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Estimation of Kinetic Rates of MAP kinase Activation from Experimental Data

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Abstract— **Mathematical model is an important tool in systems biology to study the dynamics of biological systems inside the cell. One of the significant challenges in systems biology is the lack of kinetic rates that should be measured in experiments or estimated from experimental data. This work addresses this issue by using a genetic algorithm to estimate reaction rates related to the phosphorylation and dephosphorylation of MAP kinase (ERK) in the mitogen-activated protein (MAP) kinase pathway from biological measurements. In addition, we discuss the robustness of the mathematical model with regards to the variation of kinetic rates together with external noise due to environmental fluctuations. This has been proposed as an additional criterion to choose the estimate from the candidate parameter sets that are obtained from different implementations of the genetic algorithm.**

Keywords-MAP kinase; mathematical modelling; genetic algorithm

I. INTRODUCTION

The mitogen-activated protein (MAP) kinase cell signalling transduction pathway transmits signals from activated growth factor receptors at the cell surface to transcription factors in the nucleus to regulate cellular functions including proliferation, differentiation and apoptosis. The MAP kinase pathway is among the best-characterised signal transduction pathways and has been an ideal model system for mathematical modelling because of the availability of a large amount of experimental data and a relatively comprehensive understanding of the regulatory mechanisms in operation. Since the development of the first mathematical model of the MAP kinase module [6], MAP kinase signalling has been used repeatedly as a testable paradigm for pioneering computational systems biology over the last decade [2, 7, 8, 10, 11]. Although the principal hierarchy of the signalling pathway and its activation sequence are well established, recent data have yielded additional information on critical protein-protein interactions, regulatory loops and spatio-temoral organization. Recent advances in the molecular understanding of MAP kinase signalling pose new challenges for mathematical modelling strategies [3].

The MAP kinase module comprises of a set of three protein kinases (Raf, MEK and ERK) that have highly conserved molecular architecture and act sequentially. Raf activates the dual-specificity MAP kinase kinase (or MEK) by phosphorylating serine and threonine residues. Activated MEK in turn activates MAP kinase (or ERK) by phosphorylating threonine and tyrosine residues in the activation loop. Activated MAP kinase phosphorylates multiple substrates, including transcription factors, protein kinases, phospholipases and cytoskeletal proteins. In vivo, MEK must be recruited to the plasma membrane in order to be activated by plasma membrane localized Raf, while ERK is dominantly activated in the cytosol by the activated MEK that returns to the cytosol from the plasma membrane. The majority of existing mathematical models assume that biochemical reactions of the MAP kinase pathway are homogeneous and fire in the cytosol. In order to study the critical function of the plasma membrane in signal transduction, compartmental models including reactions in different subcellular locations began to emerge [11].

One of the major challenges in systems biology is the lack of kinetic rates that ideally should be measured in experiments or estimated from experimental data. Often it is observed that the same parameter has a wide range of values in different mathematical models. This observation certainly includes the mathematical model of the MAP kinase pathway [2, 6, 7, 8, 10, 11]. In this work, we use a genetic algorithm to estimate kinetic rates in the MAP kinase signalling transduction pathway. Specifically, we will estimate kinetic rates that are related to ERK activation. Compared with the activation of other kinases in the MAP kinase pathway, the mechanisms related to the ERK activation are relatively simpler. Firstly, ERK is dominantly activated in the cytosol by MEKpp, the dual activated form of MEK. This mechanism is consistent with the assumption of the existing mathematical models. In addition, ERK is the signal output of the MAP kinase module and there are less feedback loops between the activation of ERK and the activated ERK kinase. ERK activation has been modelled by the distributively dual phosphorylation and dephosphorylation in either three distinct forms [6] or four distinct forms [8]. The kinetic rates of the mathematic model with four district forms species have been estimated from the experimental data [8]. Since the majority of mathematical models use the three distinct forms for ERK activation and deactivation [2, 6, 7, 10, 11], this work will estimate the kinetic rates of ERK activation in the framework of the three distinct forms. In the next section (II), we will present the mathematical model based on the distributive reaction mechanisms of ERK activation and deactivation. Section III estimates the kinetic rates from the experimental data by using the genetic algorithm. The robustness of the mathematical model with the estimated kinetic rates will be discussed in Section IV.

