a href="#">Muir et al., 2011</a>	no
K <sub>M</sub> SKM	0.13 mM	0.013 mM	<a href="#">García-Guevara et al., 2017</a>	no
K <sub>M</sub> SKM	0.095 mM	0.010 mM	<a href="#">Noble et al., 2006</a>	no
K <sub>M</sub> NADP <sup>+</sup>	0.056 mM	-	<a href="#">Michel et al., 2003</a>	no
K <sub>M</sub> NADP <sup>+</sup>	0.347 mM	-	<a href="#">Muir et al., 2011</a>	no
K <sub>M</sub> NADP <sup>+</sup>	0.058 mM	0.007 mM; 0.5 (chosen)	<a href="#">García-Guevara et al., 2017</a>	no
K <sub>M</sub> NADP <sup>+</sup>	0.011 mM	0.0025 mM	<a href="#">Noble et al., 2006</a>	no
K <sub>M</sub> NADPH	0.0126 mM	0.0022 mM; 0.2 (chosen)	<a href="#">Noble et al., 2006</a>	yes
K <sub>i</sub> SKM	0.16 mM	-	<a href="#">Dell and Frost, 1993</a>	no

# Shikimate - SHKK

## Reaction equation



shikimate + ATP = shikimate 5-phosphate + ADP

BIGG: -1 skm\_c -1 atp\_c +1 skm5p\_c +1 adp\_c

## Enzymes and genes

[EcoCyc SHKK I](#), [EcoCyc SHKK II](#) and [UniProt SHKK I](#), [UniProt SHKK II](#)

The SHKK (shikimate kinase) reaction consists of two isozymes: SHKK I encoded by *aroK* and SHKK II encoded by *aroL*. Both enzymes are present in the cytosol, however SHKK I has approximately 100-fold lower affinity for shikimate ([DeFeyter and Pittard, 1986](#)) and a three-fold lower specific activity than SHKK II ([Ding et al., 2016](#)), so SHKK II is the major isozyme. SHKK I is relatively easy to isolate and is expressed constitutively compared to aromatic amino acid biosynthesis, so possibly other, still unknown, function. SHKK I and II are both monomeric enzymes which require  $\text{Mg}^{2+}$  as a cofactor. A double mutant  $\Delta\text{aroK}\Delta\text{aroL}$  cannot grow on minimal medium, even when supplemented with shikimate, L-phenylalanine and L-tyrosine.

## Regulation

**Gene regulation:** L-arginine binds to ArgR (DNA-binding transcription factor) to enable inhibition and ArgR inhibits transcription initiation of *aroK*, so no gene regulation by aromatic amino acids. L-tyrosine binds to TyrR (DNA-binding transcription factor) to enable inhibition and TyrR inhibits transcription initiation of *aroL* and L-tryptophan binds to TrpR (DNA-binding transcription factor) to enable inhibition and TrpR inhibits transcription initiation of *aroL*.

**Activators:** None or unknown.

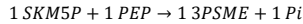
**Inhibitors:** Substrate inhibition of SHKK II by shikimate ([DeFeyter and Pittard, 1986](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
$k_{\text{cat}}$ SHKK I	6.0 1/s	-	<a href="#">Ding et al., 2016</a> (based on specific activity)	no
$k_{\text{cat}}$ SHKK II	21.8 1/s	-	<a href="#">Ding et al., 2016</a> (based on specific activity)	no
$k_{\text{cat}}$ SHKK II	32.0 1/s	0.2 (chosen)	<a href="#">DeFeyter and Pittard, 1986</a> (based on specific activity)	yes
Formation energy SKM	-403.4 kJ/mol	12.3 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy SKM5P	-1304.5 kJ/mol	11.2 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy ATP	-2280.7 kJ/mol	2.9 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy ADP	-1405.9 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	no
$K_{\text{M}}$ SKM of SHKK I	20 mM	1.5 (chosen)	<a href="#">DeFeyter and Pittard, 1986</a>	no
$K_{\text{M}}$ SKM of SHKK I	0.4 mM	-	<a href="#">Durante-Rodríguez et al., 2013</a>	no
$K_{\text{M}}$ SKM5P of SHKK I	-	-	-	no
$K_{\text{M}}$ ATP of SHKK I	-	-	-	no
$K_{\text{M}}$ ADP of SHKK I	-	-	-	no
$K_{\text{M}}$ SKM of SHKK II	0.2 mM	0.2 (chosen)	<a href="#">DeFeyter and Pittard, 1986</a>	yes
$K_{\text{M}}$ SKM5P of SHKK II	0.01 mM	2.0 (chosen)	Based on simulations	no
$K_{\text{M}}$ ATP of SHKK II	0.16 mM	0.2 (chosen)	<a href="#">DeFeyter and Pittard, 1986</a>	yes
$K_{\text{M}}$ ADP of SHKK II	0.02 mM	1.5 (chosen)	Based on other $K_{\text{M}}$ values	no

# Shikimate - PSCVT

## Reaction equation



shikimate 5-phophate + phosphoenolpyruvate = 5-O-(1-Carboxyvinyl)-3-phosphoshikimate + phosphate

BIGG: -1 skm5p\_c -1 pep\_c +1 3psme\_c +1 pi\_c

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

PSCVT (3-phosphoshikimate 1-carboxyvinyltransferase or EPSP synthase) is a monomeric enzyme encoded by the *araA* gene and is located in the cytosol. A  $\Delta araA$  mutants are auxotrophic for the aromatic amino acids and are unable to grow on minimal media.

## Regulation

**Gene regulation:** L-leucine binds to Lrp (DNA-binding transcription factor) to block either inhibition or activation and Lrp inhibits or activates transcription initiation of *araA*, depending on other factors. cAMP binds to CRP (DNA-binding transcription factor) to enable inhibition and the CRP-cAMP complex inhibits transcription initiation of *araA*.

**Activators:** Activation by PEP (phosphoenolpyruvate; [Gruys et al., 1992](#)).

**Inhibitors:** Competitive inhibition of PEP by glyphosate ([Duncan et al., 1984](#)), inhibition by pyruvate ([Huynh, 1992](#)) and 3-bromopyruvate ([Huynh, 1991](#)) and product inhibition by 5-O-(1-Carboxyvinyl)-3-phosphoshikimate (3PSME or EPSP; [Duncan et al., 1984](#)).

