

Effects of multiple parameter variations on biological system behaviors

Xiao Chang^{1,2}

Deng-Yu Liu¹

Ruiqi Wang¹

¹Institute of Systems Biology, Shanghai University, Shanghai 200444, China

²College of Statistics and Applied Mathematics, Anhui University of Finance and Economics, Bengbu, 233030, China

Abstract Biological networks often involve many parameters, and the importance of these parameters in determining system behaviors must be assessed in order to gain deep insight into designing principles of living organisms. However, many parameters cause the biological system behaviors vary extensively, therefore it is difficult to analyze the effect of individual parameters in a systematic way. In this paper, we show the effects of multiple parameter variations on bistability (or multistability) of positive feedback loop networks and further on oscillations in interlocked feedback loop networks by extending the techniques from the recently developed theory of monotone systems. This approach is based on decomposing a closed loop system into some open loops. The main ideas are illustrated through a positive feedback loop system and another system with a negative feedback loops, meanwhile, the proposed technique is valid for a general class of biological systems.

Keywords Bistability; positive feedback networks; multiple parameter variations; monotone modules; interlocked feedback networks

1 Introduction

Networks of interacting bio-molecules carry out many essential functions in living cells. It has been shown that gene regulatory networks with virtually desired properties can be constructed from simple regulatory modules. These properties, which include multistability and oscillations, have been found in specialized gene circuits such as gene switches and circadian oscillators. Here, for a wide class of feedback systems, we show that oscillations with desired properties can be deduced mathematically from the viewpoint of control theory and systems biology.

Genetic networks have been studied theoretically [1, 2] and experimentally [3, 4] for many years. So far, some techniques have been developed to construct networks with specific functions. Based on the monotone dynamical systems [5], a general design procedure by using positive feedback networks has been recently developed [6, 7]. Construction procedure of oscillators with interlocked feedback networks was also proposed [8, 9, 10]. The network structures may underlie particular functions such as genetic switches with positive feedback loop networks. However, besides the network topology, there are often many parameters involved in these networks, and the importance of these parameters in determining system behaviors need to be assessed in order to gain insight into design-

ing principles of living organisms. Systematic study on the effects of multiple parameter variations on network characteristics is still lacking.

Some techniques such as bifurcation analysis can be used to quantify the effects of single parameter variation, however, multiple parameter bifurcation is often difficult to analyze. Moreover, bifurcation analysis generally is a quantitative measure and therefore some qualitative analysis needs to be developed so some intuitive insight can be obtained. In this paper, we show the effects of multiple parameter variations on network characteristics by extending the techniques from the recently developed theory of monotone systems. The approach is based on decomposing a closed loop system into some open loop but monotone subsystems or modules. The main ideas are illustrated through a five-variable system, i.e. the Mos/MAPK kinase p42 MAPK cascade and an oscillatory network although the proposed technique holds for a general class of biological systems.

2 Methods and Results

In biology, many processes often involve many parameters, and the importance of these parameters in determining system behaviors must be assessed so as to gain deep insight into designing principles of living organisms. However, many parameters driving the biological system behaviors vary extensively, therefore it is generally difficult to analyze the effects of individual parameters in a systematic way. In our work, we first study the effects of the multiple parameter variations on positive feedback network characteristics and then on interlocked feedback networks by decomposing them into some positive feedback subnetworks. The main ideas are illustrated through a five-variable system, i.e. the Mos/MAPK kinase p42 MAPK cascade and the oscillatory Goldbeter's model although the proposed technique holds for a general class of biological systems. The equations for these two models are provided in the Appendix.

2.1 The Mos/MAPK kinase p42 MAPK cascade

Over the past years, a number of deterministic models for bistability or multistability have been proposed. Biological switches are essential for the determination of cell fate in multicellular organisms, the regulation of cell-cycle oscillations during mitosis, and the maintenance of epigenetic traits in microbes. The model captures the switch by positive-feedback loops and exhibits bistability. It encompasses five state variables for the concentrations of the components and 18 model parameters reflecting the kinetic constants of the molecular interactions.

2.2 Detection of stable equilibria

For any positive-loop system, it can be decomposed as many modules or subsystems [7]. When we decompose a system into two modules, it can be a mutually inhibitory or a double-positive feedback system. Here we just discuss the double-positive feedback system with bistability. For the mutually inhibitory feedback system or a more complicated system which needs to be decomposed as more modules, it can be studied with the same techniques. The techniques also hold for the case of multistability.

