Stochastic signaling in biochemical cascades and genetic systems in genetically engineered living cells

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Living cells, either prokaryote or eukaryote, can be integrated within whole-cell biochips (WCBCs) for various applications. We investigate WCBCs where information is extracted from the cells via a cascade of biochemical reactions that involve gene expression. The overall biological signal is weak due to small sample volume, low intrinsic cell response, and extrinsic signal loss mechanisms. The low signal-to-noise ratio problem is aggravated during initial detection stages and limits the minimum detectable signal or, alternatively, the minimum detection time. Taking into account the stochastic nature of biochemical process, we find that the signal is accompanied by relatively large noise disturbances. In this work, we use genetically engineered microbe sensors as a model to study the biochips output signal stochastic behavior. In our model, the microbes are designed to express detectable reporter proteins under external induction. We present analytical approximated expressions and numerical simulations evaluating the fluctuations of the synthesized reporter proteins population based on a set of equations modeling a cascade of biochemical and genetic reactions. We assume that the reporter proteins decay more slowly than messenger RNA molecules. We calculate the relation between the noise of the input signal (extrinsic noise) and biochemical reaction statistics (intrinsic noise). We discuss in further details two cases: (1) a cascade with large decay rates of all biochemical reactions compared to the protein decay rate. We show that in this case, the noise amplitude has a positive linear correlation with the number of stages in the cascade. (2) A cascade which includes a stable enzymatic-binding reaction with slow decay rate. We show that in this case, the noise strongly depends on the protein decay rate. Finally, a general observation is presented stating that the noise in whole-cell biochip sensors is determined mainly by the first reactions in the genetic system with weak dependence on the number of stages in the cascade.

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I. INTRODUCTION

Cascades of biochemical and genetic reactions involved in living cells are currently generating a major interest in biological systems [1–3] and biomedical devices [4–6]. Recently, several groups have reported on the use of genetically engineered living cells as the main sensing element in whole-cell biochips which converts cellular response to electrical or optical signal [7–9]. The generation process of the reporter proteins in living cells consists of a cascade of large number of biochemical and genetic reactions. For example, in environmental biosensing, processes such as DNA repair and SOS response take place [4]. The generation process is stochastic and the noise is pronounced at the initial detection stage due to low concentrations of genetically engineered living cells, slow response, extrinsic noise due to the detection system, and variations within the cell population. In this work, we present a stochastic model for the synthesized reporter proteins population in biochemical cascades and genetic system. We assume that the protein number determines the intrinsic signal and noise and the mean and deviation of the signal.

Recently, several groups have derived analytical expressions for the fluctuations of the signals in the gene expression and biochemical network [10–18]. References [11,12] present both experiment and theory describing the origin of intrinsic noise in gene expression and gene regulatory networks. They define the "noise strength" by the ratio between the signal variance and its average and show that in steady state, the noise strength of the number of proteins is greater than what could be calculated using the Poisson statistics. The noise strength is dominantly determined by the ratio between the translation rate and the decay rate of the messenger RNA (mRNA). Reference [13] analyzed the noise of an ultrasensitive signaling cascade and showed that when the ratio between the differential amplification factor and the decay rate is smaller than 1, the magnitude of fluctuations can be bounded. References [14–16] presented the relation between the extrinsic noise and the intrinsic noise in biochemical reactions. Reference [17] analyzed the noise of autoregulated gene circuits in the frequency domain and presented a simple expression for the variance of the protein population by assuming that the mRNA typically decays much faster than the protein. Using the same assumption, Ref. [18] presented an approximation that allows the estimation of distribution proteins number.

The problem that is addressed in this paper is how the fluctuations in gene expression are influenced by a series of biochemical reactions (cascade) such as in genetically engineered living cells. We derive a simple relation [Eq. (6)] between the noise strength of the number of synthesized reporter proteins and the power spectral density function of the number of mRNA molecules. This relation is referred to as the spectral intensity theorem of gene expression. The rela-

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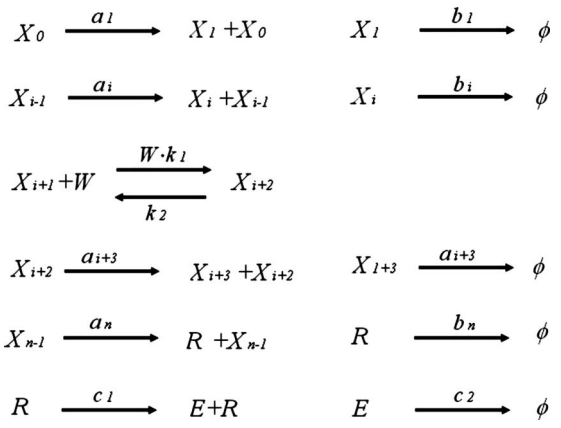


FIG. 1. Schematic model of a cascade of biochemical and genetic reactions.

tion is based on the Wiener-Khintchine theorem and on the fact that most of the synthesized proteins are rather stable and have half times much larger than that of the mRNA [17,18] and other chemical species. The model is studied in three different biological systems, including a cascade of biochemical reactions, gene expression, and enzymatic-binding reactions.

