
Integrating Metabolic, Transcriptional Regulatory and Signal Transduction Models in *Escherichia coli*

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Table S1 lists the initial conditions, flux bounds, and additional parameters of the *E. coli* central metabolism iFBA, rFBA, and ODE models. The GLCex, G6Pex, and LCTSex bounds were generally not used in the iFBA simulation. These fluxes were set to that calculated by the ODE model, except where the ODE model was found to be numerically unstable in which case the iFBA modeled defaulted to the rFBA model, and the GLCex, G6Pex, and LCTSex bounds were used. Except where noted in Table S1, bounds for reversible fluxes were -500.0 and 500.0 and bounds for non-reversible fluxes were 0.0 and 500.0. Bounds listed in Table S1 were determined experimentally by fitting the iFBA model to experimental data (Bettenbrock *et al.*, 2007).

The metabolic reactions and boolean regulation of the iFBA model are described in Covert and Palsson, 2002, with the addition of two fluxes corresponding to glucose-6-phosphate uptake by UhpT and the removal of the reaction constraints on galEKMPT, lacYZ, and pykF, which are encapsulated by the ODE model. The kinetic model of the iFBA model, and the values of its parameters, are described in Kremling *et al.*, 2007.

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Table S1. Initial conditions, flux bounds, and additional parameters for iFBA, rFBA, and ODE simulations.

Parameter	Value			
	GLC/G6P		GLC/LCTS	
Initial Condition	GLC/G6P		GLC/LCTS	
Biomass (g/L)	0.032		0.032	
External Metabolites (m mol/L)				
acetate	0.0		0.0	
carbon dioxide	100.0		100.0	
ethanol	0.0		0.0	
formate	0.0		0.0	
galactose	0.0		0.0	
glucose-6-phosphate	1.3		0.0	
glucose	12.2		1.2	
lactate	0.0		0.0	
lactose	0.0		3.4	
oxygen	100.0		100.0	
phosphate	100.0		100.0	
pyruvate	0.0		0.0	
ribose	0.0		0.0	
succinate	0.0		0.0	
Internal Metabolites (μ mol/gDCW)				
glucose-6-phosphate	0.206		0.100	
phosphoenolpyruvate	0.381		0.050	
pyruvate	2.095		0.100	
Proteins (μ mol/gDCW)				
LacZ	0.0		0.00001	
UhpT	0.0003		0.0	
PtsG	0.007		0.001	
Protein Phosphorylation States (-)				
EIIA ^{Crr}	0.004		0.010	
	GLC/G6P Bound		GLC/LCTS Bound	
Flux	Lower	Upper	Lower	Upper
ACex	0.0	500.0	-0.3	5.0
ATPM	7.6	7.6	7.6	7.6
GLCex	-4.9	500.0	-7.0	500.0
G6Pex	-2.9	500.0	0.0	500.0
LCTSex	0.0	500.0	-5.0	500.0
CO2ex	-500.0	500.0	-500.0	500.0
O2ex	-6.9	500.0	-6.5	500.0
PIex	-10.0	500.0	-10.0	500.0
Growth rate scale, β	1.2			
Protein Synthesis-degradation delay	24 min			
Time step	3 min			

Figures S1 and 2 compare the iFBA, rFBA, and ODE predicted time courses of biomass and several metabolites and proteins for *E. coli* diauxic growth on glucose/glucose-6-phosphate.

