

ATP production optimization in biochemical mass action models with protein turnover

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Abstract: We introduce a simple approach to account for the ATP consumption and production in the case of mass action models of metabolic pathways including protein turnover. Under some simplifying assumptions, the method makes it possible to characterize the optimal rate of enzyme synthesis if the substrate concentrations and other rate constants are known. Furthermore we demonstrate that the proposed approach is capable of the comparison of the efficiency of different feedback laws in dynamic environment, considering time-varying substrate concentration.

1. INTRODUCTION

Reaction kinetic systems are widely used for biochemical process modeling both on macroscopic and microscopic scale [Érdi and Tóth, 1989]. In the case of molecular biology applications [Kitano, 2002, Stelling, 2004, Tyson et al., 2003], which are more in the focus of today's research trends, two main (not necessarily disjoint) application fields can be identified. In the incredibly complex system of a cell, *signaling pathways* are responsible for efficient transmission and processing of information, while *metabolic pathways* manage the energy consumption and storage of the organism. For the modelling of signalling pathways usually the systems biology approach is used [Babu et al., 2006, Bhalla and Iyengar, 1999, Soyer et al., 2005, Weng et al., 1999]. On the other hand, in the modelling of metabolic pathways, both systems biology related methods, and approaches originating from biotechnology are used [Bailey, 1998, 2001, Gombert and Nielsen, 2000, Llaneras and Picó, 2008, Llaneras, 2010, Nielsen and Villadsen, 1992, Schilling et al., 1999].

As reviewed by Llaneras [2010], the methods used for the analysis of metabolic pathways usually assume steady-state for the intracellular concentrations, and are looking for the solutions of the general equation $Nv = 0$ where N is the stoichiometric matrix, which describes the structure of the metabolic network, and v is the vector of reaction rates or fluxes.

Since these approaches focus on the flux of metabolites in the cell, these models in general do not explicitly consider the concentration of enzymes which are converting the metabolites, only the resulting rate of conversion speed, which makes the inclusion and analysis of regulation mechanisms difficult in this representation.

Mass action kinetics [Horn and Jackson, 1972, Feinberg, 1979], dominantly used in systems biological approaches assume that the reaction rates are can be described as

$$v_j = k_j \prod_{i=1}^m [\mathbf{X}_i]^{\alpha_{ij}} = k_j \prod_{i=1}^m x_i^{\alpha_{ij}}, \quad j = 1, \dots, r \quad (1)$$

where $[\mathbf{X}_i] = x_i$ is the concentration of the component \mathbf{X}_i (also called specie), α_{ij} is the so-called *stoichiometric coefficient* of component \mathbf{X}_i in the j th reaction, and $k_j > 0$ is the *reaction rate constant* of the j th reaction, that is always positive. Even with such strict constraints on reaction rates (and without any quasi steady-state assumption), mass action systems have been shown to be still capable of producing complex dynamical phenomena like multistability. Thus, several conditions and methods have been formulated corresponding to the analysis of this property [Conradi and Flockerzi, 2012, Craciun and Feinberg, 2006]. As emphasized in [Sorribas et al., 2010], mass action and generalized mass action models do not necessary use steady state assumptions, and so are able to describe both transient and steady-state responses of metabolites and fluxes to changes in the environment of the model, and provide a more accurate description of processes than techniques based merely on the stoichiometric matrix, like Flux Balance Analysis [Orth et al., 2010].

In addition, in contrast to metabolic network models, which are focusing on metabolic fluxes, mass action systems are fully capable to include the explicit description of enzyme concentrations, which influence the conversion speed of metabolic substrates. If one would like to analyze, what is the optimal concentration of an enzyme in a reaction system under certain assumptions, this capability may bring a significant benefit. Furthermore, if some of the analyzed pathway's substrates enter the cell via active transport, the enzymatic reaction may also reflect

the activity of the transport protein which converts the extracellular compound to an intracellular one.

Regarding the thermodynamic aspects of biochemical processes [Alberty, 2002], it can be said [Nath, 1998] that the fundamental bioenergetic processes of life are all nonequilibrium processes. The energy acquired by living cells is stored in useful form mainly as molecules of adenosine triphosphate (ATP). ATP, in converting to adenosine diphosphate (ADP) releases the stored energy and supplies it to the organism for the performance of vital functions. The mathematical machinery of the classic thermodynamics, however, mainly applies to systems in equilibrium. The paper of Qian and Beard [2005] introduces thermodynamic formalism for the study of metabolic biochemical reaction networks in both time-dependent and time-independent nonequilibrium states and generalizes classical concepts in equilibrium thermodynamics (enthalpy, entropy, and Gibbs free energy of biochemical reaction systems) to nonequilibrium settings.

