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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 biopolymer reaction occurring in a dynamically heterogeneous environment. New stochastic simulation results are presented for the chemical fluctuation of a dynamically heterogeneous reaction system, which clearly show the effects of the initial state distribution on the chemical fluctuation. Our stochastic simulation results are found to be in good agreement with predictions of the analytic results obtained from the generalized master equation.

Key Words : Chemical fluctuation, Nonclassical kinetics, Single enzyme kinetics, Reaction event counting

Introduction

A chemical reaction is, in principle, a stochastic process and the number of the product molecules generated in a time interval is a random variable with a time-dependent probability distribution. While being negligible in a macroscopic 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.[1-5] 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.[6,7] 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.[8,9] 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.[10] 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,[11] catalytic turnover of a single enzyme,[8,9] the gene expression from a DNA,[10] and single molecule DNA sequencing.[12,13] Individual reaction trajectories recorded in these single molecule experiments constitute ideal data for investigation of probabilistic dynamics of the biopolymer reaction systems.

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.[9,14] 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.[15-17] Recently, a generalized MM relation is established for the mean turnover time of a general multistate enzyme reaction model, which reduces to the MM relation whenever the detailed balance condition between different states is satisfied.[18]

However, the conventional chemical kinetics is not so satisfactory in description of statistical fluctuations contained in biopolymer reaction trajectories.[8,9] 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.[9,11] 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.

exponential function.[19] Recently, a general quantitative description of fluctuations in single enzymatic turnover times was also achieved,[17,18,20] for which a generalization of chemical kinetics was made in description of non-Poisson reaction processes of enzyme-substrate (ES) complex.[16,21]

In the next section, we will review several theories given 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 general model of multistep biopolymer catalysis occurring in a dynamically heterogeneous environment. In the next section, we will introduce a new stochastic simulation method for 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 perfect 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, but the probabilistic outcome of the biopolymer reaction system with the equilibrium initial condition is qualitatively the same as that of a renewal reaction process at long times.

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.[16,17] 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, $\phi_1^0(t)$, $\phi_{-1}(t)$, and $\phi_2(t)$ for the three elementary reaction processes, $E+S \xrightarrow{\phi_1^0(t)} ES$, $E+S \xleftarrow{\phi_{-1}(t)} ES$, and $E+S \xrightarrow{\phi_2(t)} E+P$. 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 $\phi_1^0(t)dt$ 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 ϕ_1^0(t) signifies that the RTD of the enzyme substrate encounter process is normalized, i.e. $\int_0^\infty d\tau \phi_1^0(\tau) = 1$. On the other hand, $\phi_{-1(2)}(t)dt$ denotes the probability that the dissociation (catalytic) reaction of ES complex is completed in time interval $(t, t + dt)$, given that the ES complex is prepared at time 0. In contrast to $\phi_1^0(t)$, $\phi_{-1}(t)$ or $\phi_2(t)$ for the dissociation or the catalytic reaction of the ES complex does not satisfy the normalization condition; instead $\int_0^\infty \phi_{-1}(t)dt$ and $\int_0^\infty dt\phi_2(t)$ 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. $\int_0^\infty dt[\phi_{-1}(t)+\phi_2(t)] = 1$. The relation of the enzymatic turnover time distribution ψ to the reaction time distributions, ϕ_1, ϕ_{-1}, and ϕ_2, of the elementary reaction processes is given in Laplace domain as follows:

$$\hat{\psi}(u) = \frac{\hat{\phi}_1^0(u)\hat{\phi}_2(u)}{1-\hat{\phi}_1^0(u)\hat{\phi}_{-1}(u)} \tag{1}$$

