Kinetic model of *Escherichia coli* central metabolism

- Documentation

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1. Model overview

The model developed in this work represents the central metabolic network of the bacterium *Escherichia coli*. It should be noted that when developing a model, certain criteria must be decided, such as the level of detail and the boundaries within which the model can be expected to be valid. The current model simulates the metabolic operation of *E. coli* K-12 MG1655 during exponential growth phase, under aerobic condition and glucose limitation (µ = 0.1 h⁻¹). It may allow simulation of other scenarios by changing the enzyme activities to reflect the altered conditions, and/or implementing additional pathways known to be active in the other scenarios.

This model comprises three compartments: the environment and the cell which is divided in two compartments (cytoplasm and periplasm). The periplasmic volume represents 20% of the cell volume [1]. The model contains 77 species and 68 reactions constitutive of the central carbon and energy pathways of *E. coli* (Figure 1):

- transport reactions between the environment and the periplasm
- glucose phosphotransferase system (PTS)
- glycolytic and gluconeogenic pathways (EMP)
- pentose phosphate pathway (PP)
- Entner-Doudoroff pathway (ED)
- anaplerotic reactions (AR)
- tricarboxylic acids cycle (TCA)
- glyoxylate shunt (GS)
- acetate metabolism
- oxidative phosphorylation (OP)
- synthesis of biomass

The following sections describe:

- all the reactions included in the model
- the system of ODEs
- the laws for conserved moieties
- the rate laws for each reaction
- the value of all kinetic parameters
2. Model units

Model units are millimole (mmol) for amounts, litre (L) for volumes, and second (s) for time. Experimental data used for parameter estimation were converted into intracellular units (mM for concentrations and mM/s for fluxes) assuming a cytosolic volume of $1.77 \times 10^{-3}$ L/gDW [3].

3. Reactions

Table S1 lists the reactions implemented in the model. It also includes the types of kinetic equations describing each reaction and the effectors considered. When the equation was taken or adapted from the literature, the corresponding reference is given. All the equations can be found in section 5. This model is available in SBML and COPASI formats in Supplementary data and can be downloaded from the Biomodels database (http://www.ebi.ac.uk/biomodels/) with identifier <MODEL1515110000>. 

Figure 1. Central metabolic network of E. coli implemented in the model. The model comprises three compartments: the environment, the periplasm and the cytoplasm. Metabolites are shown in blue (reactants) and orange (regulators). Enzymes are shown in green. Black and orange arrows denote reactions and regulatory interactions, respectively. The diagram adopts the conventions of the Systems Biology Graphical Notation process description [2].
### Table S1. Reactions implemented in the model. When equations were taken from the literature, references are given in the Rate law column.

The following abbreviations are used: MA: Mass action; MM: Michaelis-Menten; MWC: Monod-Wyman-Changeux; OBB: Ordered Bi Bi; OUB: Ordered Uni Bi; PPTB: Ping Pong Ter Bi; PPUBBU: Ping Pong Uni Bi Bi; RBB: Random Bi Bi; RBT: Random Bi Ter; RUB: Random Uni Bi. The signs (+) and (-) denotes a positive and a negative control of reaction rates by their effectors, respectively.

