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On the calculation of signal transduction ability of signaling transduction pathways in intracellular communication: systematic approach

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ABSTRACT

Motivation: The major function of signal transduction pathways in cells is to sense signals from the environment and process the information through signaling molecules in order to regulate the activity of transcription factors. On the molecular level, the information transmitted by a small number of signal molecules is amplified in the internal signaling pathway through enzyme catalysis, molecular modification and via the activation or inhibition of interactions. However, the dynamic system behavior of a signaling pathway can be complex and, despite knowledge of the pathway components and interactions, it is still a challenge to interpret the pathways behavior. Therefore, a systematic method is proposed in this study to quantify the signal transduction ability.

Results: Based on the non-linear signal transduction system, signal transduction ability can be investigated by solving a Hamilton–Jacobi inequality (HJI)-constrained optimization problem. To avoid difficulties associated with solving a complex HJI-constrained optimization problem for signal transduction ability, the Takagi–Sugeno fuzzy model is introduced to approximate the non-linear signal transduction system by interpolating several local linear systems so that the HJI-constrained optimization problem can be replaced by a linear matrix inequality (LMI)-constrained optimization problem. The LMI problem can then be efficiently solved for measuring signal transduction ability. Finally, the signal transduction ability of two important signal transduction pathways was measured by the proposed method and confirmed using experimental data, which is useful for biotechnological and therapeutic application and drug design.

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1 INTRODUCTION

Signal transduction pathways are one of the fastest information processing networks. They enable cells to sense and transduce extracellular signals through inter- and intra-cellular communication. Signal transduction pathways consist of interactions between signaling proteins, where different external changes or events stimulate specific signaling pathways. Typical external signal stimuli are hormones, pheromones, heat, cold, light, osmotic pressure and concentration change of substances such as, K^+, Ca^{2+} or cAMP (Hohmann, 2002; Teraoka *et al.*, 2001; Xie and Askari, 2002). Metabolic processes, such as homeostasis (Hohmann, 2002), the growth (Aplin and Juliano, 1999) and differentiation of tissues through endocrine signaling (Herrington and Carter-Su, 2001), the synthesis and secretion of proteins, such as in blood clotting and immune-responses and the composition of intracellular and extracellular fluids, such as in the processes of photoreception and olfaction, are all closely regulated by specific signaling pathways (Koshland *et al.*, 1982). Hence the proper function of cellular signaling pathways is essential for life.

On the molecular level, signal transduction involves the production or degradation of substances, molecular modification and the activation or inhibition of chemical reactions. Many previous studies have focused on signaling molecules, cell-surface receptors and the second messengers of the signaling pathway. Aside from molecular transfer, signaling pathways can also be viewed as the information processing and transferring systems of cells (Klipp, 2005). Many studies have investigated the properties of signaling pathways, such as amplification (Hohmann, 2002), specificity (Pan *et al.*, 2008), adaption, ultrasensitivity (Huang and Ferrell, 1996; Kholodenko, 2000), oscillation (Kholodenko, 2000) and synchronization. However, due to the complex behavior of signaling pathways, knowledge of the components of a pathway and their interactions is often not enough to interpret the dynamic system behavior of the pathway. To the best of our knowledge, the intension to express the signal transduction ability into mathematical formula appeared in 1982 (Koshland *et al.*, 1982). It focused on the sensitivity amplification, which is defined as the ratio of the percent change in the output response to the percent change in the input stimulus, i.e. the relative change of the system output respect to a specific input. Then the mitogen-activated protein kinase (MAPK) signaling pathway in linear form is studied and signal amplitude is defined as the ratio of the total response to the signal duration, which depends on the input (Heinrich *et al.*, 2002). Next, a stochastic differential model of MAPK signaling pathway is considered and the signal amplification is defined as signal gain, which exists at least one maximum with respect to the ratio of inputs to outputs (Samoilov *et al.*, 2005). Recently, Marhl *et al.* compared the signaling pathways in biological systems with the cascade mechanisms in electrical engineering systems and then also defined the maximal amplification

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as the ratio of the maximum of output to the maximum of input, i.e. $\text{output}_{\max}/\text{input}_{\max}$ (Marhl *et al.*, 2009). Obviously, these mentioned techniques defined the signal transduction ability on case-by-case basis, that is, the results are affected not only by the structure of the system but also the input to the system. This article specifically investigates signal transduction ability, a dynamic system behavior of signaling pathways.

As a system for processing and transferring information, signaling pathways require an amplification mechanism to reduce the expense associated with the production of signaling molecules and to ensure rapid responses. From the viewpoint of energy expenditure, signal amplification can effectively reduce the quantity of initial key substances necessary to ensure upstream sensing as well as having significant effects on downstream signal transduction. Also, if a deleterious stress impacts a cell, the cell is required to ensure a response is induced as soon as possible through signaling pathways. Accordingly, there are numerous studies indicating the importance and the functional role of signal amplification. An example is the osmoadaptation of yeast under osmotic stress, where an integrated mathematical model is provided for the signaling pathway to demonstrate the relationship between the rate constants of kinases and phosphatases and signal amplification (Klipp *et al.*, 2005). For liver glycogenolysis and the release of glucose, a level of 10^{-10} M of epinephrine in blood can generate intracellular concentrations of 10^{-6} M cAMP with a signal amplification of 10^4 (Harvey *et al.*, 2000).

