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Sensitivities of Regulation Intensities in Feed-Forward Loops with Multistability

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***Abstract*— Gene regulatory networks depict the interactions among genes, proteins, and other components of the cell. These interactions are stochastic when large differences in reaction rates and small copy number of molecules are involved. Discrete Chemical Master Equation (dCME) provides a general framework for understanding the stochastic nature of these networks. Here we used the Accurate Chemical Master Equation method to directly compute the exact steady state probability landscape of the feed-forward loop motif (FFL). FFL is one of the most abundant gene regulatory networks motifs where the regulation is carried out from the top nodes to the bottom ones. We examine the behavior of stochastic FFLs under different conditions of various regulation intensities. Under the conditions with slow promoter binding, we show how FFL can exhibit different multistabilities in their landscapes. We also study the sensitivities of regulations of FFLs and introduce a new definition of stochastic sensitivity to characterize how FFLs respond in their probability distributions at the steady state to perturbations of system parameters. We show how change in gene expression under FFL regulations are sensitive to system parameters, including the state of multistability in FFLs.**

I. INTRODUCTION

Gene regulatory networks play central roles in defining molecular content and cellular phenotypes. Modeling gene regulatory networks remains challenging due to the complexity of their stochastic nature. Although gene regulatory networks in a cell might consist of dozens of genes, along with their proteins products, their functions are usually defined by smaller subnetworks, called *network motifs*. These network motif are small functional building blocks of gene regulatory networks. They occur much more frequently in real biological networks compared to random subgraphs [1].

Feed-forward loop (FFL) is one of the most prevalent motifs in nature [1]. Regulation in feed-forward loop is carried out from the top nodes towards the bottom ones. FFL are found to have wide presence in yeast [2], in bacteria [3], [4], and are also widely observed in mammals [5], [6], [7]. They are of very simple architecture, but can have a variety of functionalities.

There are many studies on the time-evolving functional characteristics of FFLs, using deterministic models. The behaviors for these models, for instance, in signal-processing, pulse generation, signal transduction, and "fold-change" detection, are well known. However, stochastic behaviors of FFLs, which occurs when copy numbers of molecules involved are small, are largely unknown [8]. Here we employ the recently developed ACME method [9], [10] to compute the exact time-evolving probability landscapes of FFLs by solving the underlying discrete Chemical Master Equation (dCME). This eliminates potential problems arising from inadequate sampling, where rare events of low probability are difficult to quantify using techniques such as the stochastic simulations algorithm (SSA) [11].

We examine *the parameters sensitivity* of FFLs, a measure of system behavior in response to perturbations of its parameters. It characterizes how changes in the network parameters can affect the network output, for example, at the steady state. Sensitivity analysis helps to understand how network output is sensitive to input, and models parameters, and has been widely used as a measure of robustness. Sensitivity of FFLs to parameters was examined in detail previously [12], [13], [14], including how FFL can carry the function of adaptation [12], [13]. However, the behavior of sensitivity of FFLs in the stochastic regime, where slow parameters binding results in highly stochastic behavior of the system is unknown.

Our models are under strong stochastic conditions of slow promoter binding. Recent studies suggest that slow promoter binding creates distinct expression levels with considerable lifetime [15], [16], [17]. We first compute accurately FFLs probability distributions. In this case FFL can be multistable and can exhibit up to three probability peaks in the copy number of the output node. We study the sensitivity of the regulation intensities of feed-forward loop to model parameters. Regulation intensities play important role as they define the strength of regulation in FFLs. We introduce a new definition of sensitivity, which characterizes the response of the steady state probability distribution to perturbation to system parameters, within a given interval. Further we show how the steady state responds to change of values of the parameters. We show how change in gene expression under FFL regulations are sensitive to system parameters, including the state of multistability in FFLs.