II. GENERAL MODEL

In this section, we model the protein expression in engineered living cells which contain engineered plasmids with promoter-reporter gene conjugation. In such systems, there are promoter genes that activate bioreporter expression in the presence of some chemical analytes. The gene promoter is transcribed into a mRNA. The information is further translated into a reporter protein which can be detected by either electrochemical, electrical, or optical methods [4]. Figure 1 shows a schematic model of a cascade of biochemical and genetic reactions in genetically engineered living cells. In our model, we consider a system with one input (X_0) and one output signal (E). X_0 is the number of the analyte molecules and E is the resulted number of the reporter proteins. The system includes a cascade of n coupled linear biochemical reactions where X_i is the number of molecules in the output of reaction i. The cascade also contains enzyme (W)-substrate binding reaction which can be described by the Michaelis-Menten kinetics. The approximated model is based on the following assumptions:

(i) The generation rate of each molecule X_i depends only on the number of the molecules of the pervious reaction X_{i-1} and in which X_i itself degrades by a first-order decay equation

$$\frac{d\overline{X_i}}{dt} = a_i\overline{X_{i-1}} - b_i\overline{X_i}, \tag{1}$$

where a_i and b_i are the differential amplification and the decay rate (both in units of [1/min]) of the ith reaction, respectively. Here, $\overline{X_i}$ is the mean value of the random variable X_i.

(ii) We assume a stable system in steady state where all coefficients are time independent.

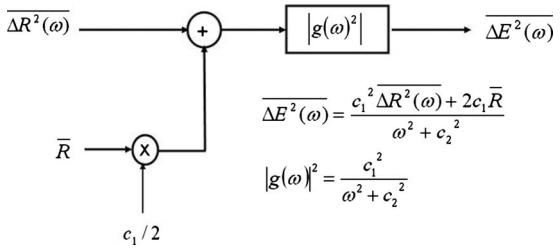


FIG. 2. Schematic model of the power spectral density function of the translation process.

(iii) The signal fluctuations are much smaller than the signal average; therefore, we can linearize the differential stochastic equations.

(iv) The noise in the input of every reaction is uncorrelated with that in the processing reaction (the intrinsic noise reaction).

(v) The intrinsic noise of each reaction is constituted with steady-state Poisson statistics.

(vi) The enzyme W constitutes a reservoir, i.e., we neglect its time variation since we assume it is rather small during a typical relevant observation period.

In this work, we use the Langevin technique [19] to model the random fluctuations. The Langevin equation can be systematically derived from the master equation [20] when random variables (signals) are treated as continues variables and the stochastic effects enter by adding a time-dependent term noise $\eta_j(t)$ to the deterministic dynamical equations. The term $\eta_j(t)$ describes the intrinsic noise of the reaction j in the cascade and is modeled as a Gaussian white-noise source where the fluctuations are uncorrelated in time. We assume the following characteristics for its statistics: $\langle \eta_j(t) \rangle = 0$ and $\langle \eta_j(t)\eta_j(t+\tau) \rangle = \langle \eta_j^2 \rangle \delta(\tau)$, with $j = i, E$ and $\delta(\tau)$ is the Dirac δ function.

The output of the genetic system in our model is simply determined by the translation process. The chemical Langevin equation of the translation process around the steady state is described by [11,12]

$$\frac{d\Delta E}{dt} = c_1\Delta R - c_2\Delta E + \eta_e(t), \tag{2}$$

where c_1 is the translation rate (1/min) and c_2 is the decay rate of the reporter protein (1/min). Here, $\Delta E = E - \bar{E}$ is the deviation of the number of reporter proteins (E) from its mean $\bar{E}$ and $\Delta R = R - \bar{R}$ is the deviation of the number of mRNA molecules (R) from its mean $\bar{R}$. In the steady state, we obtain $\bar{E} = c_1\bar{R}/c_2$. Fourier transformation of Eq. (2) yields the power spectral density function of the number of reporter proteins which is presented by the schematic model that is shown in Fig. 2 and expressed by

$$\overline{\Delta E^2(\omega)} = \frac{c_1^2\overline{\Delta R^2(\omega)} + \overline{|\eta_e(\omega)|^2}}{\omega^2 + c_2^2}. \tag{3}$$

