

UNIFIED MODELING OF SEVERAL PERTURBATION EXPERIMENTS IN SYSTEMS BIOLOGY

*A Case Study on the Glucose Uptake of *Lactococcus lactis**

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Abstract: Dynamic modeling of the metabolism is one of the main research areas of systems biology. A typical but yet unresolved problem is the modeling of glucose uptake of *Lactococcus lactis* bacteria upon in-vivo NMR measurements in perturbation experiments. Most modelers are focusing on the inverse problem, namely to identify the parameters of a set of differential equations using the available dataset. Majority of the available models suffer from the drawback that even if a perfect fit to a single experiment was achieved, they can not explain the systems' behavior in different experimental conditions.

The aim of this study is to introduce an appropriate method and a model to fit one set of parameters to several different experiments, enabling unify modeling of the glucose decay of the bacteria.

With the proposed approach a good overall fit was obtained to the dataset. The results confirm that this could be a future way towards unified modeling of data with heterogeneous experimental conditions.

1 INTRODUCTION

The aim of inverse modeling is to capture the systems' dynamic in a set of parameterized Ordinary Differential Equation (ODE). However, in systems biology the modeling task is still not certain. Problems can arise from the available dataset: the distribution of the measurement points might be uneven, and the values are often manually transcribed. The measurements are in many cases corrupted with unknown noise, and only a subset of metabolites are measurable. Other problems are caused by the the model itself: to capture the dynamic, nonlinear models are needed, but the identification of this type of models is far from trivial (Ashyraliyev et al., 2009). Too many parameters in the model may lead to overparametrisation (Vinga et al., 2008), sloppyness (Daniels et al., 2008; Vilela et al., 2009) and / or identifiability problems (Srinath and Gunawan, 2010), not to mention the need of enormous computational capacity because of the exponentially growing parameter space. Finally, many models are fitted to a single experiment only. Nevertheless, the so identified models might explain the given dataset, but in many cases lack in predictive power

to other experiments. An another consequence of the above is that even if a very close fit has been achieved to a single experiment, deterministic models might be fitted to the unknown noise as well.

The significance of good modeling of the glucose uptake of *Lactococcus lactis* bacteria is crucial, because all the other metabolite concentrations of interest depend on this process, see eg. in (Neves et al., 2005). Recently, Castro et al. (2009) revealed the pathways of glucose uptake, however to our best knowledge, no unique model fit to different experiments of the glucose uptake has been published before in the literature. This might be due to the fact that the sigmoidal shape observed in perturbation experiments is typically difficult to represent with a simple power-law function (Goel et al., 2008). Modelers often disregard a good fit to external glucose, as Goel et al. (2008), or treat the glucose as input function, eg. (Voit et al., 2006), even the models that accomplish a good fit, provide different parameters for different experiments, eg. (Vinga et al., 2008).

Here we introduce an approach based on Particle Swarm Optimization (PSO) , and a model to glucose uptake of *Lactococcus lactis* in perturbation experi-

ments. Our approach has proven useful to fit one set of model parameters to several different experimental conditions.

This paper is organized as follows: In section 2 we introduce our method of identification, in section 3 the results are described, and further discussed in section 4, finally in section 5 conclusion is drawn and we point to future work directions.

2 METHODS

In this section first we describe the used dataset, then the method of identification is introduced, finally we detail the model of the glucose uptake.

2.1 The Dataset

In vivo Nuclear Magnetic Resonance (NMR) measurements open new horizons for systems biology, allowing measurement of metabolite concentrations in the living cell (Neves et al., 2005). Unfortunately, in the case of *Lactococcus lactis* only the extracellular glucose concentration is measured, and the glucose transport should be modeled. Three perturbation datasets were used, where a bolus of ^{13}C labeled glucose was introduced of 20, 40 and 80 mM respectively to starving bacteria in anaerobe conditions. We also observed, that the multiple bolus experiments do not differ much from the single bolus regarding the shape of the glucose decay.