<table>
<thead>
<tr>
<th>Sub-system</th>
<th>Reaction name</th>
<th>EC number</th>
<th>Reaction</th>
<th>Effector(s)</th>
<th>Rate law</th>
<th>Comment</th>
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<tbody>
<tr>
<td>Exchange reactions</td>
<td>GLC_feed</td>
<td>-</td>
<td>GLC&lt;sub&gt;feed&lt;/sub&gt; → GLC&lt;sub&gt;env&lt;/sub&gt;</td>
<td>-</td>
<td>Constant flux</td>
<td>Glucose inflow into the environment</td>
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<tr>
<td></td>
<td>ACE_OUT</td>
<td>-</td>
<td>ACE&lt;sub&gt;env&lt;/sub&gt; → ACE&lt;sub&gt;out&lt;/sub&gt;</td>
<td>-</td>
<td>MA</td>
<td>Acetate output from the environment</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>PTS_0</td>
<td>2.7.3.9</td>
<td>e&lt;sub&gt;i&lt;/sub&gt; + PEP ↔ e&lt;sub&gt;i&lt;/sub&gt;P + PYR</td>
<td>-</td>
<td>MA [4]</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PTS_1</td>
<td>2.7.1.199</td>
<td>hpr + e&lt;sub&gt;i&lt;/sub&gt;P ↔ hprP + e&lt;sub&gt;i&lt;/sub&gt;</td>
<td>-</td>
<td>MA [4]</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PTS_2</td>
<td>2.7.1.199</td>
<td>e&lt;sub&gt;i&lt;/sub&gt;ia + hprP ↔ e&lt;sub&gt;i&lt;/sub&gt;iaP + hpr</td>
<td>-</td>
<td>MA [4]</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PTS_3</td>
<td>2.7.1.199</td>
<td>e&lt;sub&gt;i&lt;/sub&gt;ib + e&lt;sub&gt;i&lt;/sub&gt;iaP ↔ e&lt;sub&gt;i&lt;/sub&gt;ibP + e&lt;sub&gt;i&lt;/sub&gt;ia</td>
<td>-</td>
<td>MA [4]</td>
<td>-</td>
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<td></td>
<td>PTS_4</td>
<td>2.7.1.199</td>
<td>GLC&lt;sub&gt;per&lt;/sub&gt; + e&lt;sub&gt;i&lt;/sub&gt;ibP ↔ G6P + e&lt;sub&gt;i&lt;/sub&gt;ib</td>
<td>-</td>
<td>MA [4]</td>
<td>-</td>
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<tr>
<td>Phosphate uptake</td>
<td>PIT</td>
<td>-</td>
<td>P&lt;sub&gt;out&lt;/sub&gt; + H&lt;sub&gt;per&lt;/sub&gt; ↔ P&lt;sub&gt;in&lt;/sub&gt; + H&lt;sub&gt;per&lt;/sub&gt;</td>
<td>H&lt;sub&gt;per&lt;/sub&gt; (+)</td>
<td>MM</td>
<td>-</td>
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<tr>
<td>Glycolysis &amp; gluconeogenesis</td>
<td>PGI</td>
<td>5.3.1.9</td>
<td>G6P ↔ F6P</td>
<td>PEP (-), PGN (-)</td>
<td>MM, adapted from [5]</td>
<td>With inhibition by PGN [6, 7]</td>
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<td></td>
<td>PFK</td>
<td>2.7.1.11</td>
<td>ATP + F6P ↔ ADP + FDP</td>
<td>PEP (-)</td>
<td>MWC [5]</td>
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<td>FBP</td>
<td>3.1.3.11</td>
<td>FDP → F6P + P</td>
<td>AMP (-), P (-)</td>
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<td>FBA</td>
<td>4.1.2.13</td>
<td>FDP ↔ DAP + GAP</td>
<td>PEP (-)</td>
<td>OUB [5]</td>
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<td>TPI</td>
<td>5.3.1.1</td>
<td>DAP ↔ GAP</td>
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<td>GDH</td>
<td>1.2.1.12</td>
<td>GAP + NAD + P ↔ BPG + NADH</td>
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<td>RBT [5]</td>
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<td>PGK</td>
<td>2.7.2.3</td>
<td>ADP + BPG ↔ ATP + PGA3</td>
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<td>GPM</td>
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<td>2.7.1.40</td>
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<td>SUCCOA (-), FDP (+)</td>
<td>MWC [5]</td>
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<td>PPS</td>
<td>2.7.9.2</td>
<td>ATP + PYR ↔ AMP + PEP + P</td>
<td>ADP (-), AKG (-), OAA (-), AMP (-), P (-), PEP (-)</td>
<td>PPUBBU [5]</td>
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<td>PDH</td>
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<td>COA + NAD + PYR ↔ ACCOA + NADH + HCO&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>PPTB [5]</td>
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<td>PP pathway</td>
<td>ZWF</td>
<td>1.1.1.49</td>
<td>G6P + NADP ↔ GL6P + NADPH</td>
<td>NADPH (-)</td>
<td>RBT [5]</td>
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<td>PGL</td>
<td>3.1.3.31</td>
<td>GL6P ↔ PGN</td>
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<td>MM [5]</td>
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<td>GL6P_HYDRO</td>
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<td>GND</td>
<td>1.1.1.44</td>
<td>NADP + PGN ↔ NADPH + RUSP + HCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>FDP (-), ATP (-), NADPH (-)</td>
<td>RBT, adapted from [5]</td>
<td>Inhibition by PEP was removed (no experimental evidence)</td>
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<td>5.3.1.6</td>
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<td>E4P (-)</td>
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<td>tkt + XSP ↔ GAP + tktC2</td>
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<td>F6P_E4P_TKT</td>
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<td>5P_E4P_TAL</td>
<td>2.2.1.2</td>
<td>5P + tal ↔ E4P + talC3</td>
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<td>Enzyme</td>
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<td>Product(s)</td>
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<td><strong>ED pathway</strong></td>
<td>EDD 4.2.1.12 PGN ↔ KDPG</td>
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<td>EDA 4.1.2.14 KDPG ↔ GAP + PYR</td>
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<td><strong>Anaplerotic reactions</strong></td>
<td>PPC 4.1.1.31 PEP + HCO₃⁻ ↔ OAA + P</td>
<td>MWC [5]</td>
<td>ACCO (±), CIT (±), FDP (±), FUM (±), MAL (±), SUC (±), ASP (±), CYS (±)</td>
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<td>PCK 4.1.1.49 ATP + OAA ↔ ADP + PEP + HCO₃⁻</td>
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<td>MAD 1.1.1.39 MAL + NAD → NADH + PYR + HCO₃⁻</td>
<td>MWC [5]</td>
<td>ATP (±), ACCO (±), COA (±), ASP (±)</td>
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<td><strong>TCA cycle</strong></td>
<td>GLT 2.3.3.1 ACCO + OAA ↔ CIT + COA</td>
<td>MM [5]</td>
<td>ATP (±), AKG (±), NADH (±)</td>
<td>MWC [5]</td>
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<td>ACN_1 4.2.1.3 CIT ↔ ACO</td>
<td>MM [5]</td>
<td>ICIT (±)</td>
<td>-</td>
<td>-</td>
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<td>ACN_2 4.2.1.3 ACO ↔ ICIT</td>
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<td>CIT (±)</td>
<td>-</td>
<td>-</td>
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<td>ICD 1.1.1.42 ICIT + NADP ↔ AKG + NADPH + HCO₃⁻</td>
<td>MWC [5]</td>
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<td>ACEK_1 3.1.3.3 ATP + icd ↔ ADP + icdP</td>
<td>MA [5]</td>
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<td>ACEK_2 3.1.3.3 icdP ↔ icd + P</td>
<td>MA [5]</td>
<td>-</td>
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<td>LPD 1.2.4.2 COA + AKG + NAD → NADH + SUCOA + HCO₃⁻</td>
<td>MWC [5]</td>
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<td>SK 6.2.1.5 ADP + SUCOA + P ↔ ATP + COA + SUC</td>
<td>RBT [5]</td>
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<td>SDH 1.3.5.1 Q + SUC ↔ FUM + QH₂</td>
<td>MWC [5]</td>
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<td>FUMA 4.2.1.2 FUM ↔ MAL</td>
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<td>MQO 1.1.5.4 MAL + Q ↔ OAA + QH₂</td>
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<td><strong>Glyoxylate shunt</strong></td>
<td>MDH 1.1.1.37 QH₂ + OAA ↔ MAL + Q</td>
<td>OBB [5]</td>
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<td>ACEA 4.1.3.1 ICIT ↔ GLX + SUC</td>
<td>RUB [5]</td>
<td>PEP (±), PGA3 (±)</td>
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<td>ACEB 2.3.3.9 ACCOA + GLX ↔ COA + MAL</td>
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<td><strong>Acetate metabolism</strong></td>
<td>PTA 2.3.1.8 ACCOA + P ↔ COA + ACIP</td>
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<td>ACK 2.7.2.1 ACP + ADP ↔ ACEper + ATP</td>
<td>MM [8]</td>
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<td>ACS 6.2.1.11 ACEper + ATP + COA → ACCOA + AMP + 2 * P</td>
<td>MM [8]</td>
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<td><strong>Oxidative phosphorylation</strong></td>
<td>NDHII 1.6.5.3 NADH + Q + 4 * H⁺_cyt ↔ NAD + QH₂ + 4 * H⁺_per</td>
<td>MA</td>
<td>H⁺_per (±)</td>
<td>MA</td>
<td>Additional information are given after the table</td>
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<td>NDHIII 1.6.5.9 NADH + Q ↔ NAD + QH₂</td>
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<td>H⁺_per (±)</td>
<td>MA</td>
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<td>SQR 1.3.5.1 FADH₂ + Q ↔ FAD + QH₂</td>
<td>MA</td>
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<td>MA</td>
<td>Additional information are given after the table</td>
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<td></td>
<td>CYTBO 1.10.3.10 2 * QH₂ + 8 * H⁺_cyt + O₂ ↔ 2 * Q + 8 * H⁺_per + 2 * H₂O</td>
<td>MA</td>
<td>H⁺_per (±)</td>
<td>MA</td>
<td>Additional information are given after the table</td>
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<td>ATP_SYN 3.6.3.14 ADP + P + 4 * H⁺_cyt ↔ ATP + 4 * H⁺_per</td>
<td>MA</td>
<td>H⁺_per (±)</td>
<td>MA</td>
<td>Additional information are given after the table</td>
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<td><strong>Additional reactions for nucleotides and redox cofactors</strong></td>
<td>PNT 1.6.1.1/2/3 NAD + NADPH ↔ NADH + NADP</td>
<td>MA</td>
<td>-</td>
<td>-</td>
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<td>ADK 2.7.4.3 AMP + ATP ↔ 2 * ADP</td>
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<td>-</td>
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<td>Additional information are given after the table</td>
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<td>ATP_NGAM - ATP ↔ ADP + P</td>
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<td>CYA 4.6.1.1 ATP ↔ cAMP + 2 * P</td>
<td>MA</td>
<td>eiiaP (±)</td>
<td>MA</td>
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<td>DOS 3.1.4.53 cAMP ↔ AMP</td>
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<td><strong>Biomass synthesis</strong></td>
<td>GROWTH - 116 * G6P + 204 * E4P + 845 * PGA3 + 1010 * OAA + 610 * AKG + 1601 * PYR + 507 * RSP + 293 * PEP + 73 * GAP + 40 * F6P + 10169 * NADPH + 2118 * ACCOA + 2004 * NAD + 30508 * ATP + 10169 * NADP + 2118 * COA + 2004 * NADH + 30508 * ADP + 30508 * P</td>
<td>Random ordered</td>
<td>-</td>
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Additional information are given after the table.
All the rate laws are given in section 5. Details on the equations taken from the literature can be found in the original paper given in reference. Information on the modelling of some processes is detailed hereafter.