According to the Wiener-Khintchine theorem [21], the fluctuations value of the number of reporter proteins at steady state is given by the inverse Fourier transform of the power

spectral density function at $\tau=0$ [integrating Eq. (3)],

$$\overline{\Delta E^2} = \int_{-\infty}^{\infty} \frac{c_1^2 \overline{\Delta R^2(\omega)}}{\omega^2 + c_2^2} \frac{d\omega}{2\pi} + \frac{\overline{\eta_e^2}}{2c_2}. \tag{4}$$

We now assume that the intrinsic noise [the second term of Eq. (4)] is constituted with the steady-state Poisson statistics ($\overline{\Delta E^2}=\bar{E}$ [11]) which yields the noise $\overline{\eta_e^2}$ magnitude ($\overline{\eta_e^2}=2c_1\bar{R}$). We assume that the intrinsic noise is due to the reaction of synthesizing the reporter proteins and that the extrinsic noise is due to the contribution of the mRNA molecules. Therefore, since both sources are uncorrelated, the total power spectral density function of the number of reporter proteins can be written as a sum of the intrinsic noise (down path) and extrinsic noise (up path) contributions [14–16].

In the steady state, the translation process can be modeled as a low-pass filter amplifier with the following characteristics: $E(\omega)=g(\omega)R(\omega)$, a gain $g(\omega)=c_1/(j\omega+c_2)$, and a cutoff frequency $\omega_{cg}\approx c_2$ (−6 dB). Therefore, we consider a signal $R(t)$, with a power spectral density function $\overline{\Delta R^2(\omega)}$, which is applied to an amplifier with a gain $g(\omega)$. According to the spectral intensity theorem [21], the output signal variance can be calculated as $\overline{\Delta E^2}=\int \frac{d\omega}{2\pi} \overline{\Delta R^2(\omega)} |g(\omega)|^2$ [see Eq. (3)]. Next, we assume that the bandwidth of the effective amplifier gain, $g(\omega)$, is narrower than that of the input signal (the number of the mRNA molecules). Therefore, the variance of the output signal (the extrinsic component which is contributed by mRNA molecule fluctuations) can be calculated by the following approximation:

$$\overline{\Delta E^2} \approx \overline{\Delta R^2(\omega_{cg})} \int \frac{d\omega}{2\pi} |g(\omega)|^2. \tag{5}$$

Substituting the gain of the amplifier [$|g(\omega)|^2=c_1^2/(\omega^2+c_2^2)$] and the cutoff frequency ($\omega_{cg}=c_2$) into Eq. (5), we obtain the extrinsic noise $\overline{\Delta E^2}\approx \overline{\Delta R^2(\omega=c_2)} \frac{c_1^2}{2c_2}$ and the total noise strength of the reporter protein population can be expressed as

$$\frac{\overline{\Delta E^2}}{\bar{E}} \approx \frac{c_1 \overline{\Delta R^2(\omega=0)}}{2\bar{R}} + 1. \tag{6}$$

In Eq. (6), we used again the assumption that the power spectral density function of R is constant in the given frequency range $\overline{\Delta R^2(\omega)}\approx \overline{\Delta R^2(\omega=0)}\approx \overline{\Delta R^2(\omega=c_2)}$. The value of $\overline{\Delta E^2}/\bar{E}$ can be determined experimentally and yield the value of $\overline{\Delta R^2(\omega=0)}$ [Eq. (6)]. Usually, proteins are considered to be stable with lifetime greater than that of mRNA molecules and other chemical species, thus making the assumption $c_2 \ll BW_R$ is reasonable [17,18]. Using the previous assumption and approximation, we can describe the power spectral density function of the reporter proteins population as

$$\overline{\Delta E^2(\omega)} \approx \left(\overline{\Delta R^2(\omega=0)} + \frac{2\bar{R}}{c_1} \right) |g(\omega)|^2. \tag{7}$$