2.2 Identification Method

Particle Swarm Optimization (PSO) (Kennedy and Eberhart, 1995) is a population based stochastic optimization method inspired by the collective intelligence of simple interacting individuals. The traditional example for such systems is a bird flock seeking for food. The birds do not know the location of the food, but their distance from it. Sharing this knowledge with the other members of the flock allows them to follow the bird that is closest to the food.

In practice, PSO is initialized with a set of possible solutions, called particles (S_i) and associated random velocities (v_i). In every iteration (k) the speed and location of each particle in the parameter space is updated as

$$\begin{aligned} v_i(k) &= wv_i(k-1) + c_1r_1(pbest_i - S_i(k-1)) \\ &+ c_2r_2(gbest - S_i(k-1)) \end{aligned} \quad (1)$$

$$S_i(k) = S_i(k-1) + v_i(k), \quad (2)$$

where w is the inertia describing the impact of the previous velocity to the current one. The positive constants c_1 and c_2 correspond to the acceleration rate towards the local and global optima respectively. r_1 and r_2 are uniform distributed random variables ensuring the stochastic behavior of the method, $pbest_i$ is the best solution discovered so far by the i_{th} particle and $gbest$ is the best solution found in the iteration. The particle velocities are lower and upper bounded as $v_{min} < v_i < v_{max}$. The method can be summarized in the following steps:

1. Initialize a set of particles
2. Evaluate the objective function to all the particles
3. Update $gbest$ and $pbest_i$ for all particles
4. Count the the new velocities using eq(1)
5. Update the particles' position using eq (2)
6. Repeat from step 2. until the desired precision or the limit of iterations is reached.

The objective function here is evaluated in terms of Mean Squared Errors (MSE) of the fit. This method was already successfully put into practice for inferring metabolic networks (Naval et al., 2006). Our approach here is different in the sense that we do not aim to identify all the metabolites, but only focus on glucose, and instead of fitting to one experiment we use data of three different experiments (see section 2.1).

2.2.1 Fitting to Multiple Experiments

The trivial way to ensure a fit to multiple datasets is to use a (weighted) objective function, where the objective to all the datasets are contributing, for example by taking the sum of them. The drawback of this approach is that the summarized error surfaces might be very complex, and the particles might show very slow convergence or stuck in a local optima. Thus here we propose an extension to the method by introducing a random variables to the objective function as follows

$$G = q_1G_1 + q_2G_2 + \dots + q_nG_n, \quad (3)$$

where G is the objective function, $G_1 \dots G_n$ are the MSE values according to the datasets 1.. n , and $q_1 \dots q_n$ are random values with the following properties: $\sum_{i=1}^n q_i = 1$ and $\mathbb{E}(q_i) = \frac{1}{n}$.

2.3 The Model

The idea behind our model is to suppose a variable (θ), and the glucose concentration (x) depends from it in a power law manner as shown in eqs (4) and (5).

$$\dot{x} = -k\theta^\beta x \quad (4)$$

$$\dot{\theta} = \frac{1}{c}, \quad (5)$$

where k and β are parameters, and c is a constant. Solving eq (5) results the following simple time dependence: $\theta = \alpha + \frac{t}{c}$, introducing a new parameter: α . To make the model more flexible, we also introduce the constant fraction to the x variable. The final model is shown in eq (6).

$$\dot{x} = -k \left(\alpha + \frac{t}{c} \right)^\beta \frac{x}{c} \quad (6)$$

3 RESULTS

First we tested if our model is able to capture the different sigmoidal dynamics derived from the different glucose uptake time-series. To do this, we applied the model with the constant $c = 1$ to the single experiments separately. The model was fitted in 100 independent runs of the standard PSO algorithm to each experiment. The identified parameters and the simulated time-series are shown in figure 1. As it can be seen, this approach allows a good identification, however for the different experiments the best revealed parameters are distinct sets.