II. MODELS AND METHODS

A. Feed-forward loop can exhibit multiple phenotypes

1) Architecture of FFLs: The network motif of FFL has three nodes, consisting of three genes a, b, and c. Their protein products are denoted as A, B, and C, respectively (Fig. 1 A). Gene a expresses protein A at a constant expression rate

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s_A. Gene b has one promoter site, and turns into a gene-protein complex bA upon binding to protein A. Gene c also has one promoter site, which can be occupied by either one of its transcription factors A or B. This type of regulation is known as an "OR" gate. Competitive binding of proteins A and B to the promoter site of gene c turns it into either gene-protein complexes c_A or complex c_B, correspondingly. The biochemical reactions corresponding to the feed-forward loop model are listed below:

$$b + A \xrightarrow{r_b^A} bA; \quad bA \xrightarrow{f_b^A} b + A;$$

$$c + A \xrightarrow{r_c^A} cA; \quad cA \xrightarrow{f_c^A} c + A;$$

$$c + B \xrightarrow{r_c^B} cB; \quad cB \xrightarrow{f_c^B} c + B;$$

$$\emptyset \xrightarrow{s_A} A; \quad A \xrightarrow{d_A} \emptyset;$$

$$b \xrightarrow{s_B} B; \quad bA \xrightarrow{s_B * k_1} B; \quad B \xrightarrow{d_B = 1} \emptyset;$$

$$c \xrightarrow{s_C} C; \quad cB \xrightarrow{s_C * k_2} C; \quad cA \xrightarrow{s_C * k_3} C; \quad C \xrightarrow{d_C} \emptyset.$$

Here $r_b^A = r_c^A = r_c^B = 0.005$ are binding rates of proteins A to gene b, A to gene c, and B to gene c, respectively. $f_b^A = f_c^A = f_c^B = 0.1$ are unbinding rates of proteins A to gene b, A to gene c, and B to gene c, respectively. The rates of degradation of proteins A, B, and C are $d_A = d_B = d_C = 1$, and basal synthesis rates are $s_A = s_B = s_C = 10$, respectively. Gene b is expressed with a constant basal expression rate s_B, but once it is bound with protein A, the expression rate is reduced/increased by k_1-fold (Fig. 1 A). The basal expression rate of gene c is s_C, but it is changed k_2-fold for a complex cB, and k_3-fold for a complex c_A. We will further call the parameters k_1, k_2, and k_3 regulation intensities.

2) Types of feed-forward loop: Simplified representation of feed-forward loops consist of three nodes: a top node A, a buffer node B, and an output node C. It also has three regulatory links: the regulations of B and C by A, and the regulation of C by B. There are two regulatory paths regulating the output node C by input node A: directly from A to C, and indirectly from A to B, then from B to C. Each regulatory link is either up or a down regulation. Alltogether, there are eight types of feed-forward loops (Fig. 1 (B)). Depending on the sign of regulation (positive for activation, and negative for inhibition), either through direct or through indirect paths, FFLs can be classified into coherent and incoherent feed-forward loops. Incoherent/coherent FFLs have odd/even number of "−"signed edges, respectively. Coherent FFLs are shown as C_1, C_2, C_3, C_4 and incoherent as I_1, I_2, I_3, I_4 in the Fig. 1 B.

3) Simulations: We explore all 8 possible types of FFLs and examine their behavior over a wide range of values of k_1, k_2, and k_3. We computed probability landscapes of height types of FFLs, with the parameters k_1, k_2, and k_3 in the ranges of $k_1 \in [2.5 \times 10^{-2}, 3.0]$, $k_2 \in [2.5 \times 10^{-2}, 5.1]$ and $k_3 \in [2.5 \times 10^{-2}, 5.1]$. With these parameter ranges, we are able to observe all eight possible types of feed forward loops (Fig. 1 B, Table. (II-A.3)).