III. MODELING SINGLE STAGE OF GENE EXPRESSION

In this section, we focus on the statistics of single-stage gene expression modeling. This case was investigated experimentally and theoretically in various works [11,12,17]. We present here a different treatment using the spectral intensity theorems for describing gene expression [Eq. (6)]. Focusing only on the cell behavior, we consider here only the intrinsic noise ($\overline{\Delta X_0^2}=0$). The power spectral density function of the number of mRNA molecules can be expressed as [11]

$$\overline{\Delta R^2(\omega)} = \frac{2a\overline{X_0}}{(\omega^2 + b^2)}, \tag{8}$$

where a and b are the transcription and the decay rates of the mRNA molecule, respectively. Substituting expression (8) into Eq. (6) for $\omega=0$ yields the noise strength of E,

$$\frac{\overline{\Delta E^2}}{\bar{E}} \approx 1 + \frac{c_1}{b}. \tag{9}$$

The ratio c_1/b was defined as the burst size [11]. One further note is that in this specific example, the bandwidth of the power spectral density function $\overline{\Delta R^2(\omega)}$ is simply equal to $BW_R=b$. However, since usually protein decay rates are much smaller than mRNA molecule decay rates, we get $b \gg c_2$ and therefore $c_2/b \ll 1$.

IV. MODELING BIOCHEMICAL CASCADE AND GENE EXPRESSION

In this section, we expand the model to describe a series of linear biochemical and genetic reactions. In this genetic system, we assume that all the biochemical reactions can be described by a set of linear first-order equations. According to the general assumptions in Sec. II, we can write the chemical Langevin equations which describe our model as

$$\frac{dX_i}{dt} = a_i X_{i-1} - b_i X_i + \eta_i(t),$$

$$\frac{dR}{dt} = a_n X_{n-1} - b_n R + \eta_n(t),$$

$$\frac{dE}{dt} = c_1 R - c_2 E + \eta_e(t). \tag{10}$$

In order to simplify the analytical solution, we assume that all the differential amplification rates are equal ($a_i=a$, for $i=1\ldots n$) and all the decay rates are equal ($b_i=b$ for $i=1\ldots n$). In steady state, we obtain $\overline{X_i}=(a/b)^i\overline{X_0}$, $\bar{R}=(a/b)^n\overline{X_0}$ (for $i=1\ldots n$). The power spectral density function of variable X_i which describes the number of the molecules in the output of reaction i is given by

$$\overline{\Delta X_i^2(\omega)} = \frac{a_i^2 \overline{\Delta X_{i-1}^2(\omega)} + \overline{|\eta_i(\omega)|^2}}{\omega^2 + b_i^2}. \tag{11}$$

The fluctuations value for the number of the molecules in the output of reaction i in steady state is given by integrating Eq.

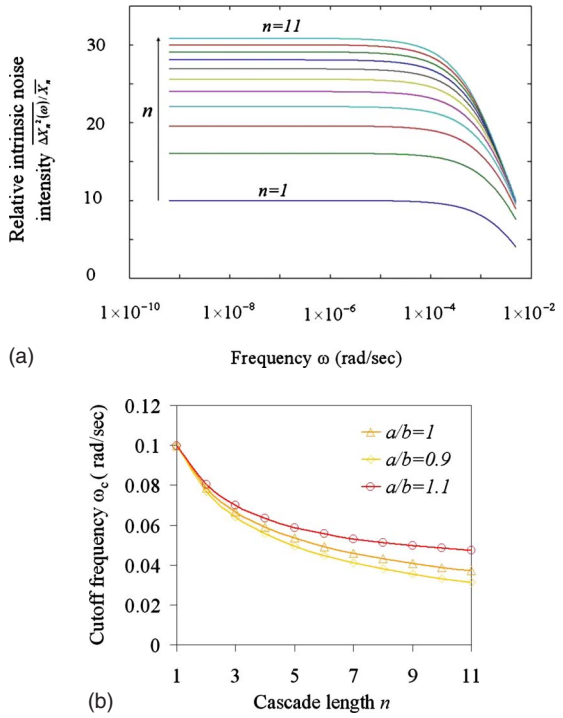


FIG. 3. (Color online) (a) Frequency-dependent intrinsic noise (power spectral density function) of the mRNA molecules number [the second term of Eq. (12)] as a function of the cascade length n when $a/b=1$. (b) Cutoff frequency ω_c (−6 dB) dependency on the cascade length as a function of the ratio a/b.

