High-frequency Noise Attenuation of a Two-component System Responding to Short-pulse Input

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ABSTRACT

Among the various biological devices developed and characterized in synthetic biology, light-sensing biological devices can serve as an input–output system owing to their light modulation property. The well-characterized devices in living systems are useful for modulating cellular sensing and transducing information. In this study, we examined short pulse responsiveness of a light-sensing two-component system (TCS), Cph8-OmpR, which was generated by replacing the sensor domain of the EnvZ-OmpR osmoregulatory system with the light sensor Cph1. We varied the input pulse width of the Cph8-OmpR system and found that an input width of <1 s was sufficient to alter the accumulation of a reporter gene upregulated by Cph8 phosphorylation of OmpR. Based on this result and the mathematical model showing that the timescale for the upstream Cph8-activity transition was much faster than that of downstream gene expression, we evaluated the merit of a TCS with such an unbalanced cascade. Our mathematical simulation of a cascade TCS suggests that high-frequency noise arising from fast transitions in kinase activity was attenuated throughout the cascade reaction. In terms of noise attenuation, these results can contribute to analyze biological cascade systems with the balance of reaction rates in each process.

CCS Concepts

• **Networks~Network dynamics**

Keywords

Cascade system; high frequency noise attenuation; light-sensing biological device; short pulse responsiveness; two-component system

1. INTRODUCTION

Characterizing the dynamic properties of biological devices developed for specific purposes in synthetic biology is essential

for their successful incorporation into organisms for advanced applications [4,7,22,31]. Incorporating the characterized device has enabled the control of organism functions such as polarization of budding yeast [18] and somite formation in embryos [15]. Such characterization also allows the system state—e.g., target protein concentration—to be modulated according to control theory [11,25,26,27,29,40,42].

Typical biological devices such as a two-component system (TCS) include multiple components with individual reaction rates in a cascade system, which can show high- or low-pass filter properties depending on the balance of timescales among cascade reaction rates of the components [23]. TCSs sense environmental signals via a histidine kinase and transduce information by phosphorylating or dephosphorylating intracellular components (response regulators) [17,37]. In addition to constituting the core system for natural bacterial survival, TCSs are useful as input–output systems in synthetic biology [28,43].

Light-sensing biological devices are useful for modulating cellular sensing and transducing information through a cascade system [33]. Optical input is superior to other types of stimulus owing to its accurate and instantaneous transmission to a target. Another strong point of such input is the absence of residual signal after the input ceases, unlike that for chemical substrates. In addition, optical input can be varied in terms of light intensity or input frequency [27,29,40]. Light-sensing biological devices from a light-sensing organism can be transplanted into others that do not possess the device. Furthermore, chimeric systems consisting of the light sensor and endogenous components have been developed [20,33].

The Cph8-OmpR light-sensing chimeric system was developed by replacing the sensor protein of the EnvZ-OmpR osmoregulatory system, which is one of the best-characterized TCSs (Figure 1) [21]. EnvZ is an osmosensory transmembrane protein that detects environmental signals and transitions between two states. The kinase domain of EnvZ in one state phosphorylates and that in the other state dephosphorylates OmpR. Phosphorylated OmpR regulates the expression of genes encoding outer membrane pore proteins for osmotic adaptation. Cph8, which is also phosphorylates and dephosphorylates OmpR, is a chimeric protein consisting of the *Escherichia coli* EnvZ kinase domain fused to the cyanobacterial red light-sensing Cph1, which functions as a sensor domain. Cph1 exists as two states, P_r and P_{fr} , in the dark and upon red light exposure, respectively [45]. The EnvZ kinase domain of P_r Cph8 phosphorylates the natural target OmpR, which then induces the expression of genes under the control of the *ompC* promoter. Although Cph8 activity is usually modulated by altering the intensity of red light [29], we varied irradiation time to determine the on/off transition rates of histidine kinase

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activity. The cyanobacterial TCS was previously studied in *E. coli* while repeatedly switching the input state at ≥ 1 -min intervals. However, the contributions of transition rates of the histidine kinase and response regulator were not elucidated because these intervals did not provide sufficient time resolution for transition-rate examination [11].