Nonequilibrium thermodynamics is used in [Qian and Regula, 2005] to describe the dynamics of a cellular signaling switch. Thermodynamics of ATP hydrolysis is addressed in articles [Alberty, 1969, Foot and Reznitz, 1974, Gajewski et al., 1986], while the thermodynamics of enzymatic regeneration of ATP from AMP and ADP is discussed in [Gardner et al., 1974].

In the context of the two detailed systematic frameworks, not surprisingly, the analysis of energetic/thermodynamic aspects of the analyzed pathways appears dominantly in the metabolic approach. Regarding the description detailed in Llaneras [2010], as the kernel of N in the general equation may contain infinitely many vectors which satisfy the general equation, various constraints may be added to the problem to define the feasible set of flux vectors. These constraints lead to various models and methods, e.g. flux balance analysis [Kauffman et al., 2003, Orth et al., 2010], where various optimality constraints are considered as well. A thermodynamics based metabolic flux analysis is proposed by Henry et al. [2007]. Optimality of metabolic networks is further studied in [Segré et al., 2002]. The constraints to take into account may include energetic considerations, as taking into account the chemical potential of the reactants [Beard et al., 2002] or assuming that the feasible flux vector(s) maximize the ATP production (see eg. Ramakrishna et al. [2001]).

If we would like to analyze the energetic aspects in the case of mass action reaction kinetic models, several questions arise. The most straightforward approach for the characterization of energetic aspects in biochemical systems is probably the quantitative analysis of energy storing molecules (ATP, NADH, FADH etc.) produced or consumed during the reactions. Although we usually have some information about these properties in the case of metabolic reactions, the explicit inclusion of these molecules in the mass action model can be problematic. In the case of explicit inclusion of eg. ATP in the model, the concentration of ATP would appear in the rate of every reaction which consumes ATP in the system. Since mass action computational models are typically made for the description of single pathways or networks, but the ATP concentration is affected by multiple additional pathways

and factors in the cell, it is generally not possible to derive an equation for the description of the change of ATP concentration. Assuming a quasi-steady state for ATP to eliminate the dependence on its concentration in the equations on the other hand would contradict to our aim, which is the analysis of energetic aspects and so the concentration change of ATP.

A possible approach is to neglect the ATP concentration dependence of reactions that consume ATP, but still take into account the consumption and production rates which affect the concentration of ATP *corresponding to the analyzed pathway*. This way, as we will see, the optimality analysis of a single pathway can be done under these simplifying assumptions.

Frameworks, in which the optimality of ATP production in mass action models can be analyzed have been defined (see eg. [Sorribas et al., 2010]), but these models usually assume constant enzyme concentrations, and neglect the turnover of enzymatic proteins and account for synthesis processes in an indirect way.

The aim of this article is propose a simple intuitive method to consider the ATP consumption/production of reactions, which can be used in mass action systems as well, and can be utilized for optimization and parameter estimation purposes.

2. MATERIALS AND METHODS

Our approach will be the following. If we are interested in the effect of a certain pathway on ATP concentration, we identify the reactions of the mass action model that directly or indirectly consume or produce ATP molecules (e.g. active transport reactions, enzyme synthesis etc.). Based on the quantity of produced/consumed ATP, we define an energy factor ϵ_i for reaction i .

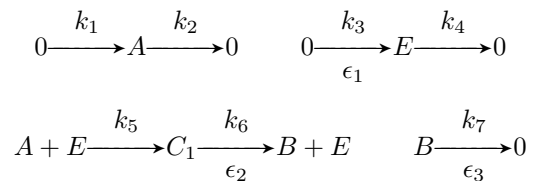
Regarding the concentration change *corresponding to the analyzed pathway* $\frac{d[ATP]}{dt}$ we may write

$$\frac{d[ATP]}{dt} = \sum_{i=1}^r \epsilon_i v_i \quad (2)$$

where v_i is the reaction rate of the i -th reaction. ϵ_i is negative for the reactions where ATP is consumed, and positive for those where ATP is generated. Furthermore, as it was previously described, we neglect the ATP consuming reaction's rate dependence on ATP concentration.

