Supporting Information

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SI Text 1: Model Describing System Without Feedforward Activation

The mathematical model containing two metabolites (X and Y) and two enzymatic reactions (E1 and E2) can be described by a set of two ordinary differential equations:

\[
\frac{dX}{dt} = \nu - v_{E1} \quad [S1]
\]

\[
\frac{dY}{dt} = v_{E1} - v_{E2} \quad [S2]
\]

where \( \nu \) denotes the influx (input of the system) and \( v_{E1} \) and \( v_{E2} \) denote the reaction rates of \( E_1 \) and \( E_2 \), respectively. At steady state, the differential expressions are zero, simplifying Eqs. S1 and S2 to the following:

\[
v_{E1} = v_{E2} = \nu \quad [S3]
\]

The rates through the two reactions \( v_{E1} \) and \( v_{E2} \) can be described with the following equations, assuming a reversible Michaelis–Menten-type kinetic for \( E_1 \) and an irreversible Michaelis–Menten-type kinetic for \( E_2 \):

\[
v_{E1} = \nu = \frac{v_{\text{max,} E1} \cdot (X - Y)}{K_{m, X, E1} \cdot (1 + \frac{Y}{K_{m, Y, E1}}) + X} \quad [S4]
\]

\[
v_{E2} = \nu = \frac{v_{\text{max,} E2} \cdot Y}{K_{m, Y, E2} + Y} \quad [S5]
\]

\( K_{m, X, E1}, K_{m, Y, E1}, \) and \( K_{m, Y, E2} \) denote the \( K_m \) values for \( X \) and \( Y \) of \( E_1 \) and for \( Y \) of \( E_2 \), respectively. \( K_{eq} \) denotes the equilibrium constant of \( E_1 \), and \( v_{\text{max,} E1} \) and \( v_{\text{max,} E2} \) denote the maximal possible fluxes of \( E_1 \) and \( E_2 \), respectively.

To obtain an analytical solution of the relationship of \( X \) and the flux \( \nu \), Eqs. S4 and S5 can be rearranged for \( X \) and \( Y \), respectively. Replacing \( Y \) in Eq. S4 (and assuming that \( K_{m, X, E1} = K_{m, Y, E1} = K_{m, Y, E2} \) to reduce the number of parameters), one obtains the following:

\[
X = \frac{v \cdot K_{m, X, E1}}{v_{\text{max,} E1} - \nu + v \cdot K_{m, Y, E2} \cdot v_{\text{max,} E1}} \quad [S6]
\]

\[
\frac{v^2 \cdot K_{m, Y, E2}}{(v_{\text{max,} E1} - \nu) \cdot (v_{\text{max,} E2} - \nu)}
\]

For \( \nu < v_{\text{max,} E1} \) and \( \nu < v_{\text{max,} E2} \) (which is equivalent to an influx that is much lower than the maximal possible fluxes for \( E_1 \) and \( E_2 \)), this equation simplifies to the following:

\[
X = \frac{v \cdot K_{m, X, E1}}{v_{\text{max,} E1}} + \frac{v \cdot K_{m, Y, E2}}{K_{eq} \cdot v_{\text{max,} E2}} \quad [S7]
\]

This equation describes the analytical solution for the relationship of \( X \) and flux \( \nu \).

SI Text 2: Model Describing System with Feedforward Activation of \( E_2 \) by \( X \)

Here, we use the same ordinary differential equations as above. In contrast to the previous model, we use a Monod–Wyman–Changeux (MWC) kinetic for \( E_2 \) in accordance with previous studies on pyruvate kinase I (PYK I) (1–3):

\[
v_{E2} = \nu = \frac{v_{\text{max,} E2} \cdot Y \cdot \left(1 + \frac{Y}{K_{m, Y, E2}}\right)^{n-1}}{L \cdot \left(1 + \frac{X}{K_{m, X, E2}}\right)^n + \left(1 + \frac{Y}{K_{m, Y, E2}}\right)^n} \quad [S8]
\]

\( L, n, \) and \( K_{m, X, E2}, K_{m, Y, E2} \) in Eq. S8 denote allosteric equilibrium constant, cooperativity constant, and affinity constant of \( X \) for \( E_2 \), respectively. We chose values for \( L \) and \( n \) in accordance to parameter values that were obtained for PYK I in previous studies: PYK I is inactive in absence of its allosteric activator FBP (1, 4), which corresponds to \( L \gg 1 \), and several studies have determined \( n \) to be equal to 4 (1, 5). Because \( n > 1 \), it is not possible to derive an analytical solution for the relationship of \( X \) and flux \( \nu \), and thus we solve this model equation as specified in the main text.

Abundance of intracellular FBP as a function of IPTG concentration (used as a proxy for pykF abundance) in glucose batch cultures of a pykF mutant strain bearing an IPTG-inducible PYK I expression plasmid, relative to the FBP concentration at 1 mM IPTG. Batch cultivations were performed in 500-mL shake flasks containing 30 mL of M9 glucose medium (5 g/L glucose) as described in the main text. Cultures were harvested in midexponential growth phase using fast filtration and extracted in hot ethanol as described previously (1). Extracts were then analyzed by LC-MS/MS as described in the main text.

Intracellular concentrations of enzymes of lower glycolysis at different glycolytic fluxes. Whereas the data from the four lower flux values stem from glucose-limited chemostat cultures, the highest flux data point was obtained from a glucose batch culture. Enzyme copy numbers were determined by targeted MS analysis and heavy reference peptides (1) and then converted to concentrations using the cell volumes measured in ref. 2.

