

# Validity of the Michaelis-Menten Approximation for the Stability Analysis in Regulatory Reaction Networks

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**Keywords:** Michaelis-Menten Approximation, Stability Analysis, Cellular Signalling Systems, Regulatory Reaction Networks.

**Abstract:** Cellular signalling systems are comprised of enzymatic reaction cascades and organized as regulatory reaction networks. The primary building block of the network is an enzymatic activation-inactivation cyclic reaction such as phosphoryl modifications. We have investigated the effects of the network architectures and kinetic parameter values on the stability such as the emergence of bi-stability or oscillations employing the canonical Michaelis-Menten equation as the approximation for Michaelis-Menten-type reaction mechanisms in each of enzymatic cyclic reaction. Although the Michaelis-Menten approximation has known to work well under an assumption of a large excess of substrate over enzyme which is usually satisfied for metabolic pathways, the approximation might not suit to regulatory reaction networks in which the required assumption might be violated. In this study, comparing the predicted stabilities from the model with the Michaelis-Menten approximation and with the full set of reaction equations derived only from the law of mass action, the validity of the Michaelis-Menten approximation was examined for the regulatory reaction networks over the possible network architectures and kinetic parameter values elucidating that employing the Michaelis-Menten approximation might not be valid even in the analysis for the steady states such as the stability analysis.

## 1 INTRODUCTION

The Michaelis-Menten-type reaction mechanism has been widely employed to construct the mathematical models for analysing the dynamics and the stability of the enzymatic reaction systems. Actually, the mechanism has been devised as its approximation form known as the Michaelis-Menten approximation or the more simplified form such as the first order equation or the higher order equation which is so-called Hill equation to formulate the co-operativity (Adler, Szekely, Mayo, & Alon, 2017; Kuwahara & Gao, 2013; Ma, Trusina, El-Samad, Lim, & Tang, 2009; Shah & Sarkar, 2011; Sueyoshi & Naka, 2017; Yao, Tan, West, Nevins, & You, 2011).

Although the Michaelis-Menten approximation has known to work well under an assumption of a large excess of substrate over enzyme which is usually satisfied for metabolic pathways, the approximation might not suit to regulatory reaction networks in which the required assumption might be

violated since the same protein could have both roles of the substrate and the enzyme simultaneously.

In this study, the cellular signalling systems are formulated as the regulatory reaction networks where the each node represents the enzymatic activation-inactivation cyclic reaction such as phosphoryl modifications and the each arc depicts their regulations. Then, the effects of the Michaelis-Menten approximation on the stability of the regulatory reaction networks comprised of two enzymes are analyzed to elucidate the validity of the approximation in construction of the mathematical models for the cellular signalling systems.

## 2 METHOD

All possible regulatory structures for the cellular signalling systems comprised of two cyclic reaction systems are formulated as the regulatory reaction networks, and the stabilities are analysed. In particular, the effects of the regulatory structures and

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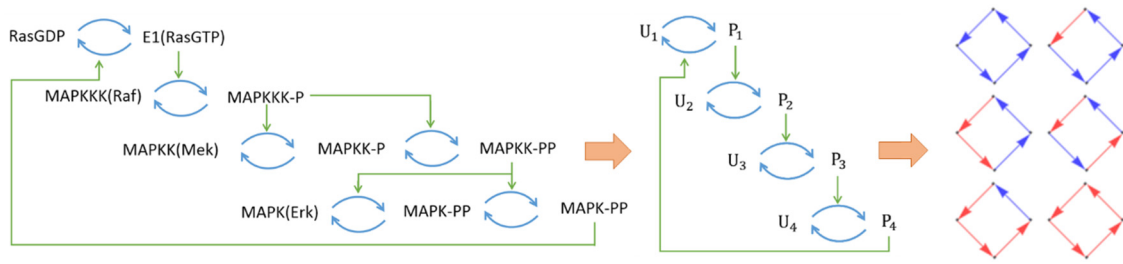


Figure 1: Regulatory reaction networks representing the MAPK cascade: the reaction scheme on the left; the simplified reaction scheme on the middle; the regulatory reaction network representations on the right where red arrows depict positive regulations, while blue arrows indicate negative regulations. The regulatory structure of the MAPK cascade is represented by the four-node regulatory reaction network at the first column and the third row in the directed graphs shown on the right.

the parameter values of the systems on the number of the stable equilibrium points are predicted.

Then, the aspects of the stability are compared between the mathematical models employing the Michaelis-Menten approximation and the models derived only from the law of mass action for each enzymatic reaction in the cyclic reaction systems.