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

The RTD, $\phi_1^0(t)$, of the Poisson enzyme substrate encounter process ($E+S \xrightarrow{k_1} ES$) is given by $\phi_1^0(t) = k_1[S]\exp(-k_1[S]t)$. The RTD, $\phi_{-1}(t)$ and $\phi_2(t)$ for the dissociation ($E + S \leftarrow ES$) and the catalytic reaction ($ES \rightarrow E + P$) of the ES complex are given by $\phi_{-1}(t) = \phi_{-1}^0(t)\int_t^\infty d\tau \phi_2^0(\tau)$ and $\phi_2(t) = \phi_2^0(t)\int_t^\infty d\tau \phi_{-1}^0(\tau)$, respectively,[21] where $\phi_{-1(2)}^0(t)$ 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, $\phi_{-1}^0(t)$ and $\phi_2^0(t)$ are given by $k_{-1}\exp(-k_{-1}t)$ 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 $\hat{\phi}_1^0(u) = k_1[S]/(u + k_1[S])$, $\hat{\phi}_{-1}(u) = k_{-1}/(u + k_{-1} + k_2)$, and $\hat{\phi}_2(u) = k_2/(u + k_{-1} + k_2)$ into Eq. (1), one gets

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

where λ and ξ are given by $\lambda = k_1[S] + k_{-1} + k_2$ and $\xi = k_2k_1[S]$. 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;

$$\psi_C(t) = \frac{\alpha\beta}{\beta-\alpha}(e^{-\alpha t}-e^{-\beta t}). \tag{3}$$

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

$$<t>_C = <t>_{\min}\left(1+\frac{K_M}{[S]}\right), \tag{4}$$

where $<t>_{\min}$ and K_M are the mean turnover time in the high substrate concentration limit and the Michaelis-Menten constant given by $<t>_{\min} = k_2^{-1}$ and $K_M = (k_{-1} + k_2)/k_1$.

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.[22] 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.[17] Depending on the microscopic reaction dynamics of the ES complex, the functional form for $\phi_{-1}(t)$ and $\phi_2(t)$ can be various. However, it is possible to obtain the expression for the mean $<t>$ and the randomness parameter R of enzymatic turnover time distribution $\psi(t)$ without assuming a particular functional form for $\phi_{-1}(t)$ and $\phi_2(t)$. The expression for the mean of enzymatic turnover time distribution $\psi(t)$ conforms to the conventional MM equation , which is given by[16,17]

$$<t> = \frac{\bar{n}+1}{k_1[S]} + \bar{n}<t_{-1}> + <t_2> . \tag{5}$$

Here $\bar{n}$ is the average number of dissociation event per each enzymatic turnover given by $\bar{n} = p_{-1}/p_2$ with $p_{-1(2)}$ being the reaction probability, $\int_0^\infty dt\phi_{-1(2)}(t)$, of ES complex for the dissociation (catalytic) reaction, $ES \rightarrow E + S(P)$. In Eq. (5), $<t_{-1(2)}>$ denotes the mean dissociation (catalytic) reaction time of the ES complex in the presence of the competing catalytic (dissociation) reaction, defined by $\int_0^\infty dt\, t\phi_{-1(2)}(t)/p_{-1(2)}$. By making comparison between Eq. (5) and Eq. (4), one can identify K_M and $<t>_{\min}$ as $K_M = (k_1 <t_{ES}>)^{-1}$ and $<t>_{\min} = <t_{ES}>/p_2$ where $<t_{ES}>$ is the mean lifetime of the ES complex, defined by $<t_{ES}> = p_{-1}<t_{-1}> + p_2<t_2>$. On the other hand, the expression for the randomness parameter R defined by $R = (<t^2>-<t>^2)/<t>^2-1$ is much different from the result of the conventional chemical kinetics[17]:

$$R = R_\infty \frac{x(x-\eta)}{(x+1)^2} . \tag{6}$$

Here R_∞, η, and x are given by $R_\infty = p_2 q_{ES}/<t_{ES}>^2$, $\eta = 2p_2<t_2>/(R_\infty<t_{ES}>)$, and $x = [S]/K_M$ with q_{ES} being defined by $q_{ES} = p_2(<t_2^2> - 2<t_2>^2) + p_{-1}(<t_{-1}^2> - 2<t_{-1}><t_2>)$. q_{ES} is the parameter representing the stochastic property of the reactions of the ES complex. When the dissociation and the catalytic reaction processes of the ES complex are Poisson processes, q_{ES} vanishes. It is known that q_{ES} appearing in R_∞ of Eq. (6) assumes a positive value if the reaction processes of ES complex is a generalized Poisson process of which rate coefficient, $k_{-1}(\mathbf{r})$ or $k_2(\mathbf{r})$, is dependent on microscopic configuration $\mathbf{r}$ of the ES complex. On the other hand, q_{ES} assumes a negative value when the reaction of ES complex is a multi-step reaction composed of consecutive Poisson reaction processes, $ES \rightleftarrows I_1 \rightleftarrows \cdots \rightleftarrows I_n \rightarrow E + S(P)$ with I_k being the k-th intermediate state during the reaction of ES complex.[17]

