

Decay times in turnover statistics of single enzymes

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(Received 9 January 2008; published 3 July 2008)

The first passage times for enzymatic turnovers in nonequilibrium steady state display a statistical symmetry property related to nonequilibrium fluctuation theorems, which makes it possible to extract the chemical driving force from single molecule trajectories in nonequilibrium steady state. Below, we show that the number of decay constants needed to describe the first passage time distribution of this system is not equal to the number of states in the first passage problem, as one would generally expect. Instead, the structure of the kinetic mechanism makes half of the decay times vanish identically from the turnover time distribution. The terms that cancel out correspond to the eigenvalues of a certain submatrix of the master equation matrix for the first exit time problem. We discuss how these results make modeling and data analysis easier for such systems, and how the turnovers can be measured.

DOI: 10.1103/PhysRevE.78.010901

PACS number(s): 87.10.-e, 82.39.-k, 02.50.-r, 05.40.-a

Enzymes are vital to most biochemical reactions, to increase reaction speed and as active components in cellular regulatory networks. Observations of the fluctuations on the single molecule level can lead to new insights into enzymatic mechanisms by revealing more detailed information than ensemble averages measured in bulk experiments [1–7]. The rapid development in single-molecule techniques has made it possible to directly observe turnover events of single enzymes in many systems (see, e.g., Ref. [8] and references therein). This motivates continuing theoretical interest in stochastic kinetics. For example, it was recently shown how nonequilibrium fluctuation theorems make it possible to extract the chemical driving force from turnover traces of single enzymes [4,5].

Here we consider the statistical properties of reversible enzymatic turnovers, and derive another useful property of the turnover times. Their distribution is a sum of exponentially decaying terms, and the number of terms is usually expected to reflect the number of states in the underlying first passage problem. We show that the number of terms in the actual distributions is only half of the expected number, due to the periodicity of the problem. Moreover, we discuss an earlier suggestion [4] of how to detect turnover events, and conclude that it does not correspond to the first passage problem for turnover times. We have previously addressed an analogous issue for stepping motor proteins, and shown that it can lead to systematic misinterpretations of experimental data [6,7]. Our results point to modifications in previously suggested experiments, and also simplify theoretical analysis of turnover time distributions.

In the next section, we introduce our model and the results. After that, we discuss how turnover times can be detected in reversible single-molecule experiments. We then derive our main result. Finally we discuss some implications.

MODEL

Following Qian and Xie [4], we start with a simple sequential kinetic model of an enzyme reaction, sketched in

Fig. 1(a), where a substrate A is converted to a product B through several intermediate states. The overall concentrations of substrate and product molecules are assumed to be kept constant, so that a nonequilibrium steady state is maintained.

As sketched in Fig. 1(b), a \pm turnover is defined as the first arrival in one of the empty states $E_{\pm n}$, after a start in state E_0 . Thus, the turnovers are equivalent to the cycle completion events associated with the work of Hill [9]. If a turnover is completed at $t=0$, the integrated turnover time distribution $w_{\pm}(t)$ is the probability that the next turnover is a \pm , and occurs at time t or earlier. Microscopic reversibility leads to a symmetry property for the forward (+) and backward (−) turnover times [10], namely, $w_+(t) = e^{\Delta\mu/k_B T} w_-(t)$ [3,4]. Here, $\frac{\Delta\mu}{k_B T} = \ln \frac{m_{10}m_{21} \cdots m_{n,n-1}}{m_{01}m_{12} \cdots m_{n-1,n}}$ is the chemical driving force, and m_{ij} is the rate of the transition $E_j \rightarrow E_i$.

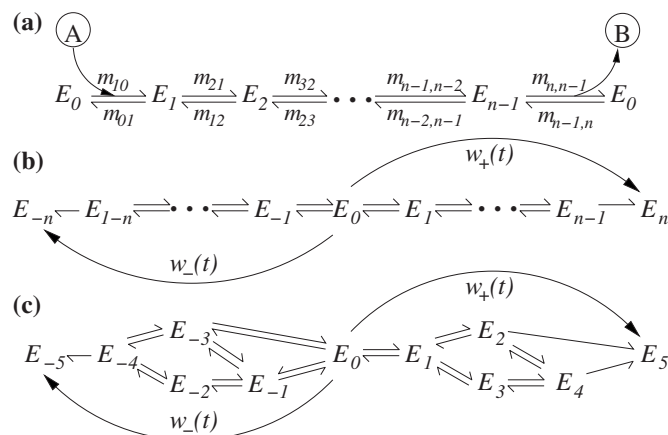


FIG. 1. (a) A multistep enzyme reaction converting A to B. The turnover time distributions $w_{\pm}(t)$ are given by the first exit time problem in (b). The enzyme starts in state E_0 and is absorbed in E_n or E_{-n} . The rate constants m_{10} and $m_{n-1,n}$ are pseudo-first-order, i.e., proportional to the concentrations of A and B, respectively. In the more general scheme studied in Ref. [3], arbitrary transitions within a cycle are allowed. An example of this, with $n=5$ different enzymatic states, is illustrated in (c). By periodicity, state E_k is equivalent to E_{k+n} , and $m_{ij} = m_{i+n,j+n}$.