## 2.1 Regulatory Reaction Networks

Figure 1 shows how the regulatory reaction networks represent the cellular signalling systems with respect to the MAPK cascade as an example, which is one of the typical and the well-studied cellular signalling systems (Ferrell, 1998; Jeschke, Baumgartner, & Legewie, 2013; Kholodenko, 2006; Mai & Liu, 2013; Qiao, Nachbar, Kevrekidis, & Shvartsman, 2007; Volinsky & Kholodenko, 2013). The dual catalytic reaction processes appeared in the third and the fourth cascades in MAPK cascade are simplified to the one reaction step processes as shown in the middle. Red arrows depict positive regulations where an activated enzyme acts on another enzyme as the activating

enzyme, while blue arrows indicate negative regulations where an activated enzyme acts on another enzyme as the inactivating enzyme. Then, the regulatory structure of the MAPK cascade is represented by the four-node regulatory reaction network at the first column and the third row in the directed graphs shown on the right.

In this study, ten variations of the two-node regulatory reaction networks shown as the legends of the graphs in Fig. 4 are analysed. These networks are all possible mutually regulatory reaction networks with at most one positive regulation and one negative regulation at each node. It should be noted that if one of the positive or negative regulation at each node is missing, a virtual regulation is added for the missing regulation which catalyse with the maximum and constant rate.

The Michaelis-Menten-type mechanisms are employed as the reaction mechanisms in the enzymatic cyclic reactions in each node. Figure 2 shows the mutual negative regulatory reaction network with auto positive regulations as an example. The representation of the network as the regulatory reaction network is shown on the top. The left graph and the right graph on the bottom depict the reaction schemes employing the Michaelis-Menten approximation and employing only the law of mass action, respectively.

The activation reaction rate  $\beta_1$  and the inactivation reaction rate  $\delta_1$  of node 1 in the model employing the Michaelis-Menten approximation are formulated as follows:

$$\beta_1 = \frac{k_1 P_1 U_1}{M_1 + U_1}, \delta_1 = \frac{l_1 P_2 P_1}{N_1 + P_1}, M_1 = \frac{d_1 + k_1}{a_1}, N_1 = \frac{e_1 + l_1}{b_1}$$

$P_1$  and  $U_1$  indicate the concentrations of the active and inactive forms of the enzyme, respectively.  $M_1$  and  $N_1$  represent the Michaelis constants for the activation and inactivation reaction, respectively. Those concentrations of two enzymes and those

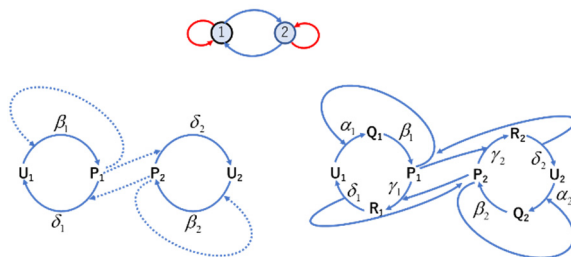


Figure 2: Reaction schemes of the above regulatory reaction network: employing the Michaelis-Menten approximation on the bottom left; employing only the law of mass action on the bottom right. Red and blue arrows on the top figure depict the positive and negative regulations, respectively. The solid lines on the bottom figures depict the associate and dissociate chemical reactions, while the dotted lines represent the enzymatic reactions formulated by Michaelis-Menten approximations.

Michaelis constants are relative, that is, normalized by the total concentrations of the respective enzymes which are assumed to be the same values for two enzymes to simplify the formulations in this study.

Supposing the steady states, that is,  $\beta_1 = \delta_1$ , with the constant  $K_1 = l_1/k_1$  leads to the following equations:

$$\frac{P_1 U_1}{M_1 + U_1} = K_1 \frac{P_2 P_1}{N_1 + P_1}, \quad P_1 + U_1 = 1$$

The same equations of the variables with the subscripts which numbers are exchanged are derived for the node 2. The enzyme concentrations at the steady state are obtained by solving these four equations.