Equations (5) and (6) for the mean turnover time and the randomness parameter hold whether or not the normalized reaction time distribution $\phi_{-1}(t)/p_{-1}$ for the dissociation reaction of ES complex is the same as $\phi_2(t)/p_2$ 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 $\phi_2(t)/p_2$. For the latter model, the physical interpretation of q_{ES} and ηR_∞ in Eq. (6) become simpler; q_{ES} is the variance $<t_{ES}^2> - <t_{ES}>^2$ of the conformation-dependent mean lifetime of the ES complex and ηR_∞ 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, $<t_{ES}>$, $<t_{ES}^2> - <t_{ES}>^2$, 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 $\phi_{-1}^{(j)}(t)$ and the forward RTD $\phi_2^{(j)}(t)$ are dependent on enzyme index j ($1 \le j \le M$). Each individual enzyme reaction system has its own RTD, $\psi_i(t)$, for enzyme reaction, which is given by Eq. (1) with ϕ_{-1} and ϕ_2 being replaced by $\phi_{-1}^{(j)}$ and $\phi_2^{(j)}$ 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 $<<t>>_M = \sum_{j=1}^{M}\langle t\rangle_j P_j$ and $<<t^2>>_M = \sum_{j=1}^{M}\langle t^2\rangle_j P_j$, 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, $\sum_{j=1}^{M} P_j = 1$. The first two moments of the enzymatic turnover time distribution of the nonergodic heterogeneous enzyme system is given by

$$<<t>>_M = <\alpha>_M + <\beta>_M \frac{1}{[S]} , \tag{7}$$

$$<<t^2>>_M = 2<\beta^2>_m [S]^{-2} + 2(2<\alpha\beta>_M - <\beta<t_2>>_M)[S]^{-1} + <\gamma>_M, \tag{8}$$

where $<x>_M$ denotes the average $\sum_{j=1}^{M} x_j P_j$ of x_j over the enzyme index j. α_j, β_j, and γ_j are defined by $\alpha_j = <t_2>_j + \bar{n}_j<t_{-1}>_j$, $\beta_j = 1/(k_1 p_2)_j$, and $\gamma_j = <t_2^2>_j + 2\bar{n}_j<t_2>_j<t_{-1}>_j + 2\bar{n}_j^2<t_{-1}>_j^2 + \bar{n}_j<t_2^{-1}>_j$. If we define $<R>$ by $<R> \equiv [<<t^2>>_M - 2<<t>>_M^2]/<<t>>_M^2$, the expression for $<R>$ is given by

$$<R> = \frac{<R>_0 + <R>_\infty x(x-\eta)}{(x+1)^2} , \tag{9}$$

where x denotes $[S]/K_M$ with K_M being equal to $<\beta>_M/<\alpha>_M$. $<R>_0$ and $<R>_\infty$ denote $\lim_{[S]\to 0}\langle R\rangle$ and $\lim_{[S]\to\infty}\langle R\rangle$, respectively given by $2(<\delta\beta^2>_M/<\beta>_M^2)$ and $<\gamma>_M/<\alpha>_M^2 - 2$. In Eq. (9), η designates $2(<\beta<t_2>>_M - 2<\delta\alpha\delta\beta>_M)/(<\alpha>_M<\beta>_M<R>_\infty)$. 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; $\lim_{[S]\to 0} R$ vanishes whereas $\lim_{[S]\to 0}\langle R\rangle\ (\equiv <R>_0)$ 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:

$$\psi_{\mathrm{GC}}(t) = \int dk_2 w(k_2)\,\psi_C(t)\,. \tag{10}$$

Noting that the n-th moment $<t^n>_{\mathrm{GC}}(\equiv \int_0^\infty t^n \psi_{\mathrm{GC}}(t)dt)$ of $\psi_{\mathrm{GC}}(t)$ is given by $<t^n>_{\mathrm{GC}} = \int dk_2 w(k_2)<t^n>_C$ with $<t^n>_{\mathrm{C}}$ being the n-th moment of $\psi_{\mathrm{C}}(t)$ given in Eq. (3), one can recover Eq. (4) for the mean $<t>_{\mathrm{GC}}$ of the enzymatic turnover distribution $\psi_{\mathrm{GC}}(t)$ with K_M in Eq. (4) being replaced by $K_M^{\mathrm{GC}} = (k_{-1} + <k_2^{-1}>_w^{-1})/k_1$. Hereafter $<k_2^{-n}>_w$ is given by $<k_2^{-n}>_w = \int dk_2 w(k_2) k_2^{-n}$. We obtain the expression of the randomness parameter R_{CG} associated with $\psi_{\mathrm{GC}}(t)$ as follows:

$$R_{\mathrm{GC}} = 2\left[\frac{\sigma_{k_2^{-1}}^2}{<k_2^{-1}>^2}\frac{(1+p_{-1}^{\mathrm{GC}}x)^2}{(1+x)^2} - \frac{p_2^{\mathrm{GC}}x}{(1+x)^2}\right]. \tag{11}$$

Here, $\sigma_{k_2^{-1}}^2$ and x respectively denote the variance of k_2^{-1} and the substrate concentration in unit of K_M^{GC}, i.e. $\sigma_{k_2^{-1}}^2 = <k_2^{-2}> - <k_2^{-1}>^2$ and $x = [S]/K_M^{\mathrm{GC}}$. p_{-1}^{GC} and p_2^{GC} in Eq. (11) are defined by $p_{-1}^{\mathrm{GC}} \equiv k_{-1}/(k_{-1} + <k_2^{-1}>^{-1})$ and $p_2^{\mathrm{GC}} \equiv <k_2^{-1}>^{-1}/(k_{-1} + <k_2^{-1}>^{-1})$.

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} $(\equiv -2p_2^0 x/(1+x)^2$ where p_2^0 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 $\psi_{\mathrm{GC}}(t)$ 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 $\psi_{\mathrm{GC}}(t)$ 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,

$$R_0\left(\equiv \lim_{[S]\to 0}\frac{<t^2> - <t>^2}{<t>^2} - 1\right) = \frac{2(<k_2^{-2}>_w - <k_2^{-1}>_w^2)}{<k_2^{-1}>_w^2}, \tag{12}$$

which could not vanish for any probability density function $w(k_2)$ with a finite variance. This fact indicates that $\psi_{\mathrm{GC}}(t)$ cannot be the correct enzymatic turnover time distribution of the β-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, $\psi_{\mathrm{GC}}(t)$ is the exact enzymatic turnover time distribution for such statically 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 $\psi_{\mathrm{C}}(t)$ 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 $\psi_{\mathrm{GC}}(t)$. However, the behavior of the randomness parameter data reported in ref. 9 is inconsistent with that of the statically 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 $R_\infty = 1.57$ and $\eta = 0.624$, and $K_M \cong 20$ μM, which yield $p_2 \cong 0.49$, and $(<t_{ES}^2> - <t_{ES}>^2)/<t_{ES}>^2 \cong 3.2$.

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:

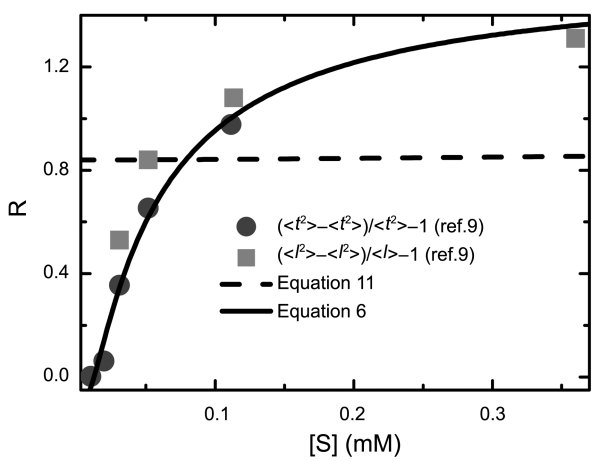

Figure 1. (circle) Randomness parameter for turnover time fluctuation of β-galactosidase enzyme. (square) Mandel's Q parameter estimated from intensity fluctuation of light emission from product molecules of β-galactosidase catalysis (Dashed line) Result of Eq. (11) (Solid line) Result of Eq. (6), both best fitted to the experimental data.