Parameter ranges for the eight types of FFL studied

FFL	k_1 range	k_2 range	k_3 range
C_1	(1.0, 3.0]	(1.0, 5.1]	(1.0, 5.1]
C_2	[0.025, 1.0)	(1.0, 5.1]	(0.025, 1.0]
C_3	(1.0, 3.0]	[0.025, 1.0)	[0.025, 1.0)
C_4	[0.025, 1.0)	[0.025, 1.0)	(1.0, 5.1]
I_1	(1.0..3.0]	[0.025..1.0)	(1.0..5.1]
I_2	[0.025, 1.0)	[0.025, 1.0)	[0.025, 1.0)
I_3	(1.0, 3.0]	(1.0, 5.1]	[0.025, 1.0)
I_4	[0.025, 1.0)	(1.0, 5.1]	(1.0, 5.1]

Our calculations uncovered six different types of multi-stabilities in these FFLs (Fig. 2) Systems with one peak with their probability landscapes are shown in red. Those with two peaks either for B, or for C are in yellow, Those with three peaks for C are in green. Those with four peaks (two for B and two for C) are in lightblue. Those with six peaks are shown in purple. Overall these FFLs have mono- or bimodality in B and up to three stable peaks in C.

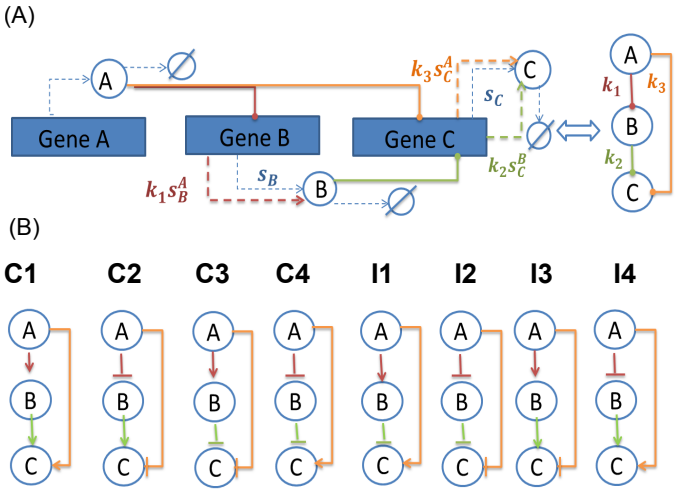

Fig. 1. The network model of FFL ans the architecture of different coherent and incoherent FFLs: (A) General network and corresponding 3-node schematic representation of the FFL containing three genes a, b, c expressing three proteins A, B, C, such that protein A regulates the expression of b and c through binding, and protein B regulates the expression of c. (B) The eight types of feed-forward loop.

B. Sensitivities of regulation intensities in FFL

1) Definition of sensitivity: In deterministic models, sensitivity of parameters simply measures changes in the output at steady state, when values of model parameters are altered. In stochastic models where the output is a probability distribution, sensitivities is often measured by changes in the expected value of the output[18].

Here we introduce a new definition of stochastic sensitivity and show it is more effective in measuring the differences and similarities in behavior of different types of FFLs.

We define the sensitivity $s'_{k_i}(k_0, k)$ to changes in k_i, $i = 1, 2, 3$, whose value is altered from k_0 to k as follows:

$$s'_{k_i}(k_0, k) = \mathbb{E}[\frac{|P_k(\mathbf{x}) - P_0(\mathbf{x})|}{P_0(\mathbf{x})} / \frac{|k - k_0|}{k_0}],$$

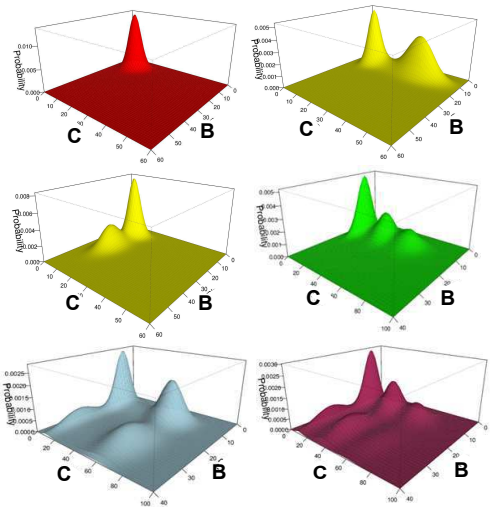

Fig. 2. Different states of multistability for Feed Forward Loops: 1 peak (red); 2 peaks, either for B, or for C (yellow); 3 peaks for C (green); 4 peaks, 2 peaks for B and 2 peaks for C, (lightblue); and 6 peaks, 2 peaks for B, and 3 peaks for C, (purple).