In the case that the Michaelis-Menten approximation is not employed corresponding to the reaction scheme on the bottom right in Fig. 2, the respective reaction rates  $\alpha_1$ ,  $\beta_1$ ,  $\gamma_1$ , and,  $\delta_1$  are formulated only from the law of mass action as follows:

$$\alpha_1 = a_1 P_1 U_1 - d_1 Q_1, \beta_1 = k_1 Q_1, \gamma_1 = b_1 P_2 P_1 - e_1 R_1, \delta_1 = l_1 R_1$$

$P_1$  and  $U_1$  represent the relative concentrations of the active and inactive forms of the enzyme, respectively.  $Q_1$  and  $R_1$  depict the relative concentrations of the substrate-enzyme complexes.  $M_1$  and  $N_1$  are the normalized Michaelis constants for the activation and inactivation reaction, respectively, as same as the case with the model employing the Michaelis-Menten approximation. Supposing the steady state drives the following equations:

$$Q_1 M_1 = P_1 U_1, R_1 N_1 = P_2 P_1, Q_1 = K_1 R_1 \\ P_1 + U_1 + 2Q_1 + R_1 + R_2 = 1$$

The same equations of the variables with the exchanged subscripts are derived for the node 2. The enzyme concentrations at the steady state are obtained by solving these equations.

## 2.2 Stability Analysis

Steady states of the two-node regulatory reaction networks are determined by the six parameters of  $K_1$ ,  $M_1$ ,  $N_1$  for the node 1 and  $K_2$ ,  $M_2$ ,  $N_2$  for the node 2. In this study, the four Michaelis constants are set to be the same value, that is,  $L = M_1 = N_1 = M_2 = N_2$  to reduce the dimension of the parameter space. The analysis is performed over 11 discrete values of  $L$  such as  $2^{-5}, 2^{-4}, \dots, 2^5$ . The remaining parameters  $K_1$  and  $K_2$  are set to be the value of  $2^p$ , for which the 1000 values of  $p$  are taken randomly over the range of  $-5 \leq p \leq 5$ . This range is determined to cover

the values of the parameters utilized in the mathematical models for MAPK cascade (Brightman & Fell, 2000; Hatakeyama et al., 2003; Huang & Ferrell, 1996; Levchenko, Bruck, & Sternberg, 2000; Schoeberl, Eichler-Jonsson, Gilles, & Muller, 2002).

The concentrations of each chemical species in the regulatory reaction networks could be obtained by solving the corresponding algebraic equations as mentioned in section 2.1. However, the analytical derivation is getting harder for higher order equations due to the nonlinearity. Furthermore, the eigen values of Jacobian matrix are required to evaluate the stability at each equilibrium points (Heinrich & Schuster, 1996). In this study, the number of stable equilibrium points are obtained by the rather practical way in which the convergent solutions are obtained by solving the differential equations formulating the dynamics of the regulatory reaction networks with a number of initial states instead of solving the corresponding algebraic equations analytically to avoid the computational complications.

The parametric robustness is employed to evaluate the stability quantitatively (Shah & Sarkar, 2011). The parametric robustness of the feature for stability is defined as the ratio of applied parameter sets exhibiting the feature. For instance, the parametric robustness of the bi-stability is defined as the ratio of the number of combinations of  $K_1$  and  $K_2$  yielding bi-stability to the total number of combinations examined which is 1000 in this study. High values of the parametric robustness imply the robustness for the parametric perturbations which is one of the important features in noisy environments such as in cells.

## 3 RESULTS

The regulatory structures examined in this study yield four types of stability, such as mono-stable, bi-stable, tri-stable, and oscillatory. Most of the examined cases exhibit mono-stability or bi-stability.

Figure 3 shows the result of the analysis for the bi-stability. The row and the column of squares correspond to the values of the Michaelis constants and to the variation of the regulatory reaction networks, respectively. Each square represents the parameter space in logarithmic scales with the abscissa of  $K_1$  and the ordinate of the  $K_2$ . The blue dots and the red dots indicate the combination of parameter values yielding bi-stability in the model with the Michaelis-Menten approximation and in the model without the Michaelis-Menten approximation, respectively.