- Exchange reactions

Three reactions enable the transport of glucose (XCH_GLC), acetate (XCH_ACE) and phosphate (XCH_P) between the environment and the periplasm. Diffusion through the outer membrane is modelled as a saturable, porin-facilitated diffusion process [9], using reversible Michaelis-Menten kinetics. The same (arbitrary) values for $V_{\text{max}}$ and $K_m$ of 100 mM/s and 10 mM were taken for all compounds.

- Oxidative phosphorylation

Oxidative phosphorylation is used by *E. coli* to generate ATP. In aerobic condition, electrons are transferred from NADH and FADH$_2$ to O$_2$. This process generates an H$^+$ gradient across the cytoplasmic membrane, which is then used to drive ATP synthesis. The main components of oxidative phosphorylation are two NADH dehydrogenases (NDHI and NDHII), the succinate dehydrogenase complex (SQR), the cytochrome bo oxidase (CYTBO) and the ATP synthase (ATP_SYN) [10]. *E. coli* also has two other cytochromes (bd1 and bd2) which are expressed under oxygen-limited condition and starvation for carbon and/or phosphate [11-13], respectively, and were not included in the model since they are not significantly active in exponential growth on glucose in aerobic condition. NDHI and NDHII catalyse the transfer of electrons from NADH to the quinone pool (Q) in the cytoplasmic membrane. In contrast to NDHII, NDHI also generates a proton gradient by translocating H$^+$ from cytoplasm to periplasm, with an H$^+$/$\text{e}^-$ ratio of 2 [10]. SQR is a complex of 4 proteins (SDHA, B, C and D). SDHA is a part of the TCA cycle (reaction SDH) and oxidizes succinate to fumarate by reducing FAD to FADH$_2$. Further transfer of electrons from FADH$_2$ to Q (reaction SQR) occurs via the three other proteins. CYTBO couples the two-electron oxidation of ubiquinol (QH$_2$) with the four-electron reduction of molecular oxygen to water. It also functions as a proton pump, with an H$^+$/$\text{e}^-$ ratio of 2 [10]. Finally, the proton gradient is used by the ATP synthase to generate ATP by translocating H$^+$ from the periplasm to the cytoplasm, with an H$^+$/ATP ratio of 4 [14].