2.1 Example 1

Let us consider the following model



A corresponds to a substrate which enters the cell at rate k_1 , and is degraded at rate k_2 . We assume that the organism is able to synthesize an enzyme E , but the synthesis

process requires ATP (ϵ_1). We denote the variable rate of synthesis by k_3 . The enzyme is degraded at rate k_4 , while k_5 and k_6 denote the rate of reactions (also energy consuming), in which the enzyme transforms the substrate A into B . We assume that B then enters into a metabolic process at rate k_7 , in which it is degraded/transformed and produces ϵ_3 number of ATP molecules per molecule.

A different interpretation may be that A denotes the extracellular concentration of a certain metabolite, while B corresponds to the intracellular value. In this case E can be regarded as the membrane transport protein, which transports the compound to the intracellular space via active transport (at the cost of ϵ_2).

The concentration of the species A, E, C_1 and B may be described with the standard mass kinetic equations obeying the mass action law (suppressing the use of brackets for denoting concentrations)

$$\begin{aligned}\frac{dA}{dt} &= k_1 - k_2A - k_5AE \\ \frac{dE}{dt} &= k_3 - k_4E - k_5AE + k_6C_1 \\ \frac{dC_1}{dt} &= k_5AE - k_6C_1 \\ \frac{dB}{dt} &= k_6C_1 - k_7B\end{aligned}\quad (3)$$

In addition, we may assume that the total ATP change corresponding to the modeled pathway can be described as

$$\frac{dATP}{dt} = \epsilon_1 k_3 + \epsilon_2 k_6 C_1 + \epsilon_3 k_7 B. \quad (4)$$

According to these equations, and assuming that every rate constant except k_3 is predetermined and known, it makes sense to analyze, what is the rate of E -synthesis, which maximizes the energy production in steady state.

Equation (3) gives the following steady-state values

$$\begin{aligned}\bar{A} &= \frac{k_1 k_4}{k_2 k_4 + k_3 k_5}, & \bar{B} &= \frac{k_1 k_3 k_5 k_6}{k_2 k_4 k_6 k_7 + k_3 k_5 k_6 k_7}, \\ \bar{C}_1 &= \frac{k_1 k_3 k_5}{k_2 k_4 k_6 + k_3 k_5 k_6}, & \bar{E} &= \frac{k_3}{k_4}\end{aligned}$$

which implies that the energy production of the pathway in steady-state is

$$\begin{aligned}\left(\frac{dATP}{dt}\right)_{ss} &= \epsilon_1 k_3 + \epsilon_2 k_6 \frac{k_1 k_3 k_5}{k_2 k_4 k_6 + k_3 k_5 k_6} \\ &+ \epsilon_3 k_7 \frac{k_1 k_3 k_5 k_6}{k_2 k_4 k_6 k_7 + k_3 k_5 k_6 k_7}\end{aligned}$$

the necessary condition of the optimum is that

$$\frac{\partial \left(\frac{dATP}{dt}\right)}{\partial k_3} = 0$$

which yields

$$\begin{aligned}0 &= \epsilon_1 + \frac{(\epsilon_2 k_1 k_5 k_6)(k_2 k_4 k_6 + k_3 k_5 k_6) - (k_5 k_6)(\epsilon_2 k_1 k_3 k_5 k_6)}{(k_2 k_4 k_6 + k_3 k_5 k_6)^2} + \\ &+ \epsilon_3 \frac{(k_1 k_5 k_6 k_7)(k_2 k_4 k_6 k_7 + k_3 k_5 k_6 k_7) - k_5 k_6 k_7 (k_1 k_3 k_5 k_6 k_7)}{(k_2 k_4 k_6 k_7 + k_3 k_5 k_6 k_7)^2}\end{aligned}$$

One may observe, that the above expression leads to a fourth order polynomial equation in k_3 . For the numerical analysis, let us use the parameter set

$$\begin{aligned}k_1 &= 5, & k_2 &= 0.2, & k_4 &= 0.2, & k_5 &= 2.1, & k_6 &= 1.6, & k_7 &= 0.3, \\ \epsilon_1 &= -5, & \epsilon_2 &= -1, & \epsilon_3 &= 3.\end{aligned}$$

The roots of the polynomial showing possible local extrema are $[-0.21, 0.18, -0.02]$ where -0.02 has a multiplicity of 2. The value of the second derivative at 0.18 is -1.15 , showing a local maximum. Fig. 1 depicts $\frac{dATP}{dt}$ as a function of the enzyme synthesis rate k_3 with the above parameters.

