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Control system with biochemical reaction network and its application to molecular robotics*

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***Abstract*— In this paper, we consider how to realize a PID controller and a signal transmission system in a biochemical reaction network, which are fundamental issues in a design of molecular robot. Since a negative feedback is implemented with an inhibition reaction in an intracellular signal transduction system instead of a negative gain or an error signal, some fundamental properties of the closed loop system such as equilibrium state and oscillation are different from a mechatronics system. We show that the input signal to a biochemical network has two aspects, the main signal to be transmitted and a noise to be rejected. Next, we computationally analyze an example model to clarify the effect of negative feedback control in a case study, calculating the input to output properties with various kinds of input signals. Then, we demonstrate that an example model is a possible candidate for a PID controller and a signal transmission system in molecular robot.**

I. INTRODUCTION

Cellular processes such as proliferation, differentiation and apoptosis are rigorously controlled by intracellular signal transduction systems that are biochemical reaction networks with proteins and genes [1]. An extracellular stimulus as a reference input is transmitted toward target genes by means of chains of activations of signaling proteins in signal transduction pathways in which the information in the flow is a concentration change of an activated protein. It is amazing that the information-communication system is reasonably reliable and robust against environmental change and disturbances in a cell. Recently, technology for controlling a biochemical actuator based on a sensor signal that is also a physical value of a molecule is needed to realize a motion control of molecular robot [2].

It has been well known that a signal transduction system in a cell is regulated by feedback control [3], [4], [1] in which a positive and a negative regulation provide a switch-like function and an enhancement of robustness, respectively This fact motivates researchers involving in control theories to perform a model-based analysis aiming for a better understanding of a complicated signal transduction pathways [5], [21], [6], [7], [8], [9]. These studies mainly focus on elucidating the mechanisms of a control system to contribute a development of a better drug for diseases such as tumors. On the other hand, there are few studies to design and implement a control system in an artificial biochemical reaction network that is an essential technology for molecular robotics.

In this paper, we consider how to realize a PID controller and a signal transmission system in a biochemical reaction network for molecular robotics. To this end, we begin with a discussion regarding functions of negative feedback control comparing with one in an engineering system. Since a negative feedback is implemented with an inhibition reaction in a signal transduction system instead of a negative gain or an error signal in mechatronics, some fundamental properties of the closed loop system such as equilibrium state and oscillation are investigated. Next, we analyze an example model in [21] to clarify the effect of negative feedback control in a case study. Since the model is highly nonlinear and it is difficult to analytically evaluate the model, we calculate the input to output properties by applying various kinds of input signal. Then, we demonstrate that the model is a possible candidate for a PID controller and a signal transmission system in molecular robot.

The subsequent sections are organized as follows. In the section II we discuss about functions of a negative feedback control in a biochemical reaction network and provide some basic results on system properties of the closed loop system. In the section III, we address a case study with an example model to investigate what information is transmitted in the signal transduction system and how we can utilize the system as a controller or a signal transmission. Then, we provide some discussions with the results in the section IV. Finally, we mention some conclusions.

II. BIO-TYPE NEGATIVE FEEDBACK

In the typical control theory for engineering systems, negative feedback is a control action with a minus feedback gain to stabilize a plant of interest or an error signal between a reference and an output signal, which is executable in practical applications since both a positive and a negative control values have physical meanings such as clockwise and anti-clockwise torques. On the other hand, signals in biochemical reaction networks are concentrations of activated proteins or expressed genes, and only positive values are available in the system, which implies that a negative feedback regulation in a biochemical reaction network should be realized by a different kind of operation without negative values (we call the operation by "bio-type" negative feedback in this paper).