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II. MATHEMATICAL MODEL

Here we are interested in the biochemical reactions for the ERK activation. In the process of distributive catalysis, the activated MEKpp binds to the substrate ERK, activates one of the sites and releases the intermediate mono-phosphorylated ERKp. Then, a new collision between MEKpp and ERKp is required for the conversion of this intermediate into the dual-phosphorylated ERKpp, the final product of the ERK activation reactions. The biological reactions of ERK activation are represented by

$$\begin{aligned}
&\text{MEKpp + ERK} \underset{d_1}{\overset{a_1}{\rightleftarrows}} \text{MEKpp-ERK}\\
&\text{MEKpp-ERK} \xrightarrow{k_1} \text{MEKpp + ERKp}\\
&\text{MEKpp + ERKp} \underset{d_2}{\overset{a_2}{\rightleftarrows}} \text{MEKpp-ERKp}\\
&\text{MEKpp-ERKp} \xrightarrow{k_2} \text{MEKpp + ERKpp}
\end{aligned} \tag{1}$$

Based on the above reactions, the mathematical model is a system of six differential equations, given by

$$\begin{aligned}
\frac{d[Mpp]}{dt} &= -a_1[Mpp][E] - a_2[Mpp][Ep]\\
&\quad + (d_1 + k_1)[Mpp - E] + (d_2 + k_2)[Mpp - Ep],\\
\frac{d[E]}{dt} &= -a_1[Mpp][E] + d_1[Mpp - E],\\
\frac{d[Mpp - E]}{dt} &= a_1[Mpp]*[E] - (d_1 + k_1)[Mpp - E],\\
\frac{d[Ep]}{dt} &= k_1[Mpp - E] - a_2[Mpp][Ep] + d_2[Mpp - Ep],\\
\frac{d[Mpp - Ep]}{dt} &= a_2[Mpp][Ep] - (d_2 + k_2)[Mpp - Ep],\\
\frac{d[Epp]}{dt} &= k_2[Mpp - Ep],
\end{aligned} \tag{2}$$

where [M] and [E] are the concentrations of MEK and ERK respectively. For example, [Mpp-E] is the concentration of complex [MEKpp-ERK].

Similarly, the deactivation of ERK by the ERK phosphatase (ERKPase) is distributive. ERKPase binds to the activated ERKpp, disphosphorylates one of the sites and releases the intermediate mono-phosphorylated ERKp. A new collision is needed for ERKPase to bind to ERKp in order to dephosphorylate the intermediate product to the unphosphorylated ERK. The biochemical reactions are represented by

$$\begin{aligned}
&\text{ERKp + ERKPase} \underset{d_3}{\overset{a_3}{\rightleftarrows}} \text{ERKp-ERKPase}\\
&\text{ERKp-ERKPase} \xrightarrow{k_3} \text{ERK + ERKPase}\\
&\text{ERKpp + ERKPase} \underset{d_4}{\overset{a_4}{\rightleftarrows}} \text{ERKpp-ERKPase}\\
&\text{ERKpp-ERKPase} \xrightarrow{k_4} \text{ERKp +ERKPase}
\end{aligned} \tag{3}$$

Based on reactions (3), the dynamics of ERK deactivation is represented by

$$\begin{aligned}
\frac{d[E]}{dt} &= k_3[Ep - EPase],\\
\frac{d[Ep]}{dt} &= -a_3[Ep][EPase] + d_3[Ep - EPase]\\
&\quad + k_4[Epp - EPase],\\
\frac{d[Epp]}{dt} &= -a_4[Epp][EPase] + d_4[Epp - EPase],\\
\frac{d[EPase]}{dt} &= -a_3[Ep][EPase] + (d_3 + k_3)[Ep - EPase]\\
&\quad - a_4[Epp][EPase] + (d_4 + k_4)[Epp - EPase],\\
\frac{d[Ep - EPase]}{dt} &= a_3[Ep][EPase] - (d_3 + k_3)[Ep - EPase],\\
\frac{d[Epp - EPase]}{dt} &= a_4[Epp][EPase] - (d_4 + k_4)[Epp - EPase],
\end{aligned} \tag{4}$$

where [E] and [EPase] are the concentrations of ERK and ERK phosphatase ERKPase respectively.

