Nonclassical Chemical Kinetics for Description of Chemical Fluctuation in a Dynamically Heterogeneous Biological System†

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We review novel chemical kinetics proposed for quantitative description of fluctuations in reaction times and in the number of product molecules in a heterogeneous biological system, and discuss quantitative interpretation of randomness parameter data in enzymatic turnover times of β-galactosidase. We discuss generalization of renewal theory for description of chemical fluctuation in product level in a multistep reaction system, stochastic nature of chemical reactions in such small reactor as a biological cell has important consequences on cell-to-cell variation in the level of important biomolecules including m-RNA and regulatory proteins that control cell’s biological function, its decision making, and its ultimate fate. For a chemical reaction with a constant reaction rate, Master equation approach or Gillespie’s stochastic simulation approach could provide a correct description for the time-evolution of the number distribution of reaction events or product molecules. However, it is not clear whether the conventional approaches within the paradigm of the rate constant concept are applicable to biological reactions with a dynamically heterogeneous distribution of reaction rates.

Modern single molecule experimental studies tell us that the rate of a biopolymer reaction keeps fluctuating in line with the conformational dynamics of the biopolymer even in a highly controlled homogeneous reaction environment. For biopolymer reactions occurring in cells, reaction rates are different from cell to cell due to heterogeneous environments posed by cells, which adds additional complexity in quantitative description for the probabilistic outcome of reactions in cells. However, with the advance of single molecule experiments, observation of individual reaction trajectories has been made possible for various biopolymer reactions including stepping of a single molecular motor, catalytic turnover of a single enzyme, the gene expression from a DNA, and single molecule DNA sequencing. As far as the average behavior of those reaction trajectories concerned, the conventional chemical kinetics provides a satisfactory description. For example, the average velocity of kinesin motors and the mean enzymatic turnover time of β-galactosidase turn out consistent with the Michaelis-Menten (MM) relation derived from the conventional chemical kinetics for the simple MM enzyme reaction scheme. It is now known that the average enzymatic turnover time obeys the MM relation for a variety of different models of enzyme reactions involving reaction rate fluctuation. Recently, a generalized MM relation is established for the mean turnover time of a general multi-state enzyme reaction model, which reduces to the MM relation whenever the detailed balance condition between different states is satisfied.

However, the conventional chemical kinetics is not so satisfactory in description of statistical fluctuations contained in biopolymer reaction trajectories. For example, the enzymatic turnover time distribution of β-galactosidase enzyme and the waiting time distribution of kinesin motors look inconsistent with the prediction of the conventional chemical kinetics for the simple MM reaction scheme. A while ago, the variance in the time-dependent positions of a kinesin motor could be successfully explained by assuming multiple intermediate biochemical states in each step of the kinesin motor, which shows that the statistical distribution of waiting times between steps of a kinesin motor is a non-

†This paper is to commemorate Professor Kook Joe Shin's honourable retirement.

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exponential function. Recently, a general quantitative description of fluctuations in single enzymatic turnover times was also achieved, for which a generalization of chemical kinetics was made in description of non-Poisson reaction processes of enzyme-substrate (ES) complex.

In the next section, we will review several theories for quantitative description of fluctuations in single enzymatic turnover time distribution, and compare predictions of these theories to experimental data obtained for single β-galactosidase enzyme. It turns out that one of the theories could provide an excellent quantitative description for the randomness parameter data reported in ref. 9. For the successful quantitative analysis of the randomness parameter data of the enzyme reaction, we find it natural to go beyond the conventional chemical kinetics in which each and every elementary reaction process composing a reaction is a simple rate process or a simple Poisson process; the randomness parameter data could not be explained with assumption that the reaction processes of enzyme-substrate (ES) complex are simple rate processes. In the subsequent section, with a brief review of reaction event counting statistics for a couple of important models of stochastic chemical reaction processes, we will present exact expressions for chemical fluctuation or the number distribution of the product molecules for a reaction process of enzyme-substrate (ES) complex.