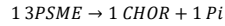
## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
$k_{cat}$	56.7 1/s	0.8 1/s	<a href="#">Gruys et al., 1992</a>	no
$k_{cat}$	17.2 1/s	-	<a href="#">Duncan et al., 1984</a> (based on specific activity)	no
$k_{cat}$	41.2 1/s	-	<a href="#">Huynh, 1987</a> (based on specific activity)	no
$k_{cat}$	50.6 1/s	0.2 (chosen)	<a href="#">Lewendon and Coggins, 1987</a> (based on specific activity)	yes
$k_{cat}$	8.2 1/s	-	<a href="#">Shuttleworth and Evans, 1996</a> (based on specific activity)	no
$k_{cat}$	26.4 1/s	-	<a href="#">Haghani et al., 2008</a>	no
$k_{cat}$	14.0 1/s	1 1/s	<a href="#">Berti and Chindemi, 2009</a>	no
$k_{cat}$	40.8 1/s	0.8 1/s	<a href="#">Healy-Fried et al., 2007</a> (based on specific activity)	no
$k_{cat}$	30.5 1/s	-	<a href="#">Eschenburg et al., 2002</a> (based on specific activity)	no
$k_{cat}$	46.6 1/s	0.8 1/s	<a href="#">Funke et al., 2009</a> (based on specific activity)	no
$k_{cat}$	43.3 1/s	2.5 1/s	<a href="#">Priestman et al., 2005</a> (based on specific activity)	no
Formation energy SKM5P	-1304.5 kJ/mol	11.2 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy PEP	-1205.1 kJ/mol	1.6 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy 3PSME	-1453.5 kJ/mol	11.8 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy Pi	-1072.6 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	no
$K_M$ SKM5P	0.0032 mM	0.0002 mM	<a href="#">Gruys et al., 1992</a>	no
$K_M$ SKM5P	0.0025 mM	-	<a href="#">Duncan et al., 1984</a>	no
$K_M$ SKM5P	0.020 mM	-	<a href="#">Huynh, 1987</a>	no
$K_M$ SKM5P	0.0025 mM	0.2 (chosen)	<a href="#">Lewendon and Coggins, 1987</a>	yes
$K_M$ SKM5P	0.135 mM	-	<a href="#">Shuttleworth and Evans, 1996</a>	no
$K_M$ SKM5P	0.14 mM	0.02 mM	<a href="#">Haghani et al., 2008</a>	no
$K_M$ SKM5P	0.060 mM	0.006 mM	<a href="#">Healy-Fried et al., 2007</a>	no
$K_M$ SKM5P	0.008 mM	0.004 mM	<a href="#">Shuttleworth et al., 1999</a>	no
$K_M$ SKM5P	0.12 mM	-	<a href="#">Eschenburg et al., 2002</a>	no
$K_M$ SKM5P	0.048 mM	0.005 mM	<a href="#">Funke et al., 2009</a>	no
$K_M$ SKM5P	0.09 mM	0.005 mM	<a href="#">Priestman et al., 2005</a>	no
$K_M$ PEP	0.021 mM	0.001 mM	<a href="#">Gruys et al., 1992</a>	no
$K_M$ PEP	0.016 mM	-	<a href="#">Duncan et al., 1984</a>	no
$K_M$ PEP	0.025 mM	-	<a href="#">Huynh, 1987</a>	no
$K_M$ PEP	0.022 mM	0.006 mM	<a href="#">He et al., 2003</a>	no
$K_M$ PEP	22.5 mM	6.32 mM	<a href="#">He et al., 2001</a>	no
$K_M$ PEP	0.016 mM	0.2 (chosen)	<a href="#">Lewendon and Coggins, 1987</a>	yes
$K_M$ PEP	0.1 mM	-	<a href="#">Shuttleworth and Evans, 1996</a>	no
$K_M$ PEP	0.1 mM	-	<a href="#">Haghani et al., 2008</a>	no
$K_M$ PEP	0.16 mM	0.02 mM	<a href="#">Haghani et al., 2008</a>	no
$K_M$ PEP	0.060 mM	0.006 mM	<a href="#">Healy-Fried et al., 2007</a>	no
$K_M$ PEP	0.013 mM	0.004 mM	<a href="#">Shuttleworth et al., 1999</a>	no
$K_M$ PEP	0.088 mM	-	<a href="#">Eschenburg et al., 2002</a>	no
$K_M$ PEP	0.045 mM	0.005 mM	<a href="#">Funke et al., 2009</a>	no

K <sub>M</sub> PEP	0.10 mM	0.004 mM	<a href="#">Priestman et al., 2005</a>	no
K <sub>M</sub> 3PSME	0.003 mM	-	<a href="#">Duncan et al., 1984</a>	no
K <sub>M</sub> 3PSME	0.003 mM	0.2 (chosen)	<a href="#">Lewendon and Coggins, 1987</a>	yes
K <sub>M</sub> 3PSME	0.011 mM	-	<a href="#">Shuttleworth and Evans, 1996</a>	no
K <sub>M</sub> 3PSME	0.010 mM	0.005 mM	<a href="#">Shuttleworth et al., 1999</a>	no
K <sub>M</sub> Pi	2.5 mM	-	<a href="#">Duncan et al., 1984</a>	no
K <sub>M</sub> Pi	2.5 mM	0.2 (chosen)	<a href="#">Lewendon and Coggins, 1987</a>	yes
K <sub>M</sub> Pi	4.6 mM	-	<a href="#">Shuttleworth and Evans, 1996</a>	no
K <sub>M</sub> Pi	5 mM	2 mM	<a href="#">Shuttleworth et al., 1999</a>	no
K <sub>i</sub> glyphosate	0.009 mM	-	<a href="#">Duncan et al., 1984</a>	no
K <sub>i</sub> glyphosate	0.0015 mM	0.0005 mM	<a href="#">He et al., 2003</a>	no
K <sub>i</sub> glyphosate	0.96 mM	0.4 mM	<a href="#">He et al., 2001</a>	no
K <sub>i</sub> glyphosate	0.009 mM	-	<a href="#">Lewendon and Coggins, 1987</a>	no
K <sub>i</sub> glyphosate	0.0012 mM	-	<a href="#">Shuttleworth and Evans, 1996</a>	no
K <sub>i</sub> glyphosate	0.0004 mM	-	<a href="#">Healy-Fried et al., 2007</a>	no
K <sub>i</sub> glyphosate	0.00013 mM	-	<a href="#">Shuttleworth et al., 1999</a>	no
K <sub>i</sub> glyphosate	0.0003 mM	-	<a href="#">Funke et al., 2009</a>	no

# Shikimate - CHORS

## Reaction equation



5-O-(1-Carboxyvinyl)-3-phosphoshikimate + phosphate = chorismate + phosphate

BIGG: -1 3psme\_c +1 chor\_c +1 pi\_c

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

CHORS (chorismate synthase) is a homotetrameric enzyme encoded by the *aroC* gene and is located in the cytosol. The flavin FMNH<sub>2</sub> is required as cofactor for CHORS, FADH<sub>2</sub> works as well but FMNH<sub>2</sub> is favoured. The CHORS enzyme is inactive under aerobic conditions, because it is oxygen sensitive. A  $\Delta$ *aroC* mutant is unable to grow in minimal medium.

## Regulation

Gene regulation: none or unknown.