III. ESTIMATION RESULTS

The genetic algorithm is an effective technique to find approximate solutions of complex search problems. We used a MATLAB toolbox [4] to estimate the six model parameters in both system (2) and (4). Since protein concentrations are zero at some time points, the estimation error is defined as the absolute error between numerical simulation and experimentally measured kinase activities. The genetic algorithm was run over 300 generations for each estimate and a population of 100 individuals was used in each generation. The estimation error generally remains unchanged after the 200th generation in each implementation of the genetic algorithm. The values of kinetic rates were taken initially to be uniformly distributed in the range $[0, W_{\max}]$ and the value of $W_{\max}$ was determined by simulation. The criterion for selecting $W_{\max}$ is that the estimated kinetic rates in all implementations are below the corresponding value of $W_{\max}$. In this work, $W_{\max}$=300 when estimating parameters in system (2) and $W_{\max}$=700 when estimating parameters in system (4). Because of the possible local maximum of the genetic algorithm, we obtained 10 sets of estimated parameters by using different sets of initial estimates.

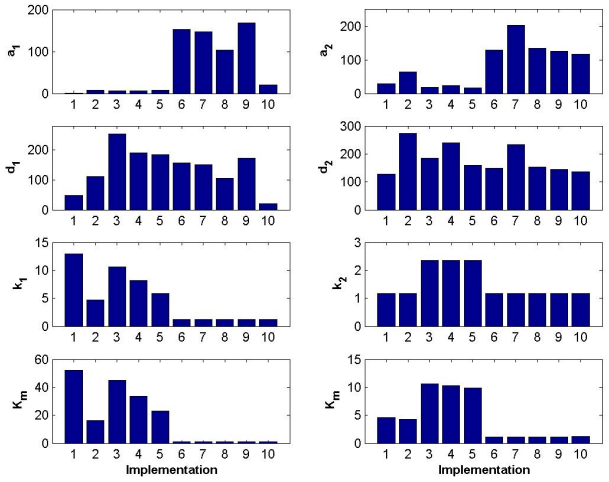

Figure 1. Estimated parameters of the kinetic rates and equilibrium constants K_m of the system (2) for ERK activation. Ten estimates were obtained from different implementations of the genetic algorithm.

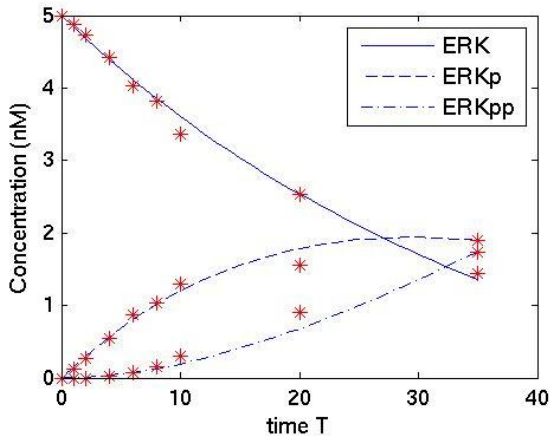

Figure 2. Experimental data (star) and simulated protein concentrations of ERK, mono-phosphorylated ERKp and dual-phosphorylated ERKpp in ERK activation by using the estimated kinetic rates (a_1 =1.1765, d_1 =48.2353, k_1 =12.9412, a_2 =28.2353, d_2 =128.2353, d_2 =1.1765).