Oxidative phosphorylation was modelled using reversible mass action kinetics. Although the oxidative phosphorylation reactions have no specific feedback regulation, experimental evidences show that kinetics of H$^+$ pumps (NDHI and CYTBO) and ATP_SYN strongly depends on the H$^+$ gradient. As the H$^+$ gradient increases, the reaction rate through NDHI and CYTBO decreases in a sigmoidal fashion. In opposite, a strongly sigmoidal increase of the rate of ATP synthesis with the increase of H$^+$ gradient was observed. We considered the dependence of reaction rates on the H$^+$ gradient using the relation proposed by [15].
E. coli maintains a cytoplasmic pH within a narrow range, approximately 7.4 to 7.8, when grown over a large range of environmental pH from pH 5 to 9 [16-18]. Thus, the cytosolic concentration of H$^+$ ions was fixed at 3.16×10^{-5} mM (pH=7.5, [18]).

- **Transport of phosphate**

Phosphate enters the cytoplasm via the PIT transporter. Transport is energised by the proton gradient with a H$^+/P$ ratio of 1 and can be abolished with uncouplers or respiration inhibitors, thus the reaction rate was modelled as function of the H$^+$ gradient, similarly to reactions of oxidative phosphorylation involved in the production of the H$^+$ gradient.

- **Additional reactions for nucleotides and redox cofactors**

Various processes strongly impact the balance of AMP, ADP, ATP and cAMP pools and had to be considered to fit the experimental data. Several reactions of non-growth associated processes which consume ATP were lumped in the reaction ATP_NGAM. Adenylate kinase (reaction ADK) catalyzes the reversible conversion of AMP and ATP to two molecules of ADP. Adenylate cyclase (CYA) catalyzes the synthesis of cAMP from ATP and is activated by the phosphorylated form of EIIA enzyme of the PTS. Finally, cAMP can be hydrolyzed into AMP by the cAMP phosphodiesterase (DOS).

The reversible reduction of NADP by NADH is catalysed by two transhydrogenases [19] lumped into the reaction PNT and modelled using reversible mass action kinetics.

- **Biomass synthesis**

In contrast to previous models where growth was function of extracellular glucose levels, we assumed that the growth rate is controlled by the intracellular concentration of the cell building blocks (G6P, E4P, PGA3, OAA, AKG, PYR, R5P, PEP, GAP, F6P, NADPH, ACCOA, NAD, ATP). Thus, we defined an overall pseudo-reaction to describe cellular growth in terms of the required metabolic precursors, with the stoichiometric coefficients taken from the biomass function published in [20] (after unit conversion from mmol/g_dry_weight/h to mmol/L_cytoplasm/s). The kinetic equation for growth is:

$$
\mu = V_{\text{max}} \cdot \prod_i \frac{S_i}{S_i + K_m^{S_i}}
$$

where $S_i$ represents the concentration of the building block $i$ and $K_m^{S_i}$ represents the saturation of the growth rate with respect to the concentration of the metabolite $i$. 
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 metabolites (glucose, phosphate and acetate)
- concentrations of intracellular metabolites
- phosphorylation states of PTS proteins
- states of transaldolases and transketolases

Metabolites:

\[
\begin{align*}
\frac{d(ACCOA)}{dt} &= v_{PDH} - v_{GLT} - v_{ACEB} - 2118 \times v_{GROWTH} + v_{ACS} - v_{PTA} \\
\frac{d(ACO)}{dt} &= v_{ACN\_1} - v_{ACN\_2} \\
\frac{d(ACE)}{dt} &= v_{ACK} - v_{ACS} - v_{XCH\_ACE1} \\
\frac{d(ACEp)}{dt} &= (v_{XCH\_ACE1} - v_{XCH\_ACE2}) \times \text{vol_cyt/\text{vol_per}} \\
\frac{d(ACEx)}{dt} &= v_{XCH\_ACE2} \times \text{vol_per/\text{vol_env}} - v_{ACE\_OUT} \\
\frac{d(ACP)}{dt} &= v_{PTA} - v_{ACK} \\
\frac{d(ADP)}{dt} &= 2 \times v_{ADK} - v_{ATP\_SYN} - v_{PGK} + v_{PFK} + v_{PYK} + v_{PCK} - v_{SK} + v_{ACEK\_1} - v_{ACK} + 30508 \times v_{GROWTH} \\
\frac{d(AKG)}{dt} &= v_{ICD} - v_{LPD} - 610 \times v_{GROWTH} \\
\frac{d(AMP)}{dt} &= v_{DOS} - v_{ADK} + v_{PPS} + v_{ACS} \\
\frac{d(ATP)}{dt} &= v_{ATP\_SYN} - v_{CYA} - v_{ADK} + v_{PGK} + v_{PFK} + v_{PYK} + v_{PCK} - v_{PPS} + v_{SK} - v_{ACEK\_1} - v_{ACS} + v_{ACK} - 30508 \times v_{GROWTH} \\
\frac{d(BPG)}{dt} &= v_{GDH} - v_{PGK} \\
\frac{d(CAMP)}{dt} &= v_{CYA} - v_{DOS} \\
\frac{d(CIT)}{dt} &= v_{GLT} - v_{ACN\_1} \\
\frac{d(DAP)}{dt} &= v_{FBA} - v_{TPI} \\
\frac{d(E4P)}{dt} &= v_{S7P\_E4P\_TAL} - v_{F6P\_E4P\_TKT} - 204 \times v_{GROWTH} \\
\frac{d(F6P)}{dt} &= v_{PGI} - v_{PFK} + v_{F6P\_E4P\_TKT} + v_{F6P\_GAP\_TAL} + v_{FBP} - 40 \times v_{GROWTH} \\
\frac{d(FAD)}{dt} &= v_{SQR} - v_{SDH} \\
\frac{d(FADH)}{dt} &= v_{SDH} - v_{SQR} \\
\frac{d(FDP)}{dt} &= v_{PFK} - v_{FBA} - v_{FBP} \\
\frac{d(FUM)}{dt} &= v_{SDH} - v_{FUMA} \\
\frac{d(G6P)}{dt} &= v_{PTS\_4} - v_{PGI} - v_{ZWF} - 116 \times v_{GROWTH} \\
\frac{d(GAP)}{dt} &= v_{FBA} + v_{TPI} - v_{GDH} + v_{X5P\_GAP\_TKT} - v_{F6P\_GAP\_TAL} + v_{EDA} - 73 \times v_{GROWTH} \\
\frac{d(GL6P)}{dt} &= v_{ZWF} - v_{PGL} - v_{GL6P\_HYDRO} \\
\frac{d(GLCp)}{dt} &= (v_{GLC\_XCH} - v_{PTS\_4}) \times \text{vol_cyt/\text{vol_per}} \\
\frac{d(GLCx)}{dt} &= (v_{GLC\_feed} - v_{GLC\_XCH}) \times \text{vol_per/\text{vol_env}} 
\end{align*}
\]
\[ \frac{d(GLX)}{dt} = v_{\text{ACEA}} - v_{\text{ACEB}} \]