Activators: none or unknown. Structural (conformational) changes upon flavin and substrate binding ([Macheroux et al., 1998](#)). "The line through the data points assumes dissociation of the active tetramer to two inactive dimers ( $K_d = 0.25$  nM) on dilution" ([Ramjee et al., 1994](#))

Inhibitors: Competitive inhibition of 3PSME by 6-fluoro-3PSME variants ([Osborne et al., 2000](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
$k_{\text{cat}}$	16.5 1/s	0.2 (chosen)	<a href="#">Ramjee et al., 1994</a>	no
$k_{\text{cat}}$	29.0 1/s	3 1/s; 0.2 (chosen)	<a href="#">Bornemann et al., 1996</a>	no
$k_{\text{app}}^{\text{max}} \times 2$	30.3 x 2 = 60.6 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations (wt)	yes
Formation energy 3PSME	-1453.5 kJ/mol	11.8 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy CHOR	-443.6 kJ/mol	15.0 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy Pi	-1072.6 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	no
$K_M$ 3PSME	0.0013 mM	0.0003 mM; 0.2 (chosen)	<a href="#">Ramjee et al., 1994</a>	yes
$K_M$ CHOR	0.01 mM	2.0 (chosen)	Based on simulations	no
$K_M$ Pi	2.5 mM	1.5 (chosen)	Based on other $K_M$ values of PSCVT	no

# Phe/Tyr - CHORM

## Reaction equation

1 CHOR → 1 PPHN

chorismate = prephenate

BIGG: -1 chor\_c +1 pphn\_c

## Enzymes and genes

[EcoCyc PheA](#), [EcoCyc TyrA](#) and [UniProt PheA](#), [UniProt TyrA](#)

The CHORM (chorismate mutase) reaction consists of two (iso)enzymes: PheA encoded by the *pheA* gene and TyrA encoded by the *tyrA* gene. Both enzymes are homodimeric and are located in the cytosol. PheA is a fused (bifunctional) chorismate mutase/prephenate dehydratase and thus catalyses both reactions. The native enzyme is a dimer of identical subunits each containing a dehydratase active site, a mutase active site and a phenylalanine binding site. Prephenate, which is formed from chorismate, dissociates from the mutase site and equilibrates with the bulk medium before combining at the dehydratase site ([Duggleby et al., 1978](#)). TyrA is a fused (bifunctional) chorismate mutase/prephenate dehydrogenase and thus catalyses both reactions. The two catalytic activities of TyrA occur in separate portions of the protein. Specifically, the chorismate mutase activity requires the amino-terminal portion of the protein, and the prephenate dehydrogenase activity is in the carboxy-terminal portion of the protein. A mol of dimerised TyrA can bind roughly one mol of NAD<sup>+</sup>, one mol of tyrosine, or one mol of prephenate. Both PheA and TyrA are common targets for metabolic engineering to increase titers of phenylalanine and tyrosine, respectively.

## Regulation

**Gene regulation:** none or unknown for PheA. L-tyrosine binds to TyrR (DNA-binding transcription factor) to enable inhibition and the tyr-TyrR complex inhibits transcription initiation of *tyrA*. This complex is in turn inhibited by L-phenylalanine, thus an increase in L-phenylalanine increases transcription of *tyrA*. SoxS (DNA-binding transcription factors) activates transcription initiation of *tyrA*.

**Activators:** none or unknown.

**Inhibitors:** Allosteric inhibition of PheA by L-phenylalanine ([Baldwin and Davidson, 1981](#); [Dopheide et al., 1972](#)) and competitive inhibition of PheA by citrate ([Baldwin and Davidson, 1983](#)) and prephenate ([Gething and Davidson, 1977](#); [Duggleby et al., 1978](#)). Allosteric inhibition of TyrA by L-tyrosine ([Hudson et al., 1983](#); [Lütke-Eversloh and Stephanopoulos, 2005](#)), competitive inhibition by prephenate to chorismate ([Koch et al., 1972](#); [Rood et al., 1982](#)) and noncompetitive inhibition by citrate ([Rood et al., 1982](#)).

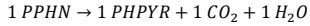
## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub> PheA	14.7 1/s	0.1 1/s; 0.2 (chosen)	<a href="#">Duggleby et al., 1978</a> (calculated from specific activity)	yes
k <sub>cat</sub> PheA	34.7 1/s	0.2 (chosen)	<a href="#">Baldwin and Davidson, 1981</a> (calculated from specific activity)	no
k <sub>cat</sub> PheA	40.0 1/s	-	<a href="#">Stewart et al., 1990</a> (calculated from specific activity; only CHORM)	no
k <sub>cat</sub> PheA	72.0 1/s	-	<a href="#">Liu et al., 1996</a> (only CHORM)	no
k <sub>cat</sub> PheA	41.4 1/s	4.8 1/s	<a href="#">Zhang et al., 1996</a> (only CHORM)	no
k <sub>cat</sub> PheA	39.0 1/s	4.3 1/s	<a href="#">Zhang et al., 1998</a>	no
k <sub>cat</sub> PheA	34.2 1/s	1.4 1/s	<a href="#">Zhang et al., 2000</a>	no
k <sub>cat</sub> PheA	38.9 1/s	5.1 1/s	<a href="#">Lassila et al., 2005</a>	no
k <sub>app</sub> <sup>max</sup> x2 PheA	31.0 x 2 = 62.0 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations (wt)	no
k <sub>cat</sub> TyrA	71.0 1/s	-	<a href="#">Hudson et al., 1983</a>	no
k <sub>cat</sub> TyrA	27.0 1/s	0.7 1/s; 0.2 (chosen)	<a href="#">Christendat et al., 1998</a>	yes
k <sub>app</sub> <sup>max</sup> x2 TyrA	29.1 x 2 = 58.2 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations ( $\Delta sdhCB$ )	no
Formation energy CHOR	-443.6 kJ/mol	15.0 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy PPHN	-492.2 kJ/mol	20.1 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> CHOR of PheA	0.045 mM	-	<a href="#">Dopheide et al., 1972</a>	no
K <sub>M</sub> CHOR of PheA	0.044 mM	0.009 mM	<a href="#">Gething and Davidson, 1977</a>	no
K <sub>M</sub> CHOR of PheA	0.024 mM	0.001 mM; 0.2 (chosen)	<a href="#">Duggleby et al., 1978</a>	yes
K <sub>M</sub> CHOR of PheA	0.031 mM	0.003 mM; 0.5 (chosen)	<a href="#">Baldwin and Davidson, 1983</a>	no
K <sub>M</sub> CHOR of PheA	0.29 mM	-	<a href="#">Stewart et al., 1990</a> (only CHORM)	no
K <sub>M</sub> CHOR of PheA	0.296 mM	0.019 mM	<a href="#">Liu et al., 1996</a> (only CHORM)	no
K <sub>M</sub> CHOR of PheA	0.3 mM	0.01 mM	<a href="#">Zhang et al., 1996</a> (only CHORM)	no
K <sub>M</sub> CHOR of PheA	0.226 mM	0.025 mM	<a href="#">Zhang et al., 1998</a>	no
K <sub>M</sub> CHOR of PheA	0.127 mM	0.019 mM	<a href="#">Zhang et al., 2000</a>	no
K <sub>M</sub> CHOR of PheA	0.304 mM	0.052 mM	<a href="#">Lassila et al., 2005</a>	no
K <sub>M</sub> PPHN of PheA	-	-	-	-
K <sub>M</sub> CHOR of TyrA	0.39 mM	-	<a href="#">Koch et al., 1971</a>	no
K <sub>M</sub> CHOR of TyrA	0.14 mM	0.01 mM	<a href="#">Rood et al., 1982</a>	no
K <sub>M</sub> CHOR of TyrA	0.092 mM	-	<a href="#">Hudson et al., 1983</a>	no
K <sub>M</sub> CHOR of TyrA	0.045 mM	0.007 mM; 0.2 (chosen)	<a href="#">Christendat et al., 1998</a>	yes
K <sub>M</sub> PPHN of TyrA	-	-	-	-
K <sub>D</sub> T PHE of PheA	0.02013 mM	0.003 mM; 0.2 (chosen)	<a href="#">Zhang et al., 1998</a>	yes
K <sub>i</sub> PPHN of PheA	0.047 mM	0.008 mM	<a href="#">Gething and Davidson, 1977</a>	no
K <sub>i</sub> PPHN of PheA	0.031 mM	0.002 mM	<a href="#">Duggleby et al., 1978</a>	no
K <sub>i</sub> CIT of PheA	1.01 mM	0.09 mM	<a href="#">Baldwin and Davidson, 1983</a>	no
K <sub>D</sub> T TYR of TyrA	0.01 mM	1.0 (chosen)	Weakly informative	yes