$\langle t_{ES}\rangle = p_2\langle t\rangle_{\min} = 0.67$ msec. In addition, the value of the bimolecular rate coefficient k_1 associated with the enzyme-substrate encounter reaction can be obtained from the MM constant by $k_1 = 1/(K_M\langle t_{ES}\rangle) \cong 7.46\times10^7$ M^{-1} sec^{-1}.

As shown in the next section, the single enzyme reaction is not really a renewal process, as the turnover time distribution of an enzyme would change in time in line with thermal fluctuations of the enzyme's conformation with a wide range of time scale. For this reason, the enzymatic turnover times are correlated, and the renewal kinetics would not be enough for a quantitative description of the turnover events counting statistics or the probability $P_m(T)$ that we observe m enzymatic turnover reactions in observation time T.[24-26] However, when the observation time is much longer than the conformational relaxation time of the ES complex, the distribution of single enzymatic turnover times can be described by Eq. (1).[17] For a particular realization of a single enzymatic turnover, the enzymatic turnover time t is given by $t = t_1 + n(t_{-1} + t_1) + t_2$ where t_1, t_{-1} and t_2 respectively denote the reaction times associated with $E + S \rightarrow ES$, $E + S \leftarrow ES$, and $ES \rightarrow E + P$, and n denotes the number of dissociation-association cycles realized during the single enzymatic turnover. As long as the probability density functions of reaction times, t_{-1} and t_2, of the ES complex are independent of the number, n, of dissociation-association cycles in the single enzymatic turnover, the joint probability $\psi_n(t)dt$ that the single enzymatic turnover time lies between t and $t + dt$ and n cycles of dissociation-association reactions occur during the single enzymatic turnover can be represented as $\hat{\psi}_n(u) = \hat{\phi}_1^0(u)[\hat{\phi}_{-1}(u)\hat{\phi}_1^0(u)]^n \hat{\phi}_2(u)$ in Laplace domain for any value of n. Note that $\int_0^\infty dt\,\psi_n(t) = \hat{\psi}_n(0) = (1-p_2)^n p_2$ is nothing but the probability that the ES complex suffers n cycles of dissociation-association reactions during a single enzymatic turnover. Note, in addition, that $\sum_{n=0}^{\infty}\psi_n(t)$ yields the single enzymatic turnover time distribution of which Laplace transform is given in Eq. (1). This result indicates that the only assumption involved in Eq. (1) is that probability density functions of reaction times, t_{-1} and t_2, of the ES complex are independent of the number, n, of dissociation-association cycles suffered by the ES complex in a single enzymatic turnover. The significant assumption in the derivation of Eq. (6) from Eq. (1) is that the substrate-enzyme association reaction has a constant steady-state reaction rate, which is widely accepted and also assumed in ref. 9. Nevertheless, when the substrate concentration is low enough, the latter assumption may not hold and the substrate-enzyme association may not be a Poisson process.

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 Γ of the reaction system and the surrounding environment.[24] We begin with introducing two fundamental probability density functions, $\psi_\Gamma^0(t)$ and $\phi_\Gamma^0(t)$, that describe the microscopic dynamics of system state Γ and the Γ-dependent reaction dynamics of the reaction system, respectively: $\psi_\Gamma^0(t)dt$ denotes the probability that a transition from state Γ to other states occurs between t and $t + dt$ for the first time after the transition to state Γ at time 0, and $\phi_\Gamma^0(t)dt$ denotes the probability that a reaction system at state Γ 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 $\psi_\Gamma^0(t)$ and $\phi_\Gamma^0(t)$ 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 Γ at time t and has had n reaction events in time t given that the first reaction event starts at time 0. $p_n(t)$ is defined by $p_n(t) = \sum_\Gamma p_n(\Gamma, t)$. The dynamics of hidden state Γ 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

$$\frac{\partial}{\partial t}p_n(\Gamma,t) = \kappa_\Gamma(t)*[p_{n-1}(\Gamma,t)-p_n(\Gamma,t)]+L(\Gamma)p_n(\Gamma,t), \quad (13)$$

where $f(t)*g(t)$ denotes the convolution integral, $\int_0^t d\tau f(t-\tau)g(\tau)$. 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 Γ. The expression for reaction rate kernel κ_Γ is simple in Laplace domain:

$$\hat{\kappa}_\Gamma(u) = \frac{u\hat{\phi}_\Gamma(u)}{1-\hat{\phi}_\Gamma(u)-\hat{\psi}_\Gamma(u)}. \quad (14)$$

