Observing optimality principles in metabolic pathways

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Abstract

Regarding transcriptional and metabolic pathways in a cell under the aspect of evolutionary development suggests an emergence of design principles, which make biological systems optimal with respect to certain constraints. Learning more about such principles can lead to a deeper understanding of the regulatory mechanisms in a cell. This essay is mainly based on the publication of *Zaslaver et al.*[1] in which optimal design principles are first investigated experimentally within the aminoacid biosynthesis (AAB) pathways and then, in a separate approach, expressed via a mathematical model. The experimental approach first demonstrates the general ability of AAB pathways to respond to changing conditions by a **dynamic adaptation of the genetic regulatory program**. In the second step the behaviour of separate AAB pathways is observed allowing for higher temporal resolution. The results reveal a pronounced **temporal and quantitative hierarchy** in the expression of enzymes involved in a pathway. The experimental results are finally compared to an optimization model of an unbranched Michaelis-Menten pathway, where a quite similar behaviour can be observed.

1 Introduction

Previously several scientific efforts have been made to develop techniques for illuminating optimal design principles in a cell. The theoretical approaches are mostly based on developing mathematical models of interactions between different components of biological networks. Definitions of optimal behaviour in such models are commonly realised through setting of **goal functions** to be optimized with respect to biologically relevant parameters, possibly subject to appropriate constraints. The justification for these goal functions arises from the assumption that the behaviour of biological systems adapts to the multitude of different conditions in a way, which constantly guarantees performance optmization. Here we will focus on unbranched metabolic pathways with Michaelis-Menten kinetics as described in *Zaslaver et al.* [1]. Prior to presenting their methods and results it is however reasonable to discuss some principles which shed light on the theoretical foundation of the experimental and modelling findings of this work.

2 Modelling via Metabolic Control Analysis

One modelling access to optimality in metabolic pathways is provided by the viewpoint of the **metabolic control analysis** (MCA). This approach is based on a model of quantitative interdependence between different parameters in a metabolic pathway. *Heinrich and Klipp* [2] deployed MCA to model an optimal behaviour both in an unbranched linear and a Michaelis-Menten pathway. Central quantities in this approach are the so called **flux controll coefficients**, which are defined as the derivatives of the steady state flux J with respect to enzyme concentrations E_j times the normalized amount of enzyme j:

$$C_j^J = \frac{\partial J}{\partial E_j}\frac{E_j}{J} \tag{1}$$

Intuitively the quantity C_j^J decribes the strength of the influence of enzyme j in a pathway on the steady state flux J. A constraint is the assumption of a **limited total enzyme amount**, expressed through the sum of all enzymes E_t bounded by the overall enzyme amount E_t^0:

$$\sum_{j=1}^{n} E_j = E_t \leq E_t^0 \tag{2}$$

Using Lagrange multipliers to maximize the steady state flux J with respect to the enzyme concentrations E_j the authors show that the flux controll coefficients can be expressed as:

$$C_j^J = \frac{E_j}{E_t^0} \tag{3}$$

implying, t*hat maximization of J leads to a distribution of control coefficients which is identic to the distribution of the enzyme concentrations* in an unbranched metabolic pathway.
Applying this model to optimization of **linear unbranched pathways** leads to the result, that the distribution of controll coefficients has a **decreasing character** from the beginning (the substrate) to the end (final product) of the reaction chain.
To model optimal behaviour in an **unbrached Michaelis-Menten pathway** the authors [2] maximize the steady-state flux not only with respect to enzyme concentrations but also with respect to the kinetic parameters of the reaction chain. The resulting distributions of controll coeffiicients are shown to be dependent on concentrations of the initial substrate and the end product. A finding important for our further considerations is, that given a high difference between standart free energy of the initial substrate and the end product, the control coefficients are again distributed in a **decreasing manner from the initial substrate to the end product**. This implies that enzymes which act close to the beginning of a pathway have a larger influence on the steady-state flux than the enzymes acting close to the end of a pathway. Their concentrations also are distributed in a decreasing manner through the reaction chain.