The system can be decomposed into some open subsystems with inputs and outputs. For the whole subsystems, we can use the open-loop technique and discuss it in the simplified 2-D plane. The system has three steady states, i.e. the three intersection points

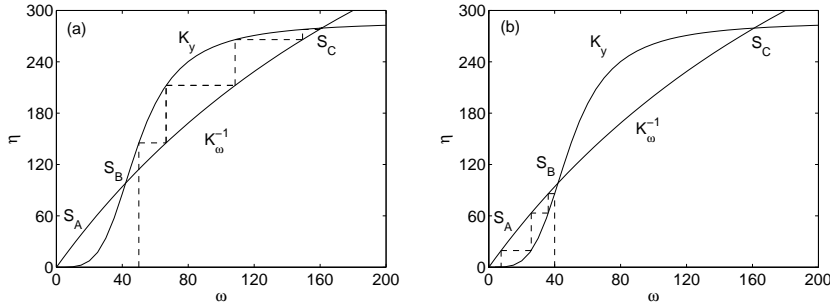


Figure 1: Bistability in a MAPK cascade (S_A and S_C are stable points, while S_B is unstable point). (a) The instability of S_B from the righthand. (b) The instability of S_B from the lefthand.

of the two characteristics curves: S_A , S_B , and S_C (which are labeled in Fig.1). We can determine the stability of each steady state as follows: for any steady state $S(\omega_s, y_s)$, we choose two initial values $(\omega_{0,1}, y_{0,1})$ and $(\omega_{0,2}, y_{0,2})$ with $\omega_{0,1} < \omega_s < \omega_{0,2}$, which are close to $S(\omega_s, y_s)$. If $\omega_{0,1} < \omega_1$ and $\omega_1 < \omega_{0,2}$ are satisfied, then $S(\omega_s, y_s)$ is stable, where ω_1 is the first iteration of the open-loop system. Otherwise it is unstable. For example, S_B is unstable, while both S_A and S_C are stable (as shown in the Fig.1). Using such a technique we just need to iterate some steps to know the stability of any steady state. In fact, the stability we obtained is global by just using two different initial values $\omega_{0,1}$ and $\omega_{0,2}$. The results hold for systems in any arbitrary dimensions, and not just in this special form.

2.3 Effect of single parameter variation on stability

A qualitative technique is developed to analyze the effects of individual parameter variations on the network characteristics. The network is decomposed into two sub-networks with nodes $\{x\}$ and $\{y_1, y_3, z_1, z_3\}$, respectively. By letting $\dot{x} = 0$, we get $x = K_2(V_1 + V_0\omega)/(V_2 - V_1 - V_0\omega) \triangleq g_1(\omega)$, i.e. $K_\omega = g_1(\omega)$. In the same way, we can also get K_y . Therefore, the effects of any single parameter variation on the stability of steady states can be analyzed directly. For example, taking K_2 as a variant parameter and others constants, the change of the characteristic curve $\eta = K_\omega \circ K_y(\omega)$ due to the variation of K_2 can be easily obtained. Because the path from x to z_2 is positive, then we have

$$\frac{\partial \eta}{\partial K_2} = \frac{\partial f_5}{\partial z_1} \frac{\partial f_4}{\partial y_3} \frac{\partial f_3}{\partial y_1} \frac{\partial f_2}{\partial x} \frac{\partial g_1}{\partial K_2} > 0. \quad (1)$$

For any change of other parameters, we can analyze its effects on stability of the two stable steady states.

From the above analysis we can know that how the stability of the steady states is affected by the parameter variations and which stable steady state will persist. The increase or decrease of the steady state due to the change of a specific parameter can also be obtained. Here we choose $\eta = z_2$ as the biological component we care about. Of course, we can choose other components and analyze the effects of single parameter on its existence and stability in a similar way. We can also analyze its change from the sign of the