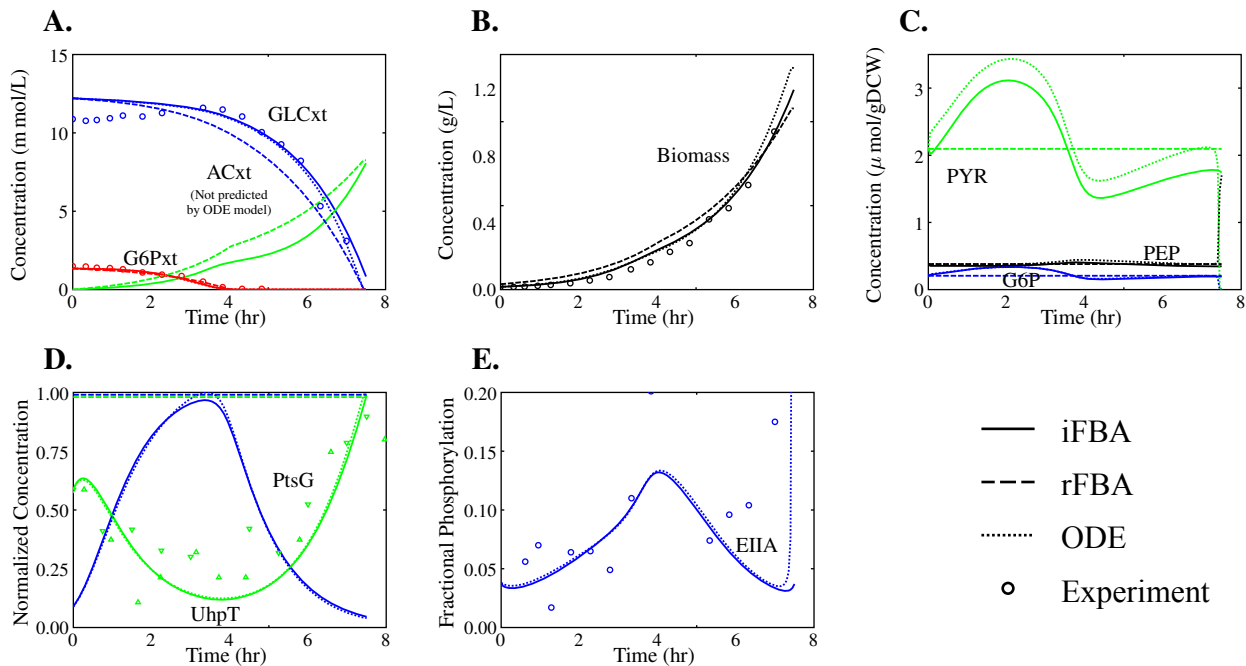
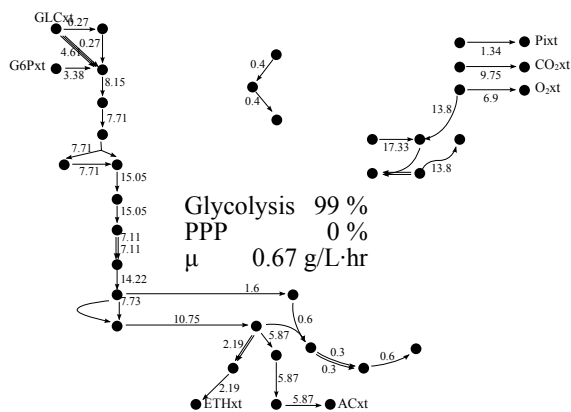


Fig. S1: Growth of the iFBA (solid lines), ODE (dotted), and rFBA (dashed) wild type models in an aerobic environment with glucose and glucose-6-phosphate as carbon sources, together with experimental data (Bettenbrock *et al.*, 2007) where available (circles). Dynamic time profiles of external (A) acetate, glucose, glucose-6-phosphate and (B) biomass concentrations; (C) internal glucose-6-phosphate, phosphoenolpyruvate, and pyruvate concentration; (D) key protein concentrations; and (E) degree of phosphorylation of regulatory protein EIIA^{Crr}.

A. Time = 1 hr



B. Time = 6 hr

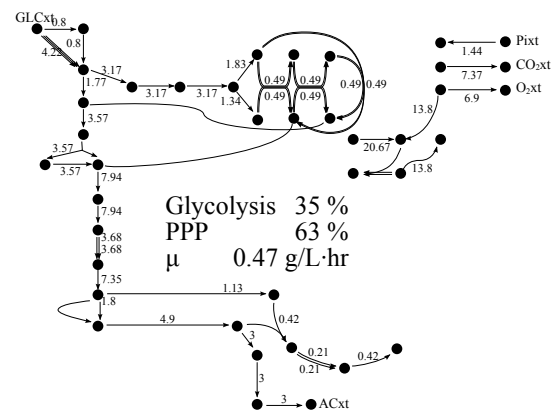


Fig. S2: Flux distributions for iFBA simulation of glucose/glucose-6-phosphate diauxic growth, at (A) one hour and (B) six hours. Detailed labels for the network are shown in Figure 1, and all values are in m mol/gDCW/hr.