3 Experimental approach

The first approach of *Zaslaver et al.* [1] to investigte optimality principles in metabolic pathways was an experimental observation of expression of genes encoding enzymes involved in aminoacid biosynthesis (AAB) reaction chains. As the transcriptional regulatory networks of these pathways are well characterized, it was possible to measure the differential gene expression at different conditions by measuring activity of the known promoters of appropriate enzymes. For this purpose a library of 52 reporter strains was constructed by cloning one of the promoter regions upstream of reporter genes Lux or GFP as shown in fig. 1.
The promoter activity in time was measured by quantifying the resulting fluorescence, luminscence and absorbance in a multiwell fluorimeter which alowed for a temporal resolution between 4 and 8 minutes.

3.1 Large scale promoter analysis of aminoacid pathway genes

In the first part of the experiment the promoter activity of AAB genes was measured after a shift from a medium with no aminoacids to a medium with one particular aminoacid added. While in the first medium all promoters were active, after the shift the promoters involved in the synthesis of the added aminoacid were downregulated (fig. 2). This results in a slight diagonal

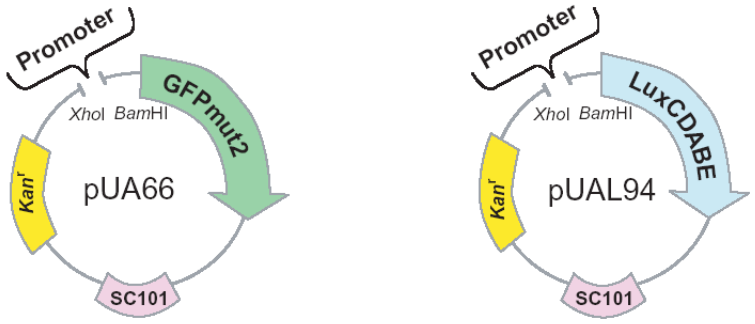


Figure 1: Construction of reporter strains by cloning AAB gene promoters upstream of reporter genes Lux or GFP (pUA66 and pUAL94 respectively). Both vectors contain BamHI and Xhol cloning site, a low copy origine and a karamycin resistance gene.

course of downregulation. A very pronounced downregulation for example can be observed for the activity of promoters involved in the arginine biosynthesis pathway after adding arginine. Furthermore several promoters from different pathways are also coregulated. For instance addition of glutamate, a precursor of argingine AAB pathway also resulted in a downregulation of arginine promoters.
The dynamic adaptivity of the AAB system is obviously an essential feature guaranteeing a **saving of resources** when their consumption is not necessary. Similar behaviour can be observed in the flagella assembly system of E. Coli, which turns on and off depending on the nutrition conditions of the medium, thus enabling a movement from locations lacking nutrition and a longer stay in a medium with nutrition abundance. This obviously displays an optimality principle of the system which has **the ability of dynamic response** to changing environmental conditions.

3.2 Separate analysis of amino acid pathways

In the second part of the experiment the promoter activity in single AAB pathways was observed after removing a single aminoacid. Here a higher temporal resolution of 4 minutes was possible which enabled a detailed analysis of activation dynamics within the pathways. As a first object of study the argingine pathway promoters were transferred into a medium containing all aminoacids except arginine. As a result a temporal and quantitative hierarchal order in the expression of enzymes involved in the pathway was observed (fig. 3 a). The synthesis pathway of arginine consists of three unbranched reaction chains. In each of the pathways a temporal hierarchy in the expression is present, reflecting the order of the enzymes in the pathway. This can be seen on the shift of the activation in the fluorimeter through the consecutive steps. For example in the pathway turning glutamate to omithine the order of promoter activation is: argA, argBC, argD, argE. This is exactly the same order in which the enzymes act in the pathway. The same observation is made for the other two unbranched pathways of the arginine biosynthesis (fig. 3 a). Observing the unnormalized expression of the enzymes in the glutamate-omithine pathway of arginine biosynthesis reveals a further result. As can be seen in (fig. 3 b) not only the order of expression of the enzymes obeys their appearance order in the pathway but also the amount of promoter activity decreases from argA to argE. These two observations have been also made for other AAB pathways like methionine and serine [1].

