Using dynamic data to test models of cellular metabolism

Introduction

Metabolic models based on flux balance analysis and other constraint-based methods have been successful in predicting metabolic fluxes inside cells under steady state growth conditions. However, they provide little insight into the cellular mechanisms that bring these flux patterns about and are unable to explain how metabolic systems are able to adapt to dynamic changes.

In recent years this has lead researchers to realize that it might be necessary to use the computationally much more demanding mechanism-based models to understand the dynamics of metabolic fluxes. The advances in computational models that followed were accompanied by advances in techniques for measuring the dynamic changes in intra-cellular metabolite concentrations. The 13C-based determination of fluxes is a particularly important example of the latter.

The key advantage of dynamic data for building mechanistic models of metabolism is that these data are particularly rich in information. This property is crucial considering the fact that mechanistic models contain a large number of adjustable parameters, hence increasing the risk of overfitting.

What drives dynamic changes in metabolic flux patterns?

The metabolic flux patterns inside a cell are not fixed. Instead, these flux patterns vary in response to both environmental and cell-internal changes. A cell growing on glucose, for example, will show other metabolic flux patterns than the same cell growing on fructose. Equally, a cell's metabolic flux pattern during the S-Phase of the cell cycle, during which a copy of the cell's DNA is synthesized, will be different from the pattern during other phases of the cell cycle, during which the production of DNA precursor metabolites is less important. But, what are the factors that produce differences in metabolic flux patterns inside a cell? The answer to this question comes in the form of a long and diverse list that includes, for example, differential expression of enzyme genes, varying availability of substrates, sequestration of enzymes in cellular compartments, allosteric enzyme inhibition/stimulation etc.

The factors driving changes in flux patterns can be mapped to changes in reaction rates

However, we have seen previously that at the most fundamental level metabolic flux patterns are determined by just two factors: The stoichiometry of the metabolic reactions and the rates, with which these reactions take place. Due to the rules of mass conservation, the stoichiometry of individual metabolic reactions is fixed, leaving reaction rates as the key mechanism for driving changes in metabolic flux patterns. Since essentially all metabolic reactions inside a cell are enzyme catalyzed and the vast majority of enzymatic reactions can be described by Michaelis-Menten kinetics, it should be possible to represent all the factors listed above in one of the variables of the Michaelis-Menten equation. The connections between these "higher level" factors and the variables of the Michaelis-Menten equation is shown in Figure 1.

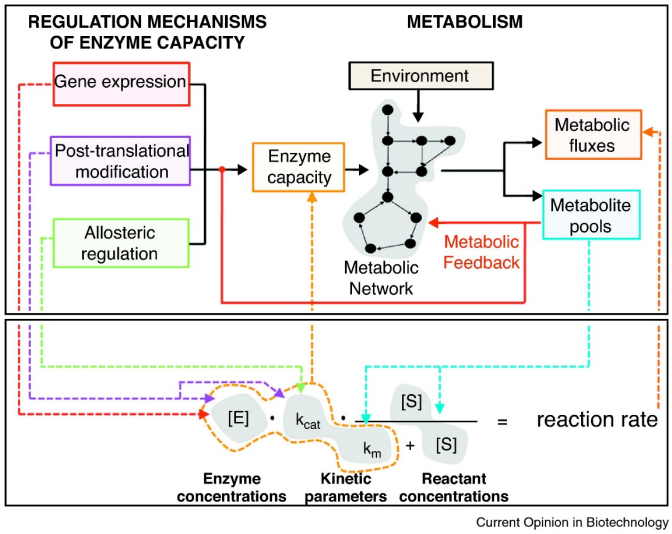


Figure 1 *Schematic representation of the link between cellular and environmental factors (top panel) that shape a cell's metabolic flux patterns and the variables in the Michaelis-Menten equation (bottom panel) through which their influence can be modeled.*

Many of the factors driving changes in flux patterns act slowly

As we can see, the network of factors and the way, in which they influence reaction rates is rather complex. Furthermore, there are a number of feedback mechanisms between these factors. The build-up of a certain metabolite, for example, may drive the repression of genes involved in its production or may trigger

regulatory changes that stimulate the sequestration or inactivation of other enzymes, which in turn will trigger further changes.