Experimental data are the measured concentrations of mono-phosphorylated ERKp and dual-phosphorylated ERKpp that are activated by ERKpp *in vitro* [5]. The estimated parameters of the system (2) are presented in Figure 1. Implementations are ranked by their estimation error from the smallest to the largest. We also presented the values of

$$K_m = \frac{k_i + d_i}{a_i}$$

which is an equilibrium constant that determines the steady-state levels of enzyme species [6]. Note that the relative values of the equilibrium constant K_m in Figure 1 is proportional to those of the corresponding rate k_i . This suggests that the phosphorylation rate may play an important role in determining the steady-state levels. Since the average of these estimates cannot reproduce the experimental data, we use the estimate with the minimal estimation error as our final estimate of the model parameters (shown in Figure 2). This estimate produces the numerical simulation in Figure 2 which matches the experimental data with good accuracy.

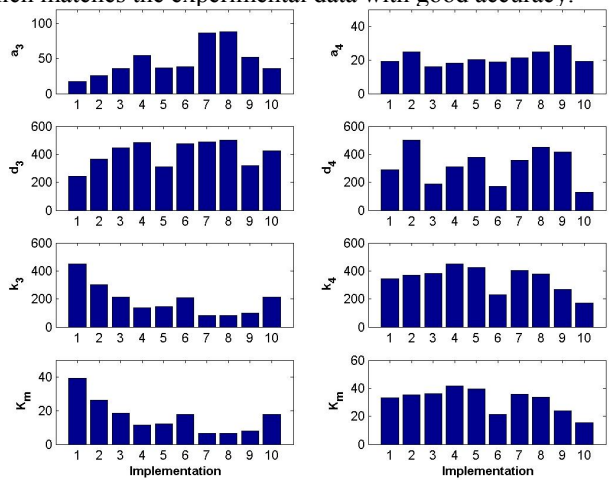

Figure 3. Estimated parameters of the kinetic rates and equilibrium constants K_m of the system (4) for ERK deactivation. Ten estimates were obtained from different implementations of the genetic algorithm.

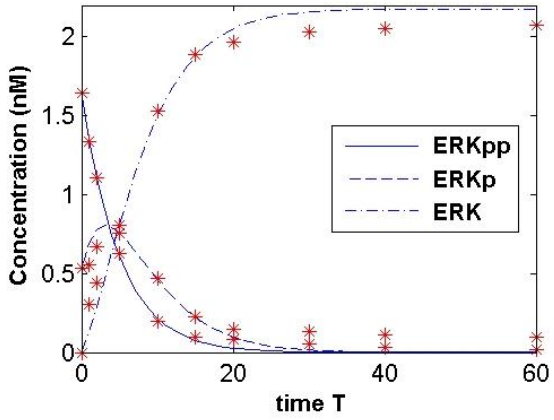

Figure 4. Experimental data (star) and simulated protein concentrations of ERK, mono-phosphorylated ERKp and dual-phosphorylated ERKpp in ERK deactivation by using the estimated kinetic rates (a_3 =17.6969, d_3 =244.8151, k_3 =448.1444, a_4 =19.2805, d_4 =289.6994, k_4 =345.9886).

We also estimated the kinetic rates of system (4) for ERK dephosphorylation based on the experimental data that include the time course of the concentrations of dual-phosphorylated ERKpp, mono-phosphorylated ERKp, and inactivated ERK, which are dephosphorylated by the ERK phosphatase MKP3 [15]. Figure 3 presents the estimated kinetic rates from 10 implementations of the genetic algorithm. Implementations are ranked by the estimation

error from the smallest to the largest. Compared with the kinetic rates of ERK activation in Figure 1, there are fewer variations between the estimated kinetic rates in Figure 3, especially for the rate k_i and equilibrium constant K_m. The values of the equilibrium constant K_m in Figure 3 again is proportional to the values of the corresponding rate k_i. The parameter set of the first implementation with the minimal estimation error was used to simulate the kinase activities in ERK deactivation that are presented in Figure 4. Numerical simulation matches the experimental data very well.