In molecular cell biology, it is well known that negative feedback is an important motif in a signal transduction pathway, which is a control system for regulating signal transduction in a cell comprised of biochemical reactions, to

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regulate an information flow [1], [10], and is accomplished by inhibition of an activator or enhancement of a deactivator in the pathways [11], [12]. Although there are some kinds of formulations for the negative feedback regulation, a typical description (e.g. [13], [14]) is defined by the following type of equations

$$\begin{aligned} \dot{x}_1(t) &= f_1(x) + \frac{v}{K + x_n^p} u(t) \\ \dot{x}_2(t) &= f_2(x) \\ &\vdots \\ \dot{x}_n(t) &= f_n(x) \end{aligned} \tag{1}$$

where $x \in \mathbb{R}^n$ is a state vector, x_i is the i-th element of x and a concentration of a signaling molecule, $f_i : \mathbb{R}^n \to \mathbb{R}$ is a rate equation such as ones described by the law of mass action and the Michaelis-Menten equation for $i = 1, 2, ..., n$. A Michaelis constant K, a catalytic constant v and a power p are positive and $u \in \mathbb{R}$ is a reference input that is also a concentration of a signaling molecule in an upper stream of the system. The negative effect is quite different from those in engineering systems where the action by input u is attenuated with a signaling molecule x_n in a down stream of the pathway.

It is well known that in a case of $n \leq 2$ any oscillation does not occur in the negative feedback system (1) for any parameters [15], and in a case of $n \geq 3$ the system (1) is equivalent to the Goodwin and the relevant model [16], [17], [18] and have an ability to oscillate with the negative feedback regulator x_n with a time delay due to a presence of a cascade $(x_2, ..., x_{n-1})$, indicating that the bio-type negative feedback has a risk to cause an oscillatory dynamics in a signal transduction system whereas an ordinary one has a risk to induce both oscillation and instability in engineering systems.

Next, we consider the equilibrium state x^* of the system (1) with $n = 1$, $p = 1$, and $f_1(x) = -kx_1$ in the simplest case where k is a degradation rate. Then, we obtain

$$x^* = \frac{-kK \pm \sqrt{k^2K^2 + 4kvu}}{2k} \tag{2}$$

It is obvious that $x^* = 0$ if and only if $u = 0$, indicating that the input should return to zero in order to terminate the signaling response, otherwise the equilibrium state is shifted to a positive value and the signaling molecule x_1 is constantly activated in a signal transaction pathway that is a risk to cause a harmful gene expression. Therefore, it is important for a bio-type negative feedback system that the reference input u surely returns to zero and/or a signal transduction system is robust against an equilibrium shift. From this simple discussion, we notice that a role of a reference input is completely different from that in engineering systems, which implies that the input u from an upper-stream system is not so much a desired value to be tracked by the output of the system as a kind of noise for the system (1) even though it is a main signal to be transmitted to a subsequent system whereas an extraneous noise is involuntarily mixed into a system in engineering applications.

In a real signal transduction pathway, a positive feedback effect is also observed and it has been reported that the action provides a switch-like function [1], [19] and is also a key regulation to produce an oscillatory dynamics in a system [12] where bistability of a positive feedback system is a fundamental property. In what follows, we discuss a difference in bistability between a positive and a negative feedback system in a simple case study. Consider the following first-order positive (PF) [20] and negative (NF) feedback system:

$$\text{(PF)} \quad \dot{x} = -kx + \frac{vx^2}{K + x^2} + bu \tag{3}$$

$$\text{(NF)} \quad \dot{x} = -kx + \frac{v}{K + x^2} + bu \tag{4}$$

where k, b, v and K are positive constants as well. For the system (3), the equilibrium states are given by solutions of the next cubic equation:

$$\frac{k}{K}x^3 - \frac{bu + v}{K}x^2 + kx - bu = 0 \tag{5}$$

The discriminant Δ for the cubic equation is calculated by

$$\Delta = -\frac{4k^4}{K} - \frac{8b^2u^2k^2 - 20buvk^2 - k^2v^2}{K^2} - \frac{4bu(bu + v)^3}{K^3} \tag{6}$$

Then, it is possible to find model parameters and input level to satisfy each of a condition $\Delta > 0$ for three different real solutions relating to bistability and a condition $\Delta < 0$ for a real solution and two complex solutions without bistability. On the other hand, for the system (4), we obtain

$$\Delta = -\frac{4k^4}{K} - \frac{8b^2u^2k^2 + 36buvk^2 + 27k^2v^2}{K^2} - \frac{4b^3u^3(b + v)}{K^3} \tag{7}$$

which implies that $\Delta < 0$ for any model parameters and input level. Hence, the negative feedback system (4) invariably has a two complex solutions and a real solution, indicating that no bistability exists in the case.