In the field of electrical engineering, there are several electrical components, such as transistors, which possess the signal amplification property. The analysis of the amplification properties of these gadgets has been undertaken (Marhl *et al.*, 2009) and the same analysis may also be performed on the amplification properties of a cellular signaling pathway (Detwiler *et al.*, 2000). However, because of the non-linearity of signaling pathways, knowledge about the systematic properties of the pathways remains limited and incomplete. In this study, signaling pathways are investigated from the dynamic system perspective for which a signal transduction ability measurement is proposed based on signals and systems theory from the systematic viewpoint. In accordance with the system gain viewpoint, the ratio of output to input signal energy of the studied signaling pathway is conducted for all possible input signals. This measurement of signal transduction ability is more dependent on the system characteristics of the studied signaling pathway rather than the input signals. Similar to an amplifier, the measure is more dependent on the systematic characteristics of the amplifier itself compared with the input signals.

The proposed signal transduction measurement scheme for non-linear signaling pathways needs to solve the corresponding non-linear Hamilton–Jacobi inequalities (HJIs). At present, except for some simple cases, there is no analytic or numerical solution for HJIs. Takagi–Sugeno (T–S) fuzzy systems have efficiently interpolated several local linear systems via a set of fuzzy bases to approximate a non-linear system, enabling the HJIs in a non-linear signal processing problem to be replaced by a set of linear matrix inequalities (LMIs; Chen *et al.*, 1999; Chen *et al.*, 2000; Takagi and Sugeno, 1985). This then allows the non-linear signal transduction ability measurement problem to be transformed into an equivalent LMIs-constrained optimization problem, which is more easily to solve. Because the measurement method is based on fuzzy system theory for signal transmission systems,

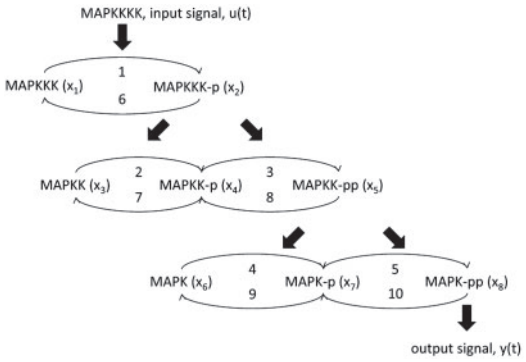

Fig. 1. The scheme of the MAPK signal transduction pathway.

the transduction ability of non-linear signal transduction pathways is able to be investigated from these fuzzy-interpolated local linear signal transduction pathways. Using this method, increasing biological transduction insights can be obtained from the linear system viewpoint. In the future, signal transduction systems may be characterized according to the proposed measurement, which is useful for biotechnological and therapeutic applications, and for the development of drug design. Synthetic biology is increasingly focusing on the rational construction of biological systems based on engineering principles in order to achieve some systematic design specifications. The proposed method described herein may hold potential for the analysis and design of synthetic gene networks or pathways for next-generation synthetic gene networks (Hasty *et al.*, 2002; Kaern *et al.*, 2003; Murphy *et al.*, 2007).

2 METHODS

2.1 Dynamic model of signaling pathways

Mathematical modeling of metabolic networks has been studied extensively and is useful for describing experimental data, deducing regulatory principles and understanding more complex dynamic phenomena, such as oscillation (Hasty *et al.*, 2002; Kholodenko 2000; Lin *et al.*, 2009). However, in modeling signaling pathways, few examples have been studied due to the unavailability of experimental data. As the structure of signaling pathways consists of consecutive activations and deactivations of proteins, the signaling pathways can be modeled as a series of chemical reactions, expressed as a system of rate aligns. The appropriate choice of rate expressions is a matter of a prior knowledge of the whole system and modeling intuition. For instance, linear and bilinear expressions or Michaelis–Menten kinetics have previously been used to model signaling pathways (Klipp, 2005; Lin *et al.*, 2009).

Independent or coupled kinase cascades participate in a variety of different intracellular signaling pathways that cover the spectrum of cellular processes, including intracellular cell growth, differentiation, transformation and apoptosis. For example, MAPK cascades transduce signals from the cell membrane to the nucleus in response to a wide range of stimuli (Fig. 1; Harvey *et al.*, 2000). The pathway consists of several levels, where the activated kinase at each level phosphorylates the kinase at the next level in the cascade (Fig. 1). The dynamic modeling of a MAPK cascade may be represented by the system in (1) with Michaelis–Menten kinetics formulation (Kholodenko, 2000).

$$\frac{\mathrm{d}}{\mathrm{d}t}x_1 = \frac{-k_1 \cdot x_1 \cdot u}{K_{m,1} + x_1} + \frac{V_{\max,2} \cdot x_2}{K_{m,2} + x_2}$$

$$
\begin{aligned}
\frac{\mathrm{d}}{\mathrm{d}t}x_2 &= \frac{k_1\cdot x_1\cdot u}{K_{m,1}+x_1}-\frac{V_{\max,2}\cdot x_2}{K_{m,2}+x_2}\\
\frac{\mathrm{d}}{\mathrm{d}t}x_3 &= \frac{-k_3\cdot x_2\cdot x_3}{K_{m,3}+x_3}+\frac{V_{\max,6}\cdot x_4}{K_{m,6}+x_4}\\
\frac{\mathrm{d}}{\mathrm{d}t}x_4 &= \frac{k_3\cdot x_2\cdot x_3}{K_{m,3}+x_3}-\frac{V_{\max,6}\cdot x_4}{K_{m,6}+x_4}-\frac{k_4\cdot x_2\cdot x_4}{K_{m,4}+x_4}+\frac{V_{\max,5}\cdot x_5}{K_{m,5}+x_5}\\
\frac{\mathrm{d}}{\mathrm{d}t}x_5 &= \frac{k_4\cdot x_2\cdot x_4}{K_{m,4}+x_4}-\frac{V_{\max,5}\cdot x_5}{K_{m,5}+x_5}\\
\frac{\mathrm{d}}{\mathrm{d}t}x_6 &= \frac{-k_7\cdot x_5\cdot x_6}{K_{m,7}+x_6}+\frac{V_{\max,10}\cdot x_7}{K_{m,10}+x_7}\\
\frac{\mathrm{d}}{\mathrm{d}t}x_7 &= \frac{k_7\cdot x_5\cdot x_6}{K_{m,7}+x_6}-\frac{V_{\max,10}\cdot x_7}{K_{m,10}+x_7}-\frac{k_8\cdot x_5\cdot x_7}{K_{m,8}+x_7}+\frac{V_{\max,9}\cdot x_8}{K_{m,9}+x_8}\\
\frac{\mathrm{d}}{\mathrm{d}t}x_8 &= \frac{k_8\cdot x_5\cdot x_7}{K_{m,8}+x_7}-\frac{V_{\max,9}\cdot x_8}{K_{m,9}+x_8}\qquad y=x_8
\end{aligned}
\tag{1}
$$