In this study, we examined the dynamic properties of the Cph8-OmpR cascade system as one of the basic biological cascade systems. Cph8 is an upstream component of the cascade system, and is here shown to have a significantly higher activity-transition rate than the protein-accumulation rate resulting from downstream gene expression. This means that a decrease in transition rate would have little effect on the time for protein accumulation until it falls below the protein-accumulation rate. A slow downstream reaction—protein accumulation in this case—must integrate and transduce upstream information within a certain time [14,23]. We therefore extended our model to evaluate perturbations in protein expression caused by fluctuations in kinase activity due to its transition. We found that high transition rates of enzymatic activity cause less noise than low transition rates. Given the noise frequency dependence on enzymatic-state transition rates and the low-pass filter characteristics of a cascade system, we propose that biological cascade systems at an upstream step favor high transition rates. For analyzing biological cascade systems, high upstream transition rates can effect noise attenuation.

Figure 1. Schematic representation of the Cph8-OmpR system.

2. MATERIALS AND METHODS

2.1 Strain and Plasmids

E. coli strain JW3367 [2] (obtained from National BioResource Project), which lacks EnvZ histidine kinase in a TCS, was used for all experiments to allow integration of red-light sensing Cph8 into the system.

Cph8, a chimera of Cph1 and EnvZ, was synthesized via the Cph1-envZ-pPROTet plasmid [21] (obtained from Christopher A. Voigt). The chromophore phycocyanobilin (PCB) biosynthetic pathway, which is required to absorb light energy, was introduced into the cell via a pPCB-gfp plasmid with a green fluorescent protein (GFP) reporter gene as the output of the Cph8-OmpR TCS. Cells were grown in basal medium (Luria-Bertani [LB] medium with 50 μ g/mL ampicillin, 30 μ g/mL kanamycin, and 25 μ g/mL chloramphenicol) at 37 $^{\circ}$ C with shaking at 100 rpm.

The pPCB-gfp plasmid is based on pPCB-AmpR [21] (obtained from Christopher A. Voigt). We inserted a fluorescent reporter positively regulated by phosphorylated OmpR. A GFP reporter (Biobrick parts: BBa_E0240) was inserted in the ompC promoter plasmid (Biobrick parts: BBa_R0082) using XbaI-PstI and SpeI-PstI restriction enzymes. The promoters and the *gfp* gene were inserted in pPCB-AmpR using an In-Fusion HD Cloning Kit

(Clontech) with forward primer (AATTCGCGGCCGCTTC), reverse primer (GACTGCAGCGGCCG), and AatII restriction enzyme.

2.2 Pulse Width Variation Assay

An overnight culture of JW3367 containing Cph1-envZ-pPROTet plasmid and pPCB-gfp plasmid was diluted 1000-fold to basal medium in 50-mL centrifuge tubes. Incubation of the cells at 37 $^{\circ}$ C with shaking at 100 rpm for 300 min under each light condition (irradiation time sets 0 s, 0.01 s, 0.05 s, 0.1 s, 0.5 s, 1 s, and 60 s in 1-min cycles) to an optical density at 590 nm (OD590) of ~ 1.0 . Next, 1.45 mL cultured cells was added to 0.55 mL basal medium, and the cells were grown for 30 min under the same light conditions as before to an OD590 of ~ 0.5 . Finally, 1 mL cultured cells was added to 1 mL basal medium, and the cells were grown under the same light condition for 30 min. The GFP fluorescence of the cells was measured using flow cytometry.