Remark 1: As discussed in [19], [12], [13], with a larger power p (Hill coefficient) the system gains an ultrasensitivity which is a system property closed to bistability, and shows a switch-like behavior even though there is no hysteresis in the dynamics.

III. The Functions: A Case Study

A. Example Model

In this section, we study functions of a bio-type negative feedback system. As mentioned in the previous section, the input u of the system (1) has two opposite aspects that are the main signal and a noise. Then, what information of u is transmitted as a key message via the system? In addition, does a bio-type negative feedback regulation contribute to enhance robustness of the system as well as an ordinary negative feedback control? In order to explore the questions, we address a case study with a mathematical model on c-Fos protein expression induced by the mitogen-activated protein kinase (MAPK) cascade via *dusp* and *c-fos* mRNAs expression [21] in which we here focus on a 10-dimensional closed loop subsystem including a negative feedback loop instead

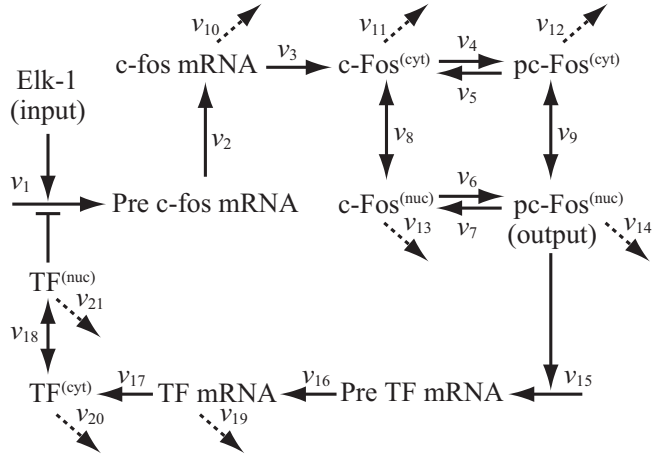

Fig. 1. 10-dimensional example model extracted from [21]

of the original 35-dimensional system (Fig. 1). The reaction scheme is summarized as follows. A transcription factor Elk-1, which is a reference input, transcribes preprocessing *c-fos* mRNA in the nucleus (reaction v_1), and the transcript is subsequently built into a *c-fos* mRNA and exported to the cytoplasm (reaction v_2). The mRNA is then translated into c-Fos protein (reaction v_3). c-Fos is activated (reactions v_4 and v_6) and deactivated (reactions v_5 and v_7), and imported into the nucleus (reactions v_8 and v_9). The degradation processes of the mRNA and the proteins are denoted by reactions v_{10} to v_{14}. In this model, activated c-Fos in the nucleus is the output of the system, and functions as a transcription factor for the subsequent system. The Elk-1 induced c-Fos expression system is negatively regulated by a transcription factor TF (reaction v_1) of which transcription is initiated by the output in a similar way (reactions v_{15} to v_{21}). The definition of notations in the model are summarized in Table I (See [21] for the detailed informations about the rate equations, the right-hand sides of the differential equations, and model parameters). In this model, the negative feedback regulation is described in

$$\dot{x}_1 = -k_1 x_1 + \frac{V_1 u^p}{K_1^p + u^p + \left(\frac{x_{10}}{K_m}\right)^q} \tag{8}$$

where k_1, V_1, K_1, K_m, p and q are positive constants. According to [21], the dynamics of the input u is also negatively regulated in the upper-stream system with another gene expression, and the response is given by a transient time-course curve.

B. Calculi of Key Characteristics

The example model is mathematically described with the Michaelis-Menten equations and the Hill functions. Since the nonlinearity of the model is too high to analytically investigate the functions, we computationally explore them. It has reported that three parameters are key characteristics in a response of a signaling molecule, signaling time τ, duration θ and amplitude S [22], [23], [24] and the calculi are typically defined in [22] as follows. For a response $x(t) \in \mathbb{R}$