On the whole cell level, these types of complex adaptive changes are very difficult to model accurately.

Reducing the number of factors under consideration would certainly help. But how is it practically possible to set up a situation, in which some of these factors can be neglected? The key to this problem is the time scale, at which the various factors act on reaction rates. As it turns out, many of the factors (up-regulation of gene expression, sequestration or degradation of enzymes etc.) exert their influence on metabolic flux patterns comparatively slowly (minute to hour scale).

By studying a metabolic system's response to short temporary perturbations, introduced for the duration of only a few seconds and then removed, we can neglect many of the slower acting adaptation processes and investigate the fast acting mechanisms in isolation. (e.g. rapid carbon source switching shown in Figure 3)

At short time scales, changes in flux patterns are primarily driven by changes in metabolite concentrations

Let's have another look at Figure 1 and eliminate all the slow-acting factors that influence reaction rates. We see that changes in reaction rates at short time scales will primarily be driven by the concentration of the metabolites themselves. This effect of metabolite concentration on reaction rates is two-fold. The first effect is that concentrations influence the reaction rate through the classical Michaels-Menten mechanism (Figure 2) in which increasing substrate concentrations increase the rate of the reaction.

$$\frac{d[P]}{dt} = [E_{total}] \cdot k_{cat} \frac{[S]}{k_M + [S]}$$

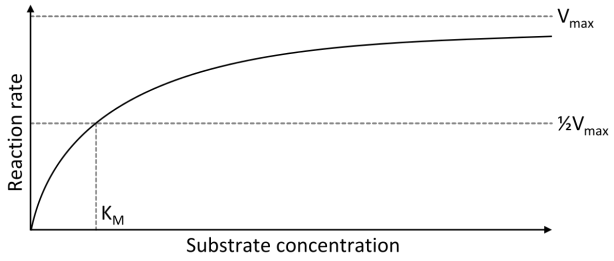


Figure 2 *Under Michaelis-Menten kinetics the rate of a reaction (d[P]/dt) (i.e. the flux through the reaction) asymptotically increases for increasing substrate concentrations [S] towards v_{max} = $[E_{total}] \cdot k_{cat}$. (image source: Wikipedia)*

The second way in which metabolite concentrations can affect reaction rates is through "non-substrate" interactions with enzymes. These include allosteric or competitive binding. Non-substrate interactions are normally instantaneous and reversible. While the first effect of metabolite concentration on reaction rate is relatively well understood, the second effect is currently an important research topic. It is important to realize that in this case the metabolite influencing the enzymes catalytic activity may or may not be a substrate of that enzyme. Thus, a potentially very large number of possible metabolite-enzyme interactions may have to be considered.

Rapid carbon source switching as an experimental tool to study metabolite-driven changes in flux patterns.

If we want to study metabolite-driven changes in metabolic fluxes without having to take into account the more complex and slower adaptive changes (e.g. gene expression and enzyme degradation), we need an experimental method that can both introduce and

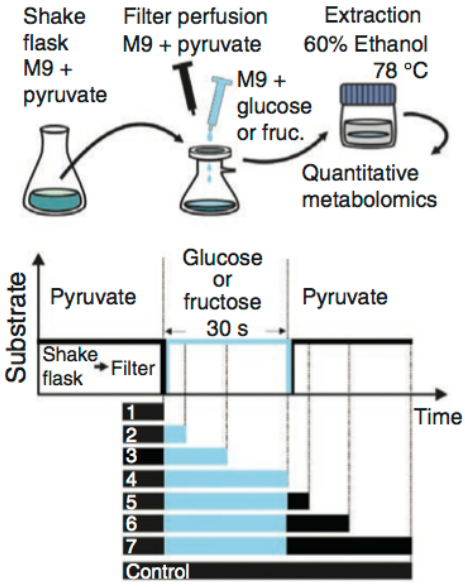
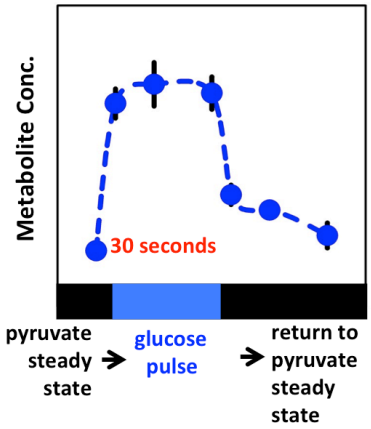