Fluctuations of Single Enzyme Turnover Times

Recently, a new type of chemical kinetics is developed for description of a single molecule reaction composed of possibly non-Poisson elementary reaction processes. In this approach, the turnover time distribution \( \psi(t) \) of the single enzyme Michaelis-Menten (MM) reaction is represented in terms of the reaction time distributions \( \phi_1(t) \), \( \phi_2(t) \), and \( \phi_0(t) \) for the three elementary reaction processes, \( E+S \xrightarrow{\phi_0(t)} ES \), \( E+S \xrightarrow{\phi_1(t)} ES \), and \( E+S \xrightarrow{\phi_2(t)} ES \). The reaction time distribution (RTD) for each of the elementary reactions represents the probability density of the time elapsed for a completion of the elementary reaction process. The precise definition of \( \phi_1(t) \) is the probability that the enzyme-substrate association reaction, \( E + S \rightarrow ES \), is completed in time interval \( (t, t + dt) \), given that the reaction begins at time 0. Here, the superscript 0 in \( \phi_0(t) \) signifies that the RTD of the enzyme substrate encounter process is normalized, i.e. \( \int_0^\infty \phi_0(t) \, dt = 1 \). On the other hand, \( \phi_1(t) \) or \( \phi_2(t) \) for the dissociation or the catalytic reaction of the ES complex is completed in time interval \( (t, t + dt) \), given that the ES complex is prepared at time 0. In contrast to \( \phi_0(t) \), \( \phi_1(t) \) or \( \phi_2(t) \) for the dissociation or the catalytic reaction of the ES complex does not satisfy the normalization condition; instead \( \int_0^\infty \phi_1(t) \, dt = 1 \) or \( \int_0^\infty \phi_2(t) \, dt = 1 \) are the probability \( p_1 \) of dissociation and the probability \( p_2 \) of catalytic reaction of the ES complex, respectively, so that their sum is normalized, i.e. \( \int_0^\infty \phi_1(t) + \phi_2(t) \, dt = 1 \). The relation of the enzymatic turnover time distribution \( \psi(t) \) to the reaction time distributions, \( \phi_1(t) \), \( \phi_2(t) \), and \( \phi_0(t) \), of the elementary reaction processes is given in Laplace domain as follows:

\[
\hat{\psi}(u) = \frac{\hat{\phi}_1(u) \hat{\phi}_2(u)}{1 - \hat{\phi}_0(u) \hat{\psi}(u)} \tag{1}
\]

In Eq. (1), \( \hat{f}(t) \) denotes the Laplace transform of \( f(t) \), defined by \( \hat{f}(u) = \int_0^\infty e^{-ut} f(t) \, dt \).

The RTD, \( \phi_0(t) \), of the Poisson enzyme substrate encounter process \( (E + S \xrightarrow{\psi} ES) \) is given by \( \psi(t) = k_1[S] \exp(-k_1t) \). The RTD, \( \phi_1(t) \) and \( \phi_2(t) \) for the dissociation \( (E + S \xrightarrow{\phi_1(t)} E) \) and the catalytic reaction \( (ES \xrightarrow{\phi_2(t)} E + P) \) of the ES complex are given by \( \phi_1(t) = \phi_0(t) \int_0^t \exp\left(-k_2u\right) \, du \) and \( \phi_2(t) = \phi_0(t) \int_0^t \exp\left(k_2u\right) \, du \), respectively, where \( \phi_0(t) \) denotes the normalized one channel reaction time distribution for the dissociation (catalytic reaction) of the ES complex in the absence of the competing catalytic reaction (dissociation). When both the dissociation and the catalytic reaction of the ES complex are Poisson processes, \( \phi_0^\prime(t) \) and \( \phi_2^\prime(t) \) are given by \( k_1 \exp(-k_1t) \) and \( k_2 \exp(-k_2t) \), respectively; therefore, \( \phi_1(t) \) and \( \phi_2(t) \) become \( \phi_1(t) = k_1 \exp[-(k_1 + k_2)t] \) and \( \phi_2(t) = k_2 \exp[-(k_1 + k_2)t] \). Substituting \( \phi_1(t) = k_1[S](u + k_1[S]), \phi_1(t) = k_2[S](u + k_1 + k_2), \) and \( \phi_1(t) = k_2[S](u + k_1 + k_2) \) into Eq. (1), one gets:

\[
\hat{\psi}(u) = \frac{\xi}{u^2 + \lambda u + \xi}, \tag{2}
\]

where \( \lambda \) and \( \xi \) are given by \( \lambda = k_1[S] + k_1 + k_2 \) and \( \xi = k_2[S] \). The inverse Laplace transform of \( \hat{\psi}(u) \) given in Eq. (2), \( \psi(t) \), is equal to \( \psi_c(t) \) predicted by the conventional chemical kinetics:

\[
\psi_c(t) = \frac{\alpha \beta}{\beta - \alpha} \left( e^{-\alpha t} - e^{-\beta t} \right). \tag{3}
\]