TABLE I

DEFINITIONS OF NOTATIONS IN THE EXAMPLE MODEL

state	notation	species
x_1	Pre *c-fos* mRNA	preprocessing *c-fos* mRNA
x_2	*c-fos* mRNA	*c-fos* mRNA
x_3	c-Fos$^{(\mathrm{cyt})}$	c-Fos protein in cytoplasm
x_4	pc-Fos$^{(\mathrm{cyt})}$	activated c-Fos protein in cytoplasm
x_5	c-Fos$^{(\mathrm{nuc})}$	c-Fos protein in nucleus
x_6	pc-Fos$^{(\mathrm{nuc})}$	activated c-Fos protein in nucleus
x_7	Pre TF mRNA	preprocessing TF mRNA
x_8	TF mRNA	TF mRNA
x_9	TF$^{(\mathrm{cyt})}$	TF protein in cytoplasm
x_{10}	TF$^{(\mathrm{nuc})}$	TF protein in nucleus
u	Elk-1	Elk-1 protein

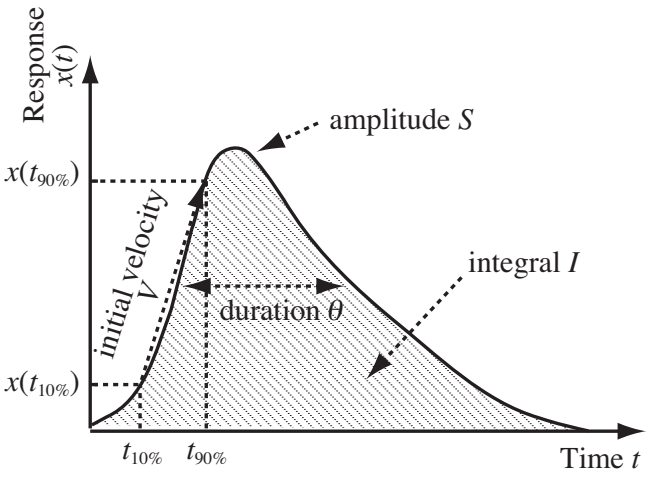

Fig. 2. Four key characteristics

and the integrals

$$I = \int_0^\infty x(t)dt, \quad T = \int_0^\infty tx(t)dt, \quad Q = \int_0^\infty t^2 x(t)dt,$$

we have

$$\tau = \frac{T}{I} \tag{9}$$

$$\theta = 2\sqrt{\frac{Q}{I} - \tau^2} \tag{10}$$

$$S = \frac{I}{\theta} \tag{11}$$

In addition, there is a result that the initial velocity of a response is regulated in a signal transduction system, and determines an amplitude of activation of a signaling molecule in down-stream pathways [8]. In this paper, we define the initial velocity by

$$V = \frac{x(t_{90\%}) - x(t_{10\%})}{t_{90\%} - t_{10\%}} \tag{12}$$

where $t_{10\%}$ and $t_{90\%}$ are times at which the response reaches the levels of 10% and 90% of the first peak of $x(t)$, respectively. Fig. 2 shows a graphical explanation of the

[1]The times are given by periods between the peak time of the input and the time at which the input returns to zero.

TABLE II

SPECIFICATION OF INPUT RANGE

characteristics	minimum	maximum	divisions
amplitude	10 [nM]	3000 [nM]	20
duration[1]	60 [s]	$3 \times 10^5 [s]$	20
initial velocity	0.01 [nM/s]	1.0 [nM/s]	20