(11), hence $\overline{\Delta X_i^2}=\int_{-\infty}^{\infty}\frac{a^2\overline{\Delta X_{i-1}^2(\omega)}}{\omega^2+b^2}\frac{d\omega}{2\pi}+\frac{\overline{\eta_i^2}}{2b}$. Next we assume that the intrinsic noise (the second term of the pervious term) is constituted with the steady-state Poisson statistics of biochemical reactions ($\overline{\Delta X_i^2}=\overline{X_i}$, $\overline{\Delta R^2}=\bar{R}$, $\overline{\Delta E^2}=\bar{E}$) which yields the noise $\overline{\eta_j^2}$ magnitudes [$\overline{\eta_i^2}=2b(a/b)^i\overline{X_0}$, $\overline{\eta_E^2}=2c_1\bar{R}$]. Substituting the value of $\overline{\eta_i^2}$ into Eq. (11) will give the power spectral density function [$\overline{\Delta R^2(\omega)}$] of the number of mRNA molecules ($X_n=R$),

$$\overline{\Delta R^2(\omega)}=\frac{a^{2n}\overline{\Delta X_0^2(\omega)}}{(\omega^2+b^2)^n}+\sum_{i=0}^{n-1}\frac{2a^{n+i}\overline{X_0}}{b^{n-i-1}(\omega^2+b^2)^{i+1}}. \quad (12)$$

A similar expression has been derived before [13]. Figure 3(a) shows the frequency-dependent intrinsic noise (power spectral density function) of the mRNA molecules number [the second term of Eq. (12)] as a function of the cascade length n when $a/b=1$. As the cascade length increases, the bandwidth of the power spectral density function decreases. Figure 3(b) shows the cutoff frequency ω_c (−6 dB) dependence on the cascade length as a function of the ratio a/b. The cutoff frequency has a power-law dependency on the cascade length ($\omega_C\propto n^{-\alpha/a/b}$), where α is a parameter that depends on both a and b.

The variance of the signal in the steady state is given by integrating Eq. (12)

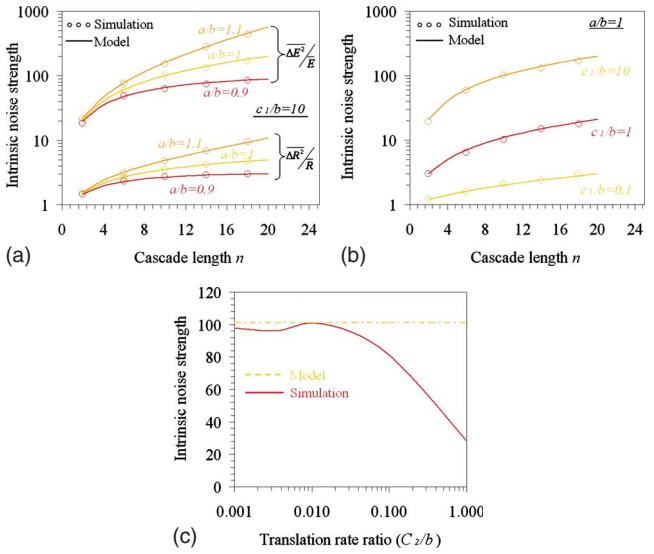


FIG. 4. (Color online) Monte Carlo simulation and theoretical model results of the intrinsic noise strength for the mRNA molecules and proteins number as a function of cascade lengths (a) $a/b=0.9,1,1.1$, $c_1/b=1$, and $c_2/b=0.01$; (b) $c_1/b=0.1,1,10$, $a/b=1$, and $c_2/b=0.01$. (c) Simulation results of the intrinsic noise strength for proteins number as a function of translation rate ratios c_2/b, $a/b=1$, $c_1/b=10$, and $n=10$.

$$\frac{\overline{\Delta R^2}}{\bar{R}}=\frac{\varepsilon_x\beta_x(a/b)^n}{b\sqrt{\pi n}}+\left(1+\sum_{j=1}^{n-1}\frac{(a/b)^j}{\sqrt{\pi j}}\right). \quad (13)$$

For simplicity, we assumed that the power spectral density function of the input signal has the form $\overline{\Delta X_0^2(\omega)}=\sigma_0^2/(\omega^2+\beta_x^2)$ and its variance $\overline{\Delta X_0^2}=2\sigma_0^2/\beta_x=\varepsilon_x\overline{X_0}$. In this case, the result that is presented in Eq. (13) is constituted with Ref. [13]. The first term represents the extrinsic noise and the second term represents the intrinsic noise of the cascade. In the case that $c_2\ll b$, the cutoff frequency (−6 dB) is very small compared to the cutoff frequency of $\overline{\Delta R^2(\omega)}$ which is given by ($\omega_C\propto n^{-\alpha/a/b}$). Hence, substituting the expression of Eq. (12) into Eq. (6) for $\omega=0$ yields the noise strength of the number of reporter proteins

$$\frac{\overline{\Delta E^2}}{\bar{E}}=\frac{\varepsilon_x c_1(a/b)^n}{\beta_x}+\left(1+\frac{c_1}{b}\sum_{j=0}^{n-1}(a/b)^j\right). \quad (14)$$