Here, $\hat{\phi}_\Gamma(u)$ and $\hat{\psi}_\Gamma(u)$ denote the Laplace transforms of $\phi_\Gamma(t) = \phi_\Gamma^0(t)\int_t^\infty d\tau\psi_\Gamma^0(\tau)$ and $\psi_\Gamma(t) = \psi_\Gamma^0(t)\int_t^\infty d\tau\phi_\Gamma^0(\tau)$, 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, $F_\lambda(\Gamma, t)$ defined by $\sum_{n=0}^{\infty}\lambda^n p_n(\Gamma,t)$:

$$\hat{F}_\lambda(\Gamma,u)=\sum_{n=0}^{\infty}(\lambda-1)^n\left[\frac{1}{u-L(\Gamma)}\hat{\kappa}_\Gamma(u)\right]^n\frac{1}{u-L(\Gamma)}P_0(\Gamma) \quad (15)$$

Here $P_0(\Gamma)$ denotes the initial distribution of Γ 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 $\langle n(n-1)\cdots(n-l+1)(t)\rangle = (\partial^l/\partial\lambda^l)F_\lambda(t)]_{\lambda=1}$ which $F_\lambda(t) = \int d\Gamma F_\lambda(\Gamma,t)$ are obtained as

$$\langle n(n-1)\cdots(n-1+1)(t)\rangle$$
$$=\mathcal{L}^{-1}\left\{\frac{l!}{u}\int d\Gamma_l\int d\Gamma_{l-1}\cdots\int d\Gamma_0\left[\prod_{j=1}^{l}\hat{\kappa}_{\Gamma_j}(u)\hat{G}(\Gamma_j,u|\Gamma_{j-1})\right]P_0(\Gamma_0)\right\}. \quad (16)$$

Here $\mathcal{L}^{-1}$ denotes the inverse Laplace transform operator, and $\hat{G}(\Gamma_j,u|\Gamma_{j-1})$ denotes the propagator defined by $[u-L(\Gamma)]^{-1}\delta(\Gamma-\Gamma_0)$.

When the relaxation dynamics of Γ occurs on a time scale much shorter than the time scale of the individual reaction event, we can assume that $G(\Gamma_j,u|\Gamma_{j-1})\cong P_{eq}(\Gamma_j)/u$ where $P_{eq}(\Gamma)$ is the equilibrium distribution satisfying $L(\Gamma)P_{eq}(\Gamma)=0$. In the latter case, Eq. (16) reduces to

$$<n(n-1)\cdots(n-l+1)(t)>=\mathcal{L}^{-1}\left\{l!\frac{\hat{\kappa}_{eq}(u)}{u^{l+1}}\right\} \tag{17}$$

where $\hat{\kappa}_{eq}(u)=\int d\Gamma\,\hat{\kappa}_\Gamma(u)P_{eq}(\Gamma)$. One can show that the probability distribution p_n corresponding to the latter moment is

$$\hat{p}_n(u)=\frac{\hat{\kappa}_{eq}(u)^n}{[u+\hat{\kappa}_{eq}(u)]^{n+1}}=\frac{1-\hat{\phi}_{eq}(u)}{u}\hat{\phi}_{eq}^n(u), \tag{18}$$

where $\hat{\phi}_{eq}(u)$ is the equilibrium reaction time distribution defined by $u\hat{\phi}_{eq}(u)/(1-\hat{\phi}_{eq}(u))=\hat{\kappa}_{eq}(u)$. Equation (18) is the same as the result of renewal theory.[28] In general, the slow dynamics of hidden state Γ makes p_n different from Eq. (18). Deviation of a stochastic process from renewal statistics can be estimated by parameter Θ, which is defined by[24]

$$\hat{\Theta}(u)=u^2\{<\hat{n}^2(u)>-<\hat{n}(u)>-2u<\hat{n}(u)>^2\}. \tag{19}$$