# Phenylalanine - PPNDH

## Reaction equation



prephenate = phenylpyruvate + CO<sub>2</sub> + H<sub>2</sub>O

BIGG: -1 pphn\_c +1 phpyr\_c (+1 co2\_c +1 h2o\_c)

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

PPNHD (prephenate dehydratase) is a homodimeric enzyme encoded by the *pheA* gene and is located in the cytosol. PheA is a fused (bifunctional) chorismate mutase/prephenate dehydratase and thus catalyses both reactions. The native enzyme is a dimer of identical subunits each containing a dehydratase active site, a mutase active site and a phenylalanine binding site. Prephenate, which is formed from chorismate, dissociates from the mutase site and equilibrates with the bulk medium before combining at the dehydratase site ([Duggleby et al., 1978](#)).

## Regulation

Gene regulation: none or unknown.

Activators: none or unknown.

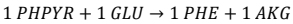
Inhibitors: Allosteric inhibition by L-phenylalanine ([Baldwin and Davidson, 1981](#); [Dopheide et al., 1972](#)), competitive inhibition by aconitate ([Baldwin and Davidson, 1983](#)) and inhibition by chorismate ([Duggleby et al., 1978](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub>	12.0 1/s	0.1 1/s	<a href="#">Duggleby et al., 1978</a> (calculated from specific activity)	no
k <sub>cat</sub>	21.3 1/s	0.2 (chosen)	<a href="#">Baldwin and Davidson, 1981</a> (calculated from specific activity)	yes
k <sub>cat</sub>	26.2 1/s	1 1/s	<a href="#">Zhang et al., 1998</a>	no
k <sub>cat</sub>	32.2 1/s	1.6 1/s	<a href="#">Zhang et al., 2000</a>	no
Formation energy PPHN	-492.2 kJ/mol	20.1 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy PHPYR	-20.0 kJ/mol	7.3 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy CO <sub>2</sub>	-403.1 kJ/mol	5.7 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> PPHN	1.0 mM	-	<a href="#">Dopheide et al., 1972</a>	no
K <sub>M</sub> PPHN	0.47 mM	0.01 mM; 0.2 (chosen)	<a href="#">Duggleby et al., 1978</a>	yes
K <sub>M</sub> PPHN	0.549 mM	0.058 mM	<a href="#">Zhang et al., 1998</a>	no
K <sub>M</sub> PPHN	0.559 mM	0.078 mM	<a href="#">Zhang et al., 2000</a>	no
K <sub>M</sub> PHPYR	-	-	-	-
K <sub>D</sub> T PHE of PheA	0.02013 mM	0.003 mM	<a href="#">Zhang et al., 1998</a>	no
K <sub>i</sub> CHOR	26.0 mM	10.4 mM	<a href="#">Duggleby et al., 1978</a>	no
K <sub>i</sub> PHPYR	4.6 mM	0.6 mM	<a href="#">Duggleby et al., 1978</a>	no

# Phenylalanine - PHETA1

## Reaction equation



phenylpyruvate + L-glutamate = L-phenylalanine + 2-oxoglutarate

BIGG: -1 phe\_l\_c -1 akg\_c +1 phpyr\_c +1 glu\_l\_c

## Enzymes and genes

[EcoCyc TyrB](#), [EcoCyc AspC](#), [EcoCyc IlvE](#) and [UniProt TyrB](#), [UniProt AspC](#), [UniProt IlvE](#)

The PHETA1 (phenylalanine transaminase) reaction consists of three isozymes: TyrB encoded by the *tyrB* gene, AspC encoded by the *aspC* gene and IlvE encoded by the *ilvE* gene. All isozymes are located in the cytosol. TyrB and AspC are homodimeric enzymes, while IlvE is a hexameric enzyme (a dimer of trimers). TyrB, AspC and IlvE are involved in catalyzing the third step of phenylalanine and tyrosine biosynthesis: all three can contribute to the synthesis of phenylalanine; only TyrB and AspC contribute to the biosynthesis of tyrosine. Under normal physiological conditions, TyrB is the primary enzyme contributing to the synthesis of tyrosine and phenylalanine. AspC contributes to their synthesis when substrate pools are large. The contribution of IlvE to phenylalanine biosynthesis was demonstrated in triple mutants of *Escherichia coli* K-12 that lacked all three aminotransferases and required both phenylalanine and tyrosine for growth. However, *tyrB* and *aspC* double mutants required only tyrosine for growth ([Gelfand and Steinberg, 1977](#)). TyrB is 1000-fold more active toward aromatic substrates than AspC ([Hayashi et al., 1993](#)). PLP (pyridoxal 5-phosphate) is a cofactor of TyrB, AspC (one per subunit) and IlvE. IlvE has very low activity for (tyrosine) and phenylalanine ([Lee-Peng et al., 1979](#)).

## Regulation

**Gene regulation:** L-tyrosine binds to TyrR (DNA-binding transcription factor) to enable inhibition and the *tyr*-TyrR complex inhibits transcription initiation of *tyrB*. Fe<sup>2+</sup> binds Fur (DNA-binding transcription factor) to enable inhibition of transcription initiation of *aspC* and FNR (DNA-binding transcription factors) activates or inhibits transcription initiation of *aspC*. L-leucine binds to Lrp (DNA-binding transcription factor) to block activation, while Lrp activates transcription initiation of *aspC*, thus an increase in L-leucine decreases transcription of *aspC*. L-leucine binds to Lrp (DNA-binding transcription factor) to enable inhibition and the Leu-Lrp complex inhibits transcription initiation of *ilvE*. ppGpp activates binding of RNA polymerase to the promoter of *ilvE* under conditions of stringent response.

**Activators:** none or unknown.

**Inhibitors:** Inhibition of TyrB by L-tyrosine ([Collier and Kohlhaw, 1972](#)), L-leucine ([Powell and Morrison, 1978](#)), and 3MOB (3-methyl-2-oxobutanoate; [Vartak et al., 1991](#)). Allosteric inhibition of AspC by 2-methylaspartate ([Okamoto et al., 1994](#)) and competitive inhibition of AspC by maleate ([Miyahara et al., 1994](#)). None or unknown for IlvE.