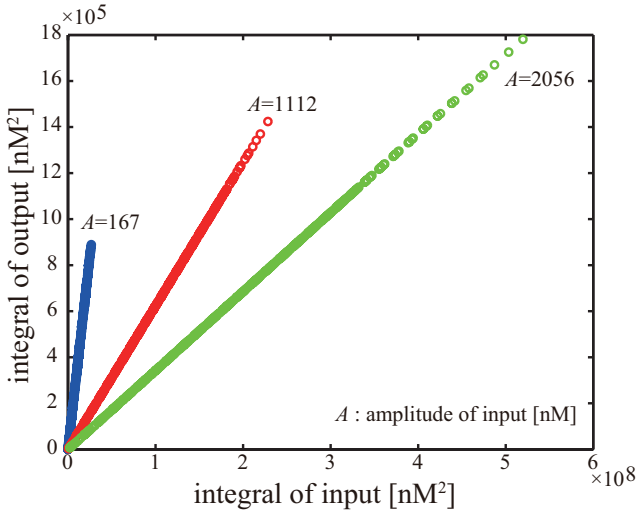

Fig. 3. Input-output relations regarding integrated responses

four characteristics. In what follows, these characteristics of the output signal x_6 are evaluated in simulations, variously changing those of the input signal u according to the fact that a rapid and a slow activations occur in a few minutes and hours with a concentration of nano molar [nM] order in a biochemical reaction in a cell. As mentioned in the previous section, since it was experimentally observed in [21] that the pattern of the input signal, which is a response of the transcription factor Elk-1, was transient with a peak, a triangular waveform with one-shot pulse is employed for the input signal. The specification of ranges of amplitude, duration, initial velocity for the input are summarized in Table II in which the values regarding duration in the table are defined by a period of time between the peak time of the input and a time at which the input returns to zero. It is noted that the net duration of the input is recalculated in analyzing the results after simulations.

C. No Negative Feedback Case

It is reasonable to evaluate the effect of negative feedback regulation by comparing with a no-feedback case in which the Eq. (8) is then replaced by

$$\dot{x}_1 = -k_1 x_1 + \frac{V_1 u^p}{K_1^p + u^p} \tag{13}$$

Table III summarizes the coefficients of correlations of amplitudes, durations, integrals and initial velocities between the input u and the output x_6 where four characteristics are calculated from response curves of u and x_6 for each of 8000 kinds of input signals (20 amplitudes $\times$ 20 durations $\times$ 20 initial velocities in Table II), indicating that there are

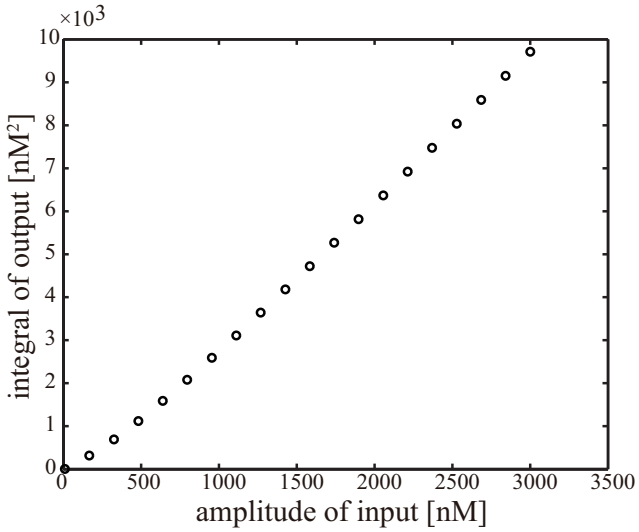

Fig. 4. Input-output relations in a weakly activated case

TABLE III

COEFFICIENTS OF CORRELATION (NO-FEEDBACK CASE)

		output			
		amplitude	duration	integral	init. velo.
input	amplitude	0.22	0.29	0.33	-0.00
	duration	0.24	**0.98**	**0.97**	-0.36
	integral	0.23	**0.79**	**0.81**	-0.28
	init. velo.	-0.12	-0.23	-0.23	0.28

high correlations between the input and the output duration or integral (indicated with a bold font). The input to output relations regarding integrated responses are depicted in Fig. 3 in which results with three amplitudes out of 20 kinds of input amplitudes are illustrated. Then, we find that the relation is almost linear and the slope depends on the input amplitude. This property is favorable since we can tune the relation by controlling the input amplitude, which has a function of a certain type of integral gain. It is reported that characteristics of a biochemical reaction network described with the Michaelis-Menten equations become closed to a linear system in a weakly activated condition since the equations can be linearized in a case with the substrate x satisfying $x \ll K$ for a Michaelis-Menten equation of $Vx/(K+x)$ [22]. In fact, if we limit the variations of input signals to induce a weakly activated condition, the input to output relation between the input amplitude and the output amplitude (or integral) show a high correlation as shown in Fig. 4 despite of a much less correlation in Table III.

Hence, it is concluded that what the system can transmit in the information flow is integral or duration, and in a weakly activated case amplitude is also transmitted.

