**Figure 1. A kinetic model of glycolysis can yield sustained oscillations**

Graphical user interface

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**Figure 1. A kinetic model of glycolysis can yield sustained oscialtions**. (**a**) Structure and stoichiometry of a simplified model of glycolysis. The outer dotted line is the model boundary. Solid arrows are reactions and dotted arrows are allosteric interactions of metabolites with enzymes. g6p: glucose-6-phosphate, fbp: fructose-1,6-biphosphate, pep: phosphoenolpyruvate, pyr: pyruvate, PFK: phophofructokinase, FBPase: fructose-1,6-bisphosphatase, PYK: pyruvate kinase, FBA: fructose-bisphosphate aldolase, PTS: phosphotransferase system, PDH: pyruvate dehydrogenase. (**b**) Two examples of simulated pyruvate concentrations with two parameter sets that produce oscillations. The model was initially at steady state and at t = 50 min the glucose uptake rate was decreased by 5%. (**c**) Boxplot showing the distribution of the periods of 393 simulations with oscillating pyruvate levels. (**d**) Comparison of parameter values in 393 sets that led to oscillations with all 2,000 parameter sets that were random sampled, with numbering of individual reactions as in panel (a) . Asterisks denote parameters that significantly (p-value < 1E-10, a = 0.01) affect the model’s propensity to produce pyruvate oscillations.

**A kinetic model of *E. coli* glycolysis predicts periodic oscillations**

To first test the likelihood of oscillations in the levels of glycolysis metabolites, we used a small kinetic model of E. coli glycolysis (Figure 1a). The model consists of four metabolites and six metabolic reactions that were simulated with Michaelis-Menten and Hill kinetics (see STAR Methods for detailed description of the model). From a multitude of allosteric metabolite-enzyme interactions that regulate the activity of glycolysis enzymes, three of the most relevant ones were included in our model. The first interaction is the feedforward activation of pyruvate kinase (PYK) by fructose-1,6-biphosphate (FBP), which plays an important role in E. coli. The other two interactions represent negative feedback from phosphoenolpyruvate (PEP) levels to the interconversion of hexose-phosphate and FBP, by respectively inhibiting phosphofructokinase (PFK) and activating fructose-bisphosphate aldolase (FBPase).

As a starting point for the model analysis, we fixed the glycolytic flux to a constant value and randomly sampled all model parameters (maximal rates and binding constants) 2,000 times such that the model was at steady state. Next, we perturbed this steady state by decreasing the glucose uptake rate by 5% and analysed whether this perturbation leads to oscillations. A forward Fourier transformation of the time dependent pyruvate levels revealed sustained oscillations of pyruvate concentrations for 393 of the tested 2000 parameters sets (Figure 1B). The typical period of oscillations across these 393 simulations was several minutes (median = 2.65 min) , although some parameter sets caused faster or slower oscillations (Figure 1C).

To identify parameters that favor pyruvate oscillations, we compared the distribution of individual model parameters across all 2000 sets with those within 393 sets that yielded oscillations (Figure 1D). This showed that allosteric activation of the pyruvate kinase by FBP (higher a3) favored oscillations, as well as a high affinity of the pyruvate dehydrogenase (PDH) for pyruvate (Km5). Thus, our simulations demonstrate that the stoichiometry and the kinetics of glycolysis can produce oscillations of intracellular metabolites on the time scale of several minutes in a broad range of parameter values and with multiple reactions being involved.

Methods

The stoichiometry of the model is shown in figure1a. Mass balancing results in a system of ordinary differential equations (ODEs), F, which is a temporal function of the state variables (g6p, fbp, pep, pyr) x and the kinetic parameters p. In total the system comprises 4 variables and 21 kinetic parameters. Dilution of metabolites by growth was not considered due to large differences between growth dilution and glycolytic flux.

The six reactions (PTS, PFK, FBP, FBA, PYK, PDH) are described by the following kinetic equations:

The PTS reaction takes up glucose from outside the system boundary depending on the ratio of pyr/pep. With an assumed glucose uptake rate of 8 mmol g-1 h-1 and the specific cell volume for *E. coli* (2 µl mg-1) the reaction rate for the PTS system is:

= 66.66 mmol min-1

Reaction 2 (PFK) follows hill-type kinetics as it was shown that the enzyme PFK1 exhibits cooperative kinetics towards its substrate. The enzyme is allosterically inhibited by pep which is modelled by a power-law where n1 is the hill-coefficient and a1 is the power-law exponent.

Reaction 3 (FBP) is modelled by a Michaelis-Menten type kinetic. The physiologically relevant activation of FBP by pep is modelled by a power-law:

The flux ratio between PFK and FBP is randomly sampled between 0.01 and 1 and constraint to a net flux of 66.66 mmol min-1. Reaction 4 (FBA) is modelled by a Michaelis Menten type kinetic. Here, glycolysis is simplified by condensing four reactions into one reaction (FBA-ENO):

Reaction 5 (PYK) follows hill-type kinetics. The allosteric feed forward activation by fbp is modelled by a power-law:

Reaction 6 (PDH) follows hill-type kinetics:

The 21 parameters were randomly sampled 2000 times from a log-uniform distribution. All state variables were set to 1. The system was set to a steady state by first setting the total reaction flux of all net reactions to the glucose uptake rate. Then, parameter values of binding constants (k1-k3, Km1-Km5), hill coefficients (n1-n3) and power-law exponents (exponents a1-a3) were inserted. Then the maximum velocities were calculated (Vmax1-Vmax6). Binding constants were sampled between 0.1 and 10, hill-coefficients were sampled between 1 and 4, where a coefficient of 1 resembles a Michaelis-Menten type kinetic, and power-law exponents for allosteric regulations were sampled between 1 and 4 for feed-forward activations and -1 and -4 for feedback inhibitions.

**Model perturbation and finding strong oscillations using fourier transformation**

The perturbation of the glucose uptake was simulated by changing the uptake rate by 5% at 50 minutes simulation time. The resulting time-course data were then processed to identify parameter sets which led to oscillations. First, a polynomial first order fit was performed to remove trends and align the time courses. Second, the data were Fourier transformed from time domain to frequency domain. Signals with amplitudes above 0.005 and the corresponding parameter sets were then selected.