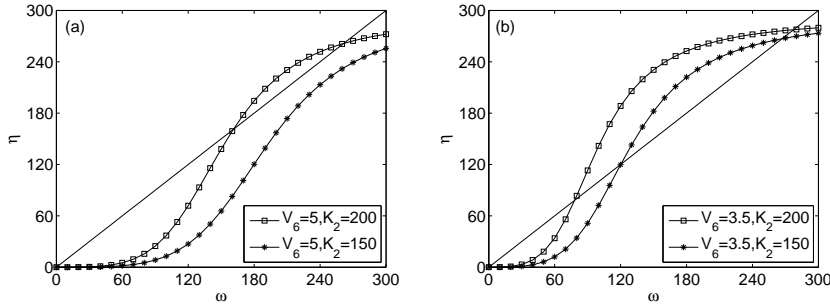


Figure 2: Effect of variation of multiple parameters on stability. (a) The effect of change of K_2 ($K_2 = 200, K_2 = 150$) when $V_6 = 5$ on stability. (b) The effect of change of K_2 ($K_2 = 200, K_2 = 150$) when $V_6 = 3.5$ on stability.

path. For example, the paths from z_2 to y_1 and y_2 are negative and positive, respectively, therefore, the increasing of η , that is z_2 will affect y_1 and y_2 negatively and positively, respectively. Thus, the components y_1 and y_2 will also decrease and increase, respectively. When there are multiple paths and all paths have the same sign, such a technique can also be used.

Please also note that the effect of the single parameter depends on not the output of its own subsystems but also the path from the input to output. For example, the effect of K_2 on z_2 depends on the sign $\frac{\partial f_5}{\partial z_1} \frac{\partial f_4}{\partial y_3} \frac{\partial f_3}{\partial y_1} \frac{\partial f_2}{\partial x}$ and $\frac{\partial g_1}{\partial K_2}$. The sign of $\frac{\partial f_5}{\partial z_1} \frac{\partial f_4}{\partial y_3} \frac{\partial f_3}{\partial y_1} \frac{\partial f_2}{\partial x}$ is the same as the sign of the path from x to z_2 . While the sign of $\frac{\partial g_1}{\partial K_2}$ is the effect of parameter variation on its own output. Although all loops are positive, negative paths still exist, such as the path from z_2 to y_1 .

2.3.1 Variations of multiple parameters

We will develop a global technique which can show the effects of multiple parameter fluctuations in a systematic way. In analogy to the single parameter analysis, we divide the parameter set into three subsets P , N , and U according to the signs of the derivative $\frac{\partial K}{\partial p}$, where $\eta = K(\omega)$ is the I/O characteristics and p is any parameter, and $p \in P, N$, or U mean that the derivative $\frac{\partial K}{\partial p}$ is positive, negative, or uncertain, respectively. The bistability arises from the positive feedback loops. The aim of the classification is to analyze the role of the regulatory mechanisms in determining system behaviors. The parameters are grouped according to their functional role, that is, activating or inhibiting the output.

We still choose z_2 as the component we care about and study the effects of multiple parameter variations on the system. According to the effect of individual parameter on each component and the sign of the path from the component to z_2 , the resulting classifications of model parameters are summarized in Table 1.

The effect of any individual parameter on the component z_2 through its direct effect on specific component and the path from the component to z_2 . Based on the above analysis, the two groups of parameters are $P = \{K_2, V_0, V_1, V_3, V_4, K_5, K_6, V_7, V_8, K_9, K_{10}\}$ and $N = \{V_2, K_3, K_4, V_5, V_6, K_7, K_8, V_9, V_{10}\}$, respectively.

Table 1: Classifications of model parameters

Para	K_2 V_0 V_1 V_2	V_3 K_3 V_6 K_6	V_4 K_4 V_5 K_5	V_7 K_7 V_{10} K_{10}	V_8 K_8 V_9 K_9	Sign
$\frac{\partial x}{\partial p}$	+ + + -					+
$\frac{\partial y_1}{\partial p}$		+ - - +				-
$\frac{\partial y_3}{\partial p}$			+ - - +			+
$\frac{\partial z_1}{\partial p}$				+ - - +		+
$\frac{\partial z_3}{\partial p}$					+ - - +	

Different parameters belong to the same set have the similar effect on the existence and stability of the steady states and increasing or decreasing parameters in the same parameter set has similar effect. For example, at the nominal value of $K_2 = 200$, the system is bistable. The parameter K_2 changes to 150 and the system becomes monostable. We can choose other parameters belonging to the same parameter set with K_2 and increase it to regain bistability. Or, we can choose parameters belong to another set and decrease it. Of course, we can choose the easiest way to regain the bistability. For example, if decreasing V_6 is plausible, we can choose and decrease it. Fig.2(a) shows that for the parameter value $V_6 = 5$ the system is bistable and monostable at $K_2 = 200$ and $K_2 = 150$, respectively. By decreasing V_6 to 3.5, the system becomes bistable for the parameter value $K_2 = 150$, as shown in Fig.2(b). Actually, the parameters belonging to the two sets P and N have the opposite effects on stability. Therefore, we can also get the similar bistability by increasing the other parameter values in the set P or decreasing the other parameter values in the set N or even both at the same time. Therefore, the effects of all parameters on the number and the stability of equilibria can be analyzed in a systematic way.