IV. Robustness analysis

The robustness of a biological system implies that a particular function or characteristic of the system is preserved despite changes in the operating environment or genetic changes in its components. For example, by a computer model and later by experiments, it has been demonstrated that the adaptation mechanism found in the chemotactic signalling pathway is robust [1]. It is expected that a mathematical model of a robust biological system should also be robust. Consequently, it has been proposed that the robustness of mathematical models can be used as a criterion for determining plausibility of candidate models [9]. A major topic of the robustness analysis of mathematical models is the model's sensitivity to parameter variations. These variations may be errors in parameter estimation or changes in the components of biological systems. Techniques used to analyse the sensitivity include the repeated simulation method and adjacent method. However, one of the challenges of parameter sensitivity analysis is studying the impact of the interactions between several parameters on the system dynamics. Systematic changes of many parameters at a time suffer from an exponential increase in the number of parameters that need to be changed.

Another important topic is the robustness of mathematical model to the noise in biological systems. As living systems are optimized to function in the presence of stochastic fluctuations, the mathematical model of a biochemical network must withstand considerable variations and random perturbations of biochemical parameters [1, 9]. To study the robustness to both parameter variation and noise, we proposed a method based on the simulation of the stochastic system that is developed from the corresponding deterministic model [12]. This is an attempt to study the robustness of mathematical model against the combined sources of fluctuations, which include, for example, errors in estimated parameters, external noise for environmental fluctuations and internal noise due to small numbers of regulatory molecules. We have demonstrated that this stochastic simulation method can study the robustness of a mathematical model not only to external noise but also to parameter variations [12].

Here we first develop a stochastic system from the corresponding deterministic system (2) for the activation of ERK kinase. It is assumed that each parameter introduce the same perturbation to different equations where it appears. The system of stochastic differential equations is given by

$$\begin{aligned}
d[E] &= (-a_1[Mpp][E](dt+\mu_1 dW_1(t)) \\
&\quad + d_1[Mpp-E])(dt+\mu_2 dW_2(t)) \\
d[Mpp] &= -a_1[Mpp][E](dt+\mu_1 dW_1(t)) \\
&\quad - a_2[Mpp][Ep](dt+\mu_4 dW_4(t)) \\
&\quad + [Mpp-E](d_1(dt+\mu_2 dW_2(t)) + k_1(dt+\mu_3 dW_3(t))) \\
&\quad + [Mpp-Ep](d_2(dt+\mu_5 dW_5(t)) + k_2(dt+\mu_6 dW_6(t))) \\
d[Mpp-E] &= a_1[Mpp][E](dt+\mu_1 dW_1(t)) \\
&\quad - [Mpp-E](d_1(dt+\mu_2 dW_2(t)) + k_1(dt+\mu_3 dW_3(t))) \\
d[Ep] &= k_1[Mpp-E](dt+\mu_3 dW_3(t)) \\
&\quad - a_2[Mpp][Ep](dt+\mu_4 dW_4(t)) \\
&\quad + d_2[Mpp-Ep](dt+\mu_5 dW_5(t)) \\
d[Mpp-Ep] &= a_2[Mpp][Ep](dt+\mu_4 dW_4(t)) \\
&\quad - [Mpp-Ep](d_2(dt+\mu_5 dW_5(t)) + k_2(dt+\mu_6 dW_6(t))) \\
d[Epp] &= k_2[Mpp-Ep](dt+\mu dW_6),
\end{aligned} \tag{5}$$

where the $\mu_i\ (i=1\sim 6)$ are the relative magnitudes of perturbation of the model parameters, and $W_i(t)$ is the Wiener process whose increment,

$$\Delta W_{in} = W_i(t+h) - W_i(t) \sim N(0,h)$$

is a Gaussian random variable. The explicit Euler method [13] with a very small step size h=0.0001 was used to simulate the above stochastic system in order to ensure the stability property of simulations. We simulated stochastic system (5) with different perturbation values $\mu = \mu_i\ (i=1\sim 6)$. For the fixed kinetic rates in Figure 5, simulation fluctuations are larger when the noise magnitude μ is larger. Notice that the total magnitude of noise is the product of the reaction rate and noise magnitude. In the following robustness analysis, we adjust the noise magnitude μ in order to obtain stable stochastic simulations for all the ten estimates of the kinetic rates in Figure 1.