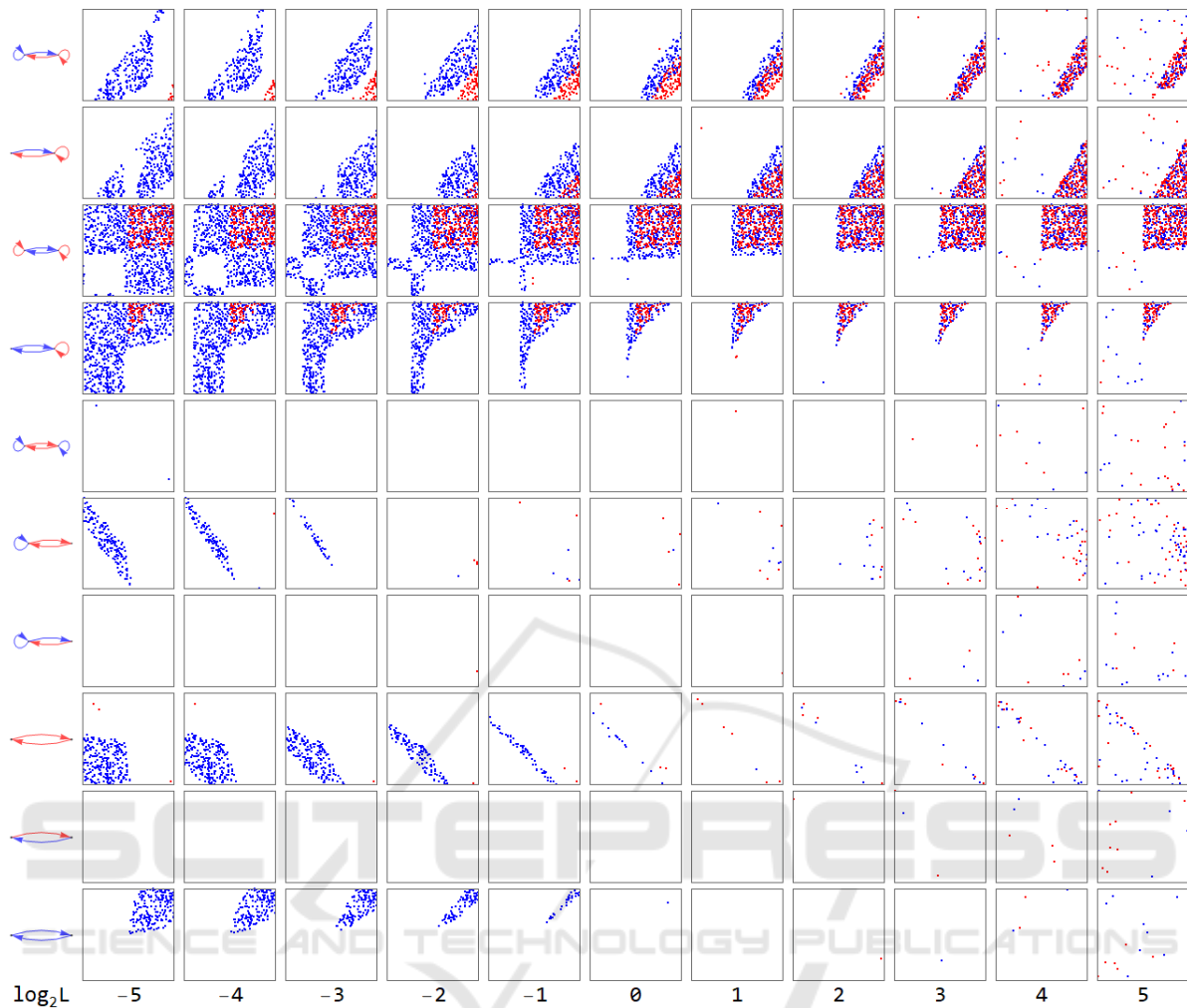


Figure 3: Parameter values yielding the bi-stabilities with respect to regulatory structures and the Michaelis constants. Each square represents the parameter space in logarithmic scales with the abscissa of  $KK1$  and the ordinate of the  $KK2$ . The row and the column of the squares correspond to the values of the Michaelis constants and to the examined variation of the two-node regulatory reaction networks, respectively. The red arrows depict positive regulations, while the blue arrows indicate negative regulations.

It is shown that the more combinations yielding bi-stability for the model with the Michaelis-Menten approximation were predicted than that for the model without the Michaelis-Menten approximation in some regulatory structures, especially in the area of small values of the Michaelis constants. Furthermore, it can be seen that each of the combination of the parameters exhibiting bi-stability for the model without the Michaelis-Menten approximation remains at the same points regardless of the value of the Michaelis constants.

Figure 4 shows the effects of Michaelis-Menten approximation on the parametric robustness for the emergence of bi-stability. The top and the bottom graphs correspond to the aspect of the emergence of

bi-stability in the model with the Michaelis-Menten approximation and in the model derived only from the law of mass action, respectively. The abscissa and the ordinate denote the values of the Michaelis constants in logarithmic scale and the parametric robustness, respectively. Each colour of the graph corresponds to the individual regulatory reaction network shown in the right side of the graph.