One can show that Θ vanishes for renewal process, whose statistics conforms to Eq. (18). When dynamics of hidden state Γ occurs in a time scale much greater than the individual reaction time scale, we can approximate Eq. (16) as

$$<n(n-1)\cdots(n-l+1)(t)>$$
$$=\mathcal{L}^{-1}\left\{\frac{l!}{u}\int d\Gamma_l\int d\Gamma_{l-1}\cdots\int d\Gamma_0\left[\prod_{j=1}^{l}k_{\Gamma_j}\hat{G}(\Gamma_j,u|\Gamma_{j-1})\right]P_0(\Gamma_0)\right\}, \tag{20}$$

where k_{Γ_j} is the effective reaction rate coefficient of the nonrenewal process, defined by $k_\Gamma=\lim_{u\to 0}\hat{\kappa}_\Gamma(u)$.

Two initial state distributions are most relevant to experiment. At first we will consider the RECS for the equilibrium initial state distribution, $P_0(\Gamma)=P_{eq}(\Gamma)$, which is the case where our experimental system is composed of a number of catalytic biopolymer. In modern single molecule spectroscopy, one can choose the initial measurement time along a single molecule reaction trajectory. When the initial measurement time is sampled homogeneously along the long enough single molecule reaction trajectory, the sampled initial state distribution would be the equilibrium state distribution given that the single molecule system is an ergodic system. When $P_0(\Gamma)=P_{eq}(\Gamma)$, we can obtain the following expression for the first two moments of p_n from Eq. (20):[29]

$$<n(t)>_{eq}=k_{eq}t, \tag{21}$$

$$<n^2(t)>_{eq}-<n(t)>_{eq}^2=<n(t)>+2\int_0^t d\tau(t-\tau)<k(\tau)k(0)>_{eq}. \tag{22}$$

Here k_{eq} denote the equilibrium reaction rate defined by $k_{eq}\equiv\int d\Gamma k_\Gamma P_{eq}(\Gamma)$. $<k(\tau)k(0)>_{eq}$ is the rate-rate autocorrelation function defined by $<k(\tau)k(0)>_{eq}=\int d\Gamma\int d\Gamma_0 k_\Gamma G(\Gamma,\tau|\Gamma_0)k_{\Gamma_0}P_{eq}(\Gamma_0)$. It is remarkable that the nonrenewal indicator Θ, defined in Eq. (19), is simply related to the rate-rate autocorrelation function:

$$\Theta(t)=2(<k(\tau)k(0)>_{eq}-k_{eq}^2)=2<\delta k(t)\delta k(0)>_{eq}. \tag{23}$$

Note that Θ(*t*) given in Eq. (23) vanishes at long times at which the relaxation of the reaction rate fluctuation occurs significantly. This tells us that nonrenewal stochastic reaction process obeys renewal statistics at long times. On the other hand, Mandel's Q parameter, which estimates the deviation from Poisson stochastic process, is given by[30]

$$\begin{aligned}Q(t)&\equiv[<n^2(t)>_{eq}-<n(t)>_{eq}^2]/<n(t)>_{eq}-1\\&=2<n(t)>_{eq}^{-1}\int_0^t d\tau(t-\tau)<\delta k(\tau)\delta k(0)>_{eq}\\&=<n(t)>_{eq}^{-1}\int_0^t d\tau(t-\tau)\Theta(\tau).\end{aligned} \tag{24}$$

It does not vanish at long times; instead, it is given by

$$Q_\infty=\lim_{t\to\infty}Q(t)=\frac{2<\delta k^2>_{eq}\xi}{k_{eq}}. \tag{25}$$

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

$$\xi=\int_0^\infty d\tau\frac{<\delta k(\tau)\delta k(0)>_{eq}}{<\delta k^2>_{eq}}. \tag{26}$$