3 An oscillatory network: the Goldbeter's model

In this section, we show how monotone modules or signaling pathways with simple dynamics can be used to construct non-monotone interlocked feedback networks functioning as oscillators and illustrate the effect of the parameters on the oscillations. By decomposing a closed-loop system which can be monotone or non-monotone into some open-loop but monotone subsystems or modules we can use the input-output characteristics to study the effects of multiple parameter variations on system behaviors in a systematic way. The proposed approach can be used to not only positive feedback loop systems but also systems with negative feedback loops. In systems with negative feedback loops, a discrete map can be used to build the correspondence between the closed and open loop systems. We use the simple circadian oscillator proposed by Goldbeter [11] as an example to illustrate our idea. The model equations can be found in the Appendix.

3.1 Analysis

From the viewpoint of control engineering, we view the model as the feedback closure of two subsystems: mRNA subsystem and protein subsystem with inputs and outputs (the equations of the decomposition of the system is provided in the Appendix). As discussed before, using the same methods we can choose P_N as the component we care about and study the effects of the variation of the parameters on the subsystems. The effect of each

individual parameter on P_N is through its direct effect on specific component and the path from the component to P_N . Based on the above analysis, using the same method, we can also separate all parameters into the following groups, $P_1 = \{v_s, K_I, K_m\}$ and $N_1 = \{v_m\}$ for Σ_1 , $P_2 = \{k_s, K_d, k_1\}$ and $N_2 = \{v_d\}$ for Σ_2 , and $U = \{V_1, K_1, V_2, K_2, V_3, K_3, V_4, K_4\}$ respectively. We can illustrate the effect of the change of the parameters on P_N through numerical simulation and the results are corresponding with the above separation. Actually, the parameters belonging to the two sets P_i and N_i have the opposite effects on the amplitudes of the sustained oscillations. The parameters belonging to set U have no effect on the amplitude of the sustained oscillations in P_N . We can also get the similar amplitude by increasing the other parameter values in the set P_i or decreasing the other parameter values in the set N_i or even both at the same time. Therefore, the effects of all parameters on the oscillation and the amplitude can be analyzed in a systematic way as discussed before.

3.2 Regulation and control method

Here, we use the output functions which are mappings of constant inputs to outputs to state for the first mRNA subsystem and the second protein subsystems, respectively. For each constant input ω we can show that there exists a globally and asymptotically stable equilibrium for the subsystem governed by Eqs.(7). Its static input-output characteristic can be obtained as follows:

$$K_y(\omega) = \frac{k_1 k_s \omega K_d}{k_2 (v_d - k_s \omega)}. \quad (2)$$

In the same way, for each constant input y , we can get

$$K_\omega(y) = \frac{v_s K_I^n K_m}{v_m (K_I^n + y^n) - v_s K_I^n} \quad (3)$$

for the subsystem governed by Eq.(6). Instead of the whole system governed by Eqs.(5), we now consider the following discrete dynamical map

$$\omega_{k+1} = (K_\omega \circ K_y)(\omega_k) \quad (4)$$

which keeps its qualitative characteristics. It is much easier to analyze, and it is also easy to carry out numerical simulation simply by iterative procedure. Two situations of the input-output characteristics in (ω, y) plan are illustrated in Fig.4 to show the convergence of Eq.(5) to different periodic orbits. Larger k_s means larger amplitude of $y = P_N$ for appropriate delays.

4 Conclusion and Discussion

In this paper, the main ideas are illustrated through a positive feedback loop system, i.e. a five-variable system Mos/MAPK kinase p42 MAPK cascade and a system with negative feedback loops, i.e. the Goldbeter's oscillatory model, and the proposed technique also hold for a general class of biological systems. The results show that the proposed approach is very effective to analyze effects of multiple parameter variations on system behaviors in a systematic way. The approach can also help us to gain insight and to design informative experiments.