Here \( \alpha \) and \( \beta \) are time-independent constants given by \( \alpha = 2^{-1}(\lambda - \sqrt{\lambda^2 - 4\lambda \xi}) \) and \( \beta = 2^{-1}(\lambda + \sqrt{\lambda^2 - 4\lambda \xi}) \). The mean, \( \langle \tau > \psi_c \) (\( = \int_0^\infty \psi_c(t) \, dt \)), of the enzymatic turnover-time distribution \( \psi_c(t) \) in Eq. (3) is

\[
\langle \tau > \psi_c = <\tau >_{\min} \left(1 + \frac{K_M}{[S]}\right), \tag{4}
\]

where \( <\tau >_{\min} \) and \( K_M \) are the mean turnover time in the high substrate concentration limit and the Michaelis-Menten constant given by \( <\tau >_{\min} = k_1^2 \) and \( K_M = (k_1 + k_2)/k_2 \).

In the single enzyme reaction, the enzyme-substrate en-
counter process may be approximated as a simple Poisson process in the steady state. However, the dissociation or the catalytic reaction of the ES complex may not be a Poisson process, as the reactivity of the ES complex is dynamically fluctuating in line with the conformational dynamics of the ES complex. For a given conformation of the ES complex, the reaction of the ES complex can still be a non-Poisson process when the substrate or product escape process out of the enzyme molecule is a complex one involving a number of different intermediate states and multiple reaction channels. Depending on the microscopic reaction dynamics of the ES complex, the functional form for the reaction rate distribution \( \phi_j(t) \) for the catalytic reaction of the ES complex is the same as \( \phi_1(t)p_1 \) for the catalytic reaction. That is to say, in the analysis of the mean turnover time and the randomness parameter, one can map the model considered above into the simpler model in which \( \phi_1(t)p_1 \) is the same as \( \phi_1(t)p_2 \). For the latter model, the physical interpretation of \( q_{ES} \) and \( \eta R \) in Eq. (6) become simpler; \( q_{ES} \) is the variance \( \langle t_{ES}^2 \rangle - \langle t_{ES} \rangle^2 \) of the conformation-dependent mean lifetime of the ES complex and \( \eta R \) is twice the success probability \( p_2 \) of the catalytic reaction of the ES complex. From the analysis of the experimental randomness parameter data along with the mean turnover time data with use of the latter model, one can separately extract values of the following physical parameters, \( p_2 \), \( <t_{ES}^2> \), \( <t_{ES}^3> \), and \( k_1 \) of the enzyme reaction system.

Until now, we discuss the case where the enzyme system is ergodic and our observation time is long enough, so that the observed RTD for every enzyme in the system is the same. When our reaction system is non-ergodic or when the observation time is not long enough, each enzyme may have different RTD from each other during the observation time. From now on, let us discuss the second moment of RTD of enzyme reaction for such nonergodic heterogeneous system. Let our system contain \( M \) enzyme molecules, each of which is under our observation at single molecule level. For the nonergodic heterogeneous system, the backward RTD \( \phi_0(\gamma) \) and the forward RTD \( \phi(\gamma) \) (dependent on enzyme index \( j \), \( 1 \leq j \leq M \)). Each individual enzyme reaction system has its own RTD, \( \psi_0(t) \), for enzyme reaction, which is given by Eq. (1) with \( \rho_1 \) and \( \rho_2 \) being replaced by \( \rho_0(\gamma) \) and \( \rho(\gamma) \) for the \( j \)-th enzyme molecule. By analyzing reaction trajectories of each single enzyme molecule, one can obtain these reaction parameters for each single enzyme. If the single enzyme reaction system is ergodic and our observation time is long enough, those reaction parameters should be the same for every single enzyme. The averages of the first two moments of RTD over the different enzymes are defined as \( \langle <t^2> \rangle = \sum_j \langle t^2 \rangle_j/P_j \) and \( \langle t^2 >_0 \rangle = \sum_j \langle t^2 \rangle_j/P_j \), where \( P_j \) is the normalized weight accounting for the contribution of the \( j \)-th enzyme. \( P_j \) is proportional to the number of reaction events observed for the \( j \)-th enzyme in experiment, and satisfies the normalization condition, \( \sum_j P_j = 1 \). The first two moments of the enzymatic turnover time distribution of the nonergodic heterogeneous enzyme system is given by