For the unified modeling of the different experiments we have chosen c proportional to the initial glucose pulse as $c_{20} = 1, c_{40} = 2, c_{80} = 4$ for the 20, 40 and 80 mMol experiment respectively. Here the extended version of PSO was used for identification to change the weights dynamically as described in the previous section. As seen on figure 2 we managed to achieve an overall good fit to all the three experiments. Using 50 particles, the method discovered the parameters seen in table 1, here the Mean Squared Errors (MSE) to the experimental data are also shown in table.

Table 1: The parameters obtained for our model on the three experiments using the extended version of PSO (left) and the MSE values to the experiment (right).

Parameter	Value	Experiment	MSE
k	0.2631	20 mM	2.0718
α	1.0557	40 mM	2.2654
β	0.8347	80 mM	2.9335

4 DISCUSSION

In connection to many models of systems biology it can be argued that the parameters may not be unique even within the same experiments. This phenomena can be interpreted either from the side of the biological system as sloppiness, or from the modelers point

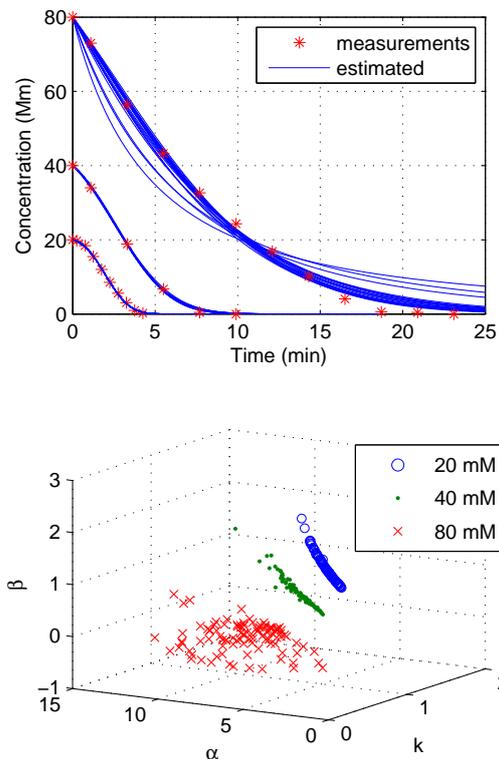


Figure 1: The fit to the time-series (top) stars are the measured values, solid lines denotes for the estimates via the 100 identified parameter sets on each experiment (bottom).

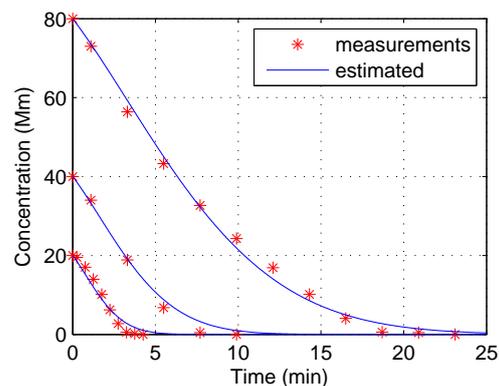


Figure 2: The original data points and the estimations with the best fitting the model.

of view a poorly constrained model resulting in bad identifiability. As (Daniels et al., 2008) points out, different conditions may attract different sets of parameters of the same biological model. The param-

eters of interest should be chosen from the intersections of these sets, since using those ensures the good description of the system within the different conditions. Unfortunately, in the case of using the same constant $c = 1$ in the model for different glucose bolus, the explored parameter sets to the single experiments do not overlap, see figure 1. In our interpretation this indicates the inadequacy of this approach. However the model fits remarkably to individual experiments, we would like to point out that this only proves the models flexibility to capture the different sigmoidal shapes.