\[ \frac{d(Hp)}{dt} = (4 \cdot v_{\text{NDHI}} + 8 \cdot v_{\text{CYTBO}} - 4 \cdot v_{\text{ATP\_SYN}} - v_{\text{PIT}}) \cdot \frac{vol_{\text{cyt}}}{vol_{\text{per}}} \]

\[ \frac{d(ICIT)}{dt} = v_{\text{ACN\_2}} - v_{\text{ICD}} - v_{\text{ACEA}} \]

\[ \frac{d(KDPG)}{dt} = v_{\text{EDD}} - v_{\text{EDA}} \]

\[ \frac{d(MAL)}{dt} = v_{\text{FUMA}} - v_{\text{MAD}} + v_{\text{MDH}} - v_{\text{MQO}} + v_{\text{ACEB}} \]

\[ \frac{d(NAD)}{dt} = v_{\text{MDH}} - v_{\text{PNT}} - v_{\text{GDH}} - v_{\text{MAD}} - v_{\text{PDH}} - v_{\text{LPD}} - 2004 \cdot v_{\text{GROWTH}} \]

\[ \frac{d(NADH)}{dt} = v_{\text{GDH}} + v_{\text{MAD}} + v_{\text{PDH}} + v_{\text{LPD}} - v_{\text{MDH}} + v_{\text{NADH\_req}} + v_{\text{PNT}} - 2004 \cdot v_{\text{GROWTH}} \]

\[ \frac{d(NADPH)}{dt} = v_{\text{PNT}} - v_{\text{GDH}} - v_{\text{ZWF}} - v_{\text{ICD}} - v_{\text{PNT}} - 10169 \cdot v_{\text{GROWTH}} \]

\[ \frac{d(NADPH)}{dt} = v_{\text{GND}} + v_{\text{ZWF}} + v_{\text{ICD}} - v_{\text{PNT}} - 10169 \cdot v_{\text{GROWTH}} \]

\[ \frac{d(OAA)}{dt} = v_{\text{PPC}} - v_{\text{PCK}} - v_{\text{GLT}} + v_{\text{MQO}} - v_{\text{MDH}} - 1010 \cdot v_{\text{GROWTH}} \]

\[ \frac{d(Pp)}{dt} = (v_{\text{P\_XCH}} - v_{\text{PIT}}) \cdot \frac{vol_{\text{cyt}}}{vol_{\text{per}}} \]

\[ \frac{d(Pc)}{dt} = v_{\text{FBP}} - v_{\text{GDH}} + v_{\text{PPC}} + v_{\text{PPS}} - v_{\text{SK}} + v_{\text{ACEK\_2}} - v_{\text{ATP\_SYN}} + 2 \cdot v_{\text{CYA}} + 2 \cdot v_{\text{ACS}} - v_{\text{PTA}} + v_{\text{ATP\_MAINTENANCE}} + 30508 \cdot v_{\text{GROWTH}} + v_{\text{PIT}} \]

\[ \frac{d(Pep)}{dt} = v_{\text{ENO}} - v_{\text{PYK}} - v_{\text{PPC}} + v_{\text{PCK}} + v_{\text{PPS}} - v_{\text{PTS\_0}} - 293 \cdot v_{\text{GROWTH}} \]

\[ \frac{d(PG2)}{dt} = v_{\text{GPM}} - v_{\text{ENO}} \]

\[ \frac{d(PGA3)}{dt} = v_{\text{PGK}} - v_{\text{GPM}} - 845 \cdot v_{\text{GROWTH}} \]

\[ \frac{d(PGN)}{dt} = v_{\text{PGL}} - v_{\text{GND}} - v_{\text{EDD}} \]

\[ \frac{d(PYR)}{dt} = v_{\text{PYK}} - v_{\text{PPS}} + v_{\text{MAD}} - v_{\text{PDH}} + v_{\text{EDA}} + v_{\text{PTS\_0}} - 1601 \cdot v_{\text{GROWTH}} \]

\[ \frac{d(Q)}{dt} = v_{\text{MDH}} + 2 \cdot v_{\text{CYTBO}} - v_{\text{SQR}} - v_{\text{NDHI}} - v_{\text{NDHII}} - v_{\text{MQO}} \]

\[ \frac{d(QH2)}{dt} = v_{\text{SQR}} + v_{\text{NDHI}} + v_{\text{NDHII}} + v_{\text{MQO}} - v_{\text{MDH}} - 2 \cdot v_{\text{CYTBO}} \]

\[ \frac{d(R5P)}{dt} = v_{\text{RPI}} - v_{\text{S7P\_R5P\_TKT}} - 507 \cdot v_{\text{GROWTH}} \]

\[ \frac{d(RU5P)}{dt} = v_{\text{GND}} - v_{\text{RPE}} + v_{\text{RPI}} \]

\[ \frac{d(S7P)}{dt} = v_{\text{S7P\_R5P\_TKT}} - v_{\text{S7P\_E4P\_TAL}} \]

\[ \frac{d(SUC)}{dt} = v_{\text{SK}} - v_{\text{SDH}} + v_{\text{ACEA}} \]

\[ \frac{d(SUCCOA)}{dt} = v_{\text{LPD}} - v_{\text{SK}} \]

\[ \frac{d(X5P)}{dt} = v_{\text{RPE}} - v_{\text{X5P\_GAP\_TKT}} \]