The first term in Eq. (14) represents the extrinsic noise and the second term represents the intrinsic noise of the system. The simulation results were calculated by Gillespie's algorithm for stochastic coupled chemical reactions [22], which is a based on Monte Carlo simulation. In this simulation, each chemical reaction follows Poisson statistics: (1) the probability of a reaction with rate k happening in a time dt is given by kdt and (2) the waiting times between successive reactions are exponentially distributed. Figure 4 demonstrates the approximated theoretical model and the simulation results of the intrinsic noise strength of the mRNA molecules number and the proteins number as a function of the cascade length n [Eqs. (13) and (14)]. The theoretical model results are in good agreement with the simulation results. We

find that the intrinsic noise strength $\overline{\Delta E^2}/\bar{E}$ shows a strong positive dependence on the transcription rate ratio (c_1/b) [Fig. 4(b)] and on the differential amplification rate ratio (a/b) [Fig. 4(a)]. This is in contrast to a weak dependence of the intrinsic noise strength on the translation rate ratio (c_2/b). Figure 4(c) shows that for $\frac{c_2}{b} < \frac{1}{30}$ $(n=10)$, there is a very weak dependence on the translation rate ratio (c_2/b) and the approximated theoretical model results (dotted line) are matching the simulation results. However, when the ratio $c_2/b \sim 1$, the approximated theoretical model results are different from the simulation results.

We discuss two cases describing the biochemical cascade and genetic system in two relevant situations:

(1) "Buffer signaling cascade"—$\bar{R}=\overline{X_0}$. Substituting $a/b=1$ into the relevant terms in Eqs. (13) and (14) yields

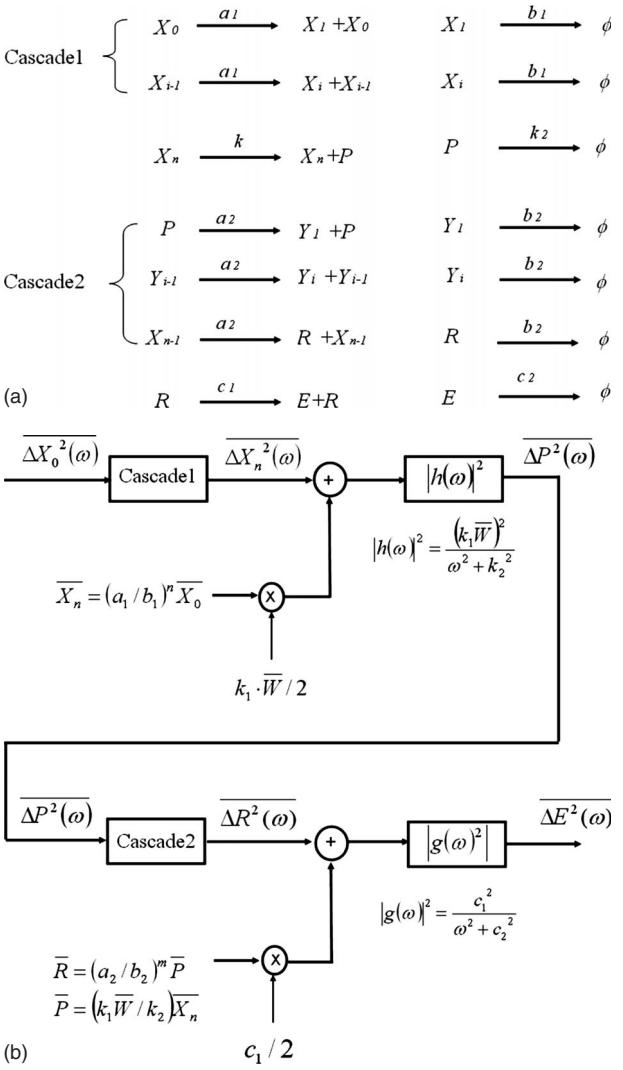
$$\frac{\overline{\Delta R^2}}{\bar{R}} \approx 1+\sqrt{n}, \quad \frac{\overline{\Delta E^2}}{\bar{E}} \approx 1+\frac{c_1}{b}n. \tag{15}$$

The relation of the intrinsic noise strength of the number of reporter proteins indicates that the burst size increases n times compared to the "single stage." We also found that the intrinsic noise strength of the number of mRNA molecules has a root square dependence on the cascade length.

(2) "Ultrasensitive signaling cascade" [13,15], where $a/b<1$ and $\bar{R}<\overline{X_0}$. In this case, the intrinsic noise strength of the number of mRNA molecules and reporter proteins increases as the cascade length n increases and reaches a limit for large n. Following Eq. (13) and assuming $\beta_x/b \gg 1$, the extrinsic noise becomes the dominant source in the fluctuations value of the number of mRNA molecules $(1/\beta_x$ is the time constant of the input signal). However, taking Eq. (14) and assuming $a/\beta_x \gg 1$, the intrinsic noise becomes the dominant source in the fluctuations value of the number of reporter proteins.