2.3 Pulse Frequency Variation Assay

The overnight culture and 300 min culture steps described for pulse width variation assay were followed. After 300 min culturing under photoirradiation or dark conditions, 4 mL cultured cells was added to 6 mL basal medium, and the cells were grown for 30 min under the same condition as before. This dilution step was repeated four times at 30-min intervals for cell density adjustment. After pre-processing, 1 mL cultured cells was added to 1 mL basal medium, and the cells were grown under each light frequency condition for 30 min. For an additional 30 min culture, 1 mL cultured cells was added to 1 mL basal medium. For the 60-min culture, 0.55 mL cultured cells was added to 1.45 mL basal medium. The OD590 of cultured cells was ~ 0.5 before dilution. We measured the GFP fluorescence of the cells using flow cytometry after each dilution.

2.4 Dynamical Control Assay

The steps described for pulse frequency variation were repeated through the four rounds of 30-min cultures for cell density adjustment. After culturing, 1 mL cultured cells was added to 1 mL basal medium, and the cells were grown under the light-controlled condition for 30 min. This dilution and cultivation were repeated. After each cultivation, we measured the GFP fluorescence of the cells, entered the result in the closed-loop control simulation, and modified the input sequence according to the output.

2.5 Fluorescence Measurement

All fluorescence data were collected using a Becton-Dickinson FACSCalibur flow cytometer with a 488 nm laser and a 515–545 nm emission filter [35]. The fluorescence measurements were normalized with respect to forward scatter and median value as reported by Miliias-Argeitis *et al.* [27]. Fluorescence measurements of 100,000 cells were averaged.

2.6 Mathematical Model of the Cph8-OmpR System

$$\frac{d[P_r]}{dt} = -v_1 \cdot [P_r] \cdot u + v_2 \cdot (P_{tot} - [P_r]) \quad (1)$$

$$\frac{d[R_p]}{dt} = v_{p1} \cdot [P_r] \cdot (R_{tot} - [R_p]) - v_{p2} \cdot (P_{tot} - [P_r]) \cdot [R_p] - \gamma \cdot [R_p] \quad (2)$$

$$\frac{d[mRNA]}{dt} = \frac{\alpha_m \cdot [R_p]^n}{[R_p]^n + K_d^n} - \gamma_m \cdot [mRNA] + leak \quad (3)$$

$$\frac{d[GFP]}{dt} = \alpha_G \cdot [mRNA] - \gamma \cdot [GFP] \quad (4)$$

To identify the Cph8 transition rate, we considered four variables: concentration of Cph8 in the P_r state, phosphorylated OmpR (R_p), mRNA, and protein (GFP). We have developed ordinary differential equations consist of the four variables. Equation (1) indicating Cph8-state transition and equation (2) indicating OmpR phosphorylation were newly developed based on the Cph8-OmpR system network (Figure 1). Equation (3) and (4) indicating mRNA and protein synthesis–degradation were developed based on Hill equation which is often used for gene expression [13]. By dividing the number of Cph8 molecules by the cell volume, we determined the concentration of Cph8. P_{tot} and R_{tot} represent the constant total concentrations of Cph8 and OmpR, respectively. Cph8 undergoes a state transition with rates v_1 and v_2 with light input u (0 or 1, where 0 indicates no light input). OmpR is phosphorylated with rate v_{p1} and dephosphorylated with rate v_{p2} . We assumed that the concentration of phosphorylated OmpR decreases with rate γ upon cell growth (increased volume). R_p activates synthesis of GFP mRNA, which is the only mRNA included in our model among all the included proteins, with maximum rate α_m . The mRNA is also synthesized without R_p by leaky transcription (*leak*). The mRNA degradation rate γ_m is significantly faster than γ , and the dilution effect on mRNA is therefore not considered. GFP is synthesized from mRNA with rate α_G .