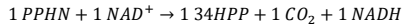
## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
k <sub>cat</sub> TyrB	26.7 1/s	-	<a href="#">Mavrides and Orr, 1974</a> (calculated from specific activity)	no
k <sub>cat</sub> TyrB	29.3 1/s	-	<a href="#">Gelfand and Steinberg, 1977</a> (calculated from specific activity)	no
k <sub>cat</sub> TyrB	13.2 1/s	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a> (calculated from specific activity)	yes
k <sub>cat</sub> TyrB	250.0 1/s	-	<a href="#">Hayashi et al., 1993</a>	no
k <sub>cat</sub> TyrB	520.0 1/s	-	<a href="#">Onuffer et al., 1995</a>	no
k <sub>cat</sub> TyrB	180.0 1/s	34 1/s	<a href="#">Luong and Kirsch, 1997</a>	no
k <sub>cat</sub> AspC	9.8 1/s	-	<a href="#">Mavrides and Orr, 1974</a> (calculated from specific activity)	no
k <sub>cat</sub> AspC	52.1 1/s	-	<a href="#">Gelfand and Steinberg, 1977</a> (probably non-aromatic substrate; calculated from specific activity)	no
k <sub>cat</sub> AspC	6.6 1/s	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a> (calculated from specific activity)	yes
k <sub>cat</sub> AspC	13.8 1/s	2.3 1/s	<a href="#">Han et al., 2001</a>	no
k <sub>cat</sub> IlvE	0.8 1/s	0.2 (chosen)	<a href="#">Lee-Peng et al., 1979</a> (calculated from specific activity)	yes
Formation energy PHPYR	-20.0 kJ/mol	7.3 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy GLU	-361.7 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy PHE	255.3 kJ/mol	5.7 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy AKG	-637.0 kJ/mol	2.6 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> PHPYR of TyrB	0.012 mM	-	<a href="#">Gelfand and Steinberg, 1977</a>	no
K <sub>M</sub> PHPYR of TyrB	0.056 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> GLU of TyrB	0.28 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> PHE of TyrB	0.333 mM	-	<a href="#">Mavrides and Orr, 1975</a>	no
K <sub>M</sub> PHE of TyrB	0.06 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> PHE of TyrB	0.26 mM	-	<a href="#">Hayashi et al., 1993</a>	no
K <sub>M</sub> PHE of TyrB	0.56 mM	0.03 mM	<a href="#">Luong and Kirsch, 1997</a>	no
K <sub>M</sub> AKG of TyrB	0.23 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> AKG of TyrB	1.7 mM	-	<a href="#">Hayashi et al., 1993</a>	no
K <sub>M</sub> AKG of TyrB	5 mM	1 mM	<a href="#">Luong and Kirsch, 1997</a>	no
K <sub>M</sub> PHPYR of AspC	3.9 mM	-	<a href="#">Gelfand and Steinberg, 1977</a>	no
K <sub>M</sub> PHPYR of AspC	0.65 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> GLU of AspC	0.9 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> GLU of AspC	15 mM		<a href="#">Yagi et al., 1985</a>	no
K <sub>M</sub> GLU of AspC	0.6 mM	-	<a href="#">Miyahara et al., 1994</a>	no
K <sub>M</sub> PHE of AspC	2.17 mM	-	<a href="#">Mavrides and Orr, 1975</a>	no
K <sub>M</sub> PHE of AspC	0.55 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> PHE of AspC	8 mM	3.6 mM	<a href="#">Han et al., 2001</a>	no
K <sub>M</sub> AKG of AspC	0.15 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes

K <sub>M</sub> AKG of AspC	0.24 mM		<a href="#">Yagi et al., 1985</a>	no
K <sub>M</sub> AKG of AspC	0.47 mM	0.01 mM	<a href="#">Toney and Kirsch, 1991</a>	no
K <sub>M</sub> AKG of AspC	0.59 mM	0.05 mM	<a href="#">Deu et al., 2002</a>	no
K <sub>M</sub> PHPYR of IlvE	-	-	-	no
K <sub>M</sub> GLU of IlvE	-	-	-	no
K <sub>M</sub> PHE of IlvE	-	-	-	no
K <sub>M</sub> AKG of IlvE	1.28 mM	-	<a href="#">Lee-Peng et al., 1979</a>	no
K <sub>i</sub> maleate	5.6 mM	0.5 mM	<a href="#">Toney and Kirsch, 1991</a>	no

# Tyrosine - PPND

## Reaction equation



prephenate + NAD<sup>+</sup> = 3-(4-hydroxyphenyl)pyruvate + CO<sub>2</sub> + NADH

BIGG: -1 pphn\_c -1 nad\_c +1 34hpp\_c +1 nadh\_c (+1 co2\_c)

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

PPND (prephenate dehydrogenase) is a homodimeric enzyme encoded by the *tyrA* gene and is located in the cytosol. TyrA is a fused (bifunctional) chorismate mutase/prephenate dehydrogenase and thus catalyses both reactions. The two catalytic activities of TyrA occur in separate portions of the protein. Specifically, the chorismate mutase activity requires the amino-terminal portion of the protein, and the prephenate dehydrogenase activity is in the carboxy-terminal portion of the protein. A mol of dimerised TyrA can bind roughly one mol of NAD<sup>+</sup>, one mol of tyrosine, or one mol of prephenate. Both PheA and TyrA are common targets for metabolic engineering to increase titers of phenylalanine and tyrosine, respectively.

## Regulation

**Gene regulation:** L-tyrosine binds to TyrR (DNA-binding transcription factor) to enable inhibition and the tyr-TyrR complex inhibits transcription initiation of *tyrA*. This complex is in turn inhibited by L-phenylalanine, thus an increase in L-phenylalanine increases transcription of *tyrA*. SoxS (DNA-binding transcription factors) activates transcription initiation of *tyrA*.

**Activators:** none or unknown.

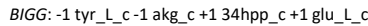
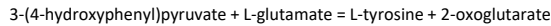
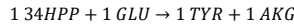
**Inhibitors:** Competitive inhibition by L-tyrosine to prephenate ([Hudson et al., 1983](#); [Lütke-Eversloh and Stephanopoulos, 2005](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub> TyrA	71.0 1/s	-	<a href="#">Hudson et al., 1983</a>	no
k <sub>cat</sub> TyrA	27.0 1/s	1 1/s; 0.2 (chosen)	<a href="#">Christendat et al., 1998</a>	yes
Formation energy PPHN	-492.2 kJ/mol	20.1 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy 34HPP	-183.2 kJ/mol	7.3 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy NAD <sup>+</sup>	-1146.0 kJ/mol	13.1 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy NADH	-1079.8 kJ/mol	13.1 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy CO <sub>2</sub>	-403.1 kJ/mol	5.7 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> PPHN	0.37 mM	-	<a href="#">Koch et al., 1971</a>	no
K <sub>M</sub> PPHN	0.13 mM	0.01 mM	<a href="#">Rood et al., 1982</a>	no
K <sub>M</sub> PPHN	0.044 mM	0.008 mM; 0.2 (chosen)	<a href="#">Christendat et al., 1998</a>	yes
K <sub>M</sub> 34HPP	-	-	-	-
K <sub>M</sub> NAD <sup>+</sup>	0.33 mM	-	<a href="#">Koch et al., 1971</a>	no
K <sub>M</sub> NAD <sup>+</sup>	0.05 mM	-	<a href="#">Hudson et al., 1983</a>	no
K <sub>M</sub> NAD <sup>+</sup>	0.103 mM	0.011 mM; 0.2 (chosen)	<a href="#">Christendat et al., 1998</a>	yes
K <sub>M</sub> NADH	-	-	-	-
K <sub>i</sub> TYR	0.1 mM	-	<a href="#">Hudson et al., 1983</a>	no

# Tyrosine - TYRTA

## Reaction equation



## Enzymes and genes

[EcoCyc TyrB](#), [EcoCyc AspC](#) and [UniProt TyrB](#), [UniProt AspC](#)

The TYRTA (tyrosine aminotransferase) reaction consists in two isozymes: TyrB encoded by the *tyrB* gene and AspC encoded by the *aspC* gene. Both isozymes are homodimeric enzymes and located in the cytosol. TyrB, AspC and IlvE are involved in catalyzing the third step of phenylalanine and tyrosine biosynthesis: all three can contribute to the synthesis of phenylalanine; only TyrB and AspC contribute to the biosynthesis of tyrosine. Under normal physiological conditions, TyrB is the primary enzyme contributing to the synthesis of tyrosine and phenylalanine. AspC contributes to their synthesis when substrate pools are large. The contribution of IlvE to phenylalanine biosynthesis was demonstrated in triple mutants of *Escherichia coli* K-12 that lacked all three aminotransferases and required both phenylalanine and tyrosine for growth. However, *tyrB* and *aspC* double mutants required only tyrosine for growth ([Gelfand and Steinberg, 1977](#)). TyrB is 1000-fold more active toward aromatic substrates than AspC ([Hayashi et al., 1993](#)). PLP (pyridoxal 5-phosphate) is a cofactor of TyrB and AspC (one per subunit).