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

Up to now, we discuss RECS for reaction system of which initial distribution is the equilibrium distribution $P_{eq}(\Gamma)$ that satisfies $L(\Gamma)P_{eq}(\Gamma)=0$, 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 $P_0^*(\Gamma)$ of the single molecule system state sampled only at the moment when single molecule reaction begins, which is given by

$$P_0^*(\Gamma)\equiv k(\Gamma)P_{eq}(\Gamma)/\int d\Gamma' k(\Gamma')P_{eq}(\Gamma'). \tag{27}$$

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

$$<n(n-1)\cdots(n-l+1)(t)>_0^*=k_{eq}^{-1}\frac{d}{dt}<n(n-1)\cdots(n-l)(t)>_{eq}. \tag{28}$$

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 $<n(t)>_0^*$ of RECS for the case with

nonequilibrium initial state distribution $P_0^*(\Gamma)$:

$$\begin{aligned} <n(t)>_0^* &= k_{eq}^{-1}\frac{d}{dt}<n(n-1)(t)>_{eq} \\ &= k_{eq}^{-1}\int_0^t d\tau <k(\tau)k(0)>_{eq} \\ &= k_{eq}\left(t + k_{eq}^{-2}\int_0^t d\tau <\delta k(\tau)\delta k(0)>_{eq}\right) \end{aligned} \tag{29}$$

Note that $<n(t)>_0^*$ is nonlinear in time in contrast to the mean reaction number $<n(t)>_{eq}$ given in Eq. (21); the time derivative of $<n(t)>_0^*$ is a monotonically decreasing function of the size t of measurement time,

$$\frac{d<n(t)>_0^*}{dt} = k_{eq}\left[1 + \frac{<\delta k(t)\delta k(0)>_{eq}}{k_{eq}^2}\right], \tag{30}$$

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 $<n(t)>_0^*$ 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, $k(\Gamma)=k_0+\kappa\Gamma^2$, where $\Gamma(t)$ is Gaussian process with the time correlation function being given by $<\Gamma(t+t_0)\Gamma(t_0)>_{eq}=b^2\exp(-\lambda t/2)$. k_0 and κ are time-independent constants. b^2 denotes the variance $<\Gamma^2>_{eq}$ and λ 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 $P_0^*(\Gamma)$ as well as for the case with the equilibrium initial state distribution. Figure 2 clearly shows that the mean product number $<n(t)>_{eq}$ 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 $<n(t)>_0$ has nonlinear time dependence, as given in Eq. (29), for the reaction system with nonequilibrium initial state distribution $P_0^*(\Gamma)$ defined in Eq. (27). In calculation of the results shown in Figure 2, b and k_0^{-1} are chosen to be units of length and time. The values of other parameters used are chosen to be $\kappa b^2/k_0 = 1$ and $\lambda/k_0=2\times10^{-3}$.

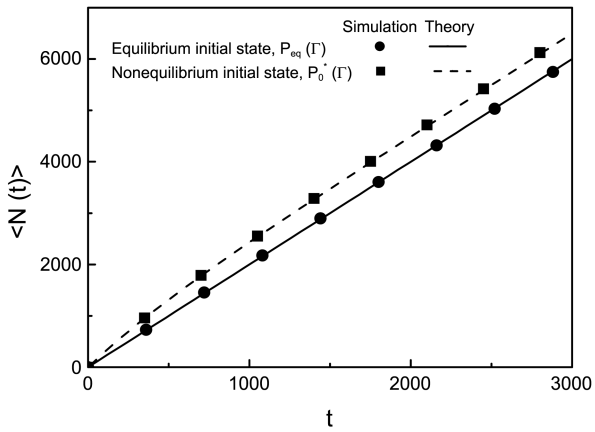

Figure 2. Dependence of the mean number of product molecules for the elementary reaction with fluctuating reaction rate, $k(\Gamma) = k_0 + \kappa\Gamma^2$. $\Gamma(t)$ is Gaussian variable of which time correlation function is given by $<\Gamma(t)\Gamma(0)>_{eq} = b^2\exp(-\lambda t/2)$. (Solid line) Prediction of Eq. (21) (circle) simulation results for the system with equilibrium initial state distribution (Dashed line) Prediction of Eq. (29) (square) simulation results for the system with nonequilibrium state distribution $P_0^*(\Gamma)$.

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 β-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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