V. MODELING BIOCHEMICAL CASCADE AND GENE EXPRESSION WITH STABLE REACTIONS

In this section, we model a system with a biochemical cascade and gene expression including stable reactions. Here, we call a reaction a stable reaction when the product of the reaction X_i has a decay rate which is much smaller than the mRNA molecules decay rate. In this case, the assumption $c_2 \ll max\{b_i\}$ is no longer valid and the noise treatment should be different from the pervious example. For the sake of simplicity, we assume that there are two stable reactions in the genetic system including the translation process [Fig. 5(a)]. We divide the cascade to two subcascades [Fig. 5(b)]. The first one with a length n including all the reactions from X_0 until the first stable reaction $(i=1\ldots n)$. The second cascade with a length n_2 and it includes all the reactions which occur after the stable reaction until generating the mRNA molecules $(j=1-m)$, when $n+m=n'$ (n' is the length of the original cascade). We assume that all the differential amplification rates for every cascade are equal $(a_i=a_1,\ a_j=a_2)$ and also all the decay rates for every cascade are equal $(b_i=b_1,\ b_j=b_2)$ when $k_2,k_1 \ll b_1,b_2$. We consider the total



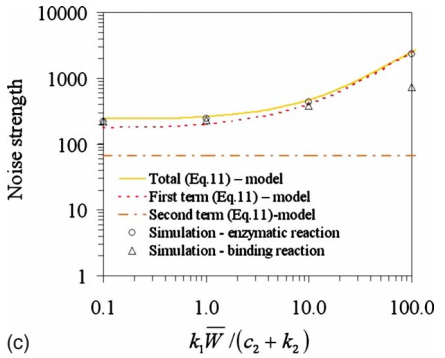


FIG. 5. (Color online) Modeling of a biochemical cascade genetic system and gene expression including a stable reaction. (a) Schematic model of biochemical reactions. (b) Schematic model for the power spectral density function. (c) Simulation results and theoretical model results of the intrinsic noise strength for proteins number as a function of the binding-enzymatic rates $b_1=b_2=1$, $a_1/b_1=0.9$, $a_2/b_2=0.9$, $n=m=10$, $c_1/b_2=10$, and $(c_2+k_2)/b_2=0.02$. Solid line is the analytical model [Eq. (16)], dotted line is the analytical model of the first term of Eq. (16), dashed line is the analytical model of second term of Eq. (16), circles are the simulation results of the total noise in the case of enzymatic reaction, and triangles are the simulation results of the total noise in the case of binding reaction.

noise of the first cascade and the stable reaction as the input noise of the second cascade [Fig. 5(b)]. Using directly Eq. (14) when $\varepsilon_x \equiv \frac{k}{b_1}\sum_{i=0}^{n-1}(\frac{a_1}{b_1})^i+1$ is the total intrinsic noise strength of the first cascade and the stable reaction, and using $\beta_x \equiv k_2$, one could express the intrinsic noise strength of the reporter proteins number as

$$\frac{\overline{\Delta E^2}}{\bar{E}} = \frac{c_1}{c_2+k_2}\left(\frac{a_2}{b_2}\right)^m\left[\frac{k}{b_1}\sum_{i=0}^{n-1}\left(\frac{a_1}{b_1}\right)^i+1\right]+\frac{c_1}{b_2}\sum_{j=0}^{m-1}\left(\frac{a_2}{b_2}\right)^j+1 \tag{16}$$

[in Eq. (14), we assumed that $\beta_x \gg c_2$]. The first term represents the total noise contributed by the first cascade and the stable reaction and the second term represents the noise contributed by the second cascade and synthesizing the reporter proteins (the second stable reaction). The relation between the output and the input of every cascade [Fig. 5(b)] is determined by Eq. (12),

$$\overline{\Delta X_n^2(\omega)} = \frac{a_1^{2n}\overline{\Delta X_0^2(\omega)}}{(\omega^2+b_1^2)^n}+\sum_{i=0}^{n-1}\frac{2a_1^{n+i}\overline{X_0}}{b_1^{n-i-1}(\omega^2+b_1^2)^{i+1}}, \tag{17}$$

$$\overline{\Delta R^2(\omega)} = \frac{a_2^{2m}\overline{\Delta P^2(\omega)}}{(\omega^2+b_2^2)^m}+\sum_{j=0}^{m-1}\frac{2a_2^{m+j}\bar{R}}{b_2^{m-j-1}(\omega^2+b_2^2)^{j+1}}, \tag{18}$$