The described results gain a special relevance when considered in the light of the theoreti-

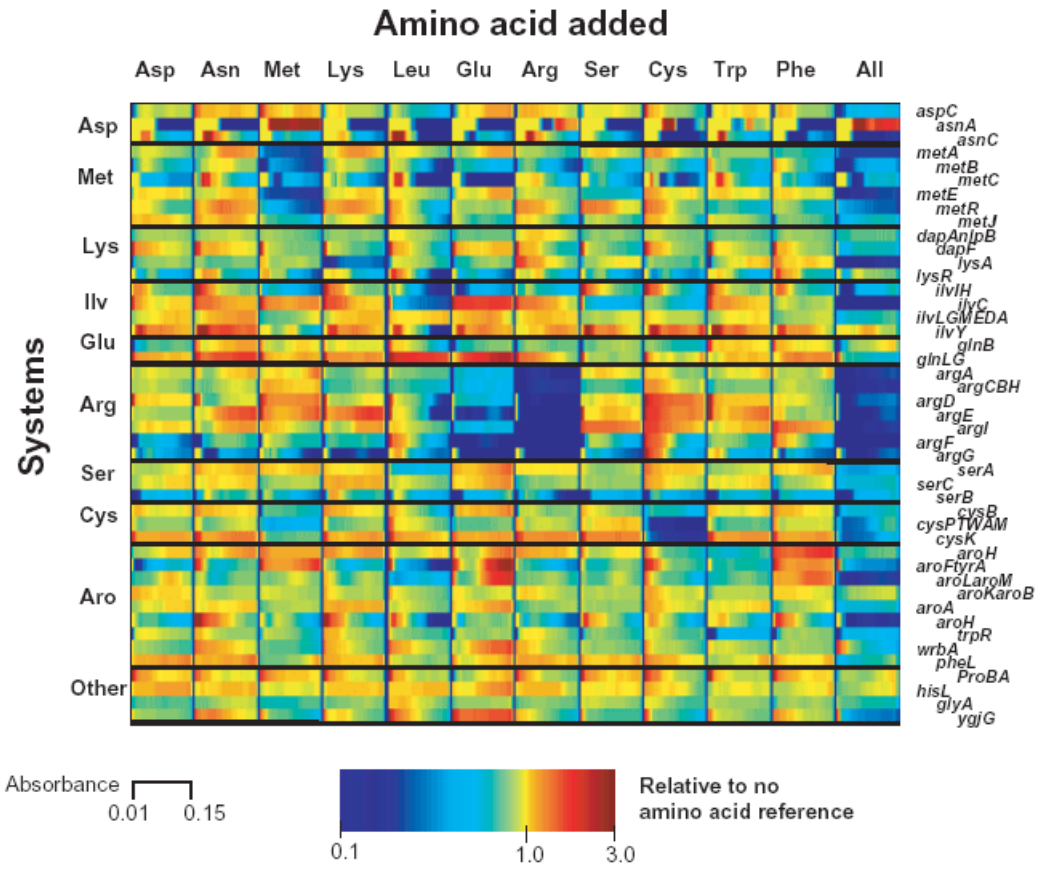
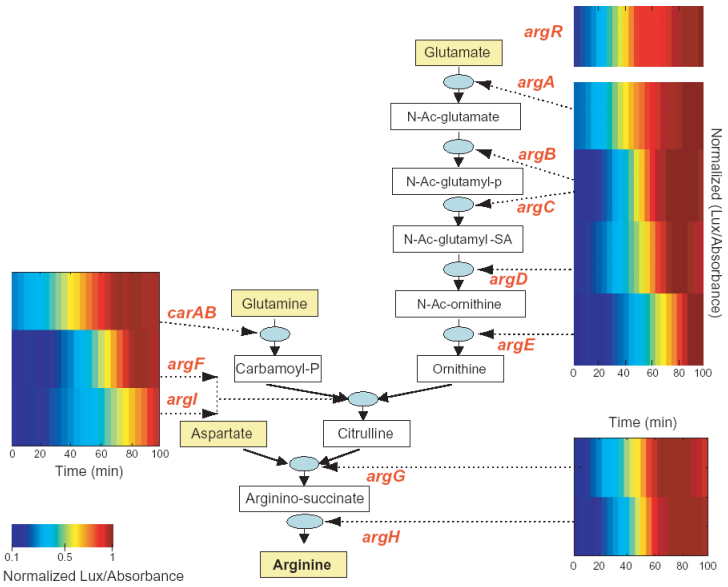
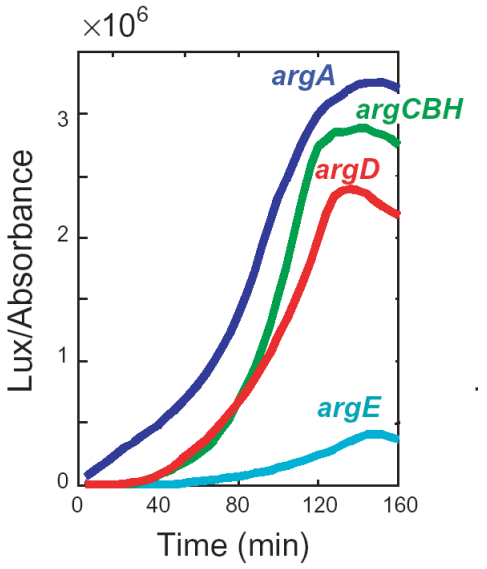