Let us denote the state variable vector $x(t)$, protein interaction vector $f(x)$, input signal $u(t)$, output signal $y(t)$, input coupling vector $g(x)$ and output coupling matrix C in (2).

$$
x(t)=\begin{bmatrix}x_1(t)\\x_2(t)\\x_3(t)\\x_4(t)\\x_5(t)\\x_6(t)\\x_7(t)\\x_8(t)\end{bmatrix}=\begin{bmatrix}\text{MAPKKK}(t)\\\text{MAPKKK-p}(t)\\\text{MAPKK}(t)\\\text{MAPKK-p}(t)\\\text{MAPKK-pp}(t)\\\text{MAPK}(t)\\\text{MAPK-p}(t)\\\text{MAPK-pp}(t)\end{bmatrix},
$$

$$
f(x)=\begin{bmatrix}
\dfrac{V_{\max,2}\cdot x_2}{K_{m,2}+x_2}\\
-\dfrac{V_{\max,2}\cdot x_2}{K_{m,2}+x_2}\\
\dfrac{-k_3\cdot x_2\cdot x_3}{K_{m,3}+x_3}+\dfrac{V_{\max,6}\cdot x_4}{K_{m,6}+x_4}\\
\dfrac{k_3\cdot x_2\cdot x_3}{K_{m,3}+x_3}-\dfrac{V_{\max,6}\cdot x_4}{K_{m,6}+x_4}-\dfrac{k_4\cdot x_2\cdot x_4}{K_{m,4}+x_4}+\dfrac{V_{\max,5}\cdot x_5}{K_{m,5}+x_5}\\
\dfrac{k_4\cdot x_2\cdot x_4}{K_{m,4}+x_4}-\dfrac{V_{\max,5}\cdot x_5}{K_{m,5}+x_5}\\
\dfrac{-k_7\cdot x_5\cdot x_6}{K_{m,7}+x_6}+\dfrac{V_{\max,10}\cdot x_7}{K_{m,10}+x_7}\\
\dfrac{k_7\cdot x_5\cdot x_6}{K_{m,7}+x_6}-\dfrac{V_{\max,10}\cdot x_7}{K_{m,10}+x_7}-\dfrac{k_8\cdot x_5\cdot x_7}{K_{m,8}+x_7}+\dfrac{V_{\max,9}\cdot x_8}{K_{m,9}+x_8}\\
\dfrac{k_8\cdot x_5\cdot x_7}{K_{m,8}+x_7}-\dfrac{V_{\max,9}\cdot x_8}{K_{m,9}+x_8}
\end{bmatrix},
\tag{2}
$$

$$
u(t)=\text{MAPKKKK}(t), y(t)=\text{MAPK-pp}(t),
$$

$$
g(x)=\begin{bmatrix}\frac{-k_1\cdot x_1}{K_{m,1}+x_1} & \frac{k_1\cdot x_1}{K_{m,1}+x_1} & 0 & 0 & 0 & 0 & 0\end{bmatrix}^{\mathrm{T}},
$$

$$
C=\begin{bmatrix}0 & 0 & 0 & 0 & 0 & 0 & 0 & 1\end{bmatrix}.
$$

The dynamic system of the MAPK cascade signal transduction in (1) can then be represented in (3)

$$
\begin{aligned}
\dot{x}(t) &= f(x)+g(x)u(t)\\
y(t) &= Cx(t)
\end{aligned}
\tag{3}
$$

where $x(t), f(x), g(x)$ and C are defined in (2).

In general, a signal transduction pathway may consist of m input signals, $u(t)=[u_1(t)\cdots u_m(t)]^T$, n proteins, $x(t)=[x_1(t)\cdots x_n(t)]^T$ in a signal transduction cascade, $f(x)$ and p output signals, $y(t)=[y_1(t)\cdots y_p(t)]^T$, where $u_i(t)$ denotes the i-th input signal, $x_i(t)$ denotes the concentration of the i-th protein in the signal cascade and $y_i(t)$ denotes the concentration of the i-th output signal. In this situation, $f(x)$, $g(x)$ and C in (2) should be modified accordingly. For example, if $x_n(t)$ is the output signal to be measured then $C=[0\cdots 0\ 1]$, i.e. C is the row vector of n elements with 0 expressed at all elements except 1 at the last entry.

System knowledge resides in models and data. A model is a construction, usually validated by data. Data validate models, and models give system meaning of data. They are opposite of the same coin. Actually, in this study, we assume the system models of signaling pathways have been identified by experimental data. Then, we use the system models of signaling pathways to investigate their signal transduction abilities. Since the signal transduction ability is a system property, it is not easy to investigate from input/output data directly. Further, from the signal transduction ability, we could get insight into the system meaning of signaling pathways. In this study, we assume the system dynamic model of signaling pathways in (3) has been well identified by rich input/output signals.