2.7 Stochastic Differential Equation to Examine Noise Attenuation

$$\frac{dN_{on}}{dt} = -v_{ina} \cdot N_{on} + v_{act} \cdot (N_{tot} - N_{on}) + \sqrt{v_{ina} \cdot N_{on} + v_{act} \cdot (N_{tot} - N_{on})} \cdot W(t) \quad (5)$$

$$\frac{d[R_p]}{dt} = v_{p1} \cdot C \cdot N_{on} \cdot (R_{tot} - [R_p]) - v_{p2} \cdot C \cdot (N_{tot} - N_{on}) \cdot [R_p] - \gamma \cdot [R_p] \quad (6)$$

$$\frac{d[mRNA]}{dt} = \frac{\alpha_m \cdot [R_p]^n}{[R_p]^n + K_d^n} - \gamma_m \cdot [mRNA] \quad (7)$$

$$\frac{d[protein]}{dt} = \alpha_G \cdot [mRNA] - \gamma \cdot [protein] \quad (8)$$

Here we consider a histidine kinase such as EnvZ, which transitions between the phosphorylating and dephosphorylating states with transition rates v_{act} and v_{ina} . N_{on} represents the number of histidine kinases in the phosphorylating state. N_{tot} is the total number of histidine kinase molecules and is assumed to be 100 [39]. C is a factor for conversion between the number and concentration of histidine kinases; we approximated one molecule in a cell as 1 nM [1]. Other parameters are the same as those of the Cph8-OmpR model. $W(t)$ indicates a Wiener process.

2.8 Genetic Algorithm for Parameter Identification

To identify parameters in equations (1)–(4), we used a genetic algorithm with the simplex crossover method (SPX) [41] and the minimal generation gap (MGG) generation alternation model [32]. Genetic algorithm parameters are as follows: population size, 30; generation alternation counts, 3000; evaluation value, squared error between the number calculated with a parameter set and the experimental data shown in Figure 3. The variable regions for parameters are defined in Table 1. Igoshin *et al.* previously compiled the reaction rates of EnvZ autophosphorylation, EnvZ-OmpR binding, and other steps [6]. The parameters of our model integrate some reactions and reflect the values reported by Igoshin *et al* [6].

3. RESULTS AND DISCUSSION

3.1 Different Timescales for Cph8 Response and Accumulation of Downstream Gene Product

To compare the response timescale for the upstream Cph8 in the TCS with that for accumulation of a downstream gene product, we examined GFP reporter expression downstream of Cph8-OmpR using pulse stimuli with various widths and an interval of 60 s, which is shorter than the timescale of product accumulation (Figure 2). The interval needs to be shorter than the timescale of product accumulation to identify Cph8 transition rates. On the other hand, complete dark transition of Cph1, one of the two domains of Cph8, takes minutes [16]. The interval therefore needs a certain time which we set 60 s. A pulse width of 60 s (continuous light input) stops reporter GFP expression and decreases cellular fluorescence to one-eighth of that observed for

Table 1. Parameter range of genetic algorithm

Parameter	Lower limit	Upper limit	Unit	Description
v_1	0.1	10	s ⁻¹	Transition rate from P _r to P _{fr}
v_2	0.00001	0.1	s ⁻¹	Transition rate from P _{fr} to P _r
P_{tot}	0.005	1	μM	Cph8 concentration The number of Cph8 is divided by cell volume 1 μm ³
v_{p1}	0.0001	1	s ⁻¹ μM ⁻¹	OmpR phosphorylation rate by Cph8
v_{p2}	0.0001	1	s ⁻¹ μM ⁻¹	OmpR dephosphorylation rate by Cph8
γ	0.0002	0.001	s ⁻¹	Dilution rate upon growth
R_{tot}	0.5	5	μM	OmpR concentration
α_m	0.001	0.1	s ⁻¹	mRNA synthesis rate
n	1	10		Hill coefficient
K_d	0.01	2	μM ⁻¹	Dissociation constant
γ_m	0.001	0.05	s ⁻¹	mRNA degradation rate
α_G	0.00001	0.05	s ⁻¹	Protein synthesis rate
<i>leak</i>	0.0002	0.05	s ⁻¹	Leak of mRNA synthesis

a 0-s pulse width (no input). We further observed a trend in which increasing pulse width caused a corresponding decrease in fluorescence. In this trend, the fluorescence intensity sharply changed for pulse widths between 0.1 s and 0.5 s. The results imply that membrane-bound Cph8 responds to light input and decreases its kinase domain activity in less than 1 s. Because the light-induced conformational change of Cph1 occurs in milliseconds [10], we presume that the activity transition of the EnvZ kinase domain, the other domain of Cph8, occurs in the order of milliseconds to 1 s, which is much less than the time required for the accumulation of gene products for either detection of the GFP reporter or response to changes in osmotic pressure [8,9].