\[
\langle <t^2> \rangle = \sum_j <t^2 >_j \rho_j(\gamma)/\rho_j(\gamma) \rho_0(\gamma)
\]

where \( x_M \) denotes the average \( \sum_j x_j P_j \) of \( x_j \) over the enzyme index \( j \). \( \alpha, \beta, \gamma \) are defined by \( \alpha_1 = \langle t^2 >_1 \rho_1(\gamma) \rho_0(\gamma) \rho_1(\gamma) \rangle \) and \( \gamma_j = \langle t^2 >_j \rho_j(\gamma)/\rho_j(\gamma) \rho_0(\gamma) \rho_j(\gamma) \rangle + 2\eta_j <t^2 >_j \rho_j(\gamma)/\rho_j(\gamma) \rho_0(\gamma) \rho_j(\gamma) \rangle \). If we define \( \langle <t^2> \rangle \) by \( \langle <t^2> \rangle = \sum_j <t^2 >_j \rho_j(\gamma)/\rho_j(\gamma) \rho_0(\gamma) \rho_j(\gamma) \rangle \), the expression for \( \langle <t^2> \rangle \) is given by

\[
\langle <t^2> \rangle = \frac{<R>_0 + <R>_1 x(x-\eta)}{(x+1)^2}
\]
where $x$ denotes $[S]/K_M$ with $K_M$ being equal to $<\beta>_M<\gamma>_M$, $<R>_{\alpha}$ and $<R>_{\beta}$ denote $\lim_{[M] \to 0} R$ and $\lim_{[M] \to \infty} R$, respectively given by $2(<\phi\beta>_M<\gamma>_M)$ and $<\gamma>_M<\alpha>_M$. In Eq. (9), $\eta$ designates $2(<\phi\gamma>_M<\beta>_M)$ and $2(<\alpha>_M<\phi>_{M}<\beta>_M)$. Note that the dependence of $<R>$ on $[S]$ given in Eq. (9) for the non-ergodic, heterogeneous enzyme system is qualitatively different from $R$ given in Eq. (6) for the homogeneous ergodic system, especially in the small $[S]$ limit; $\lim_{[M] \to 0} R$ vanishes whereas $\lim_{[M] \to \infty} R$ does not.

We note here that the formula proposed in ref. 9 for the single enzyme turnover time distribution is correct only for a heterogeneous nonergodic enzyme reaction system, and this prediction for the randomness parameter conforms to Eq. (9). In ref. 9, a generalization of the conventional chemical kinetics is made, in which a probability distribution $w(k_2)$ of the rate coefficient $k_2$ of the catalytic reaction of the ES complex is assumed, and the following form of enzymatic turnover time distribution is suggested:

$$\psi_{GC}(t) = \int dk_2 w(k_2) \psi(t).$$

(10)

Noting that the n-th moment $<t^{n-2}> = \int dx \psi(x,t) x^{n-2}$ of $\psi(t)$ is given by $<t^{n-2}> = \int dx \psi(x,t) x^{n-2}$, with $<t>$ being the n-th moment of $\psi(t)$ given in Eq. (3), one can recover Eq. (4) for the mean $<t>_{GC}$ of the enzymatic turnover time distribution $\psi_{GC}(t)$ with $K_S$ in Eq. (4) being replaced by $K_S^{GC} = (k_2 + + k_2^2 > ) / k_2$. Hereafter $<k_2^2>$ is given by $<k_2^2> = \int dx w(k_2) k_2^2$. We obtain the expression of the randomness parameter $R_{GC}$ associated with $\psi_{GC}(t)$ as follows:

$$R_{GC} = 2 \left[ \frac{\sigma^2_{k_2}}{<k_2^2>-2} \left( \frac{1 + p_{GC}^2}{x+1} - \frac{p_{GC}^2}{x+2} \right) \right].$$

(11)

Here, $\sigma^2_{k_2}$ and $x$ respectively denote the variance of $k_2$ and the substrate concentration in unit of $K_M^{GC}$, i.e. $\sigma^2_{k_2} = <k_2^2>-2 <k_2^2>^2$ and $x = [S]/K_M^{GC}$. $p_{GC}^2$ and $p_{GC}$ are given by $p_{GC}^2 = \int dx / (k_2 + k_2^2 > )$ and $p_{GC}^2 = <k_2^2>-1 / (k_2 + k_2^2 > )$.