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The turnover time distributions are of the general form

$$w_{\pm}(t) = \alpha_0^{\pm} + \sum_{k=1}^N \alpha_k^{\pm} e^{\lambda_k t}. \quad (1)$$

The characteristic decay times $\tau_k = -1/\lambda_k$ and prefactors α_k^{\pm} depend on the transition rates and topology of the underlying kinetic mechanism. Hence, this mechanism can be studied by fitting theoretically predicted distributions to experimental data.

The underlying first passage problem is governed by a system of linear master equations [11], one equation for each state from which the systems escapes. Since one generally expects a matrix of dimension N to have N eigenvalues, a simple and common way to estimate the number of states is to count how many exponential terms are needed to fit the first passage time distribution. As illustrated in Fig. 1, the turnover events correspond to escape events from states $E_{1-n}, E_{2-n}, \dots, E_{n-1}$. Hence, the number of states in the first passage problem is $N=2n-1$ in this case, where n is the number of intermediate states of the enzyme-substrate complex. As shown below, the structure of this first passage problem, as well as the more general one studied by Wang and Quan [3], makes $n-1$ of the coefficients α_k^{\pm} in $w_{\pm}(t)$ vanish. This leaves only n terms in the distribution, i.e., the above estimate fails by a factor 2. Before we derive this, we discuss how turnover times can be detected in reversible single molecule experiments.

DETECTING ENZYMATIC TURNOVERS

Single enzyme experiments using fluorescence techniques often probe the state of an enzyme-substrate complex [12–15], but do not report directly on the number of turnovers. In our example model, a realistic possibility is that the empty states ($E_0, E_{\pm n}, \dots$) can be experimentally distinguished from the other states, but not from each other. If the product concentration is kept very low, it is safe to assume that each departure from an empty state starts a new forward turnover. However, detecting individual forward and backward turnovers in conditions where backward turnovers are possible is more complicated, as the following discussion will show.

In their proposal to measure $\Delta\mu$ directly from turnover traces, Qian and Xie [4] suggested that individual turnover times could be measured by monitoring the net number $\nu_B(t)$ of product molecules, as they are released and absorbed by the reaction $E_{n-1} \rightleftharpoons E_n$. However, this is equivalent to monitoring the position of a processive motor protein. A closer examination reveals that this situation corresponds to a different first passage problem, with quite different statistical properties. This discrepancy can lead to large systematic errors in the estimate for $\Delta\mu$ [6,7].

To see why that measurement will not detect turnovers, note that the turnover event starts in state E_0 and finishes when an enzymatic cycle is completed, i.e., when either E_n or E_{-n} is reached for the first time [2–4]. However, $\nu_B(t)$ does not change during the reaction $E_{1-n} \rightarrow E_{-n}$. Therefore,

backward turnovers cannot be detected by only monitoring changes in ν_B .

The attractive statistical properties of turnover times [3–5] motivate a consideration of how they could be measured, using slightly different experimental setups. One possibility would be to monitor both substrate and product molecules, but one could also imagine various setups involving fluorescence techniques with multiple fluorescence levels, in the spirit of the experiment that demonstrated bidirectional rotation in ATP synthase [16]. To summarize, it is important to make sure that the theoretical first passage problem describes the actual experimental situation.

NUMBER OF DECAY TIMES IN $w_{\pm}(t)$

We now derive our main result, i.e., that the number of exponential terms in the turnover time distributions $w_{\pm}(t)$ are not given by the number of states in the first passage problem, $2n-1$, as one might expect [4]. Instead, $w_{\pm}(t)$ only contains n terms. The decay constants that drop out are the eigenvalues of a certain submatrix of the master equation matrix for the first exit time problem. This result may simplify practical calculations considerably.