Figure 2. Input pulse responsiveness with changing pulse width. The input pulse is applied to *E. coli* with 1-min intervals. The pulse width (X) is indicated for each measurement. Light intensity is maximal light-emitting diode intensity (approximately 190 W/m^2), and was sufficient for Cph8 switching [38].

3.2 Modeling of the Cph8-OmpR System and Identification of its Parameters

To estimate the transition timescale of Cph8 in the Cph8-OmpR system, we modeled the following reaction steps of the system: Cph8 activity transition, phosphorylation and dephosphorylation of OmpR, and reporter gene product accumulation (Figure 3a), (1)–(4) (Equations in Materials and Methods). Because Cph8 was continuously synthesized, degraded, and diluted by cell growth, the total level of P_r and P_{fr} Cph8 was represented as a constant. Although the total concentration of phosphorylated and non-phosphorylated OmpR was also assumed to be constant [39], each form of OmpR was assigned its own variable since newly produced OmpR protein is non-phosphorylated. The concentrations of the mRNA and protein reporter gene products were also described by variables.

Key parameters for reactions in the Cph8-OmpR system, including one for the state transition of Cph8 kinase, were identified from time-course results of GFP reporter accumulation in cell cultures with varied frequency of light inputs (Figure 3b). We also examined two initial conditions: with and without light input. Reporter gene accumulation was monitored from culture with various input pulse frequencies (60–5 s) and a 0.1-s pulse width. A 0.1-s pulse width in a 1-min period had high sensitivity and efficiency for embodying the Cph8-OmpR system property (Figure 2). The GFP fluorescence of a culture with one pulse per 60 s was slightly lower than that without light input. One pulse per 5 s decreased GFP production close to the level obtained with continuous light input. To fit the experimental data and mathematical model parameters, we used a genetic algorithm.

Figure 3. Model of the Cph8-OmpR system and parameter identification. (a) Schematic model of the Cph8-OmpR system. P_r and P_{fr} are Cph8 states determined by light input u (0 or 1, where 0 indicates no light input) with transition rates v_1 and v_2 . OmpR (R) is synthesized at a constant rate and can be phosphorylated by P_r Cph8. Phosphorylated OmpR (R_p) is dephosphorylated by P_{fr} Cph8. R and R_p are diluted with rate γ upon cell growth. R_p activates synthesis of GFP mRNA, which is the only mRNA included in our model among all the included proteins, with maximum rate α_m . The mRNA is also synthesized without R_p by leaky transcription (*leak*). The mRNA degradation rate γ_m is significantly faster than γ [1], and the dilution effect on mRNA is therefore not considered. GFP is synthesized from mRNA with rate α_G . (b) Input pulse responsiveness with changing pulse frequency. Six samples were treated with different pulse cycles: 60 s, 30 s, 10 s, 5 s, constitutive input, and no input. Markers represent experimental data, and lines indicate simulation data from the mathematical model with optimum parameter values. Cell culture was pre-incubated with

(center) and without light (right).

Among the identified parameters shown in Table 2, the OmpR concentration is consistent with the result of single-molecule measurements [39]. The protein half-life was about 38 min, which was close to the *E. coli* doubling time of about 30 min in our study (as described in Materials and Methods, we diluted *E. coli* by half every 30 min to maintain cell density within the desired range). The GFP reporter proteins lacked degradation tags, and therefore the dilution of GFP concentration was determined by the offset of *E. coli* growth and GFP synthesis. The estimated mRNA half-life of 30 s was also close to the 1–3 min reported from analysis of living *E. coli* [5]. Although the parameters may correspond to a local (not global) solution, most parameters agree with experimental observations. The estimated transition rate of Cph8 from P_r to P_{fr} was about 0.5 s^{-1} .