## Regulation

**Gene regulation:** L-tyrosine binds to TyrR (DNA-binding transcription factor) to enable inhibition and the *tyr*-TyrR complex inhibits transcription initiation of *tyrB*. Fe<sup>2+</sup> binds Fur (DNA-binding transcription factor) to enable inhibition of transcription initiation of *aspC* and FNR (DNA-binding transcription factors) activates or inhibits transcription initiation of *aspC*. L-leucine binds to Lrp (DNA-binding transcription factor) to block activation, while Lrp activates transcription initiation of *aspC*, thus an increase in L-leucine decreases transcription of *aspC*.

**Activators:** none or unknown.

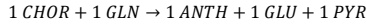
**Inhibitors:** Inhibition of TyrB by L-tyrosine ([Collier and Kohlhaw, 1972](#)), L-leucine ([Powell and Morrison, 1978](#)), and 3MOB (3-methyl-2-oxobutanoate; [Vartak et al., 1991](#)). Allosteric inhibition of AspC by 2-methylaspartate ([Okamoto et al., 1994](#)) and competitive inhibition of AspC by maleate ([Miyahara et al., 1994](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	yes	-	-	-
k <sub>cat</sub> TyrB	31.1 1/s	-	<a href="#">Mavrides and Orr, 1974</a> (calculated from specific activity)	no
k <sub>cat</sub> TyrB	31.5 1/s	-	<a href="#">Gelfand and Steinberg, 1977</a> (calculated from specific activity)	no
k <sub>cat</sub> TyrB	18.3 1/s	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a> (calculated from specific activity)	yes
k <sub>cat</sub> TyrB	210.0 1/s	-	<a href="#">Hayashi et al., 1993</a>	no
k <sub>cat</sub> TyrB	660.0 1/s	-	<a href="#">Onuffer et al., 1995</a>	no
k <sub>cat</sub> AspC	10.1 1/s	-	<a href="#">Mavrides and Orr, 1974</a> (calculated from specific activity)	no
k <sub>cat</sub> AspC	88.0 1/s	-	<a href="#">Gelfand and Steinberg, 1977</a> (probably non-aromatic substrate; calculated from specific activity)	no
k <sub>cat</sub> AspC	6.6 1/s	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a> (calculated from specific activity)	yes
Formation energy 34HPP	-183.2 kJ/mol	7.3 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy GLU	-361.7 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy TYR	91.7 kJ/mol	5.7 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy AKG	-637.0 kJ/mol	2.6 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> 34HPP of TyrB	0.013 mM	-	<a href="#">Gelfand and Steinberg, 1977</a>	no
K <sub>M</sub> 34HPP of TyrB	0.032 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> GLU of TyrB	0.28 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> TYR of TyrB	0.625 mM	-	<a href="#">Mavrides and Orr, 1975</a>	no
K <sub>M</sub> TYR of TyrB	0.042 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> TYR of TyrB	0.32 mM	-	<a href="#">Hayashi et al., 1993</a>	no
K <sub>M</sub> AKG of TyrB	0.23 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> AKG of TyrB	1.3 mM	-	<a href="#">Hayashi et al., 1993</a>	no
K <sub>M</sub> 34HPP of AspC	3.9 mM	-	<a href="#">Gelfand and Steinberg, 1977</a>	no
K <sub>M</sub> 34HPP of AspC	0.4 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> GLU of AspC	0.9 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> GLU of AspC	15 mM	-	<a href="#">Yagi et al., 1985</a>	no
K <sub>M</sub> GLU of AspC	0.6 mM	-	<a href="#">Miyahara et al., 1994</a>	no
K <sub>M</sub> TYR of AspC	1.43 mM	-	<a href="#">Mavrides and Orr, 1975</a>	no
K <sub>M</sub> TYR of AspC	0.45 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> AKG of AspC	0.15 mM	0.2 (chosen)	<a href="#">Powell and Morrison, 1978</a>	yes
K <sub>M</sub> AKG of AspC	0.24 mM	-	<a href="#">Yagi et al., 1985</a>	no
K <sub>M</sub> AKG of AspC	0.47 mM	0.01 mM	<a href="#">Toney and Kirsch, 1991</a>	no
K <sub>i</sub> maleate	5.6 mM	0.5 mM	<a href="#">Toney and Kirsch, 1991</a>	no

# Tryptophan - ANS

## Reaction equation



chorismate + L-glutamine = anthranilate + L-glutamate + pyruvate

BIGG: -1 chor\_c -1 gln\_L\_c +1 anth\_c +1 glu\_L\_c + pyr\_c

## Enzymes and genes

[EcoCyc](#) and [UniProt trpE](#), [UniProt trpGD](#)

The ANS (anthranilate synthase) reaction is catalysed by the TrpDE (TrpGDE) complex, a heterotetrameric enzyme (two trpD and two trpE subunits) located in the cytosol and encoded by the *trpD* and *trpE* genes. TrpE on its own can carry out an alternate version of this reaction, using ammonium sulfate rather than glutamine as an amino donor ([Ito et al., 1969](#)). However, TrpD dramatically increases the affinity of TrpE for glutamine over TrpE alone ([Ito and Yanofsky, 1969](#)). Mg<sup>2+</sup> is preferred as cofactor, but Co<sup>2+</sup> and Fe<sup>2+</sup> work as well. Both mutants are not viable on minimal medium. The complex is more thermostable for both the ANS and ANPRT reactions, than the corresponding individual components. Drawing of subunits and conformational changes in [Pabst et al., 1973](#).

## Regulation

**Gene regulation:** L-tryptophan binds to TrpR (DNA-binding transcription factor) to enable inhibition and TrpR inhibits transcription initiation of *trpE* and *trpD*. L-tryptophanyl (tRNA for L-tryptophan promotes premature termination of transcription. Translation of TrpE and TrpD is coordinated via a specialized intercistronic sequence between trpE and trpD. If the latter portion of the trpE mRNA is not translated, trpD mRNA translation is markedly reduced.