Figure 3 *Schematic representation of a rapid carbon source switching experiment. In this experiment yeast cells are grown in a filter chamber that allows rapid exchange of the growth medium. In this case cells are initially grown on a medium containing pyruvate as a carbon source. The carbon source is then briefly switched to ^{13}C fructose or ^{13}C glucose before being switched back to pyruvate again. Samples of the yeast are extracted at short time intervals. Plunging samples into hot ethanol, which kills the yeast cells, halts enzymatic activity and thus preserves the metabolites they contain. Metabolite concentrations are then measured offline. M9 is a mix of essential salts and buffering agents contained in the growth medium. (from Link et al. Nature Biotechnology, 2013)*

measure changes in metabolite concentrations on a time scale of tens of seconds. The rapid carbon source switching experiments developed by the Sauer group here at the ETH provide a means therefore. Figure 3

This method generates time course data for a large number of metabolites in the central metabolism. The result is a complex data set that is very rich in information. Figure 4

a)



b)

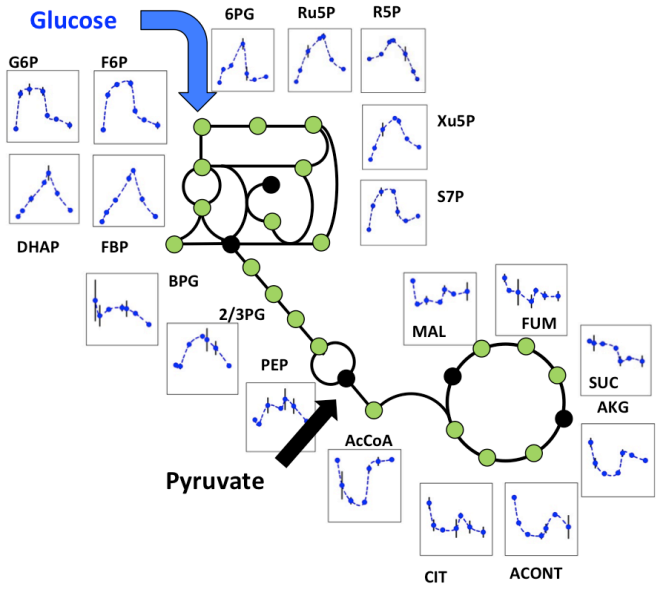


Figure 4 *The rapid carbon source switching experiment provides dynamic data on the changes in metabolite concentrations triggered by short (30 sec.) changes in the provided carbon source. Panel* ***a*** *shows this data for a single metabolite but this data is collected simultaneously for many of the other key metabolites of the central metabolism.*

Connecting Models and experimental data

The data sets we obtain from rapid carbon source switching experiments certainly contain information about the cells' metabolic responses to changes in metabolite concentrations. But how can we actually establish and exploit a link between these data and a mathematical model of metabolism?

Flux balance analysis models are certainly not suitable, because they only model steady states and the system we are investigating is clearly not in a steady state. The natural choice for the dynamic data we have is an ODE based mechanistic model that can be solved numerically.