**Proteins:**

\[ \frac{d(ei)}{dt} = v_{\text{PTS\_1}} - v_{\text{PTS\_0}} \]

\[ \frac{d(eiia)}{dt} = v_{\text{PTS\_3}} - v_{\text{PTS\_2}} \]

\[ \frac{d(eiiaP)}{dt} = v_{\text{PTS\_2}} - v_{\text{PTS\_3}} \]

\[ \frac{d(eiicb)}{dt} = v_{\text{PTS\_4}} - v_{\text{PTS\_3}} \]

\[ \frac{d(eiicBP)}{dt} = v_{\text{PTS\_3}} - v_{\text{PTS\_4}} \]

\[ \frac{d(eiP)}{dt} = v_{\text{PTS\_0}} - v_{\text{PTS\_1}} \]

\[ \frac{d(hpr)}{dt} = v_{\text{PTS\_2}} - v_{\text{PTS\_1}} \]

\[ \frac{d(hprP)}{dt} = v_{\text{PTS\_1}} - v_{\text{PTS\_2}} \]

\[ \frac{d(icd)}{dt} = v_{\text{ACEK\_2}} - v_{\text{ACEK\_1}} \]
\[ \frac{d(icdP)}{dt} = v_{\text{ACEK}_1} - v_{\text{ACEK}_2} \]
\[ \frac{d(tal)}{dt} = v_{\text{F6P}_\text{GAP}_TAL} - v_{\text{S7P}_\text{E4P}_TAL} \]
\[ \frac{d(talC3)}{dt} = v_{\text{S7P}_\text{E4P}_TAL} - v_{\text{F6P}_\text{GAP}_TAL} \]
\[ \frac{d(tk)}{dt} = v_{\text{S7P}_\text{R5P}_TKT} - v_{\text{X5P}_\text{GAP}_TKT} + v_{\text{F6P}_\text{E4P}_TKT} \]
\[ \frac{d(tkC2)}{dt} = v_{\text{X5P}_\text{GAP}_TKT} - v_{\text{F6P}_\text{E4P}_TKT} - v_{\text{S7P}_\text{R5P}_TKT} \]

5. Rate laws

This section contains the rate laws for each reaction.
<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFKase</td>
<td>15.5 - 4F-PEP - PEP - PFKase</td>
</tr>
</tbody>
</table>
6. Conservation laws

The following equations describe the conservation laws of conserved moieties:

\[
\begin{align*}
\text{tal}_{\text{total}} &= \text{tal} + \text{talC3} \\
\text{tkt}_{\text{total}} &= \text{tkt} + \text{tktC2} \\
\text{icd}_{\text{total}} &= \text{icd} + \text{icdP} \\
\text{ei}_{\text{total}} &= \text{ei} + \text{eiP} \\
\text{eiia}_{\text{total}} &= \text{eiia} + \text{eiiaP} \\
\text{eiicb}_{\text{total}} &= \text{eiicb} + \text{eiicbP} \\
\text{hpr}_{\text{total}} &= \text{hpr} + \text{hprP} \\
\text{Q}_{\text{total}} &= \text{Q} + \text{QH}_2 \\
\text{NAD}_{\text{total}} &= \text{NAD} + \text{NADH} \\
\text{NADP}_{\text{total}} &= \text{NADP} + \text{NADPH} \\
\text{FAD}_{\text{total}} &= \text{FAD} + \text{FADH}_2 \\
\text{AxP}_{\text{total}} &= \text{AMP} + \text{ADP} + \text{ATP} + \text{cAMP} 
\end{align*}
\]

7. Concentrations of cofactors and conserved moieties

Concentrations of cofactors, metal ions and conserved moieties were taken from the literature and are given in table S2.

Table S2. Initial intracellular concentrations of cofactors, metal ions and conserved moieties.

<table>
<thead>
<tr>
<th>Specie</th>
<th>Concentration (mM)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCO\textsubscript{3}\textsuperscript{-}</td>
<td>1.4</td>
<td>saturation concentration in water at 298 K, 1 atm, pH=7.5</td>
</tr>
<tr>
<td>O\textsubscript{2}</td>
<td>0.21</td>
<td>saturation concentration in water at 298 K, 1 atm</td>
</tr>
<tr>
<td>H\textsuperscript{+}\textsubscript{cytoplasm}</td>
<td>3.16 × 10\textsuperscript{-5}</td>
<td>from [18], pH\textsubscript{cytoplasm} = 7.5</td>
</tr>
<tr>
<td>Mg\textsuperscript{2+}</td>
<td>1</td>
<td>from [21]</td>
</tr>
<tr>
<td>Mn\textsuperscript{2+}</td>
<td>0.3</td>
<td>from [22]</td>
</tr>
<tr>
<td>Asp</td>
<td>1.17</td>
<td>from [23]</td>
</tr>
<tr>
<td>Cys</td>
<td>0.085</td>
<td>from [23]</td>
</tr>
<tr>
<td>CoA</td>
<td>0.5</td>
<td>from [24]</td>
</tr>
<tr>
<td>AxP\textsubscript{total}</td>
<td>4.28</td>
<td>from [23]</td>
</tr>
<tr>
<td>ICD\textsubscript{total}</td>
<td>0.043</td>
<td>from [25]</td>
</tr>
<tr>
<td>Q\textsubscript{total}</td>
<td>1</td>
<td>from [24]</td>
</tr>
<tr>
<td>TAL\textsubscript{total}</td>
<td>0.006</td>
<td>from [26]</td>
</tr>
<tr>
<td>TKT\textsubscript{total}</td>
<td>0.007</td>
<td>from [27]</td>
</tr>
<tr>
<td>NAD\textsubscript{total}</td>
<td>1.57</td>
<td>from [3], in agreement with [28]</td>
</tr>
<tr>
<td>NADP\textsubscript{total}</td>
<td>0.257</td>
<td>from [3]</td>
</tr>
<tr>
<td>FAD\textsubscript{total}</td>
<td>1</td>
<td>arbitrary</td>
</tr>
</tbody>
</table>
8. Magnesium complexes

