Isotopic model of the extracellular gluconolactone-gluconate by-pass in *Escherichia coli*

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Documentation

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1. Model overview

This model contains a coarse-grained representation of the central metabolism and of the extracellular gluconolactone-gluconate (GL-GNT) by-pass of *Escherichia coli*.

This model was developed with COPASI (http://copasi.org). All models are available in SBML and COPASI formats at https://github.com/MetaSys-LISBP/GL_GNT_bypass. The model can also be downloaded from the Biomodels database (http://www.ebi.ac.uk/biomodels) with identifier MODEL2310250001.

This model comprises 2 compartments (the environment and the cell), 9 species (8 metabolites and biomass) and 9 reactions that represent the following processes (Figure 1):

- glucose uptake
- glycolysis
- pentose phosphate pathway
- extracellular GL-GNT by-pass
- growth

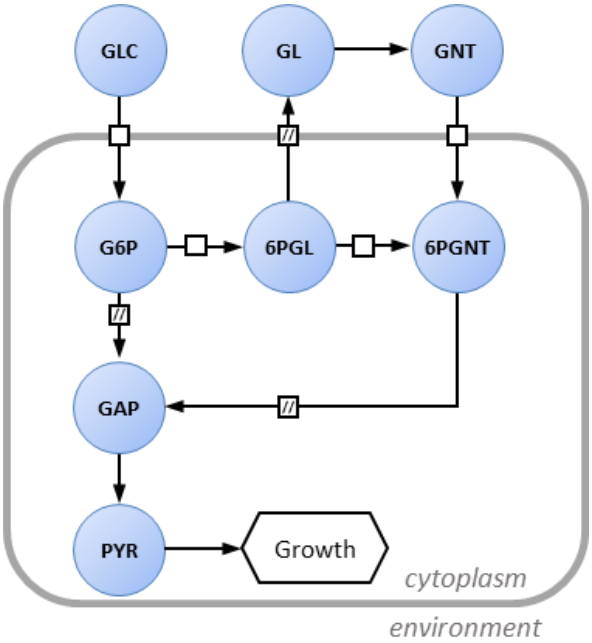

Figure 1. Metabolic network of *E. coli* metabolism. The diagram follows the conventions of the Systems Biology Graphical Notation process description.

2. Model units

Model units are millimole (mmol) for amounts of metabolites, litre (L) for volumes, and hour (h) for time. Amount of biomass is expressed as gram dry weight (g_{DW}).

3. Reactions

The reactions included in the model are listed in the table below.

Name	***Reaction***	***Flux***
r1_PTS	GLC → G6P	v1
r2_EMP_high	G6P → GAP	v2
r3_EMP_low	GAP → PYR	v3
r4_PYR_out	PYR → ∅	v4
r5_G6PDH	G6P → 6PGL	v5
r6_PGL	6PGL → 6PG	v6
r7_PPP	6PG → 1.66 * GAP	v7
r8_GL_export	6PGL → GL_{env}	v8
r9_GL_degradation	GL_{env} → GNT_{env}	v9
r10_GNT_uptake	GNT_{env} → 6PG	v10
r11_growth	X → 2 * X	v11

4. ODEs system

The differential equations, which describe the progression of the variables over time as a function of the system's rates, balance the concentrations of extracellular (biomass, glucose, gluconolactone and gluconate) and intracellular (G6P, 6PGL, 6PG, GAP and PYR) species.

Extracellular species:

$$\frac{dGLC}{dt} = v_1 \cdot X$$

$$\frac{dGL}{dt} = v_8 \cdot X - v_9$$

$$\frac{dGNT}{dt} = v_9 - X \cdot v_{10}$$

$$\frac{dX}{dt} = v_{11} \cdot X$$

Intracellular species:

$$\frac{dG6P}{dt} = v_1 - v_2 - v_5$$

$$\frac{d6PGL}{dt} = v_5 - v_6 - v_8$$

$$\frac{d6PG}{dt} = v_6 + v_{10} - v_7$$

$$\frac{dGAP}{dt} = 1.66 \cdot v_7 + 2 \cdot v_2 - v_3$$

$$\frac{dPYR}{dt} = v_3 - v_4$$

5. Reaction rates

Non-enzymatic degradation of extracellular gluconolactone was modelled using a first-order degradation rate law ($v_9 = k_{deg} \cdot [GL]$), as observed experimentally. Glucose uptake rate (v_1) and growth rate (v_{11}) were defined as constant. All other reaction rates were constrained to match the steady-state conditions (exponential growth) and balance all intracellular species. The following constraints were used:

$$v_2 = (1 - R_{glycolysis\_PPP}) \cdot v_1$$

$$v_3 = 2 \cdot v_2 + 1.66 \cdot v_7$$

$$v_4 = v_3$$

$$v_5 = R_{glycolysis\_PPP} \cdot v_1$$

$$v_6 = R_{bypass\_PPPintra} \cdot v_5$$

$$v_7 = v_{11} + v_6$$

$$v_8 = (1 - R_{bypass\_PGL}) \cdot v_5$$

where $R_{glycolysis\_PPP}$ is the flux partition between glycolysis and the oxidative pentose phosphate pathway (i.e. $\frac{v_5}{v_2+v_5}$) and R_{bypass_PGL} is the flux partition between PGL and the extracellular GL-GNT bypass (i.e. $\frac{v_6}{v_6+v_8}$).

Since no accumulation of gluconate was detected, we also balanced its concentration:

$$v_{10} = v_9$$

6. Extension with isotopic equations

This dynamic model was extended with isotopic equations as detailed previously (doi: 10.1186/s12918-015-0213-8). Briefly, all reactions (except biomass synthesis) were considered separately for unlabeled and labeled metabolites. For instance, the isotopically extended balance of G6P corresponds to:

$$\frac{dG6P_0}{dt} = \frac{GLC_0}{GLC_0 + GLC_1} \cdot v_1 - \frac{G6P_0}{G6P_0 + G6P_1} \cdot (v_2 + v_5)$$

$$\frac{dG6P_1}{dt} = \frac{GLC_1}{GLC_0 + GLC_1} \cdot v_1 - \frac{G6P_1}{G6P_0 + G6P_1} \cdot (v_2 + v_5)$$

where subscripts 0 and 1 refers to the unlabeled and labeled metabolite, respectively.

7. Pulse of ^{13}C-glucose

To simulate the isotopic dynamics in response to a pulse of ^{13}C-labeled glucose, we added an event which sets the concentration of ^{13}C-glucose (GLC_1) at time t_{pulse}.

8. Flux calculation

Initial concentrations of unlabelled intracellular metabolites were set to unity, and initial concentrations of labelled metabolites were set to zero. The degradation constant of GL was set to its experimental value ($k_{deg} = 2.58\ h^{-1}$). The flux partition between glycolysis and the oxidative pentose phosphate pathway was set to its experimental value ($R_{glycolysis\_PPP} = 0.17$, calculated by summing the relative contribution of the PPP to PYR biosynthesis determined by stationary ^{13}C-labeling experiments – 0.15 – and the accumulation rate of extracellular GL relative to glucose uptake rate – 0.17/8.52 = 0.02).

This model therefore contains 7 free parameters:

- initial concentrations of extracellular glucose (GLC_0) and biomass (X)
- time at which the pulse of ^{13}C-glucose is performed (t_{pulse})
- concentration of ^{13}C-glucose added during the pulse (GLC_1)
- glucose uptake rate (v_1) and growth rate (v_{11})
- flux partition between the extracellular GL-GNT by-pass and PGL (R_{bypass_PGL}).

These parameters were estimated by fitting experimental data (time course concentrations of unlabelled and labelled glucose and gluconolactone, and time course concentration of biomass), as detailed in the publication. Values and standard deviations obtained for each of the three independent biological replicates are provided below. All parameters could be determined with a good precision in each biological replicate. We recalculated all fluxes from the estimated parameters.

Parameter	*Replicate 1*		*Replicate 2*		*Replicate 3*		***mean[c]***	***sd[c]***
	value[a]	*sd[b]*	*value[a]*	*sd[b]*	*value[a]*	*sd[b]*		
GLC_0	15.59	0.13	15.00	0.10	15.04	0.09	**15.21**	**0.33**
X	0.057	0.003	0.052	0.002	0.051	0.002	**0.053**	**0.004**
GLC_1	12.2	0.2	9.3	0.2	10.2	0.2	**10.6**	**1.5**
t_{pulse}	5.2	0.1	5.0	0.1	5.0	0.1	**5.0**	**0.1**
R_{bypass_PGL}	0.001	0.027	0.001	0.026	0.001	0.026	**0.001**	**0.001**
v_1	7.75	0.15	7.04	0.12	7.10	0.13	**7.29**	**0.39**
v_{11}	0.395	0.007	0.412	0.006	0.410	0.006	**0.406**	**0.009**

[a]best fit obtained with COPASI

[b]standard deviation estimated by COPASI from the best fit

[c]mean and standard deviation of the three independent biological replicates