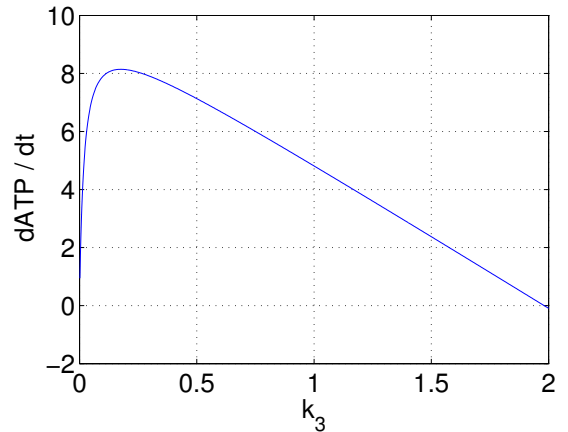
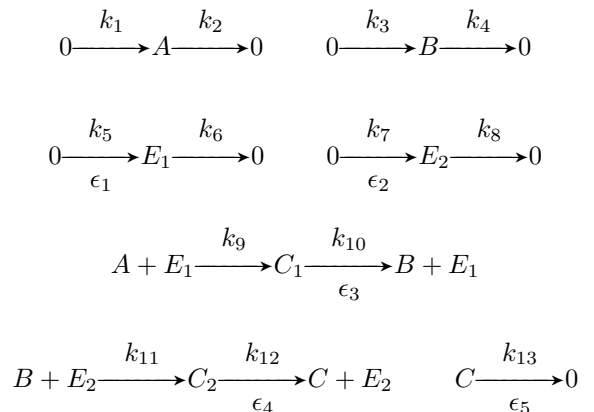


Fig. 1. $\frac{dATP}{dt}$ as the function of k_3 in the case of example 1.

2.2 Example 2

In our second example, we have two substrates: A and B . E_1 is able to transform A into B , while E_2 transforms B into C , which is the source of energy. In this case, we have to determine the synthesis rate of E_1 and E_2 . The reactions are as follows.



The change of ATP in steady-state can be described by the following equation

$$\left(\frac{dATP}{dt}\right)_{ss} = \epsilon_1 k_5 + \epsilon_1 k_7 + \epsilon_3 k_{10} \bar{C}_1 + \epsilon_4 k_{12} \bar{C}_2 + \epsilon_5 k_{13} \bar{C}$$

Again, we analyze the energy production in steady state. The steady state values of the compounds will be as follows.

$$\bar{A} = \frac{k_1 k_6}{k_2 k_6 + k_5 k_9}, \quad \bar{E}_1 = \frac{k_5}{k_6}, \quad \bar{C}_1 = \frac{k_1 k_5 k_9}{k_2 k_6 k_{10} + k_5 k_9 k_{10}}, \quad \bar{E}_2 = \frac{k_7}{k_8},$$

$$\bar{B} = \frac{k_2 k_3 k_6 k_8 + k_3 k_5 k_8 k_9 + k_1 k_5 k_8 k_9}{k_2 k_4 k_6 k_8 + k_2 k_6 k_7 k_{11} + k_4 k_5 k_8 k_9 + k_5 k_7 k_9 k_{11}},$$

$$\bar{C}_2 = \frac{k_2 k_3 k_6 k_7 k_8 k_{11} + k_3 k_5 k_7 k_8 k_9 k_{11} + k_1 k_5 k_7 k_8 k_9 k_{11}}{k_2 k_6 k_{12} (k_4 k_8^2 + k_7 k_8 k_{11}) + k_5 k_9 k_{12} (k_4 k_8^2 + k_7 k_8 k_{11})},$$

$$\bar{C} = \frac{k_2 k_3 k_6 k_7 k_8 k_{11} k_{12} + k_3 k_5 k_7 k_8 k_9 k_{11} k_{12} + k_1 k_5 k_7 k_8 k_9 k_{11} k_{12}}{k_{12} k_{13} (k_2 k_6 k_8 (k_4 k_8 + k_7 k_{11}) + k_5 k_8 k_9 (k_4 k_8 + k_7 k_{11}))}$$

Let us use the following parameter values

$$k_1 = 3, \quad k_2 = 0.7, \quad k_3 = 1, \quad k_4 = 0.5, \quad k_6 = 1.1, \quad k_8 = 0.6, \\ k_9 = 1.3, \quad k_{10} = 1, \quad k_{11} = 1.4, \quad k_{12} = 1.5, \quad k_{13} = 1.9 \\ \epsilon_1 = \epsilon_2 = -5, \quad \epsilon_3 = -1, \quad \epsilon_4 = -2, \quad \epsilon_5 = 10$$

The resulting $\left(\frac{dATP}{dt}\right)_{ss}$ as a function of k_5 and k_7 is depicted in Fig. 2. The optimum is at $[k_5 \ k_7] = [0.77 \ 0.75]$, where the value of the Hessian matrix is

$$\begin{pmatrix} -7.23 & 1.67 \\ 1.67 & -10.27 \end{pmatrix},$$

which is negative definite, justifying the local maximum.