D. Negative Feedback Case

Table IV summarizes the coefficients of correlations in the negative feedback case with Eq. 8 in a similar way to the previous calculations. Then, we find that there are high correlations between initial velocity of the input and integral, amplitude or initial velocity of the output (indicated with

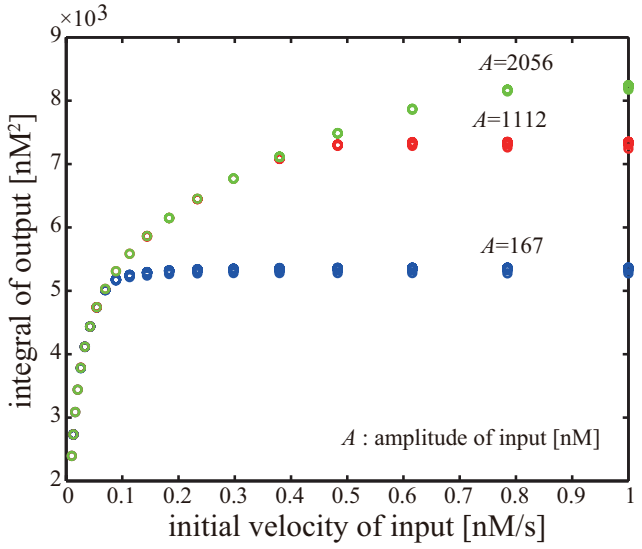

Fig. 5. Input-output relations regarding initial velocity vs integral

TABLE IV

COEFFICIENTS OF CORRELATION (NEGATIVE FEEDBACK CASE)

		output			
		amplitude	duration	integral	init. velo.
input	amplitude	0.17	0.29	0.19	0.10
	duration	-0.30	0.10	-0.29	-0.33
	integral	-0.18	0.28	-0.16	-0.24
	init. velo.	**0.75**	-0.05	**0.77**	**0.70**

a bold font), which is completely different from Table III although the high correlation between the initial velocity and the amplitude has been reported in [8]. The input to output correlations regarding the initial velocity vs the integral are given in Fig. 5 as well in which a larger initial velocity of the input induces a larger integral of the output and the relation curves are almost the same in the range from 0.01 [nM/s] to 0.1 [nM/s], and different depending on the input amplitudes. This property is also favorable since we can utilize the amplitude of the input to determine the relation curve.

Hence, it is concluded that what the system can transmit in the information flow is the initial velocity of the input. Then, to determine the input amplitude is similar to tune a derivative gain.

IV. DISCUSSIONS

A. Application to PID control in Molecular Robot

We now consider the example model as a controller. Then, from the results, the input signals with a larger amplitude, integral and initial velocity induce the output signal with a larger integral in cases of no-feedback under a weakly activated condition, no-feedback and negative feedback, respectively. These input to output properties remind us a proportional, an integral and a derivative gain of a PID controller. In a molecular robot [2], since a feedback controller is implemented in a biochemical reaction network and therefore only operations without negative values are available, we have to design one by using bio-type negative feedback control where the control objective is to drive a biochemical actuator such as a molecular motor based on a reference input. Then, the structures as shown in Fig. 6 is a possible candidate for a PID controller in a molecular robot (bio-type PID controller). It should be noted that the bio-type PID controller can induce a harmful oscillation in the system depending on the parameters as shown in the Section II.

B. Application to Signal Transmission in Molecular Robot

We next consider the example model as a signal transmission system. Then, it is desirable to chain ones in a cascade in order to transmit an information to a far system, and to achieve a reliable and robust transmission against noise. Regarding the former, it would be reasonable that the input and the output employ the same characteristics to realize a connectivity among transmissions. Then, from Tables III and IV, a signal transmission with duration or integral of the no-feedback system, or initial velocity of the negative feedback system is suitable. It might be easier to design the initial velocity of a reaction since a higher concentration of a signaling molecule results in a higher initial velocity of a reaction. For example, consider a third-order biochemical reaction.

$$\begin{aligned} \dot{x}_1(t) &= -k_p x_1(t)x_2(t) + k_m x_3(t) \\ \dot{x}_2(t) &= -k_p x_1(t)x_2(t) + k_m x_3(t) \\ \dot{x}_3(t) &= -k_m x_3(t) + k_p x_1(t)x_2(t) \end{aligned} \tag{14}$$

Then, it is obvious that a larger x_1 (or x_2) induces a larger initial velocity of the reaction for x_3. Hence, it is reasonable to utilize the bio-type negative feedback system as a signal transmission in which what the system transmits in the pathway is the information about the initial velocity of the input signal. Regarding robustness, we test a cascade system with the example model as shown in Fig. 7. Then, the simulations with and without random noise indicate that the cascade is robust against the disturbance.