The turnover times studied by [4] are the solutions of the first exit problem illustrated in Fig. 1(b). If we label the states $-n, -n+1, \dots, n-1, n$, the system starts in state 0 and is absorbed in states $\pm n$. Let $q_k(t)$ be the probability of being in state k a time t after starting in state 0. The $q_k(t)$ are governed by the master equation

$$\partial_t q_i(t) = \sum_{j \neq i} [m_{ij} q_j(t) - m_{ji} q_i(t)], \quad -n < i < n, \quad (2)$$

with initial condition $q_j(0) = \delta_{j,0}$. Since $\pm n$ are absorbing states, the integrated turnover time distribution functions are given by

$$w_{\pm}(t) = q_{\pm n}(t) = \int_0^t \frac{dq_{\pm n}(t)}{dt} dt = \sum_{j=1-n}^{n-1} m_{\pm n, j} \int_0^t q_j(t) dt. \quad (3)$$

Note that $w_{\pm}(t)$ are not normalized to unity. Instead, the fractions p_{\pm} of \pm turnovers are given by $p_{\pm} = \lim_{t \rightarrow \infty} w_{\pm}(t)$. Introducing the matrix \mathbf{M} and vector $\vec{q}(t)$ with elements

$$M_{ij} = m_{ij} - \delta_{ij} \sum_{k=1-n}^{n-1} m_{ik}, \quad -n < i, j < n, \quad (4)$$

$$\vec{q}(t) = [q_{-n+1}(t), q_{-n+2}(t), \dots, q_{n-1}(t)]^T, \quad (5)$$

the solution of Eq. (2) can be written $\vec{q}(t) = e^{t\mathbf{M}} \vec{q}(0)$.

We restrict our attention to the generic situation in which \mathbf{M} can be diagonalized [17]. In this case, $\vec{q}(t)$ can be expressed in terms of right and left eigenvectors $\vec{a}^{(k)}$ and $\vec{b}^{(k)}$ of \mathbf{M} , namely,

$$\vec{q}(t) = \sum_{k=1}^{2n-1} e^{t\lambda_k} \vec{a}^{(k)} [\vec{b}^{(k)} \cdot \vec{q}(0)]. \quad (6)$$

Note that the eigenvalues λ_k need not all be distinct [18]. Among the $2n-1$ terms, we look for left eigenvectors $\vec{b}^{(k)}$

that are orthogonal to the initial condition $\vec{q}(0)$. Those terms drop out of Eq. (6), and hence from $w_{\pm}(t)$ as well. We introduce

$$\begin{aligned}\vec{y}_+ &= [m_{1-n,0}, \dots, m_{-1,0}]^T, & \vec{y}_- &= [m_{1,0}, \dots, m_{n-1,0}]^T, \\ \vec{v}_+ &= [m_{0,1-n}, \dots, m_{0,-1}]^T, & \vec{v}_- &= [m_{0,1}, \dots, m_{0,n-1}]^T, \\ \vec{b}_-^{(k)} &= [b_{1-n}^{(k)}, b_{2-n}^{(k)}, \dots, b_{-1}^{(k)}]^T, & \vec{b}_+^{(k)} &= [b_1^{(k)}, b_2^{(k)}, \dots, b_{n-1}^{(k)}]^T,\end{aligned}\quad (7)$$

and take \mathbf{Y} as the $(n-1) \times (n-1)$ matrix with elements $Y_{ij} = M_{ij}$ for $0 < i, j < n$, i.e., the master equation matrix for the first exit problem from states $1, 2, \dots, n-1$. Using the periodicity of the transition rates, $m_{i,j} = m_{i+n,j+n}$, and a ‘‘bottle-neck’’ property of state 0, $m_{i,j-n} = m_{j-n,i} = 0$ for $0 < i, j < n$, the left eigenvalue problem for \mathbf{M} can be written

$$\mathbf{M}^T \vec{b}^{(k)} = \begin{bmatrix} \mathbf{Y}^T & \vec{y}_- & \mathbf{0} \\ \vec{v}_-^T & M_{00} & \vec{v}_+^T \\ \mathbf{0} & \vec{y}_+ & \mathbf{Y}^T \end{bmatrix} \begin{bmatrix} \vec{b}_-^{(k)} \\ b_0^{(k)} \\ \vec{b}_+^{(k)} \end{bmatrix} = \lambda_k \vec{b}^{(k)}, \quad (8)$$

where $\mathbf{0}$ is the $(n-1) \times (n-1)$ zero matrix. This structure of \mathbf{M} also holds for the more general turnover time problem studied in Ref. [3] and illustrated in Fig. 1(c). For the sequential model of Fig. 1(b), studied in Ref. [4], \mathbf{M} and \mathbf{Y} are tridiagonal, in which case \vec{y}_{\pm} , \vec{v}_{\pm} have only one nonzero element each. Setting $\vec{b}^{(k)} \cdot \vec{q}(0) = b_0^{(k)} = 0$ in Eq. (8) gives

$$\mathbf{Y}^T \vec{b}_-^{(k)} = \lambda_k \vec{b}_-^{(k)}, \quad (9)$$

$$\vec{v}_- \cdot \vec{b}_-^{(k)} + \vec{v}_+ \cdot \vec{b}_+^{(k)} = 0, \quad (10)$$

$$\mathbf{Y}^T \vec{b}_+^{(k)} = \lambda_k \vec{b}_+^{(k)}. \quad (11)$$

The solutions are given by the eigenvalues of \mathbf{Y}^T , which are also eigenvalues of \mathbf{M} . Since these have equal algebraic and geometric multiplicity by assumption, there are $n-1$ solutions, corresponding to terms that do not contribute to the turnover time distributions in Eq. (6). Hence, $w_{\pm}(t)$ contains at most n exponential terms: those where λ_k is an eigenvalue of \mathbf{M} , but not of \mathbf{Y} .