**Activators:** None or unknown.

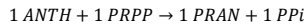
**Inhibitors:** Competitive inhibition of chorismate by L-tryptophan ([Baker and Crawford, 1966](#), [Pabst et al., 1973](#)) and 7-mehtyl-L-tryptophan ([Held and Smith, 1970](#)). Noncompetitive inhibition with respect to ammonium sulfate or L-glutamine by L-tryptophan ([Baker and Crawford, 1966](#)), trpE alone is inhibited by L-tryptophan (competitive to chorismate; [Ito et al., 1969](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>app</sub> <sup>max</sup> x2	2.27 x2 = 4.54 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations (ΔsdhCB)	yes
Formation energy CHOR	-443.6 kJ/mol	15.0 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy GLN	-104.9 kJ/mol	3.6 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy ANTH	-42.3 kJ/mol	5.7 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy GLU	-361.7 kJ/mol	2.4 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy PYR	-355.2 kJ/mol	1.5 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> CHOR	0.0055 mM	-	<a href="#">Ito and Yanofsky, 1969</a>	no
K <sub>M</sub> CHOR	0.005 mM	-	<a href="#">Pabst et al., 1973</a>	no
K <sub>M</sub> CHOR	0.0012 mM	0.2 (chosen)	<a href="#">Baker and Crawford, 1966</a>	yes
K <sub>M</sub> GLN	0.36 mM	0.2 (chosen)	<a href="#">Baker and Crawford, 1966</a>	yes
K <sub>M</sub> ANTH	-	-	-	-
K <sub>M</sub> GLU	-	-	-	-
K <sub>M</sub> PYR	-	-	-	-
K <sub>M</sub> NH <sub>4</sub> <sup>+</sup>	39 mM	-	<a href="#">Ito and Yanofsky, 1969</a>	no
K <sub>M</sub> CHOR of trpE	0.03 mM	-	<a href="#">Ito and Yanofsky, 1969</a>	no
K <sub>M</sub> NH <sub>4</sub> <sup>+</sup> of trpE	15 mM	-	<a href="#">Ito et al., 1969</a>	no
K <sub>M</sub> NH <sub>4</sub> <sup>+</sup> of trpE	25 mM	-	<a href="#">Ito and Yanofsky, 1969</a>	no
K <sub>i</sub> (K <sub>D</sub> T) TRP	0.001 mM	0.2(chosen)	<a href="#">Pabst et al., 1973</a>	no

# Tryptophan - ANPRT

## Reaction equation



anthranilate + 5-phosphoribose 1-diphosphate = N-(5-phosphoribosyl)anthranilate + diphosphate

BIGG: -1 anth\_c -1 prpp\_c +1 pran\_c +1 ppi\_c

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

ANPRT (anthranilate phosphoribosyltransferase) is a monomeric (?) enzyme encoded by the *trpD* gene or the TrpDE complex ([Jackson and Yanofsky, 1974](#)) and is located in the cytosol. The phosphoribosyl transferase and anthranilate synthase contributing portions of TrpD are present in different portions of the protein. The anthranilate synthase reaction requires the amino-terminal portion of the protein, whereas the phosphoribosyltransferase reaction requires the carboxy-terminal region ([Jackson and Yanofsky, 1974](#)). A *ΔtrpD* mutant is not viable in minimal medium.

## Regulation

**Gene regulation:** L-tryptophan binds to TrpR (DNA-binding transcription factor) to enable inhibition and TrpR inhibits transcription initiation of *trpE* and *trpD*. L-tryptophanyl (tRNA for L-tryptophan promotes premature termination of transcription. Translation of TrpE and TrpD is coordinated via a specialized intercistronic sequence between *trpE* and *trpD*. If the latter portion of the *trpE* mRNA is not translated, *trpD* mRNA translation is markedly reduced.

**Activators:** None or unknown.

**Inhibitors:** Competitive inhibition of PRPP by L-tryptophan ([Gonzalez and Somerville, 1986](#)) or noncompetitive inhibition of PRPP by L-tryptophan ([Ito and Yanofsky, 1969](#)) and of anthranilate by L-tryptophan ([Gonzalez and Somerville, 1986](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
k <sub>cat</sub>	6.6 1/s	-	<a href="#">Jackson and Yanofsky, 1974</a> (calculated from specific activity)	no
k <sub>cat</sub>	4.4 1/s	0.2 (chosen)	<a href="#">Gonzalez and Somerville, 1986</a> (calculated from specific activity)	yes
k <sub>appt</sub> <sup>max</sup> x2	2.27 x2 = 4.54 1/s	0.2 (chosen)	Calculated from experimental fluxes and protein concentrations (ΔsdhCB)	no
Formation energy ANTH	-42.3 kJ/mol	5.7 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy PRPP	-2978.9 kJ/mol	3.3 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy PRAN	-1038.7 kJ/mol	7.5 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy PPI	-1960.7 kJ/mol	2.2 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> ANTH	0.00058 mM	0.2 (chosen)	<a href="#">Gonzalez and Somerville, 1986</a>	yes
K <sub>M</sub> PRPP	0.1 mM	-	<a href="#">Ito and Yanofsky, 1969</a>	no
K <sub>M</sub> PRPP	0.05 mM	0.2 (chosen)	<a href="#">Gonzalez and Somerville, 1986</a>	yes
K <sub>M</sub> PRAN	-	-	-	-
K <sub>M</sub> PPI	-	-	-	-
K <sub>M</sub> PRPP of trpD	0.2 mM	-	<a href="#">Ito and Yanofsky, 1969</a>	no
K <sub>i</sub> TRP	0.0005 mM	-	<a href="#">Pabst et al., 1973</a>	no
K <sub>i</sub> TRP	0.0005 mM	-	<a href="#">Gonzalez and Somerville, 1986</a>	no

# Tryptophan - PRAI

## Reaction equation

1 PRAN → 1 2CPR5P

N-(5-phosphoribosyl)anthranilate = 1-(2-carboxyphenylamino)-1-deoxyribose 5-phosphate

BIGG: -1 pran\_c +1 2cpr5p\_c

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

PRAI (phosphoribosylanthranilate isomerase) is a monomeric enzyme encoded by the *trpC* gene and is located in the cytosol. TrpC catalyses both the PRAI and IGPS reaction and mutant complementation studies demonstrated that the two reactions occur at two distinct, non-overlapping sites on the polypeptide and that 2CPR5P is a free intermediate ([Creighton, 1970](#)). The amino-terminal domain carries out the synthase activity and the carboxy-terminal domain carries out the isomerase activity ([Eberhard and Kirschner, 1989](#)), so channeling of the intermediate substrate is not likely. TrpC is unique among the five enzymes in the tryptophan biosynthesis pathway in that it is not part of a multisubunit enzyme complex ([Christie and Platt, 1980](#)). A  $\Delta trpC$  mutant is not viable in minimal medium.

## Regulation

**Gene regulation:** L-tryptophan binds to TrpR (DNA-binding transcription factor) to enable inhibition and TrpR inhibits transcription initiation of *trpC*. L-tryptophanyl (tRNA for L-tryptophan promotes premature termination of transcription.