2.2 Signal transduction ability measurement

Many properties of signaling pathways are investigated, including signaling time, signal duration or signal amplitude (Heinrich *et al.*, 2002). The focus of this study is on the signal transduction ability of signaling pathways. The transduction ability is defined from the system gain viewpoint as follows (Boyd, 1994):

$$
\rho_a=\sup_{u(t)\in L_2[0,t_f]}\frac{\left(\int_0^{t_f}y^{\mathrm{T}}(t)y(t)\mathrm{d}t\Big/t_f\right)^{1/2}}{\left(\int_0^{t_f}u^{\mathrm{T}}(t)u(t)\mathrm{d}t\Big/t_f\right)^{1/2}}=\sup_{u(t)\in L_2[0,t_f]}\frac{\|y(t)\|_2}{\|u(t)\|_2}
\tag{4}
$$

where $u(t)$ and $y(t)$ are the input and output signals of the signaling pathway, respectively. The physical meaning of transduction ability is the maximum ratio of the output signal to the input signal from the root mean square sense for all of the possible bounded input signals within the time interval $[0,t_f]$, where $L_2[0,t_f]$ denotes the set of all possible bounded signals in $[0,t_f]$. The reason for employing the maximum root mean square ratio of the output signal to all possible input signals is that input signals may change under different conditions, causing the signal energy ratio to vary. However, transduction ability should be based on the maximum effect of all the possible input signals on the output signals and exhibit greater dependency on the characteristics of the dynamic system, in accordance with the system gain viewpoint. Theoretically, the transduction ability can be measured directly by testing all possible bounded input signals to get the maximum root mean square ratio. In general, the maximization problem described in (4) for measuring the signal transduction ability of a signaling pathway cannot be solved directly. It can only be solved indirectly by applying a systematic analysis method on the signal transduction dynamic in (3), as described in the following expressions.

Let us denote an upper bound of transduction ability as follows:

$$
\|y(t)\|_2^2\Big/\|u(t)\|_2^2\leqslant\rho^2,\forall u(t)\in L_2[0,t_f]
\tag{5}
$$

or

$$
\|y(t)\|_2^2\leqslant\rho^2\|u(t)\|_2^2,\forall u(t)\in L_2[0,t_f]
\tag{6}
$$

In (5) or (6), if ρ^2 is the upper bound of $\|y(t)\|_2^2/\|u(t)\|_2^2$ for every $u(t)\in L_2[0,t_f]$, then ρ^2 is also the upper bound of ρ_a^2 in (4), as every $u(t)\in L_2[0,t_f]$ should include the input signal which leads to the maximum mean square ratio ρ_a in (4). Therefore, we can find an upper bound ρ for the signal transduction ability ρ_a and then minimize the upper bound to approach the transduction ability, i.e. the signal transduction ability ρ_a is estimated by minimizing the upper bound ρ.

PROPOSITION 1. If a positive function $V(x)$ with $V(0)=0$ is able to satisfy the following HJI:

$$
\left(\frac{\partial V(x)}{\partial x}\right)^{\mathrm{T}}f(x)+x^{\mathrm{T}}C^{\mathrm{T}}Cx+\frac{1}{4\rho^2}\left(\frac{\partial V(x)}{\partial x}\right)^{\mathrm{T}}g(x)g^{\mathrm{T}}(x)\frac{\partial V(x)}{\partial x}\leqslant 0
\tag{7}
$$

then the signal transduction ability of the signal pathway is $\leqslant\rho$.

PROOF. See Appendix A in Supplementary Materials. ■

Since ρ is the upper bound of transduction ability ρ_a, the transduction ability can be obtained by solving the following constrained optimization problem:

$$\begin{aligned} &\rho_a = \min \rho \\ &\text{subject to HJI in (7) and } V(x) > 0 \end{aligned} \tag{8}$$

It means to make ρ as small as possible without violating HJI constraint in (7). After solving the transduction ability ρ_a from the HJI constraint optimization expression (8) and substituting ρ_a for ρ in (7), we find:

$$\left(\frac{\partial V(x)}{\partial x}\right)^{\mathrm{T}} f(x) + x^{\mathrm{T}} C^{\mathrm{T}} C x + \frac{1}{4\rho_a^2}\left(\frac{\partial V(x)}{\partial x}\right)^{\mathrm{T}} g(x) g(x)^{\mathrm{T}} \frac{\partial V(x)}{\partial x} \leqslant 0 \tag{9}$$

If the transduction ability ρ_a is small, the last term in (9) is positively large. In this situation, the first term $(\partial V(x)/\partial x)^T f(x)$ should be more negative (more stable) so that the HJI in (9) still holds. On the other hand, if $(\partial V(x)/\partial x)^T f(x)$ is less negative (less stable), then the transduction ability ρ_a should be large enough to satisfy the HJI in (9). From the HJI in (9), it can be seen that the signal transduction ability is more closely related to the system characteristics $f(x)$, $g(x)$ and C of signaling pathway than to the external stimuli. This is a similar case to a low-pass filter having greater dependence on the system characteristic of the filter than the incoming noise.

In general, it is still very difficult to solve the non-linear HJI-constrained optimization problem for the measurement of signal transduction ability described in (8). Thus, a fuzzy interpolation method is employed to overcome the non-linear difficulties associated with solving the HJI problem in (7) and to simplify the constrained optimization procedures required to solve the transduction ability measurement problem in (8).