where $P_0(\mathbf{x})$ is the system probability landscape at parameter value of $k_i = k_0$ and $P_k(\mathbf{x})$ is the landscape at $k_i = k \neq k_0$. The value of parameter k_i belongs to a finite interval (a,b), which is $(0,1)$ for inhibition and $(1,A)$ for activation, where A is some finite number. The sensitivity of s_{k_i} on the interval (a,b) is defined as:

$$s_{k_i} = \max_{k_0,k\in(a,b)} s'_{k_i}(k_0,k). \tag{1}$$

Our stochastic sensitivity is specific to the intervals of regulation intensities, which is FFL specific.

2) Sensitivity of regulation intensity k_1: We examined the stochastic sensitivity of regulation intensity k_1 using Eqn. (1). We consider the cases when gene b is inhibited by protein A, with $k_1 < 1$, and gene b is activated by protein A, with $k_1 > 1$ (Fig. 3).

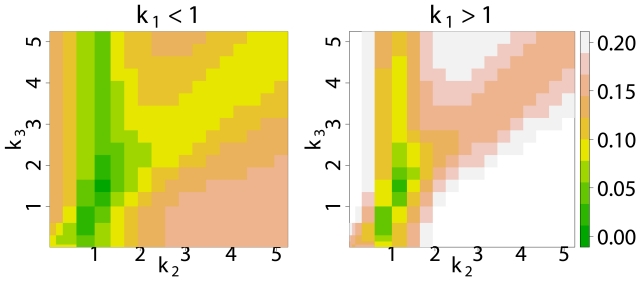

Fig. 3. The sensitivity of regulation intensity $s(k_1)$ on the inhibition of gene b by protein A ($k_1 < 1$), and on the activation of gene b by protein A ($k_1 > 1$).

The sensitivity of k_1 is smaller in the green and yellow regions of Fig. 3, and larger in the white and pink regions (Fig. 3). There are two situations when the sensitivity of k_1 is smallest, and the system is most robust to change in k_1. The first situation is when $k_2 = 1$, where the regulation of the gene c by B is weak, such that the expression of c does not depend on B copy number. The other region is where $k_2 = k_3$, both the rates of activation/inhibition of c by B and c by A are of similar values. It means that the system is robust to k_1 change when the proteins A and B regulate the output node C with the same intensity. The sensitivity of k_1 is also small for the smallest values of k_1 (i.e., $k_1 < 1$), and larger for larger values of k_1 (i.e., $k_1 > 1$).

3) Sensitivity of regulation intensity k_2: We examined the stochastic sensitivity of regulation intensity k_2 using Eqn. (1). We consider the cases when gene c is inhibited by protein B, with $k_2 < 1$, and gene c is activated by protein B, with $k_2 > 1$ (Fig. 4).

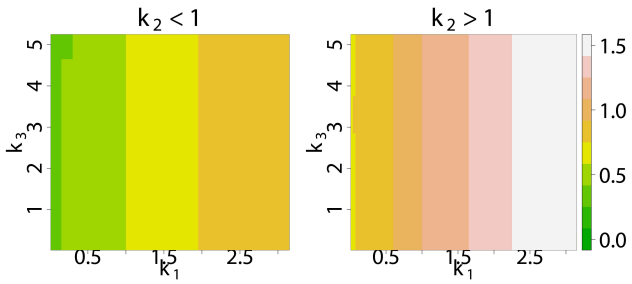

Fig. 4. The sensitivity of regulation intensity $s(k_2)$ on the inhibition of gene c by protein B ($k_2 < 1$), and on the activation of gene c by protein B ($k_2 > 1$).