The following functions were used to estimate the concentrations of magnesium complexes taking part as substrates in particular enzyme reactions:

\[
MgADP = \frac{Mg \cdot ADP}{K_{dADP}Mg + Mg}
\]

\[
MgATP = \frac{Mg \cdot ATP}{K_{dATP}Mg + Mg}
\]

\[
MgFDP = \frac{Mg \cdot FDP}{K_{dFDP}Mg + Mg}
\]

where MgADP, MgATP and MgFDP are the concentrations of magnesium complexes, ATP, ADP and FDP are the concentrations of free metabolites, Mg is the concentration of free magnesium ions, and KdADPMg, KdATPMg and KdFDPm are the respective dissociation constants.

9. Model calibration

This section outlines the followed model calibration strategy and lists the values of all the parameters.

To the extent possible, values of the biochemical parameters were taken from the literature. This was the case for 56% of the parameters (253/449, Table S3). Parameters not available in the literature, which do not have a real biochemical meaning (e.g. Michaelis constants of the biomass function), or for which biochemical estimates are generally not indicative of cellular conditions (e.g. Vmax) were estimated to reproduce in the best possible way various experimental data obtained from a unique E. coli strain (the model strain K-12 MG1655 wild-type) grown in a unique reference condition (M9 minimal medium, dilution rate = 0.1 h\(^{-1}\), temperature = 37°C, pH = 7.0, pO\(_2\) > 20%). This was critical to prevent biases during parameter estimation since fluxes and metabolite concentrations depends on environmental conditions and strains [29-32]. Experimental data used for parameter estimation were steady state reaction rates and metabolite concentrations [23, 33-35] and time-course concentrations of intracellular metabolites in response to a glucose pulse [23] (S1 Dataset). A total of 276 data points was used to estimate the remaining 196 parameters. Parameter estimation problem was formulated as a constrained optimization problem:

\[
\text{minimize } f(p)
\]

\[
\text{subject to } g(p) \geq c
\]
where \( p \) is the parameter vector, \( f \) is the objective function which evaluates the deviation between the simulated and measured data, \( g(p) \) is the constraint function vector, and \( c \) is the constraint vector. The objective function \( f \) is defined as the sum of squared weighted errors:

\[
f(p) = \sum_i \left( \frac{x_i - y_i(p)}{\sigma_i} \right)^2
\]

where \( x_i \) is the experimental value of the data point \( i \) with standard deviation \( \sigma_i \) and \( y_i(p) \) is the corresponding simulated value.

Constraints were defined on estimated parameters (\( 10^{-4} \) mM \( \leq K_M \leq 10^3 \) mM; \( 10^{-2} \) mM/s \( \leq V_{max} \leq 10^3 \) mM/s; \( 10^{-4} \leq K_{eq} \leq 10^3 \)) to ensure they are kept within a biologically reasonable range.