where $P(t)$ is the signal with a power spectral density function $\overline{\Delta P^2(\omega)}$ which is applied to an amplifier with a gain $h(\omega)$ and can be expressed as $\overline{\Delta P^2(\omega)}=[\overline{\Delta X_n^2(\omega)}+\overline{X_n}k/2]|h(\omega)|^2$. In the case where $k_1 \ll b_1$, the power spectral density function of P can be approximated using the spectral intensity theorems [Eq. (7)]

$$\overline{\Delta P^2(\omega)} = [\overline{\Delta X_n^2(\omega=0)}+2\overline{X_n}/k]|h(\omega)|^2. \tag{19}$$

In Sec. II, we assumed [see Eq. (6)] that the bandwidth of the power spectral density function of R $[\overline{\Delta R^2(\omega)}]$ is much smaller than the cutoff frequency $(\omega_{gc}=c_2)$ of the amplifier $g(\omega)$. However, in the case where there are multiple stable reactions, the cutoff frequency $(\omega_{hc}=k_2)$ of the amplifier $h(\omega)$ can be of the same order as the cutoff frequency of R. Therefore, we use a modified equation which is given by

$$\overline{\Delta E^2} = \left(\frac{a_2}{b_2}\right)^{2m}\int\frac{d\omega}{2\pi}\overline{\Delta P^2(\omega)}|g(\omega)|^2+\bar{E}\frac{c_1}{b_2}\sum_{j=0}^{m-1}\left(\frac{a_2}{b_2}\right)^j+\bar{E}. \tag{20}$$

Substituting the relevant expressions in Eq. (20) and solving the analytical integral, we will obtain Eq. (16). The noise strength which is expressed in Eq. (16) strongly depends on the decay rate of the reporter protein in contrast to the biochemical cascade which was given in Sec. IV and the single stage modeling in Sec. III. In the case where $a_2/b_2<1$, the first term [Eq. (16)] decreases as the cascade length increases, however, the second term [Eq. (16)] increases. Figure 5(c) compares between the approximated analytical model [Eq. (16)] and the simulation results. We simulate two types of stable reactions: enzymatic reaction [Fig. 5(a)] and enzyme-substrate binding reaction (Fig. 1) when $k=k_1\bar{W}$. In the first type, we assumed that extrinsic and intrinsic noises are uncorrelated sources; however, in the binding reaction, the sources are correlated [16]. This effect is shown in the simulation results of Fig. 5(c); for the case $k \gg b$, the binding reaction contributes strongly the signal X_n and decreases the noise strength [Fig. 5(c), triangles plot]. For very fast enzymatic-binding rate, the noise is determined mainly by the first cascade. For very slow enzymatic-binding rate, the first term is constant and is independent on the first cascade parameters (n,a_1,b_1).

VI. CONCLUSION

Engineered living cells can be integrated with electronic circuits on the same microchip, which is often referred to as a micrototal analysis system (μ-TAS) or "lab on a chip" such as whole-cell bioluminescent-electrochemical biosensors. These living cells are considered to be the main element on the integrated system and play a major role in determining the signal-to-noise ratio of the full integrated system. In this work, we present a stochastic model for cascades that include biochemical and genetic reactions in genetically engineered living cells. The theory is referred to the spectral intensity theorems of gene expression [17]. Our approach is valid when the protein decay rate is smaller than mRNA molecules and other chemical species decay rates. We showed that in biochemical cascade and gene expression systems, the fluctuations value in the number of reporter proteins has a linear dependence on the cascade length $(\propto n)$ and the fluctuations value in the number of mRNA molecules has a root square dependence on the cascade length $(\propto\sqrt{n})$. We also showed that the burst size increases approximately n times compared to a single stage of gene expression. It is possible to design the genetic system such that the intrinsic noise of the reporter proteins number is bounded and in some cases, the noise of the input signal will be the dominant source.

We also analyzed that a cascade of biochemical and genetic reactions includes a stable enzymatic-binding reaction. This example describes many biological systems such as analyte-receptor binding or enzyme-substrate binding reactions. We observed that for very fast binding rate, the noise is mainly determined by the first biochemical reactions in the cascade with a weak dependence on the cascade length. In such systems, the fluctuations in the gene expression strongly depend on the decay rate of the synthesized protein. Finally, we have built a model based on the first-order rate equations and we believe that such model should deal also with other biosystems such as repressor interactions [23] and feedback biological circuits [17,24].

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