The sensitivity of k_2 is smaller in the green and yellow regions of Fig. 4, and larger in the white and pink regions (Fig. 4). The sensitivity is smaller, when the values of k_1 are small, specifically, A inhibits the expression of b and the overall copy number of B in the system is reduced. Hence, the regulation of output C by B is less prominent, and k_2 has smaller sensitivity. The sensitivity of k_2 is also smaller for the small values of k_2 (i.e., $k_2 < 1$), and larger for larger values of k_2 ($k_2 > 1$). The dependence of the sensitivity of k_2 to the value of k_3 is negligible.

4) Sensitivity of regulation intensity k_3: We examined the stochastic sensitivity of regulation intensity k_3 using Eqn. (1). We consider the cases when gene c is inhibited by protein A, with $k_3 < 1$, and gene c is activated by protein A, with $k_3 > 1$ (Fig. 5).

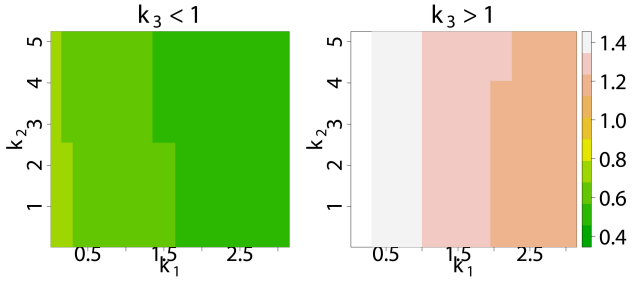

Fig. 5. The sensitivity of regulation intensity $s(k_3)$ on the inhibition of gene c by protein A ($k_3 < 1$), and activation of gene c by protein A ($k_3 > 1$).

The sensitivity of k_3 is smaller in the green and yellow regions of the Fig. 5, and larger in the white and pink regions (Fig. 5). Smaller k_3 sensitivities correspond to larger values of k_1. In this situation A activates the expression of c, therefore the effect of the regulation of c by direct path is more prominent. There is also a weak dependence of the sensitivity of k_3 of the value of k_2. Smaller k_3 sensitivities correspond to larger values of k_2 in the case of $k_3 < 1$, where the inhibition is more prominent with larger k_2. Smaller k_3 sensitivities correspond to smaller values of k_2 in the case of $k_3 > 1$, where the activation is more prominent with larger k_2. The sensitivity of k_3 is also smaller for the small values of k_3 ($k_3 < 1$), and larger for larger values of k_3 ($k_3 > 1$).

5) Dependence of sensitivity on multistability of FFLs: We now examine the dependence of the values of the sensitivity on the number of peaks in the system Table. (II-B.5).

k_1, k_2, and k_3 sensitivities of coherent and incoherent FFLs with different numbers of peaks

Type of FFL	Number of peaks	Mean k_1	Mean k_2	Mean k_3
Coherent	3	0.16	0.80	0.94
	2	0.15	0.77	0.69
	1	0.12	0.44	0.47
Incoherent	3	0.20	1.02	0.89
	2	0.15	0.72	0.75
	1	0.11	0.57	0.46

Our results show that the number of peaks is correlated with the sensitivities of k_1, k_2, and k_3. FFLs with three peaks are the least robust to changes in the parameters for both coherent and incoherent FFLs. In contrast, systems with one peak are the most robust to the change of the parameters for both case of coherent and incoherent loops.

III. Conclusion

In this work, we studied the sensitivities of regulation intensities of feed-forward loops (FFLs) under the conditions of slow promoter binding. We first computed the precise steady state probability distributions of eight types of FFLs under a wide range of conditions. Our results reveal the overall of multistable behavior of FFLs in the copy number of C. We introduced a new definition of the stochastic sensitivity, to quantify the sensitivity of different parameters of stochastic FFL. We showed how the steady state distribution responds to changes in model parameters. Specifically, we quantified how sensitivities of regulation intensities depend on the values of other regulation intensities and the state of multistability of the system. We found that the sensitivity of regulation intensity k_1 depends on the values of k_2 and k_3, whereas the sensitivities of k_2 and k_3 strongly depend on k_1. The FFL with more peaks of protein C copy number is less robust.

The results of our work could be used in construction of synthetic feed-forward loop, and choosing parameters of the system according to particular programmed phenotypic behavior.

IV. Acknowledgments

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