Comparison to Experimental Randomness Parameter Data

We make a direct comparison between the predictions of the above-mentioned theories for the randomness parameter and the experimental randomness parameter data reported in ref. 9. Randomness parameter calculated from the conventional chemical kinetics or from Eq. (3), $R_c = -2p_0^2 x (1+x)^2$ where $p_2$ is given by $k_2 / (k_1 + k_2)$, yields a negative value for any substrate concentration, inconsistent with the experimental randomness parameter data. In comparison, $R_{GC}$ in Eq. (11) yields a positive value for the randomness parameter. However, the dependence of $R_{GC}$ on substrate concentration appears much different from the experimental randomness parameter data. The randomness parameter $R_{GC}$ calculated from $\psi_{GC}(t)$ looks nearly constant at all substrate concentrations investigated in ref. 9, whereas the experimental randomness parameter data exhibit a strongly nonlinear behavior. Particularly, the behavior of the randomness parameter $R_{GC}$ predicted by $\psi_{GC}(t)$ is qualitatively different from the experimental data in the low substrate concentration regime. $R_{GC}$ yields the following expression for the randomness parameter $R_0$ in the low substrate concentration limit,

$$R_0 = \lim_{[S] \to 0} \frac{R_0}{<k_2^2>-1} = \frac{2(<k_2^2>-<k_2^2>^2)}{<k_2^2>-1},$$

(12)

which could not vanish for any probability density function $w(k_2)$ with a finite variance. This fact indicates that $\psi_{GC}(t)$ cannot be the correct enzymatic turnover time distribution of the $\beta$-galactosidase enzyme investigated in ref. 9, for any choice of $w(k_2)$. In producing the curves for $R_{GC}$, the values of the adjustable parameters and the functional form of $w(k_2)$ are chosen as given in ref. 9.

As a matter of fact, $\psi_{GC}(t)$ is the exact enzymatic turnover time distribution for such stochastically heterogeneous enzymes in which each enzyme has constant values for rate constants, $k_1$, $k_1$, and $k_2$ throughout the experiment but the value of $k_2$ is different from enzyme to enzyme, distributed over the enzymes according to $w(k_2)$. In the latter system, each enzyme has turnover time distribution $\psi(t)$ with different value of $k_2$ from each other, and the average of the turnover time distribution over the enzymes with equal weight for every enzyme results in $\psi_{GC}(t)$. However, the behavior of the randomness parameter data reported in ref. 9 is inconsistent with that of the stochastically heterogeneous enzyme model.

We find that $R$ given in Eq. (6) provides an excellent quantitative description of the randomness parameter data unless we set the value of the MM constant, $K_M$, to be the same as that reported in ref. 9, 23. The values of the extracted parameters are given by $R_c = 1.57$ and $\eta = 0.624$, and $K_M \approx 20$ mM, which yield $p_2 \approx 0.49$, and $<t>_{GC} = <t>_{12} \approx 3.2$.

Using the value of 1/<$t>_{\min}$ as 730 sec$^{-1}$, extracted from the mean turnover time analysis in ref. 9, we can determine the value of the mean lifetime $<t>_{ES}$ of the ES complex.

![Figure 1](image)

Figure 1. (circle) Randomness parameter for turnover time fluctuation of $\beta$-galactosidase enzyme. (square) Mandel’s Q parameter estimated from intensity fluctuation of light emission from product molecules of $\beta$-galactosidase catalysis (Dashed line) Result of Eq. (11) (Solid line) Result of Eq. (6), both best fitted to the experimental data.
functions, \( \psi_E(t) \) and \( \phi_E(t) \), that describe the microscopic dynamics of system state \( \Gamma \) and the \( \Gamma \)-dependent reaction dynamics of the reaction system, respectively. \( \psi_E(t)dt \) denotes the probability that a transition from state \( \Gamma \) to other states occurs between \( t \) and \( t + dt \) for the first time after the transition to state \( \Gamma \) at time 0, and \( \phi_E(t)dt \) denotes the probability that a reaction system at state \( \Gamma \) completes a single reaction event between \( t \) and \( t + dt \) given that the event began at time 0. In this work, we are interested in a general relation of \( \psi_E(t) \) and \( \phi_E(t) \) to the number distribution \( p_n(t) \) of product molecules for the reaction system at time \( t \).