By introducing a constant to the model, proportional to the initial glucose bolus we managed to achieve a good overall fit of the model with the same set of parameters on different experiments (figure 2). The trade-off is that MSE values to particular experiments are moderately high. We found that our approach was adequate in fitting several experiments. The random variables in the objective function resulted that the algorithm is dynamically changing the weights between the experiments, and ensures a good convergence even if the sum of the error surfaces would get difficult.

The sigmoid shape of the glucose uptake was found slightly varying on the different experiments. This might be a consequence of the different activity of the glucose uptake systems revealed by Castro et al. (2009), or the differences between the transport of the glucose monomers. We are also aware of that the glucose uptake might be influenced by other factors, for example the biomass, a feedback mechanism from the inside of the cell or the energy level of the cell according to Papagianni et al. (2007). The model could be extended to involve these aspects.

5 CONCLUSION AND FUTURE WORK

Here we introduced a model to *Lactococcus lactis* glucose uptake, and an approach based on PSO to fit it to three glucose perturbation experiments with different glucose input. With our approach a good overall fit was achieved to the data using one set of parameters. We think that this could be a future way towards unified modeling of data with different experimental conditions.

We can not exclude that our model is not complete, and additional terms might be missing from it. Our future work will aim to identify these terms. We are also considering the distinct modeling of the glucose monomers and to extend the identification of the model to aerobic conditions.

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REFERENCES

- Ashyraliyev, M., Fomekong-Nanfack, Y., Kaandorp, J. a., and Blom, J. G. (2009). Systems biology: parameter estimation for biochemical models. *The FEBS journal*, 276(4):886–902.
- Castro, R., Neves, A. R., Fonseca, L. L., Pool, W. a., Kok, J., Kuipers, O. P., and Santos, H. (2009). Characterization of the individual glucose uptake systems of *Lactococcus lactis*: mannose-PTS, cellobiose-PTS and the novel GlcU permease. *Molecular microbiology*, 71(3):795–806.
- Daniels, B. C., Chen, Y.-J., Sethna, J. P., Gutenkunst, R. N., and Myers, C. R. (2008). Sloppiness, robustness, and evolvability in systems biology. *Current opinion in biotechnology*, 19(4):389–95.
- Goel, G., Chou, I.-C., and Voit, E. (2008). System estimation from metabolic time-series data. *Bioinformatics*, 24(21):2505.
- Kennedy, J. and Eberhart, R. (1995). Particle swarm optimization. *of IEEE international conference on neural*, 4:1942–1948.
- Naval, P., Sison, L., and Mendoza, E. (2006). Metabolic network parameter inference using particle swarm optimization. In *International Conference on Molecular Systems Biology*, pages 3–4.
- Neves, A. R., Pool, W. a., Kok, J., Kuipers, O. P., and Santos, H. (2005). Overview on sugar metabolism and its control in *Lactococcus lactis* - the input from in vivo NMR. *FEMS microbiology reviews*, 29(3):531–54.
- Papagianni, M., Avramidis, N., and Filiouis, G. (2007). Glycolysis and the regulation of glucose transport in *Lactococcus lactis* spp. *lactis* in batch and fed-batch culture. *Microbial cell factories*, 6:16.
- Srinath, S. and Gunawan, R. (2010). Parameter identifiability of power-law biochemical system models. *Journal of biotechnology*.
- Vilela, M., Vinga, S., Maia, M. a. G. M., Voit, E. O., and Almeida, J. S. (2009). Identification of neutral biochemical network models from time series data. *BMC systems biology*, 3:47.
- Vinga, S., Thomaseth, K., Lemos, J., Neves, A., Santos, H., and Freitas, A. (2008). Structural analysis of metabolic networks: a case study on *Lactococcus lactis*. In *8th Portuguese Conference on Automatic Control (CONTROLO2008)*, pages 566–571.
- Voit, E., Neves, A., and Santos, H. (2006). The intricate side of systems biology. *Proceedings of the National Academy of Sciences*, 103(25):9452.