2.3 Signal transduction measurement via fuzzy method

Fuzzy dynamic modeling has been widely employed to approximate non-linear dynamic systems and to simplify non-linear control problems by interpolating several local linear dynamic systems (Chen *et al.*, 1999; Chen *et al.*, 2000). The T–S fuzzy dynamic model (Takagi and Sugeno, 1985) is described by fuzzy 'If–Then' rules and employed here to solve the transduction ability measurement problem for the signaling pathway. The i-th rule of the fuzzy model for the non-linear signal transduction pathway in (3) is proposed as follows (Chen *et al.*, 1999; Chen *et al.*, 2000; Tseng and Chen 2001; Tseng *et al.*, 2001):

$$\begin{aligned} &\text{Rule } i\text{: If } x_1(t) \text{ is } F_{i1},\ x_2(t) \text{ is } F_{i2},\ \ldots,\ x_l(t) \text{ is } F_{il}, \\ &\text{Then } \begin{cases} \dot{x} = A_i x(t) + B_i u(t) \\ y = Cx(t) \end{cases} \end{aligned} \tag{10}$$

where i= 1,2, ⋯, L. $x_i(t)$ is the premise variable, F_{ij} is the fuzzy set, A_i and B_i are the local system matrices, L is the number of fuzzy rules and l is the number of premise variables. If all state variables are used as premise variables then $l = n$. The physical meaning of fuzzy rule i in (10) is that if the state variables $x_1(t)$, $x_2(t)$, ⋯, $x_l(t)$ are with the local fuzzy sets F_{i1}, F_{i2}, ⋯, F_{il}, then the non-linear signaling pathway in (3) could be represented by the linear system in the 'Then' part of (10). The fuzzy inference system of (10) can then be described as follows (Chen *et al.*, 1999; Chen *et al.*, 2000; Takagi and Sugeno, 1985):

$$\begin{aligned} &\dot{x} = \frac{\sum_{i=1}^{L} \mu_i\big(x(t)\big)[A_i x(t) + B_i u(t)]}{\sum_{i=1}^{L} \mu_i\big(x(t)\big)} = \sum_{i=1}^{L} m_i\big(x(t)\big)[A_i x(t) + B_i u(t)] \\ &y = Cx(t) \end{aligned} \tag{11}$$

where $m_i(x(t)) = \mu_i(x(t))/\sum_{i=1}^{L} \mu_i(x(t))$ and $\mu_i(x(t)) = \prod_{i=1}^{L} F_{ij}(x_i(t))$, with $F_{ij}(x_i(t))$ describing the grade of membership of $x_i(t)$ in F_{ij} and $m_i(x(t))$, i= 1, 2, ⋯, L describing the fuzzy bases. The denominator $\sum_{i=1}^{L} \mu_i(x(t))$ is applied to enable normalization so that the total sum of the fuzzy bases $\sum_{i=1}^{L} m_i(x(t))$=1. The physical meaning of the fuzzy inference system in (11) is that L local linear signaling pathways are interpolated through non-linear fuzzy bases $m_i(x(t))$ to approximate the non-linear signaling pathway in (3).

Aside from the fuzzy bases, other interpolation bases could also be employed to interpolate several linear signaling pathways to approximate a non-linear signaling pathway.

By fuzzy approximation, the non-linear signaling pathway can be represented by the following fuzzy interpolated signaling pathway:

$$\dot{x} = f(x) + g(x)u = \sum_{i=1}^{L} m_i\big(x(t)\big)[A_i x(t) + B_i u(t)] \tag{12}$$

There are many system identification methods available to determine the local system matrixes A_i and B_i for the fuzzy model (Takagi and Sugeno, 1985), such as the fuzzy toolbox in MATLAB.

PROPOSITION 2. For the non-linear signaling pathway in (12), if a positive definite symmetric matrix P exists, satisfying the following Riccati-like inequalities:

$$PA_i + A_i^{\mathrm{T}} P + C^{\mathrm{T}} C + \frac{1}{\rho^2} P B_i B_i^{\mathrm{T}} P \leqslant 0, i = 1, 2, \cdots, L \tag{13}$$

then the signal transduction ability is bounded by ρ.

PROOF. see Appendix B in Supplementary Materials. ■

The inequality in (13) can be considered as the local linearization of HJI in (7). In order to solve the above Riccati-like inequalities by the conventional LMI method, the positive definite symmetric matrix $Q = P^{-1}$ is multiplied at both sides of (13). The Riccati-like inequalities in (13) can be described as:

$$A_i Q + Q A_i^{\mathrm{T}} + \frac{1}{\rho^2} B_i B_i^{\mathrm{T}} + Q C^{\mathrm{T}} C Q \leqslant 0, i = 1, 2, \ldots, L \tag{14}$$

By the Schur complement method (Boyd, 1994), the Riccati-like inequalities in (14) are then equivalent to the following LMIs:

$$\begin{bmatrix} A_i Q + Q A_i^{\mathrm{T}} + \frac{1}{\rho^2} B_i B_i^{\mathrm{T}} & Q C^{\mathrm{T}} \\ CQ & -1 \end{bmatrix} \leqslant 0, i = 1, 2, \ldots, L \tag{15}$$

If the LMIs in (15) hold for $Q > 0$, the signal transduction ability of non-linear signaling pathway is $< \rho$ and ρ is the upper bound of the signal transduction ability.

Using the fuzzy approximation method, the HJI in (7) is interpolated by a set of Riccati-like algebraic inequalities in (13) or (14). Therefore, the signal transduction ability of the non-linear signaling pathway could be obtained by solving the following LMIs-constrained optimization problem:

$$\begin{aligned} &\rho_a = \min \rho \\ &\text{subject to LMIs in (15) and } Q > 0 \end{aligned} \tag{16}$$

which can then be solved by decreasing ρ until no positive definite symmetric matrix Q is able to satisfy the inequalities in (13) or (14).