To find the relation, we should first obtain the generalized master equation (GME) for \( p_n(\Gamma, t) \), the probability density that the reaction system is in hidden state \( \Gamma \) at time \( t \) and has had \( n \) reaction events in time \( t \) given that the first reaction event starts at time 0, \( p(0, t) \) is defined by \( p_n(\Gamma, t) = \sum_{\Gamma_0} \mathcal{G}(\Gamma_0, \Gamma, t) \). The dynamics of hidden state \( \Gamma \) can be modeled by the continuous time random walk in the presence of arbitrary potential,\(^{21,27} \) which can describe a variety of dynamical phenomena. The derivation of the GME is a straightforward generalization of that presented in ref. 21. The result is

\[
\frac{\partial}{\partial t} p_n(\Gamma, t) = \kappa_i(t)[p_{n-1}(\Gamma, t) - p_n(\Gamma, t)] + L(\Gamma) p_n(\Gamma, t),
\]

where \( f(t) * g(t) \) denotes the convolution integral, \( \int_0^t f(\tau) g(t-\tau) d\tau \). The first term in the right side of Eq. (13) describes the time evolution of \( p_n(\Gamma, t) \) due to the reaction of the system in state \( \Gamma \). The expression for reaction rate kernel \( \kappa_i \) is simple in Laplace domain:

\[
k_i(u) = \frac{u\hat{\phi}_i(u)}{1 - \hat{\phi}_i(u) - \hat{\psi}_i(u)}. \tag{14}
\]

Here, \( \hat{\phi}_i(u) \) and \( \hat{\psi}_i(u) \) denote the Laplace transforms of \( \phi_i(t) \) and \( \psi_i(t) \), respectively. \( L(\Gamma) \) in Eq. (13) is the operator describing the dynamics of hidden state.\(^{24} \)

We can obtain the expression for the moments of \( p_n(t) \) from the characteristic function method. From Eq. (14), one can obtain the following expression for the characteristic function, \( F_{\Gamma}(\mu, t) \) defined by \( \sum_{\Gamma_0} \mathcal{G}(\Gamma_0, \Gamma, t) \):

\[
F_{\Gamma}(\mu, t) = \sum_{n=0}^{\infty} (\mu - 1)^n \frac{1}{u - L(\Gamma)} \kappa_i(u) \left[ \frac{1}{u - L(\Gamma)} \right]^n P_0(\Gamma). \tag{15}
\]

Here \( P_0(\Gamma) \) denotes the initial distribution of \( \Gamma \) sampled at the initial time of each measurement bin. Expressions for the moments of \( p_n(t) \) can be obtained from the well-known property of the characteristic function, i.e. with \( <n(n-1)\cdots(n-l+1)(t)> = (e^{\mu t} / \mu^l) F_{\Gamma}(\mu, t) \mid_{\mu=1} \) which \( F_{\Gamma}(\mu, t) \) are obtained as

\[
<\sum_{n=0}^{\infty} (\mu - 1)^n \frac{1}{u - L(\Gamma)} \kappa_i(u) \left[ \frac{1}{u - L(\Gamma)} \right]^n P_0(\Gamma) \operatorname{d} \mathcal{G}(\Gamma, u | \Gamma_{t-1}) \rangle_{\mu=1} \tag{16}
\]

Here \( \mathcal{L}^{-1} \) denotes the inverse Laplace transform operator, and \( \mathcal{G}(\Gamma, u | \Gamma_{t-1}) \) denotes the propagator defined by \( [u - L(\Gamma)]^{-1} \partial / \partial \Gamma \).