Figure 2: Decrease of AAB promoter activity of involved genes when adding the respective aminoacid to the medium and the decrease of activity of all promoters when adding all aminoacids. The promoter activity was measured with reporter strains in a defined medium. The rows represent promoters invloved in a appropriate AAB pathway, and columns represent respective conditions (addition of the respective aminoacids). The last column indicates adding of all amino acids. The logarithm of the ratio of the promoter activity to the promoter activity in the absence of all aminoacids is shown. Blue means downregulation and red indicates upregulation.

cal conclusions of the metabolic control analysis by *Heinrich and Klipp* [2]. The decrease of control coefficients from the beginning to the end of an unbranched pathway is anticipated for an unbranched linear metabolic pathway and some cases of unbranched Michaelis-Menten pathways (depending on initial substrate and product concentrations). *Klipp et al.* [3] furthermore suggested that time dependent gene expression enables cells to adapt the metabolic capabilities to varying external conditions. Both of these optimality principles were observed here experimentally within different AAB pathways.



(a)



(b)

Figure 3: (a) Activation order of promoters involved in the arginine biosynthesis pathway is shown as a result of a shift of the reporter strains from a medium containing all aminoacids to a medium lacking arginine. (b) Unnormalized promoter activity profiles for arginine AAB genes after transfer to a medium lacking argignine.

4 Mathematical modelling of optimality in a Michaelis-Menten reaction pathway

The second approach by *Zaslaver at al.* [1] used a mathematical modelling of an unbranched reaction chain obeying Michaelis-Menten kinetics:

$$\mathbf{S_0} \xrightarrow{\mathbf{E_1}} \mathbf{S_1} \xrightarrow{\mathbf{E_2}} \mathbf{S_2} \xrightarrow{\mathbf{E_3}} \mathbf{P} \tag{4}$$

where S_i represent the concentrations of metabolites and E_i are the concentrations of enzymes. The rate of production, utilization and dilution of the metabolites is described by a set of following ODEs:

$$\frac{dS_i}{dt} = V_i E_i \frac{S_{i-1}}{S_{i-1} + Km_i} - V_{i+1} E_{i+1} \frac{S_i}{S_i + Km_{i+1}} - \alpha S_i \tag{5}$$

where V_i are the enzyme velocities, set equally to the same value $V_i = V$. The rate of concentration change of enzymes is modelled by a set of ODEs as follows:

$$\frac{dE_i}{dt} = \beta_i \frac{1}{1 + R(t)/k_i} - \alpha E_i \tag{6}$$

The values α and β_i in equations (5) and (6) represent the cell growth rate and the maximal promoter activity of the gene coding for enzyme i respectively. $R(t)$ is the amount of repressor bound to the product P. A further ODE, not shown here, describes the change of total repressor concentration R_t.
An optimal behaviour of this system was modelled by minimizing the following cost function:

$$C = a \sum_i \int_0^T \beta_i \frac{1}{1 + R(t)/k_i} dt \; + \; \int_0^T \left| \frac{dP}{dt} - \frac{dP}{dt}_{\text{goal}} \right| dt = \min \tag{7}$$

The first summand is a sum of integrals of enzyme concentration rates, where a is a relative cost of producing enzymes and k_i is a concentration of a repressor needed for 50% repression. Minimizing this term constrains the total enzyme amount used. This corresponds to the constraint of a limited total enzyme amount, which we encountered in *Heinrich et al* [2] (see Section 2). Minimizing the second summand, induces as fast as possible product synthesis. This criterion can be observed as equivalent to the maximization of the total steady state flux also applied in the cited work.

The modelled system was optimized numerically with respect to the maximal promoter activity β_i and repression coefficients k_i. The full model and the resulting optimal parameters where reproduced for this essay. For this purpose a system of eight ordinary differential equations was solved using the Matlab ODE solver `ode23`. Figure 4 shows the solution functions of product synthesis-dillution rates. It can be seen, that with increasing cell growth parameters the decay of the product rate gets higher. The resulting optimal enzyme rates for $T = 60$ are depicted in figure 5. The hierarchy of enzyme rates from the first to the third enzyme in the reaction chain resembles the hierarchical order in the promoter activity of enzymes found in the aminoacid biosynthesis pathways (Section 3). The resulting parameters β_i are distributed in a decreasing manner from the first to the third enzyme. *Zaslaver et al* claim that this result can be observed for a wide span of values of T and enzyme production cost a as shown in figure 6. Generally an increasing integration time T of the cost function leads to a smaller difference between times needed to achieve 50 % of the maximal concentration.
The matlab code for solving the system of ODEs can be found in the Appendix.

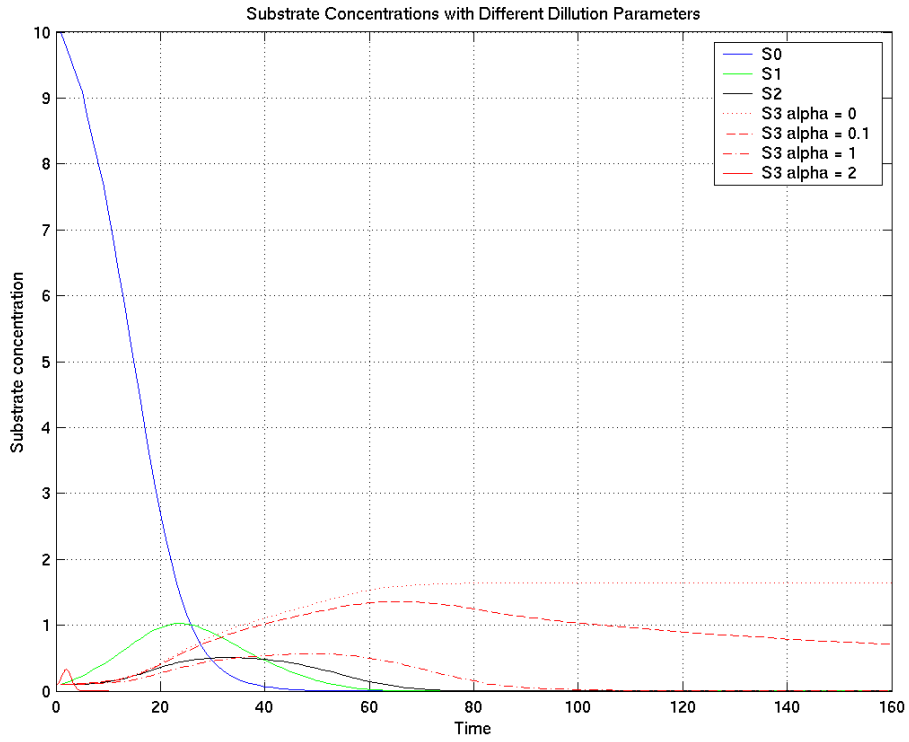


Figure 4: Substrate rates as a function of time obtained by solving the system of ODEs as desribed by *Zaslaver et al.* Different functions of the product concentration rate can be observed with varying cell growth rates.