REMARK 1. The Riccati-like inequalities in (13) or (14) are an approximation of HJI with L local linear systems. Similarly, the LMIs-constrained optimization problem in (16), describing the signal transduction ability measurement of a non-linear signaling pathway, is also an efficient approach based on a fuzzy interpolation method to replace the very difficult HJI-constrained optimization problem in (8). The LMIs-constrained optimization problem for the signal transduction ability measurement is known as an "genvalue problem", which can be efficiently solved by the LMI toolbox in MATLAB.

REMARK 2. After substituting ρ_a for ρ in (13), we get

$$PA_i + A_i^{\mathrm{T}} P + C^{\mathrm{T}} C + \frac{1}{\rho_a^2} P B_i B_i^{\mathrm{T}} P \leqslant 0, i = 1, 2, \cdots, L \tag{17}$$

If ρ_a is smaller, the last term will be larger so that the eigenvalues of A_i would be in the far left-hand complex plane (i.e. with more negative real part or more stable) and (17) would hold. If ρ_a is larger, the last term in (17) will be smaller, meaning that the eigenvalues of A_i would be closer to the imaginary axis (i.e. with smaller negative real part or less stable). In general,

the signaling pathways of the cell amplify the input signals (i.e. $\rho_a > 1$). In this situation, the eigenvalues of A_i would approach the imaginary axis (less stable) in order to amplify the extracellular signals so as to enable the downstream activation of the corresponding genes within the cell. The signal transduction ability of the signaling pathway is more dependent on the system characteristics A_i, B_i and C than on the external signal $u(t)$, just as a low-pass filter is more dependent on the low-pass characteristic (poles and zeros) of the system than on the filtered noise.

REMARK 3. In this study, we assume the system dynamic model of signaling pathways in (3) has been well identified. However, there are some parameter estimation errors due to the incorrect structure, stochastic noises (Chen and Zhang, 2004; Zhang and Chen, 2006), infeasible parameter region and solution method. The effect of these parameter estimation errors may lead to an overestimate of signal transduction ability ρ_a and is discussed in Supplementary Materials.

3 RESULTS

To demonstrate the procedure of our proposed measurement of the signal transduction ability, two signaling pathway examples are described using the simulation method.

3.1 MAPK signal transduction pathway

The MAPK signal transduction pathway is one of the most well-known and well-studied signaling pathways. The pathway is highly conserved in eukaryotes, including humans, *Drosophila* and yeast. A wide variety of membrane receptors, including RTK and cytokine receptors, utilize the MAPK cascade to transfer external upstream signals to the nucleus (Harvey *et al.*, 2000). As soon as MAPK is activated by the signaling pathway, it is translocated into the nucleus, promoting the transcription of hundreds of genes. Based on these properties, the MAPK cascade was chosen as the first simulation example.

In mammalian cells, the MAPK cascade is operated in a sequential fashion downstream from an activated Ras protein. Active Ras binds to a Raf protein (MAPKKK), thereby activating it. The active Raf phosphorylates and thereby activates MEK (MAPKK). Active MEK then phosphorylates and activates MAP kinase. Finally MAP kinase phosphorylates many different proteins, including nuclear transcription factors that mediate cellular responses. In summary, activation of Ras by RTK induces a kinase cascade that includes Raf, MEK and MAPK (RTK → Ras → Raf → MEK → MAPK; Harvey *et al.*, 2000).

The dynamics of a MAPK cascade can be represented by the ODE system in (1). The input signal $u(t)$ is MAPKKK and the output signal $y(t)$ is MAPK-pp. The values for the rate constants, the Michaelis–Menten constants and enzyme rates are adopted from (Kholodenko, 2000), which were obtained from fitting the experimental data (Supplementary Table S1). The performance of the MAPK kinase cascade is measured in the ability to transmit and amplify the signal and then to notably enhance the concentration of double-phosphorylated MAPK which is dependent on the dynamic system of the signaling pathway and their constants. The simulation procedure was initiated by selecting a fuzzy model to approximate the non-linear model. Selection of the model required the approximation of the parameters of the fuzzy model, A_i and B_i in (10) or (11), which can be found by the least mean square parameter estimation algorithm based on a system identification method (Takagi and Sugeno, 1985). The LMIs in (15) can then be solved via the LMI toolbox in MATLAB. Finally, the signal transduction ability in (16) can be obtained by decreasing ρ until no positive definite symmetric matrix Q is able to satisfy (15).

Using the least-squares parameter estimation method (Takagi and Sugeno, 1985), the parameters A_i and B_i of the fuzzy model can be identified to achieve the minimum square error (simulation results were shown in Fig. 2). Since there are 8 state variables in the model, and 2 fuzzy sets for each state variable, a total of 256 fuzzy rules are applied to the fuzzy model. The fuzzy approximation error of the MAPK signaling pathway in Figure 2 is $\sim 10^{-10}$ with 256 fuzzy rules. Solving the optimization problem in (16), the signal transduction ability of the fuzzy signaling pathway is measured as $\rho_a = 2.73 \times 10^6$ with the corresponding positive definite symmetric matrix Q (shown in Supplementary Materials). This value implies the best ability of the MAPK signaling pathway to transduce input signals, that is, to amplify input signals. We have calculated ratio of the output signal to the input signal from the root mean square sense with a few kinds of input signals (shown in Supplementary Fig. S1). From Equations (4)–(6), it is seen that ρ_a is the least upper bound of these ratios and could be confirmed by the results in Figure S1. However, it seems not an easy task to find an adequate input signals to achieve the transduction ability.