**Reaction Event Counting Statistics of a Dynamically Heterogeneous Biological Reaction System**

**Theory.** In this section, we introduce RECS of a general nonrenewal reaction process, in which the reaction time distribution is dependent on hidden dynamical state \( \Gamma \) of the reaction system and the surrounding environment.\(^{24} \) We begin with introducing two fundamental probability density

<image>
When the relaxation dynamics of $\Gamma$ occurs on a time scale much shorter than the time scale of the individual reaction event, we can assume that $G(t_{\Gamma}; t_{\Gamma}) = P_{\Theta}(\Gamma)/\mu$ where $P_{\Theta}(\Gamma)$ is the equilibrium distribution satisfying $L(\Gamma)P_{\Theta}(\Gamma) = 0$. In the latter case, Eq. (16) reduces to

$$<n(n-1)\cdots(n-l+1)(t)>=L^{-1}\left\{\frac{\Gamma_{eq}(u)}{u}\right\} (17)$$

where $\Gamma_{eq}(u) = \int dt \Gamma_{eq}(u) P_{\Theta}(\Gamma)$. One can show that the probability distribution $p_\Theta$ corresponding to the latter moment is

$$\hat{p}_\Theta(u) = \int dt \hat{\Theta}(u) P_{\Theta}(\Gamma)$$

where $\hat{\Theta}(u)$ is the equilibrium reaction time distribution defined by $u \hat{\Theta}(u)(1-\hat{\Theta}(u)) = \Gamma_{eq}(u)$. Equation (18) is the same as the result of renewal theory.28 In general, the slow dynamics of hidden state $\Gamma$ makes $p_\Theta$ different from Eq. (18). Deviation of a stochastic process from renewal statistics can be estimated by parameter $\Theta$, which is defined by

$$\Theta(u) = \int dt \frac{\hat{\Theta}(u)}{u^2} = \int dt \frac{\hat{\Theta}(u)}{u}$$

where $u$ denotes the characteristic time scale of the reaction rate fluctuation, defined by

$$<\xi^2> = \int dt d\tau \frac{\hat{\Theta}(\tau)\hat{\Theta}(0)}{<\delta \hat{\Theta}^2>_{eq}}.$$

Equation (25) indicates that deviation $\xi$ from Poisson statistics increases with both the magnitude $<\delta \hat{\Theta}^2>_{eq}$ of the reaction rate fluctuation and the relaxation time scale $\xi$ of the reaction rate fluctuation.

Up to now, we discuss RECS for reaction system of which initial distribution is the equilibrium distribution $P_{\Theta}(\Gamma)$ that satisfies $L(\Gamma)P_{\Theta}(\Gamma) = 0$, and the results presented above are applicable to the reaction system composed of a number of biopolymers at equilibrium state. In the analysis of single molecule reaction trajectories, the equilibrium initial state distribution can be prepared by sampling the initial measurement time homogeneously in time along each single molecule reaction trajectory. For a system with a nonequilibrium initial distribution, the moments of reaction event number distribution are given by Eq. (20). A particularly important nonequilibrium initial state distribution in the analysis of single molecule reaction trajectories is the distribution $P_{\Theta}(\Gamma)$ of the single molecule system state sampled only at the moment when single molecule reaction begins, which is given by

$$P_{\Theta}(\Gamma) = k(\Gamma)P_{\Theta}(\Gamma)/\int d\Gamma' k(\Gamma')P_{\Theta}(\Gamma').$$

For the latter initial condition, Eq. (20) yields,

$$<n(n-1)\cdots(n-l+1)(t)>=\int d\Gamma' k(\Gamma')P_{\Theta}(\Gamma)/\int d\Gamma' k(\Gamma')P_{\Theta}(\Gamma').$$

Equation (28) with $l$ being equal to 1 tells us that the information about the rate-rate autocorrelation function contained $<n(n-1)(t)>$ can be obtained directly from the mean reaction number $<n(t)>$ of RECS for the case with...
nonequilibrium initial state distribution \( P_0^e(\Gamma) \):

\[
< n(t)^e >_0 = k_{eq} \frac{d}{dt} < n(n-1)(t)^e >_eq \\
= k_{eq} \int_0^t d\tau < k(\tau) k(0)^e >_eq \\
= k_{eq} \left( t + k_{eq}^2 \int_0^t d\tau < \delta k(\tau) \delta k(0)^e >_eq \right) 
\]

(29)