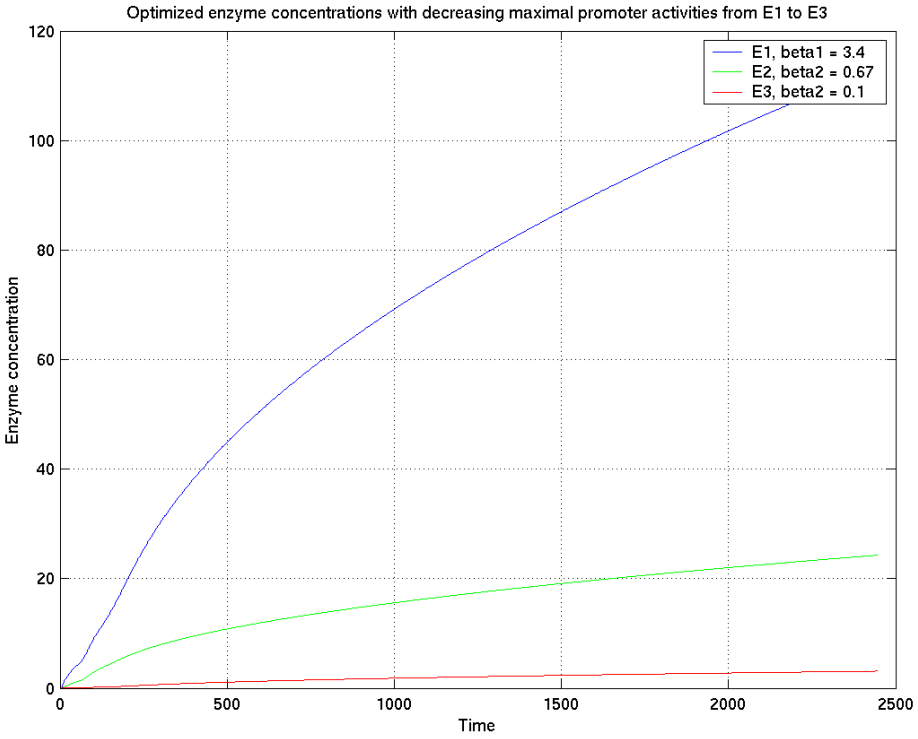


Figure 5: Optimal enzyme rate profiles for an unbranched three-step Michaelis-Menten reaction chain modelled by equations (5) and (6) and optimized with respect to the cost function (7). The x-axis reaches from 0 to 2500 ODE solving steps corresponding to a time span of $T = 60$.

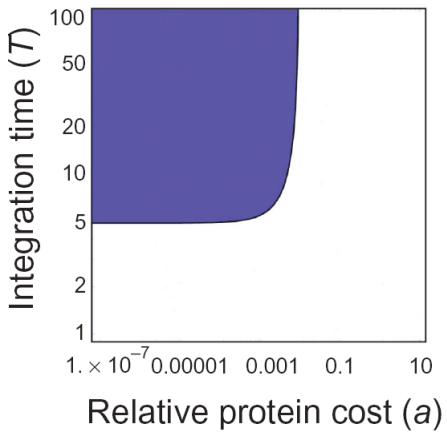


Figure 6: Properties of the optimal solution for various integration times T and enzyme production costs a. The solutions in the blue region show a decreasing maximal enzyme rate $\max(E_1) > \max(E_2) > \max(E_3)$ and increasing response time (time needed to achieve 50 % of maximal concentration)

.