Conceptually such a model begins with a set of initial conditions (i.e. vector $\mathbf{X}_{to}$ that represents the metabolite concentrations at t_0) and then uses Michaelis-Menten kinetics (figure 2) to calculate the rate of all reactions included in the model, based on these concentrations. The concentrations of the metabolites certainly enter here as substrate concentrations ([S]) but could also play a role as a non-substrate activators or inhibitors altering reaction rates. Based on these rates and on the reactions' stoichiometries the concentration of all metabolites $\mathbf{X}_{t1}$ at time $t_1 = t_0+\Delta t$ is calculated. This set of new concentrations is subsequently used as an input for the next computational cycle. The output of the process is a time series of $\mathbf{X}$, which corresponds to predicted time courses for the concentrations of all metabolites.

In the process of model fitting the software adjusts the models' parameters in order to minimize the difference between the predicted time course of metabolite concentration and the experimentally observed. In the case discussed above these parameters would be the $[E_{total}]$, k_m and k_{cat} for each enzymatic reaction plus the parameters describing the non-substrate effects of metabolites on reaction rates.

Both the processes of solving the ODEs of the model numerically and of model fitting were described here in a rather vaguely manner. In reality of course both of them employ highly sophisticated algorithms. Particularly in the case of fitting mechanistic models of metabolism to dynamic metabolite concentration data the development and optimization of these algorithms is still a topic of active research.

What if the model cannot describe the data?

If the structure of the model with which we seek to describe a certain metabolic process is correct, then it should be possible to fit the model so that we obtain a set of model parameters generating a reasonable match between the predicted and the observed metabolite concentration time-courses.

Not always can a good match be achieved. What to do in such a situation? Clearly, one might first check if the experimental setup is working properly etc. But, if the experimental data is solid, we will have to consider the

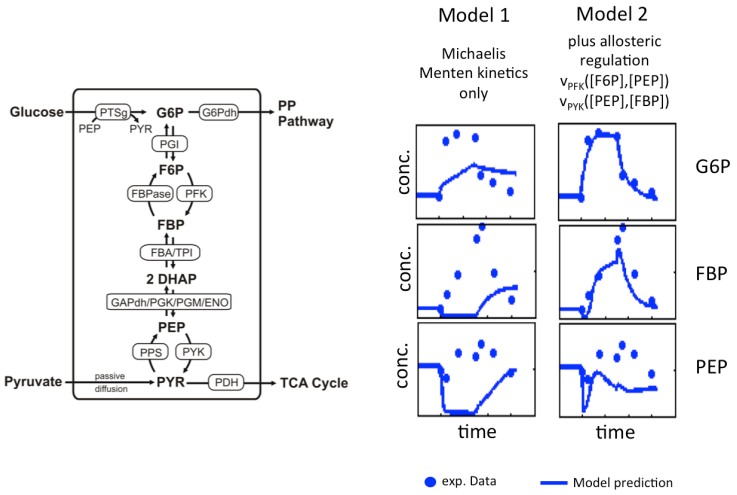


Figure 5 *Effect of model structure on the fit between experimentally observed and model-predicted metabolite concentration time courses. The metabolic network being modeled is shown in the left panel. The right panel shows the experimentally determined (blue circles) concentration of three metabolites as a function of time as well as the corresponding predictions (solid blue line) derived from two mechanistic models of this metabolic network after model fitting. The fit achieved by the model 1, which is based purely on Michaelis-Menten kinetics, is clearly unsatisfactory. Model 2 however, which also incorporates non-substrate effects of three metabolites on the rate of two of the enzymatic reactions achieves a clearly superior fit.*

possibility that the structure of our model is not correct. It becomes necessary to think about ways in which we can improve the structure of our model so that it is able to capture the actual metabolic process.

0 shows an example of a case where a model based on straightforward Michaelis-Menten kinetics was not able to recapitulate the experimentally observed time-course of several metabolite concentrations - even after fitting all model parameters. This lead to a reconsideration of the models structure and various alternative models were tested. Finally, it became clear, that a reasonable fit could only be achieved when the model's structure was adapted to include two -previously unknown- non-substrate effects of metabolites on enzymatic rates.