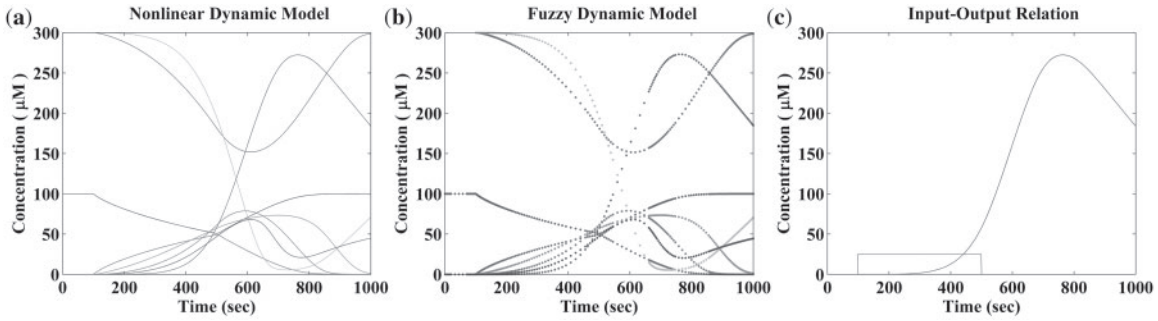

Fig. 2. The simulation example for the MAPK signaling pathway. (**a**) The time profile of the state variables (MAPKKK:blue, MAPKK-p:dark green, MAPKK:red, MAPKK-p:cyan, MAPKK-pp:magenta, MAPK:gold, MAPK-p:gray and MAPK-pp:brown) for the non-linear dynamic model of the MAPK signal transduction pathway in (1). (**b**) The time profile of the state variables for the fuzzy dynamic model of the MAPK signal transduction pathway in (11). (**c**) An input (red) and output (blue) signal of the MAPK signal transduction pathway.

In this example, we focused on the sequential phosphorylation of MAPKKK, MAPKK and MAPK to investigate the transduction ability of on the MAPK signal transduction (Fig. 1). The whole signaling pathway can be divided into three levels: (i) MAPKKK →MAPKKK-p, (ii) MAPKK→MAPKK-pp and (iii) MAPK→ MAPK-pp. According to our measurements, the signal transduction ability of the entire signaling pathway is $\sim 10^6$, which closely resembles the value source from the literature (Harvey *et al.*, 2000). Moreover, the measurement of transduction abilities of the above three stages by the proposed method were found to be ∼80, 120 and 120, respectively. The results from measuring the MAPK signaling pathway as a whole or as separate pathways are similar, i.e. $10^6 \backsimeq 80 \times 120 \times 120$ through three separate sub-pathways. This finding implies that the measurement for the signaling pathway can be obtained as a whole or by measuring the separate pathways if the size of signaling pathway is very large and the computation of signal transduction ability in (16) becomes difficult by a whole calculation way (Zumsande and Gross, 2010).

3.2 NF-κB signal transduction pathway

According to research on the evolution of the NF-κB pathway, it has existed for millions of years. The NF-κB pathway is referred to as the master of transcription factors for immune-responses (Hoffmann *et al.*, 2002). It is rapidly activated in mammalian immune-system cells in response to infection, inflammation and a number of other stressful situations, such as ionizing radiation (Karin and Greten, 2005). In contrast to the previous MAPK pathway, which is a reversible signaling pathway, the NF-κB is an irreversible pathway where a component is proteolytically cleaved. The NF-κB pathway in Figure 3 with dynamic model in (18) is investigated as the second simulation example to demonstrate our proposed measurement method.

$$\begin{aligned}
\mathrm{dIKKK}_a(t)/\mathrm{d}t &= k_a \times u(t) \times \left(K_N - \mathrm{IKKK}_a(t)\right) - k_i \times \mathrm{IKKK}_a(t) \\
\mathrm{dIKK}_n(t)/\mathrm{d}t &= k_4 \times \left(K_{NN} - \mathrm{IKK}_n - \mathrm{IKK}_a - \mathrm{IKK}_i\right) - k_1 \\
&\quad \times \mathrm{IKKK}_a^2(t) \times \mathrm{IKK}_n(t) \qquad (18) \\
\mathrm{dIKK}_a(t)/\mathrm{d}t &= k_1 \times \mathrm{IKKK}_a^2(t) \times \mathrm{IKK}_n(t) - k_3 \times \mathrm{IKK}_a(t) \\
\mathrm{dIKK}_i(t)/\mathrm{d}t &= k_3 \times \mathrm{IKK}_a(t) - k_4 \times \mathrm{IKK}_i(t)
\end{aligned}$$

In resting cells, the dimeric transcription factor NF-κB, composed of p50 and p65, is sequestered in the cytosol and bound to the inhibitor I-κB (Hacker and Karin, 2006; Hoffmann and Baltimore, 2006). Stimulation by TNF-α or IL-1 induces activation of TAK1 kinase (IKKK), leading to activation of the trimeric I-κB kinase (IKK). Ionizing radiation and other stresses are able to directly activate I-κB. Following phosphorylation of I-κB by IKK and the binding of ubiquitin ligase, the polyubiquitination of I-κB leads to degradation by proteasomes. The removal of I-κB unmasks the nuclear-localization signals in both subunits (p65 and p50) of NF-κB, allowing their translocation into the nucleus.