Table S2 lists the kinetic parameter-gene correspondences of the *E. coli* central metabolism iFBA model.

Table S2. Genes required for full activity of each enzyme in the ODE model.

Enzyme/Parameter	Gene Products
$dEIIA/dt$	crp
k_1	uhpT
k_2	ptsG crr (galP & glk)
k_3	lacY & lacZ & (glk & (galE & galK & galM & galT & galU & pgm))
k_{gly}	pgi & (pfkA pfkB) & fbaA & gapA & pgk & (gpmA gpmB) & eno
k_{pdh}	(aceE & aceF & lpd) (pflA & pflB) (pflC & pflD)
k_{pts}	ptsG crr (galP & glk)
k_{pyk}	pykA pykF
x_o	crp

Table S3 lists the predicted phenotypes of all single gene perturbations for which all three models qualitatively predicted the same phenotype – healthy or unhealthy. Single gene perturbations with qualitatively different phenotypes predicted by the three models are illustrated in Figure 5.

Table S3. Single gene perturbations with the same predicted phenotype in each of the iFBA, rFBA, and ODE models. All enzymatic perturbations are knockdowns.

Healthy (> 80% wild type growth)				Unhealthy (< 80% wild type growth)			
Enzymes		Transcription Factors		Enzymes		Transcription Factors	
GLC/G6P	GLC/LCTS	GLC/G6P	GLC/LCTS	GLC/G6P	GLC/LCTS	GLC/G6P	GLC/LCTS
aceAB	aceAB	arcA=0,1	arcA=0,1	eno		crp=0	crp=0
ackA	ackA	cra=0,1	cra=0,1	fbaA	fbaA		
acnAB	acnAB	crp=1	crp=1		galEKMTU		
acs	acs	dcuRS=0,1	dcuRS=0,1	gapA			
actP	actP	fadR=0,1	fadR=0,1		glk		
adhE	adhE	galRS=0,1	galRS=0		lacYZ		
adk	adk	glpR=0,1	glpR=0,1	pgk			
crr	crr	iclR=0,1	iclR=0,1		pgm		
cydAB	cydAB	lacI=0,1	lacI=0				
cyoABCD	cyoABCD	mle=0,1	mle=0,1				
dctA	dctA	rbsR=0,1	rbsR=0,1				
dcuABC	dcuABC	rpiR=0,1	rpiR=0,1				
dld	dld						
fdnGHI	fdnGHI						
fdoGHI	fdoGHI						
fdp	fdp						
fnr	fnr						
focA	focA						
frdABCD	frdABCD						
fumABC	fumABC						
galEKMTU	galP						
glpABCDKF	glpABCDKF						
gnd	gnd						
gpmAB	gpmAB						
gpsA	gpsA						
lacYZ							
ldhA	ldhA						
maeAB	maeAB						
mdh	mdh						
ndh	ndh						
pck	pck						
pdhR	pdhR						
pfkAB	pfkAB						
pflABCD	pflABCD						
pgl	pgl						
pgm							
pntAB	pntAB						
ppa	ppa						
ppsA	ppsA						
pta	pta						
ptsGHI	ptsGHI						
pykAF	pykF						
rbsABCK	rbsABCK						
rpe	rpe						
rpiB	rpiB						
sdhABCD	sdhABCD						
sucABCD	sucABCD						
talAB	talAB						
tktAB	tktAB						
	uhpT						
zwf	zwf						

REFERENCES

- Bettenbrock, K., Sauter, T., Jahreis, K., Kremling, A., Lengeler, J.W., Gilles, E.D. (2007) Correlation between growth rates, EIIA^{Crr} phosphorylation, and intracellular cyclic AMP levels in *Escherichia coli* K-12, *J Bacteriol*, **189**, 6891-6900.
- Covert, M.W., Palsson, B.O. (2002) Transcriptional regulation in constraints-based metabolic models of *Escherichia coli*, *J Biol Chem*, **277**, 28058-28064.
- Kremling, A., Bettenbrock, K., Gilles, E.D. (2007) Analysis of global control of *Escherichia coli* carbohydrate uptake, *BMC systems biology*, **1**, 42.