In the models utilizing the Michaelis-Menten approximation, the parametric robustness for the negative mutual regulatory network with two positive auto-regulations or one positive auto-regulation is quite high especially in the area for the small Michaelis constants, which was reported in the previous study (Sueyoshi & Naka, 2017). On the

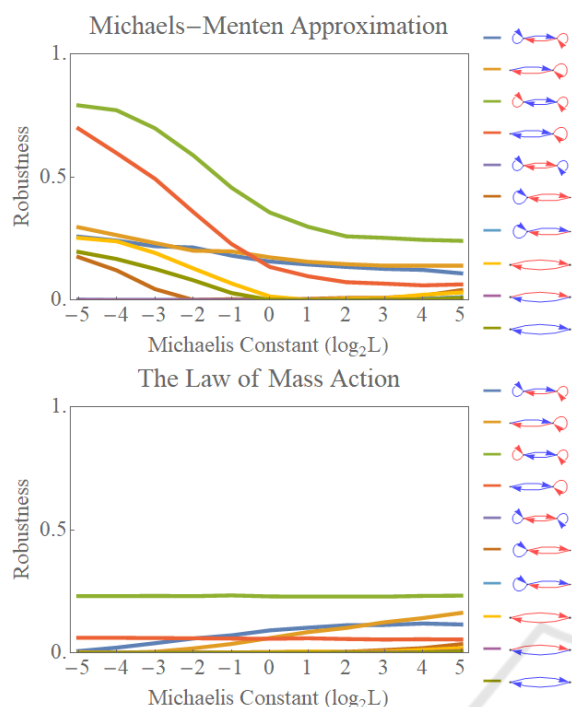


Figure 4: The effects of Michaelis-Menten approximation on the parametric robustness for the emergence of bi-stabilities. The robustness for the model with the Michaelis-Menten approximation and with the law of mass action is shown on the top, and on the bottom, respectively. Each colour of the graph corresponds to the regulatory reaction network shown in the right side of the graphs where the red arrows depict positive regulations, while the blue arrows indicate negative regulations.

contrary, in the models not utilizing the Michaelis-Menten approximation, the parametric robustness has hardly changed with respect to the values of the Michaelis constants in both regulatory reaction networks. The unchanged value of the parametric robustness is as almost the same value as one for the large value of the Michaelis constant in the model employing the Michaelis-Menten approximation. Concerning the other regulatory structures, the similar tendencies emanate while the parametric robustness is much less on the whole.

Figure 5 shows the effects of Michaelis-Menten approximation on the parametric robustness for the emergence of tri-stability. The top and the bottom graphs correspond to the aspect of the emergence of tri-stability in the model with the Michaelis-Menten approximation and in the model derived only from the law of mass action, respectively. High parametric robustness appears in the area of the small Michaelis constants for the negative mutual regulatory network with two positive auto-regulations which yields quite high parametric robustness for bi-stability as

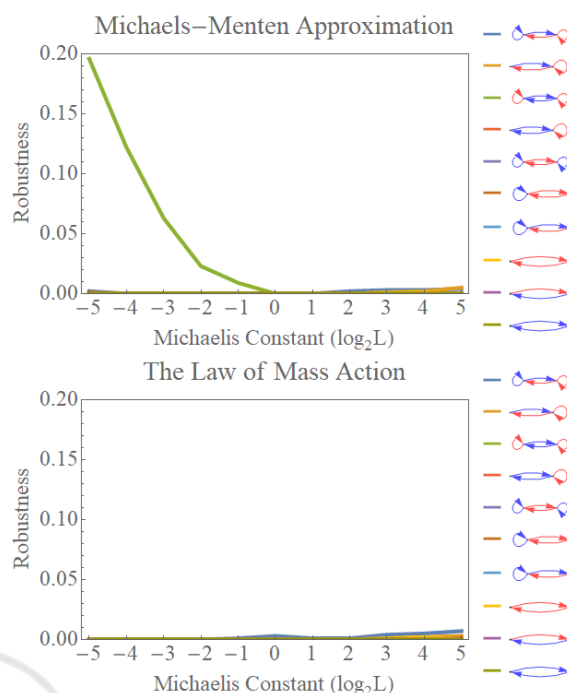


Figure 5: The effects of Michaelis-Menten approximation on the parametric robustness for the emergence of tri-stabilities. The robustness for the model with the Michaelis-Menten approximation and with the law of mass action is shown on the top, and on the bottom, respectively. Each colour of the graph corresponds to the regulatory reaction network shown in the right side of the graphs where the red arrows depict positive regulations, while the blue arrows indicate negative regulations.

mentioned before. However, the part of high parametric robustness has vanished in the models not employing the Michaelis-Menten approximation. The slight emergence of tri-stability for the model with the Michaelis-Menten approximation in the area of large Michaelis constants is seen and the aspect of the parametric robustness is as the almost same as for the model without the approximation.