Similarly, the stochastic system for ERK deactivation can be developed from the corresponding deterministic system (4), given by

$$\begin{aligned}
d[E] &= k_3[Ep-EPase](dt+\mu_3 dW_3(t)), \\
d[Ep] &= -a_3[Ep][EPase](dt+\mu_1 dW_1(t)) \\
&\quad + d_3[Ep-EPase](dt+\mu_2 dW_2(t)) \\
&\quad + k_4[Epp-EPase](dt+\mu_6 dW_6(t)) \\
d[Epp] &= -a_4[Epp][EPase](dt+\mu_4 dW_4(t)) \\
&\quad + d_4[Epp-EPase](dt+\mu_5 dW_5(t)) \\
\frac{d[Ep-EPase]}{dt} &= a_3[Ep][EPase](dt+\mu_1 dW_1(t)) \\
&\quad - (d_3(dt+\mu_2 dW_2(t)) + k_3(dt+\mu_3 dW_3(t)))[Ep-EPase]
\end{aligned} \tag{6}$$

$$\frac{d[Epp-EPase]}{dt} = a_4[Epp][EPase](dt+\mu_4 dW_4(t))$$
$$-(d_4(dt+\mu_5 dW_5(t))+k_4(dt+\mu_6 dW_6(t)))[Epp-EPase]$$
$$\frac{d[EPase]}{dt} = -a_3[Ep][EPase](dt+\mu_1 dW_1(t))$$
$$-a_4[Epp][EPase](dt+\mu_4 dW_4(t))$$
$$+[Ep-EPase](d_3(dt+\mu_2 dW_2(t))+k_3(dt+\mu_3 dW_3(t)))$$
$$+[Epp-EPase](d_4(dt+\mu_5 dW_5(t))+k_4(dt+\mu_6 dW_6(t)))$$

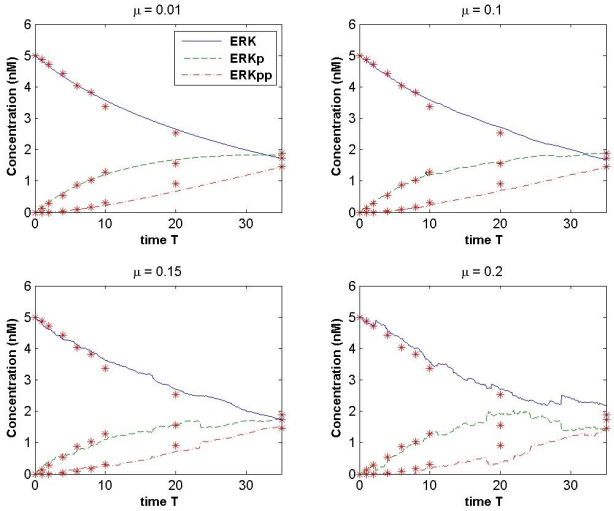

Figure 5. Simulations of the stochastic system (5) with different values of perturbation $\mu = \mu_i\,(i = 1 \sim 6)$. The corresponding deterministic simulation is presented in Figure 2.

Here we are interested in choosing a set of estimated parameters in Fig 1 from which the stochastic system (5) can generate the most stable stochastic simulations together with the best accuracy. To address this issue, we obtained 1,000 simulations of the stochastic model (5) for each set of estimated model parameters. To compare the stability and accuracy of simulations from different parameter sets, we used the same noise magnitude $\mu = 0.05$ for all the ten sets of estimates and this value was selected to ensure that stochastic model (5) with each of the ten sets of parameters could generate stable stochastic simulations. We calculated the mean and standard derivation of stochastic simulations, which are presented in Figure 6. Similar work has also been carried out for stochastic system (6). The same noise magnitude $\mu = 0.065$ was used for each of the ten sets of estimated parameters in Figure 3.