APPLICATION TO THE SEQUENTIAL MODELS

Analytical expressions for the turnover time distributions are useful for efficient parameter extraction. Our result for the number of exponential terms in $w_{\pm}(t)$ makes the derivation of such expressions easier, and extends the range of system sizes that can be treated analytically.

To illustrate this, we analyze the sequential model in Fig. 1(a), using the ansatz

$$w_{\pm}(t) = p_{\pm}(1 + \alpha_1 e^{\lambda_1 t} + \dots + \alpha_n e^{\lambda_n t}), \quad (12)$$

with $\alpha_k = \alpha_k^{\pm} / p_{\pm}$, together with the initial conditions for $w_{\pm}(t)$. As shown by Qian and Xie [4] (their Ref. [24]), the sequential models satisfy $p_{\pm}^{-1} w_{\pm}(0) = 1 + \sum_k \alpha_k = 0$ and $p_{\pm}^{-1} \partial_t^m w_{\pm}(0) = \sum_k \lambda_k^m \alpha_k = 0$ for $1 \leq m \leq n-1$. This leads to a Vandermonde-type system of equations,

$$\begin{bmatrix} 1 & 1 & \dots & 1 \\ \lambda_1 & \lambda_2 & \dots & \lambda_n \\ \vdots & \vdots & \ddots & \vdots \\ \lambda_1^{n-1} & \lambda_2^{n-1} & \dots & \lambda_n^{n-1} \end{bmatrix} \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \vdots \\ \alpha_n \end{bmatrix} = \begin{bmatrix} -1 \\ 0 \\ \vdots \\ 0 \end{bmatrix}. \quad (13)$$

Solving with Cramer’s rule for the normalized distribution $p_{\pm}^{-1} w_{\pm}(t)$, we get

$$p_{\pm}^{-1} w_{\pm}(t) = 1 + (-1)^n \sum_{k=1}^n e^{\lambda_k t} \prod_{m \neq k} \left(\frac{\lambda_m}{\lambda_k - \lambda_m} \right). \quad (14)$$

This is the distribution of a sum N of independent exponential random variables with mean values $|\lambda_1|^{-1}, |\lambda_2|^{-1}, \dots, |\lambda_n|^{-1}$. The reduced number of unknown coefficients α_k simplifies the analytical computation significantly, especially so for sequential models, where $\sum_k \lambda_k^m \alpha_k \neq 0$ for $m \geq n$ [4].

Analytical calculation of the eigenvalues λ_k means finding the roots of a characteristic polynomial. (For large systems, the eigenvalues must be found numerically. In these cases, root-finding in the characteristic polynomial is usually not the best method [19].) Since the $n-1$ noncontributing time constants are the eigenvalues of \mathbf{Y} , these can be removed from the eigenvalue equation in advance, hence reducing the problem from root-finding in the characteristic polynomial of \mathbf{M} , $P_{\mathbf{M}}(\lambda) = \det(\mathbf{M} - \lambda \mathbf{I})$, which has degree $2n-1$, to root-finding in the polynomial $P_{\mathbf{M}}(\lambda) / P_{\mathbf{Y}}(\lambda)$, which has degree n . This makes it feasible to compute decay constants analytically for larger systems.

CONCLUSION

We have demonstrated that enzymatic turnover times constitute a counterexample to the expectation that the number of states in a first passage time problem is equal to the number of exponential terms in the first passage time distribution. Instead, the number of terms is in this case equal to the number of states per cycle. This number is an important characteristic of the kinetic mechanism of an enzyme, and our results make it possible to estimate it correctly from time series of turnover times.

Furthermore, our results make it easier to derive (semi-)analytical expressions for the turnover time distributions, thus simplifying modeling and data analysis. The approach demonstrated above for a sequential model also works for large systems, if the eigenvalues are computed numerically.

Finally, we have supplied an important clarification to an earlier suggestion [4] on how to detect turnover events. This should make our results, together with earlier predictions [3–5], into useful analysis tools for future experiments, and a further reason to study enzymatic turnovers under reversible conditions.

ACKNOWLEDGMENTS

The author is grateful for constructive input from Hong Qian, Mats Wallin, Michael E. Fisher, and Denis Tsygankov, and financial support from the Royal Institute of Technology and the Wallenberg Foundation.

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