Next, we consider the core system to give the system property of the example model.

$$\begin{aligned} \dot{x}_1(t) &= -k_1 x_1(t) + \frac{v_1 u(t)}{K + x_2(t)^q} \\ \dot{x}_2(t) &= -k_2 x_2(t) + v_2 x_1(t) \end{aligned} \tag{15}$$

where k_1, k_2, v_1, v_2, K and q are positive constants. In a case with $x_2 \ll K$, x_1-system is given by

$$\dot{x}_1 = -k_1 x_1 + \frac{v_1 u}{K} \tag{16}$$

which implies that x_1 aims to the steady-state value with a time constant, and the initial velocity of x_1 increases depending on an increasing of the initial velocity of the input u. If $x_2 \gg K$ and x_2 is enough large to fully attenuate the effect of u, we have

$$\dot{x}_1 = -k_1 x_1 \tag{17}$$

which implies that x_1 is exponentially decreasing from a initial value. From the two cases, there is a trend that x_1

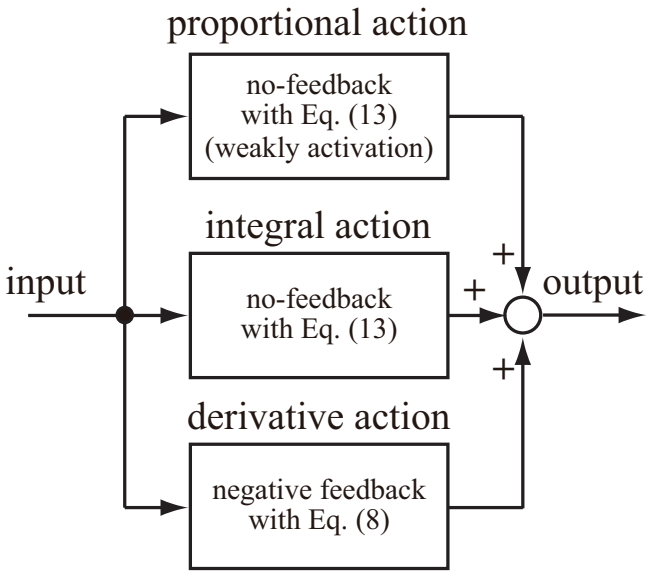

Fig. 6. Bio-type PID controller

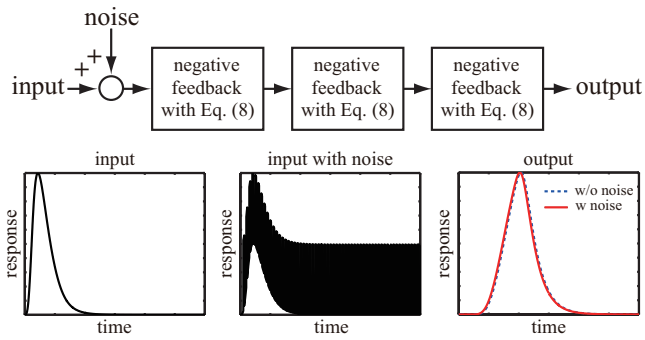

Fig. 7. Simulation on signal transmission

can response with an input u with less effect of x_2. Then, the function of the negative feedback seems to be a selector between the two structures (16) and (17) and the effect is enhanced with a larger Hill coefficient q.

V. Conclusions

In this paper, we consider functions of bio-type negative feedback system by using the example model. First, we discuss about the difference in the mechanics of negative feedback between an engineering system and a biochemical reaction system. Next, in a case study, we investigate the example model with bio-type negative feedback loop. We show that the model is a possible solution to realize a bio-type PID controller and a signal transmission system in molecular robot.

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