Table 2. Optimum parameter values

Parameter	Value	Description
v_1	$0.499883 \text{ [s}^{-1}\text{]}$	Transition rate from P_r to P_{fr}
v_2	$0.000905 \text{ [s}^{-1}\text{]}$	Transition rate from P_{fr} to P_r
P_{tot}	$0.125480 \text{ [}\mu\text{M}\text{]}$	Cph8 concentration The number of Cph8 is divided by cell volume $1 \mu\text{m}^3$
v_{p1}	$0.134366 \text{ [s}^{-1}\mu\text{M}^{-1}\text{]}$	OmpR phosphorylation rate by Cph8
v_{p2}	$0.036881 \text{ [s}^{-1}\mu\text{M}^{-1}\text{]}$	OmpR dephosphorylation rate by Cph8
γ	$0.000305 \text{ [s}^{-1}\text{]}$	Dilution rate upon growth
R_{tot}	$0.082036 \text{ [}\mu\text{M}\text{]}$	OmpR concentration
α_m	$0.015811 \text{ [s}^{-1}\text{]}$	mRNA synthesis rate
n	2.541060	Hill coefficient
K_d	$0.05827 \text{ [}\mu\text{M}^{-1}\text{]}$	Dissociation constant
γ_m	$0.030053 \text{ [s}^{-1}\text{]}$	mRNA degradation rate
α_G	$0.004921 \text{ [s}^{-1}\text{]}$	Protein synthesis rate
$Leak$	$0.000764 \text{ [s}^{-1}\text{]}$	Leak of mRNA synthesis

3.3 Validation of the Mathematical Model through Dynamical Control

To test our model and estimate parameters, we employed dynamical control of the output protein amount to the desired value, calculating the optimal input frequency with the mathematical model and the current amount of output protein. We first applied open-loop control, which uses a set input sequence determined before the culture experiment. During cell culture after pre-incubation with light, the measured reporter values (Figure 4a, black diamonds) were only slightly different from the desired values (Figure 4a, dashed line), especially in the second half of the time course. We further applied closed-loop control, which uses the measured reporter value at one time point to calculate the next input frequency using the mathematical model (Figure 4a, white diamonds and solid line). The average error between the desired values and the measured reporter values of the closed-loop control after 120 min was 4%. This quantitative agreement indicates that the estimate parameters are appropriate. The result of pre-incubation without light was almost the same as that with light (Figure 4b). The results of dynamical control indicate that the mathematical model is acceptable.

3.4 Output Noise Attenuation in Fast Protein Activity Transition

According to the production process design method based on the theory of constraints [30,34], balanced cascade systems, which consist of similar reaction timescales without significant pauses (buffer time) between processes, at equilibrium show higher accumulation rates for the final product of the cascade but are also more susceptible to fluctuation than unbalanced cascade systems. Our measurements of the different timescales of upstream Cph8-activity transition and accumulation of downstream gene product evoke the trade-off described in the theory. A cascade system with balanced reaction timescales transfers information with less wasteful resource consumption than a system with imbalanced timescales [44]. In turn, a system with a fast upstream response and slow downstream gene expression is resistant to reaction fluctuations at short timescales. From another perspective, signal transductions in biological systems have a low-pass filter property [14,23]. Considering this trade-off between efficiency of output

Figure 4. Output control for the desired value (dashed lines) with open-loop and closed-loop controls for cells pre-incubated (a) with light or (b) without light. Measured values with the open-loop control (black diamonds) are controlled based on the model-predicted values (dotted lines) close to the desired value. Light input is shown as pulses in the subjacent boxes. Measured values of GFP fluorescence strength with the closed-loop control (white diamonds) are controlled based on the model-predicted values (solid lines). The measured values with the closed-loop control are reflected in the model-predicted values with some time delay.