Figure 6 shows the effects of Michaelis-Menten approximation on the parametric robustness for the emergence of oscillations. The oscillations occur in some regulatory reaction networks while their parametric robustnesses are quite small. In the case for the model with the Michaelis-Menten approximation, the oscillations occur in the area of the small Michaelis constants for the positive and negative mutual regulations with a positive auto-regulation. Furthermore, the oscillation appears in the area of the large Michaelis constants for the mutual positive regulations without auto-regulations. However, the oscillations emerged in the area of small Michaelis constant vanish in the models



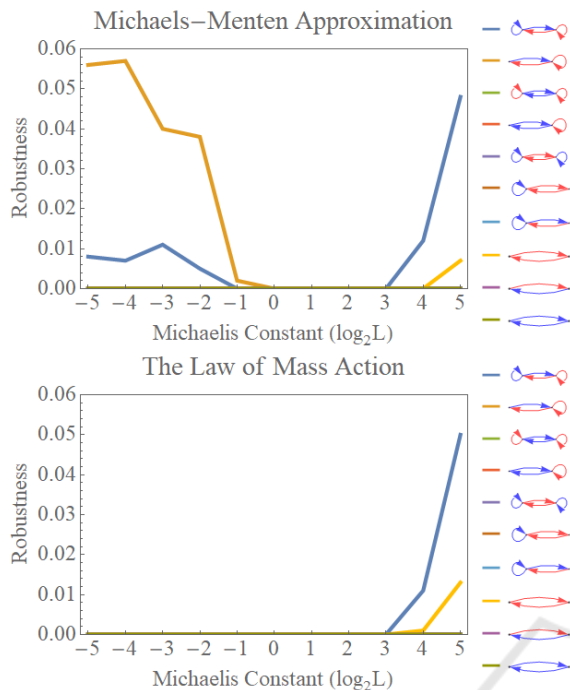


Figure 6: The effects of Michaelis-Menten approximation on the parametric robustness for the emergence of oscillations. The robustness for the model with the Michaelis-Menten approximation and with the law of mass action is shown on the top, and on the bottom, respectively. Each colour of the graph corresponds to the regulatory reaction network shown in the right side of the graphs where the red arrows depict positive regulations, while the blue arrows indicate negative regulations.

without the Michaelis-Menten approximation. The aspect of the parametric robustnesses is almost the same in two models.

Taken together, it is suggested that the parametric robustness of stability might be overestimated on the mathematical model employing the Michaelis-Menten approximation. This bias seems to be dominant in the condition of the small Michaelis constants. On the other hand, almost the same parametric robustnesses are predicted in the area of the large Michaelis constants. Therefore, it might be not valid to utilize the Michaelis-Menten approximation for analysing the properties even at the steady states. The validity depends on the values of the Michaelis constants of the enzymes comprising the cellular signalling systems.

The quite large value of the Michaelis constant implicates the much less associate rate than the dissociate and catalytic rate, which means that the concentrations of substrate-enzyme complex are much less than the concentrations of the free substrates and the enzymes. The Michaelis

approximation makes the substrate-enzyme complexes not exist in the conservative laws. Therefore, the large Michaelis constants might make the effect of the absence of the complexes less. This implication may be reason why the similar aspects are observed about the emergence of stability for the two models in the area of the large Michaelis constants.

## 4 CONCLUSIONS

In this study, the validity of the Michaelis-Menten approximation was examined for a set of regulatory reaction networks comprised of the two enzymatic cyclic reactions, in which each enzyme also works as the substrate each other such like cellular signalling systems. As a result, it is suggested that the mathematical models utilizing the Michaelis-Menten approximation for an enzyme which has the small Michaelis constant might overestimate the emergence of the bi-stability and the oscillations even for analysing the properties at the steady state.

Although it might be safer to construct a mathematical model derived only from the law of mass action without the Michaelis-Menten approximation, it may cause a problem of high computing cost. Furthermore, utilizing the Michaelis-Menten approximations often makes it possible to divide the target system into a number of sub-systems due to omitting the substrate-enzyme complexes. On the contrary, utilizing only the law of mass action often cause the computational difficulty due to intra-connections of each dynamics in the entire system caused by the substrate-enzyme complexes.

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