Note that \( < n(t)^e >_0 \) is nonlinear in time in contrast to the mean reaction number \( < n(t)^e >_eq \) given in Eq. (21); the time derivative of \( < n(t)^e >_0 \) is a monotonically decreasing function of the size \( t \) of measurement time,

\[
\frac{d<n(t)^e>_0}{dt} = k_{eq} \left[ 1 + \frac{<\delta k(t)\delta k(0)^e>_e}{k_{eq}^2} \right],
\]

(30)

which varies from the initial value, \( k_{eq} <\delta k^2>/k_{eq} \), to the final value, \( k_{eq} \) the equilibrium reaction rate. The time derivative of \( <n(t)^e>_0 \) is exactly the same as two reaction event density obtained by Yang and Cao.31

Comparison to Stochastic Simulation Results

Gillespie’s stochastic simulation method provides the numerical solution of the master equation, which provides numerical results for the RECS of a reaction with a constant reaction rate.7 In our recent work we generalize Gillespie’s stochastic simulation method to investigate RECS of biopolymer reaction with reaction rate fluctuation.29 Our simulation method is particularly useful when the propagator \( G(\Gamma,t|\Gamma_0) \) of the system state is available, and it gives the numerical results for the RECS of a reaction system with a state-dependent reaction rate much more efficiently compared to the stochastic simulation method involving an explicit simulation of the system dynamics in the state space.

In Figure 2, comparison is made between the analytic results, Eqs. (21) and (29), and the stochastic simulation results for the time-dependence of the mean number of the product molecules generated by an elementary reaction with the following rate fluctuation, \( k(\Gamma) = k_0 + \sigma^2 \Gamma(t) \), where \( \Gamma(t) \) is Gaussian process with the time correlation function being given by \( < \Gamma(t+\tau)\Gamma(0)^e >_e = b^2 \exp(-\lambda \tau/2) \). \( k_0 \) and \( \kappa \) are time-independent constants. \( b^2 \) denotes the variance \( <\Gamma^2>_e \) and \( \lambda \) denotes the relaxation rate of dynamical variable \( \Gamma(t) \). As shown in Figure 2, the predictions of the analytic results are in perfect agreement with the simulation results for the reaction system with nonequilibrium state distribution \( P_0^e(\Gamma) \) as well as for the case with the equilibrium initial state distribution. Figure 2 clearly shows that the mean product number \( <n(t)^e>_e \) of the reaction system with equilibrium initial state distribution increases linearly in measurement time \( t \), as given in Eq. (21); in contrast, the mean product number \( <n(t)^e>_0 \) has nonlinear time dependence, as given in Eq. (29), for the reaction system with nonequilibrium initial state distribution \( P_0^e(\Gamma) \) defined in Eq. (27). In calculation of the results shown in Figure 2, \( b \) and \( k_0^e \) are chosen to be units of length and time. The values of other parameters used are chosen to be \( k\sigma^2/k_0 = 1 \) and \( \lambda/k_0^e = 2 \times 10^{-3} \).

Our stochastic simulation results are in good agreement with predictions of the analytic results also for a higher order moment of the number distribution of the product molecules, which will appear somewhere else shortly.

Summary

We introduce novel chemical kinetics recently proposed for description of the fluctuations in reaction times and in the number of product molecules in a small and heterogeneous biological system. We review renewal chemical kinetics that could provide successful quantitative interpretation of randomness parameter data in fluctuating enzymatic turnover times of \( \beta \)-galactosidase and discuss the information that can be extracted from the analysis of single enzyme turnover time fluctuations. We discuss generalization of renewal theory for description of chemical fluctuation or the number distribution of the product molecules of a multistep biopolymer reactions occurring in a dynamically heterogeneous environment. We also present new stochastic simulation results for the chemical fluctuation of a dynamically heterogeneous reaction system and investigate the effects of the initial state distribution on the probabilistic outcome of the dynamically heterogeneous reaction system. The simulation results are found to be in good agreement with predictions of the analytic results obtained from the generalized master equation. The time-dependence of the chemical fluctuation of the biopolymer system with a nonequilibrium initial condition turns out qualitatively different from that predicted by renewal statistics.

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References

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22. The value of $K_M$ reported in ref. 9 is 380 mM. With use of the latter value of $K_M$, Eq. (6) could not provide an excellent quantitative interpretation of the randomness parameter data. See Figure 5 of ref. 17.