For biochemical reactions of ERK activation, the first set of estimated parameters in Figure 1, which has the minimal estimation error in the genetic algorithm, can produce stochastic simulations with the smallest error of the averaged ERKpp concentration as well as the smallest standard derivation. Therefore, this set of estimated kinetic rates is recommended for further research in mathematical modelling. For biochemical reactions of ERK deactivation, the first set of estimated parameters in Figure 3 can produce stochastic simulations that have the smallest error of the averaged ERKpp concentration as well as the smallest standard derivation. Therefore, this set of estimated kinetic rates is also recommended for further research.

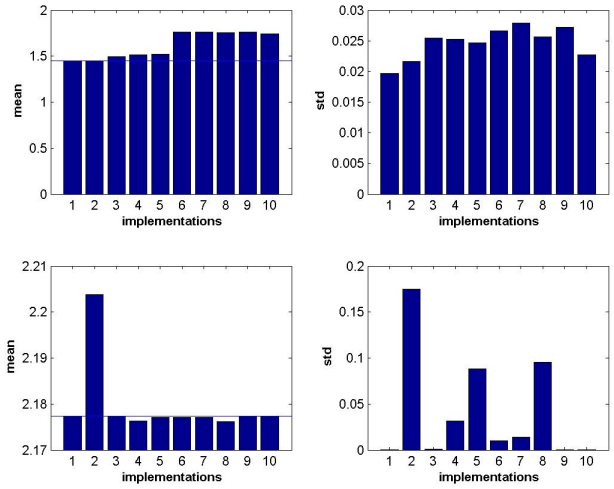

Figure 6. The mean and standard deviation (std) of stochastic simulations of the stochastic system (5) with $\mu = 0.05$ and ten sets of estimated parameters in Figure 1 (the top two figures) and stochastic system (6) with $\mu = 0.065$ and ten sets of estimated parameters in Figure 3 (the bottom two figures).

In addition, we analyzed the robustness of the mathematical model with regards to the perturbation of a single parameter. We simulated stochastic system (5) with the model parameters presented in Figure 2 and introduced perturbations to each of the six model parameters. For each of the six model parameters, we let the corresponding noise magnitude be $\mu_i = 0.15$ and all other noise magnitudes are zero. For each model parameter, we obtained 1000 simulations and calculated the mean and standard deviation of stochastic simulations. Results in Table 1 suggest that the fluctuations introduced to the binding rate a_i may cause larger computational error while the noise in the process of mono-phosphorylation may cause larger fluctuations because the noise in the process of mono-phosphorylation propagates to the process of dual phosphorylation and therefore may have larger impact on the system dynamics.

TABLE I. SIMULATION RESULTS OF THE STOCHASTIC SYSTEM (5) BY INTRODUCING NOISE TO EACH OF THE SIX MODEL PARAMETERS. (RATE: VALUES OF ESTIMATED MODEL PARAMETER; MEAN AND STD: THE MEAN AND STANDARD DERIVATION OF ERKPP CONCENTRATION AT T=35 FROM 1000 SIMULATIONS; ERROR: THE DIFFERENCE BETWEEN THE MEAN AND DETERMINISTIC SIMULATION ERKPP(T=35)=1.4474)

	a1	d1	k1	a2	d2	k2
rate	1.176	48.23	12.94	28.23	128.2	1.176
mean	1.500	1.468	1.475	1.501	1.482	1.496
error	0.052	0.021	0.027	0.053	0.035	0.022
std	0.150	0.160	0.143	0.143	0.146	0.134

V. CONCLUSIONS

In this work, we have applied the genetic algorithm to estimate kinetic rates of mathematical models for biological systems. Focused on the MAP kinase signalling transduction pathway, which is an important model system in molecular cell biology and systems biology, we estimated the reactions rates related to MAP kinase (ERK) activation and deactivation from experimentally measured mono-phosphorylated and dual-phosphorylated ERK. In addition, we analysed the robustness of the mathematical model with the estimated model parameters using the stochastic simulation method. The robustness of the mathematical model is used as an additional criterion to choose the model parameters from the candidate estimates obtained from different implementations of the genetic algorithm. It is expected this principle will be extended to the estimation of kinetic rates of stochastic models [14]. This research provides useful information of reaction rates that is recommended to further research about the mathematical modelling of the MAP kinase cell signalling transduction pathway.

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