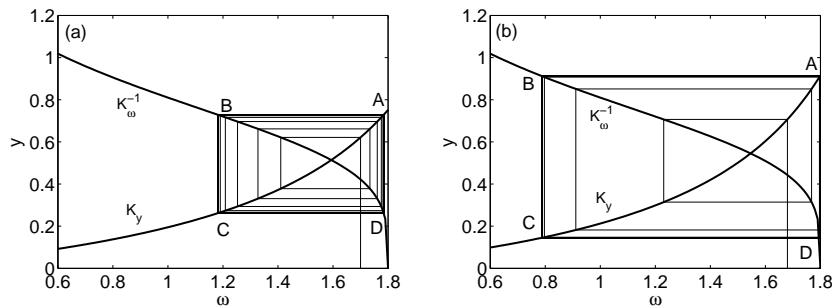


Figure 3: Different types of input-output characteristics in (ω, y) plane. (a) $k_s = 0.38$; (b) $k_s = 0.40$.

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Appendix

(1). The Mos/MAPK kinase p42 MAPK cascade:

$$\begin{aligned}\Sigma_1: \dot{x} &= -\frac{V_2x}{K_2+x} + V_0\omega x + V_1, \\ \Sigma_2: \dot{y}_1 &= \frac{V_6(1200-y_1-y_3)}{K_6+(1200+y_1+y_3)} - \frac{V_3xy_1}{K_3+y_1}, \\ \dot{y}_3 &= \frac{V_4x(1200-y_1-y_3)}{K_4+(1200-y_1-y_3)} - \frac{V_5y_3}{K_5+y_3}, \\ \dot{z}_1 &= \frac{V_{10}(300-z_1-z_3)}{K_{10}+(300-z_1-z_3)} - \frac{V_7y_3z_1}{K_7+z_1}, \\ \dot{z}_3 &= \frac{V_8y_3(300-z_1-z_3)}{K_8+(300-z_1-z_3)} - \frac{V_9z_3}{K_9+z_3},\end{aligned}$$

with input ω and output x for Σ_1 and input x and output $\eta = z_3$ for Σ_2 .

(2). Minimal model for circadian oscillations of PER and per mRNA with delay

$$\begin{aligned}\frac{dM}{dt} &= v_s \frac{K_I^n}{K_I^n + P_N^n(t-\tau)} - v_m \frac{M}{K_m + M} \\ \frac{dP_0}{dt} &= k_s M - V_1 \frac{P_0}{K_1 + P_0} + V_2 \frac{P_1}{K_2 + P_1} \\ \frac{dP_1}{dt} &= V_1 \frac{P_0}{K_1 + P_0} - V_2 \frac{P_1}{K_2 + P_1} - V_3 \frac{P_1}{K_3 + P_1} + V_4 \frac{P_2}{K_4 + P_2} \\ \frac{dP_2}{dt} &= V_3 \frac{P_1}{K_3 + P_1} - V_4 \frac{P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_N - v_d \frac{P_2}{K_d + P_2} \\ \frac{dP_N}{dt} &= k_1 P_2 - k_2 P_N\end{aligned}\quad (5)$$

(3). Decomposition of the preceding systems

The first subsystem is the mRNA subsystem and described by

$$\Sigma_1: \frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + y^n} - v_m \frac{M}{K_m + M}\quad (6)$$

with input y and output ω .

The second subsystem is the protein subsystem and described by

$$\begin{aligned}\Sigma_2: \frac{dP_0}{dt} &= k_s \omega - V_1 \frac{P_0}{K_1 + P_0} + V_2 \frac{P_1}{K_2 + P_1} \\ \frac{dP_1}{dt} &= V_1 \frac{P_0}{K_1 + P_0} - V_2 \frac{P_1}{K_2 + P_1} - V_3 \frac{P_1}{K_3 + P_1} + V_4 \frac{P_2}{K_4 + P_2} \\ \frac{dP_2}{dt} &= V_3 \frac{P_1}{K_3 + P_1} - V_4 \frac{P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_N - v_d \frac{P_2}{K_d + P_2} \\ \frac{dP_N}{dt} &= k_1 P_2 - k_2 P_N\end{aligned}\quad (7)$$

with input ω and output y .