Figure 5. The influence of the noise in histidine kinase activation through phosphorylation, transcription, and translation in the TCS. Transition rates of histidine kinase activation (dephosphorylating to phosphorylating state, v_{act}) and inactivation (v_{ina}) are set to relatively high values ($v_{act} = 0.15, v_{ina} = 0.5$; gray) and low values ($v_{act} = 0.015, v_{ina} = 0.05$; black). Top left: time series of the number of histidine kinase molecules in the phosphorylating state. Top right: time series of phosphorylated response regulator concentration. Lower left: time series of mRNA concentration. Lower right: time series of protein concentration.

Figure 6. Distributions of noise frequency in histidine kinase.

The data between 0 min and 55 min in Figure 5 (top left) are transformed into frequency space using a Fourier transform. Lines represent the moving average lines of twenty data points. Line colors correspond to those in Figure 5, where transition rates in black are slower than those in gray.

Production and noise resistance, we hypothesized that the TCS with a high kinase-activity transition rate has the ability to reduce noise in sensing of environmental conditions.

To confirm that the histidine kinase of a TCS exhibits robustness, which is an important property for an engineering tool, we extended our model to examine the noise-resistant advantage of a cascade system unbalanced by a fast transition rate for histidine kinase activity. To test noise attenuation through signal transduction and gene expression, we modeled a TCS with noise generation through kinase activity transitions (5)–(8) (Equations in Materials and Methods). The extended model has the same variables and parameters as the Cph8-OmpR model except the transition rates of histidine kinase. Here we focused on the state transition itself as a source of noise with saturating input [24]. Note that the ratio of transition rates v_{act} to v_{ina} (from inactive to

active kinase state and vice versa) determines the active fraction of histidine kinase. In our extended model, the rates are set to give a 50% ratio of phosphorylated to non-phosphorylated response regulator, which is the same as that of OmpR in the BW25114 strain expressing wild-type *ompR* and *envZ* genes [3]. This ratio may differ depending on the TCS and the strain, but it is independent of the trend of noise attenuation through the cascade process. Furthermore, the noise-resistant advantage deduced from our finding prompted us to evaluate changes in v_{act} and v_{ina} values with a constant ratio. Using the stochastic differential equation with most of the parameters listed in Table 2, we confirmed that faster transitions in kinase activity (Figure 5, gray line) yield less fluctuation in target protein concentration than slower transitions (Figure 5, black line). The noise in histidine kinase induced by a slow transition rate is distributed in the lower frequency band (Figure 6, black line). Combining the noise from enzymatic state transitions and the low-pass filter characteristics of cellular signal transduction, we propose that biological systems favor higher transition rates for upstream processes to reduce output noise.

Although any transition of a component in a biological system inevitably generates noise with a frequency equal to that of the transition [24], this noise can be cancelled when such a component is in an upstream position in the a cascade and has a high-frequency transition. Cascade systems have a low-pass filter property through signal transduction [23,36] and can reduce the high-frequency noise generated in the upstream process. These mechanisms of high-frequency noise generation and attenuation suggest the importance of gradually slowing reaction timescales along the cellular signal transduction pathway. In synthetic biology, engineering-based methods for designing and constructing gene-network systems combining well-characterized biological devices have been appreciated [12,19]. For example, in electronic circuit design, noise attenuation is one of the most important problems and one of criteria for choosing electronic

parts. In designing gene-network systems, noise, which arises from the reaction rates of the devices, could also become one of the criteria for choosing devices.

4. CONCLUSION

We examined a TCS regulating gene expression that responds to pulse stimuli shorter than 1 s. By estimating each reaction rate from a mathematical model, we found that the upstream transition rate is far faster than the downstream reaction rate. We propose that allocation of the upstream-reaction noise at high frequency is advantageous for noise attenuation, which is in agreement with the principles of production process design. Although the noise caused in the fast upstream transition cannot be repressed completely, it can reach high frequencies where it is attenuated through cellular reaction processes. This strategy for noise attenuation can be applicable to the design method of biological cascade systems.

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