Here NF-κB activates the transcription of numerous target genes, including the gene encoding the alpha subunit of I-κB, which acts to terminate signaling (Hayden and Ghosh, 2008). In this case, we adopted the mathematical model in (18) (Hoffmann *et al.*, 2002), and considered the IKK_a as the output, which directly affects the transcription factors (Fig. 3 and Supplementary Table S2). Using similar procedures employed in the previous example, we first

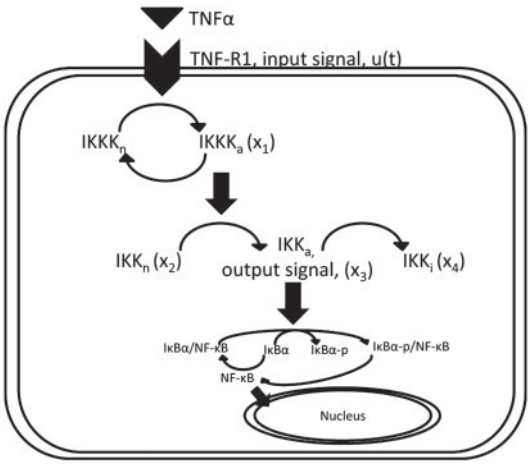

Fig. 3. The scheme of NF-κB signal transduction pathway.

constructed the fuzzy model and then solved the corresponding LMIs (simulation results are shown in Fig. 4). We found the signal transduction ability of the fuzzy signaling pathway to be $\rho_a = 6.48 \times 10^5$ with the corresponding positive definite symmetric matrix shown in Supplementary Materials.

4 DISCUSSION

In order to measure the signal transduction ability in biological experiments, it is necessary to test a multitude of input signals and then compute the ratio of the output to the input signals. This process requires numerous experiments and is not a realistic method of obtaining data. Therefore, a method that is independent of the measurement of output and input signals, which can be used to quantify the signal transduction ability based on the system dynamic model, is required. According to the concept of a system gain, we proposed a new method to measure the signal transduction ability of a signaling pathway, which is dependent only on the structure of the signaling pathway and the kinetic parameters of the components involved. Thus, the signal transduction ability can be viewed as a quantity to characterize a signaling pathway. Considering the important role of the MAPK and the NF-κB signaling pathways in a cell, an immediate and strong enough response to environmental changes or stresses is necessary. The simulation results describing a large system gain in concentration are unsurprising.

Here, we extend the concept of signal amplitude to the non-linear signaling pathway through a fuzzy modeling method, compared with the linear case (Heinrich *et al.*, 2002). Moreover, the most of previous studies considered the relationship between the output and the inputs on case-by-case basis (Heinrich *et al.*, 2002; Koshland *et al.*, 1982; Marhl *et al.*, 2009; Samoilov *et al.*, 2005). But from the system point of view, the transduction ability is a systematic characteristic of a signaling pathway, which should be invariant unless the system has been changed. The value can be referred to as the best ability of the signaling pathway to transduce input signals, that is, to amplify input signals. However, the simulated signal transduction ability might be not achieved easily without testing all possible inputs for the least upper bound ratio ρ_a in (4) (see Supplementary Fig. S1). That is, infinite L_2-bounded input

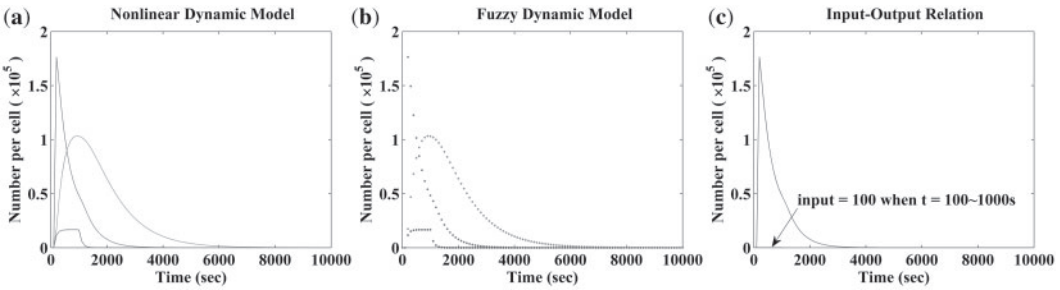

Fig. 4. The simulation example of the NF-κB signaling pathway. (**a**) The state variables ($IKKK_a$:blue, IKK_n:green, IKK_a:red and IKK_i:cyan) of the non-linear dynamic model of the NF-κB signal transduction pathway in (17). (**b**) The state variables of the fuzzy dynamic model of the NF-κB signal transduction pathway in the same form of (11). (**c**) An input (red) and output (blue) signal of the NF-κB signal transduction pathway.

signals are required to measure the transduction ability of a signaling pathway, which could be an open question.

The simulation results also show that the proposed measurement method can be applied in two different ways. A signaling pathway can be viewed as a composition of several sub-signaling pathways. The total signal transduction ability of a signaling pathway is equal to the product of the signal transduction abilities of each of the sub-signaling pathways. Each sub-signaling pathway can be referred to as a basic element of the general signaling pathway. Hence, if the signal transduction abilities of sub-signaling pathway are known, we can design a signaling pathway with the desired signal transduction ability. A similar concept is utilized in synthetic biology (Hasty *et al.*, 2002; Kaern *et al.*, 2003; Murphy *et al.*, 2007), which designate DNA sequences as the bricks to construct an artificial function in a cell. It is possible, through the analysis of signal transduction ability, to analyze and calculate each sub-signaling pathway so that a synthetic signal pathway can be engineered with the desired signal transduction ability. In this situation, greater biological transduction insights can be obtained from the linear system viewpoint. In the future, characterization of signal transduction systems by the proposed transduction ability measurement may be useful for biotechnological and therapeutic applications as well as the development of drug design.

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