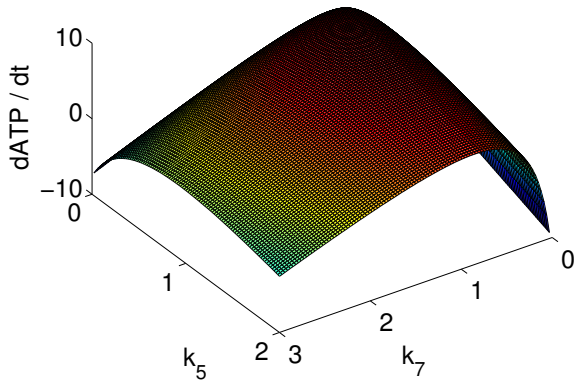
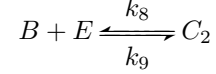


Fig. 2. $\left(\frac{dATP}{dt}\right)_{ss}$ as a function of k_5 and k_7 in the case of example 2. The maximum is at $[k_5 \ k_7] = [0.77 \ 0.75]$.

2.3 Example 3

Now we analyze the dynamic behavior of the system defined in 2.1 under a perturbation. We assume a significant fluctuation of the substrate A around its former steady state. We model this fluctuation by an additional $1.8 \sin(2\pi t/30)$ term in the kinetic equation of A. The following three cases are compared.

- The value of k_3 is constant, and it is equal to its former value (0.18).
- A very simple linear feedback applied. In this case, k_3 depends on the actual concentration of A as follows: $k_3 = 0.072A$. It is easy to check that this feedback law provides the optimal k_3 if A is in steady state as in section 2.1.
- The well-known enzymatical regulation mechanism of product inhibition is assumed. In addition to the reactions described in section 2.1, the reaction



is assumed, which inhibits the enzymatic catalysis at higher product concentrations, and also stabilizes the enzyme. We assume $k_8 = k_9 = 0.7$. As it can be seen after some simple calculation, this additional regulation mechanism does not affect the steady state values of A, E, C₁ and B, which means that the optimal value of k_3 is still equal to 0.18.

We compare the ATP production in the case of substrate fluctuation for the above three cases. The resulting ATP production is depicted in Fig. 3.

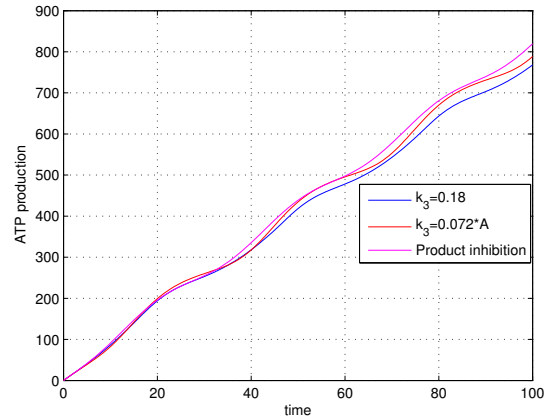


Fig. 3. ATP production in the case of constant and adaptive enzyme synthesis in the case of example 1.

It can be seen in the figure, that as time elapses, the difference between the static and the dynamic cases grows. Even the simple linear feedback law is able to increase the ATP production corresponding to the analyzed pathway. Further questions may arise if we take into account that to implement such a feedback in biological systems requires some kind of sensory mechanism. The synthesis of e.g. proteins which are capable to play this role may require further ATP, which in turn also affects the resulting ATP production.

It also turns out from Fig. 3 that the mechanism of product inhibition gives the best performance regarding the resulting ATP balance.

3. CONCLUSIONS

In this paper, a simple approach was introduced to account for the ATP consumption and production of mass action pathway models. The elements of the computation were

shown through illustrative examples. The approach allows us to characterize the optimal rate of enzyme synthesis if other rate constants and parameters describing ATP need or production are known. Taking into account the ATP need/production of reactions and assuming ATP-optimality (as it is often done in literature), the method has the potential to support parameter estimation in the form of additional constraints. Furthermore, as demonstrated, the presented approach is suitable for the comparison of the efficiency of different feedbacks even in a dynamic environment, considering changes in substrate concentration.

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