The objective function was minimized with the Particle Swarm Optimization algorithm [36] (with a swarm size of 50 and 20,000 iterations), using the Condor-COPASI system [37] on a pool of 2500 CPU cores. Values of all the parameters (and the corresponding reference for values taken from the literature) are listed in Table S3. The experimental and fitted data are provided in S1 Dataset.
Table S3. Parameters of the kinetic model.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Equation</th>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACEA</td>
<td>[5]</td>
<td>KdICITsuc</td>
<td>0.0049</td>
<td>mM</td>
<td>Estimated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KdPEP</td>
<td>1.05</td>
<td>mM</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KdPEPglx</td>
<td>0.0312</td>
<td>mM</td>
<td>Estimated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KdPEPicit</td>
<td>0.164</td>
<td>mM</td>
<td>Estimated</td>
</tr>
<tr>
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<td></td>
<td>KdPGA3</td>
<td>0.8</td>
<td>mM</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KdSUC</td>
<td>0.53</td>
<td>mM</td>
<td>[38]</td>
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<tr>
<td></td>
<td></td>
<td>Keq</td>
<td>8.8</td>
<td>1</td>
<td>[38]</td>
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<td></td>
<td></td>
<td>KmGLX</td>
<td>0.13</td>
<td>mM</td>
<td>[38]</td>
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<td></td>
<td>KmICIT</td>
<td>0.063</td>
<td>mM</td>
<td>[38]</td>
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<td>mM</td>
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<td>Vmax</td>
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<td>mM/s</td>
<td>Estimated</td>
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<td>mM</td>
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<td></td>
<td>KmCOA</td>
<td>10</td>
<td>mM</td>
<td>[5]</td>
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<td></td>
<td>KmGLX</td>
<td>0.021</td>
<td>mM</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KmMAL</td>
<td>15.1</td>
<td>mM</td>
<td>Estimated, in agreement with the value of 10 estimated in [5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vmax</td>
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<td>mM/s</td>
<td>Estimated</td>
</tr>
<tr>
<td>ACEK_1</td>
<td>[5]</td>
<td>k</td>
<td>1.25</td>
<td>mM/s</td>
<td>Estimated</td>
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<td>ACEK_2</td>
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<td>Estimated</td>
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<td></td>
<td>Keq</td>
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| **FBP** [5] | **KdFDP|Mg** | 5.81 mM | Estimated, in agreement with the value of 10 estimated in [5] |
|-------------|---------|--------|----------------------------------|
| KirAMP | 0.0012 mM | [56] |
| KirAMPFDP | 0.256 mM | [56] |
| KirF6P | 1.12 mM | [56] |
| KirF6PMg | 0.385 mM | [57] |
| KirFDP | 1.35 mM | Estimated, in agreement with the value of 1.16 estimated in [5] |
| KirFDP|Mg | 0.76 mM | [56-59] |
| KirFDP|MgMg | 0.356 mM | [56-59] |
| KirP | 3.16 mM | [57] |
| KirPF6P | 6.6 mM | [57] |
| KirPF6PMg | 48.4 mM | [57] |
| KirPMg | 0.856 mM | [57] |
| KirKAMP | 0.000255 mM | [56-59] |
| KirKAMPFDP | 690 mM | [56-59] |
| KirF6P | 0.304 mM | [57] |
| KirF6PMg | 315 mM | [57] |
| KirFDP | 0.043 mM | Estimated |
| KirFDP|Mg | 0.00642 mM | [56-59] |
| KirFDP|MgMg | 100 mM | [56-59] |
| KirP | 0.642 mM | [57] |
| KirPF6P | 0.00689 mM | [56-59] |
| KirPF6PMg | 16.5 mM | [57] |
| KirPMg | 539 mM | [57] |
| KmrFDP | 0.064 ? | Estimated |
| KmrMg | 0.039 ? | [56-59] |
| KmtFDP | 1.0e-05 ? | [56-59] |
| KmtMg | 55 ? | [57] |
| LO | 0.000815 ? | [56-59] |
| n | 4 ? | [60] |
| Vmax | 0.216 ? | Estimated |

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<tr>
<td>PPC</td>
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<td>Estimated, in agreement with the value of 6.6 estimated in [5]</td>
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<tr>
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<td>PPC</td>
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<td>21.4</td>
<td>? Estimated</td>
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| PPS | alpha | 38900 | ? Estimated |
| PPS | KdADPMg | 1.28 | ? [5] |
| PPS | KdAMP | 1480 | ? [5] |
| PPS | KdATPMg | 0.085 | ? [5] |
| PPS | KdATPMgPPS | 0.0549 | ? [5] |
| PPS | KdMg | 36.9 mM | [5]  |
| PPS | KdP | 346 | ? [5] |
| PPS | KdPEP | 95.7 | ? [5] |
| PPS | KdPYR | 2740 | ? [5] |
| PPS | KefADP | 0.0283 | ? [5] |
| PPS | KefAKG | 0.274 | ? [5] |
| PPS | KefATP | 0.000628 | ? [5] |
| PPS | KefOAA | 0.796 | ? [5] |
| PPS | Keq | 2.00E+05 mmol^2/l^2 | [5]  |
| PPS | KmAMP | 0.000384 | ? [5] |
| PPS | KmATPMg | 0.0549 | ? [5] |
| PPS | KmP | 85 | ? [5] |
| PPS | KmPEP | 20.7 | ? [5] |
| PPS | KmPYR | 0.229 | ? [5] |
| PPS | Vmax | 0.0164 | ? Estimated |
| PPS | W | 10 | ? [5] |

<p>| PTA | Keq | 0.005 | 1 Estimated |
| PTA | KiACCOA | 0.2 mM | [77] |
| PTA | KiACP | 0.2 mM | [77] |
| PTA | KICOA | 0.029 mM | [77] |
| PTA | KIP | 13.5 mM | [77] |
| PTA | KmACP | 0.7 mM | [77] |
| PTA | KmP | 6.1 mM | Estimated |
| PTA | Vmax | 2.7 mM/s | Estimated |</p>
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[1] Estimated, in agreement with the value of 25.3 estimated in [5] |
[2] Estimated, in agreement with the value of 25.3 estimated in [5] |
[3] Estimated, in agreement with the value of 25.3 estimated in [5] |
[5] Estimated, in agreement with the value of 25.3 estimated in [5] |
10. Model validation

We first assessed the stability of the model by checking the stability of the Jacobian matrix under two different conditions, namely: the reference state condition (glucose limitation at a growth rate of 0.1 h⁻¹), and glucose excess condition (by fixing extracellular glucose concentration at 10 mM). In both situations the model demonstrates stable steady states with strictly negative Jacobian eigenvalues.

Then, we evaluated the metabolic control analysis results by comparing the predicted flux control to observations. The model predictions were in line with the literature, as detailed in the manuscript.

Finally, we assessed the ability of the model to identify conserved functional couplings that are independent of gene expression. As detailed in the manuscript, we collected 778 flux data from some 266 experiments, where different *E. coli* K-12 wild-type and mutant strains were cultivated under different conditions (in batch, chemostat, or shake flask). It is important to note that these data were not used to calibrate the model, they were used only for validation purpose. This data set is very different from the data set used for parameter estimation, which were from a single *E. coli* strain grown in a unique condition. The 778 data used for validation (growth rates, glucose uptake rates, biomass yields, oxygen uptake rates, and fluxes through the TCA cycle) are provided in Dataset S2. The simulations and measurements are in excellent agreement (Figures 4, 5, and 6 of the manuscript), which indicates the model yielded fairly accurate predictions of the metabolic states that can be expressed by *E. coli* growing on glucose. All the
experimental data support the model-driven hypothesis that metabolic regulation is sufficient to maintain the tight coordination between these key metabolic processes.

11. References