**Activators:** None or unknown.

**Inhibitors:** None or unknown.

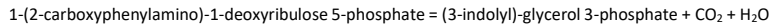
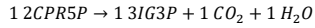
## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
$k_{cat}$	38.9 1/s	0.2 (chosen)	<a href="#">Eberhard and Kirschner, 1989</a>	yes
$k_{cat}$	40.0 1/s	-	<a href="#">Hommel et al., 1989</a>	no
$k_{cat}$	40.0 1/s	-	<a href="#">Eberhard et al., 1995</a>	no
Formation energy PRAN	-1038.7 kJ/mol	7.5 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy 2CPR5P	-1048.5 kJ/mol	7.1 kJ/mol	<a href="#">eQuilibrator</a>	no
$K_M$ PRAN	0.005 - 0.01 mM	-	<a href="#">Creighton, 1970</a>	no
$K_M$ PRAN	0.0071 mM	0.2 (chosen)	<a href="#">Eberhard and Kirschner, 1989</a>	yes
$K_M$ PRAN	0.0049 mM	-	<a href="#">Hommel et al., 1989</a>	no
$K_M$ PRAN	0.0049 mM	-	<a href="#">Eberhard et al., 1995</a>	no
$K_M$ 2CPR5P	-	-	-	-



# Tryptophan - IGPS

## Reaction equation



BIGG: -1 2cpr5p\_c +1 3ig3p\_c (+1 co2\_c +1 h2o\_c)

## Enzymes and genes

[EcoCyc](#) and [UniProt](#)

ISPS (indole-3-glycerol-phosphate synthase) is a monomeric enzyme encoded by the *trpC* gene and is located in the cytosol. TrpC catalyses both the PRAI and IGPS reaction and mutant complementation studies demonstrated that the two reactions occur at two distinct, non-overlapping sites on the polypeptide and that 2CPR5P is a free intermediate ([Creighton, 1970](#)). The amino-terminal domain carries out the synthase activity and the carboxy-terminal domain carries out the isomerase activity ([Eberhard and Kirschner, 1989](#)), so channeling of the intermediate substrate is not likely. TrpC is unique among the five enzymes in the tryptophan biosynthesis pathway in that it is not part of a multisubunit enzyme complex ([Christie and Platt, 1980](#)). A  $\Delta trpC$  mutant is not viable in minimal medium.

## Regulation

**Gene regulation:** L-tryptophan binds to TrpR (DNA-binding transcription factor) to enable inhibition and TrpR inhibits transcription initiation of *trpC*. L-tryptophanyl (tRNA for L-tryptophan promotes premature termination of transcription.

**Activators:** None or unknown.

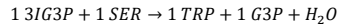
**Inhibitors:** Inhibition by anthranilate and derivatives ([Smith and Yanofsky, 1962](#)) and by rCdRP ([Priestle et al., 1987](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	-	-
$k_{cat}$	7.2 1/s	0.2 (chosen)	<a href="#">Eberhard and Kirschner, 1989</a>	yes
$k_{cat}$	3.6 1/s	-	<a href="#">Eberhard et al., 1995</a>	no
$k_{cat}$	2.7 1/s	-	<a href="#">Darimont et al., 1998</a>	no
Formation energy 2CPR5P	-1048.5 kJ/mol	7.1 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy 3IG3P	-584.0 kJ/mol	7.3 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy CO <sub>2</sub>	-403.1 kJ/mol	5.7 kJ/mol	<a href="#">eQuilibrator</a>	no
$K_M$ 2CPR5P	0.005 mM	-	<a href="#">Creighton and Yanofsky, 1966</a>	no
$K_M$ 2CPR5P	0.0012 mM	0.2 (chosen)	<a href="#">Eberhard and Kirschner, 1989</a>	yes
$K_M$ 2CPR5P	0.00042 mM	-	<a href="#">Eberhard et al., 1995</a>	no
$K_M$ 2CPR5P	0.0003 mM	-	<a href="#">Darimont et al., 1998</a>	no
$K_M$ 3IG3P	-	-	-	-
$K_M$ CO <sub>2</sub>	-	-	-	-

# Tryptophan - TRPS1

## Reaction equation



(3-indolyl)-glycerol 3-phosphate + L-serine = L-tryptophan + glyceraldehyde 3-phosphate + H<sub>2</sub>O

BIGG: -1 3ig3p\_c -1 ser\_l\_c +1 trp\_l\_c +1 g3p\_c (+1 h2o\_c)

## Enzymes and genes

[EcoCyc](#) and [UniProt trpA](#), [UniProt trpB](#)

The TRPS (tryptophan synthase) reaction is catalysed by the TrpAB complex, a heterotetrameric enzyme (two A subunits and one dimer of B) located in the cytosol and encoded by the *trpA* and *trpB* genes. The overall tryptophan synthase reaction consists of a sequence of two partial reactions. The α subunit of the complex carries out the aldol cleavage of 3IG3P to indole and G3P. The β subunit is responsible for the synthesis of L-tryptophan from indole and L-serine. The intermediate substrate (indole) is channeled through the enzyme complex and does not appear in solution. ([Miles et al., 1999](#)) The TrpA monomer is able to catalyse the first part of the reaction, however within the physiological complex with the B subunit, the reaction rate is increased by 1-2 orders of magnitude ([Lim et al., 1991](#), [Kirschner et al., 1991](#)). The TrpB dimer is seems to be able to catalyse the second part of the reaction ([Kaufmann et al., 1991](#)). PLP (pyridoxal 5-phosphate) is a cofactor of the enzyme complex, because two PLP molecules bind to the B dimer. Both mutants are not viable on minimal medium.

## Regulation

**Gene regulation:** L-tryptophan binds to TrpR (DNA-binding transcription factor) to enable inhibition and TrpR inhibits transcription initiation of *trpA* and *trpB*. L-tryptophanyl (tRNA for L-tryptophan promotes premature termination of transcription.

**Activators:** None or unknown.

**Inhibitors:** The partial reaction of the A subunit is competitively inhibited by indolepropanol phosphate ([Kirschner et al., 1975](#)).

## Reaction and enzyme constants

Parameter	Value	Uncertainty	Reference	In current model
Reversible	no	-	(only A part is reversible)	-
k <sub>cat</sub>	23.4 1/s	-	<a href="#">Jackson and Yanofsky, 1974</a> (calculated from specific activity)	no
k <sub>cat</sub>	1.5 1/s	-	<a href="#">Kirschner et al., 1991</a>	no
k <sub>cat</sub>	3.8 1/s	-	<a href="#">Banik et al., 1995</a>	no
k <sub>cat</sub>	1.4 1/s	0.2 (chosen)	<a href="#">Lane and Kirschner, 1991</a>	no
k <sub>cat</sub>	4.7 1/s	0.2 (chosen)	<a href="#">Anderson et al., 1995</a>	yes
k <sub>app</sub> <sup>max</sup> x2		-	Calculated from experimental fluxes and protein concentrations (WT)	no
Formation energy 3IG3P	-584.0 kJ/mol	7.3 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy SER	-233.5 kJ/mol	2.2 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy TRP	-393.5 kJ/mol	4.2 kJ/mol	<a href="#">eQuilibrator</a>	no
Formation energy G3P	-1091.5 kJ/mol	1.3 kJ/mol	<a href="#">eQuilibrator</a>	no
K <sub>M</sub> 3IG3P	0.03 mM	-	<a href="#">Kirschner et al., 1991</a>	no
K <sub>M</sub> 3IG3P	0.03 mM	-	<a href="#">Banik et al., 1995</a>	no
K <sub>M</sub> 3IG3P	0.069 mM	0.2 (chosen)	<a href="#">Lane and Kirschner, 1991</a>	yes
K <sub>M</sub> SER	14.5 mM	-	<a href="#">Crawford and Ito, 1964</a>	no
K <sub>M</sub> SER	0.34 mM	0.2 (chosen)	<a href="#">Lane and Kirschner, 1991</a>	yes
K <sub>M</sub> TRP	-	-	-	-
K <sub>M</sub> G3P	0.7 mM	-	<a href="#">Kirschner et al., 1991</a>	no