5 Conclusions and Discussion

An experimental approach and a mathematical model for investigating optimal behaviour in unbranched metabolic pathways are presented. In the first approach several general design prin-

ciples can be observed. The first principle is the dynamic adaptivity of a metabolic pathway of aminoacid biosynthesis enabling a reasonable consumption rate of available recources in the cell depending on environmental conditions. The mechanism of such optimal adaptation processes can be elucidated when regarding the separate AAB pathways with higher temporal resolution. Enzymes acting closer to the beginning of an AAB pathway show a higher maximal promoter activity and a shorter time needed for reaching this acitvity level. Furthermore the general principle of **just-in-time production** can be observed, expressing a sequential activation of promoters in the order of appearence in the pathway.
The hierarchical expression level distribution was also observed in an optimization model based on Michaelis-Menten kinetics, suggesting that the mathematical model to a certain level explains the real AAB metabolic pathway. Nevertheless it should be kept in mind that the presented system of ODEs is at most a simplification of the real biochemical system, where certain factors are not taken into account or are simply still unknown. It is intriguing how close the results of the MCA modelling (Section 1) are to the presented experimental observations and to the optimization results of the ODE model. This approves the combination of the different procedures. For proving that a system behaves in an optimal way a **goal function** is needed. Optimizing it in mathematical terms substitutes the process of biological evolutionary refinement. The experimental approach in turn helps to verify if a certain behaviour is biologically reasonable. Commonly in all approaches optimization of goal functions used leads to a maximization of the steady-state flux or to a minimization of the total enzyme amount used.

References

[1] Zaslaver, A., Mayo, A. E., Rosenberg, R., Bashkin, P., Sberro, H., Tsalyuk, M., Surette, M. G., and Alon, U. *Just-in-time transcription program in metabolic pathways.* Nature Genetics, 36(5):486–91, 2004

[2] R. Heinrich and E. Klipp, *Control analysis of unbrached enzymatic chains in states of maximal activity.* J. theor. Biol. 182, 243-252, 1996

[3] E. Klipp, R. Heinrich and H.-G. Holzhütter, *Prediction of temporal gene expression*, Eur. J. Biochem. 269, 5406-5413, 2002

A Appendix

A.1 Matlab code

Solving the ODE system:

```
[T,Y]=ode23(@mmpathway,[0 20],[20 0 0 0.1 0.1 0.1 0.1 0])
```

A.1.1 mmpathway.m

```
%mmpathway.m
%Models an unbranched Michaelis-Menten pathway
function dy = mmpathway(t,y)
V=1;
Km=1;
Kr=0.0001;
alpha=1;
alpha_end=2;
beta0 = 1.3;
beta1=3.4;
beta2=0.67;
beta3=0.1;
k0= 0.23;
k1=0.23;
k2=0.68;
k3=2.1;
dy = zeros(8,1);

%ODEs for metabolites
dy(1) =                            - V*y(5) * y(1)/(y(1)+Km) - alpha*y(1)
dy(2) = V*y(5) * y(1)/ (y(1)+Km )- V*y(6)*  y(2)/(y(2)+Km) - alpha*y(2)
dy(3) = V*y(6) * y(2)/ (y(2)+Km )- V*y(7)*y(3)/(y(3)+Km)   - alpha*y(3)
dy(4) = V*y(7) * y(3)/ (y(3)+Km )- alpha_end*y(4)

%ODEs for enzymes
dy(5) = beta1 * 1/(1+Rfunc(y(4), y(8), Kr) / k1)
dy(6) = beta2 * 1/(1+Rfunc(y(4), y(8), Kr) / k2)
dy(7) = beta3 * 1/(1+Rfunc(y(4), y(8), Kr) / k3)

%ODE for total repressor concentration
dy(8) = beta0 * 1/(1+Rfunc(y(4), y(8), Kr)/k0) - alpha*y(8)
```

A.1.2 Rfunc.m

```
%Rfunc.m
%Function of active repressor level
function sol = Rfunc(S3,Rt,Kr)
sol = Rt*S3/(Kr + S3)
```