Generalized Haldane equation and fluctuation theorem in the steady-state cycle kinetics of single enzymes

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Enzyme kinetics are cyclic. We study a Markov renewal process model of single-enzyme turnover in nonequilibrium steady state (NESS) with sustained concentrations for substrates and products. We show that the forward and backward cycle times have identical nonexponential distributions: $\Theta_+(t) = \Theta_-(t)$. This equation generalizes the Haldane relation in reversible enzyme kinetics. In terms of the probabilities for the forward ($p_+$) and backward ($p_-$) cycles, $k_B T \ln(p_+/p_-)$ is shown to be the chemical driving force of the NESS, $\Delta \mu$. More interestingly, the moment generating function of the stochastic number of substrate cycle $\nu(t)$, \(e^{-\nu(t)k_B T}\), follows the fluctuation theorem in the form of Kurchan-Lebowitz-Spohn-type symmetry. When $\lambda = \Delta \mu/k_B T$, we obtain the Jarzynski-Hatano-Sasa-type equality \(e^{-\nu(t)k_B T} = 1\) for all $t$, where $\nu \Delta \mu$ is the fluctuating chemical work done for sustaining the NESS. This theory suggests possible methods to experimentally determine the nonequilibrium driving force in situ from turnover data via single-molecule enzymology.

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Most biochemical reactions in a living cell have nonzero flux $J$ and nonzero chemical driving force $\Delta \mu$. The nonequilibrium state of such a reaction is sustained by continuous material and energy exchange with and heat dissipation into its environment [1]. Hence, to understand the state of a biochemical network in an open environment, it is necessary to be able to experimentally measure both $J$ and $\Delta \mu$ in situ. A large literature exists on measuring $J$, but none exists on directly measuring $\Delta \mu$. One could in principle compute $\Delta \mu$ from in situ measurements of the concentrations of the substrate and product of a reaction if its equilibrium constant is known [2]. Alternatively, one should be able to obtain $\Delta \mu$ from fluctuating cycle kinetics of a single enzyme directly. This possibility has been recently investigated in term of stochastic simulations [3]. Here we exam this idea through an analytical model.

Enzyme kinetics are complex mainly due to the many possible intermediates in the form of enzyme-substrate complexes. Recent laboratory measurements with high resolution at the single-molecule level give the waiting time distributions for enzyme cycles [4]. This motivated the present Markov renewal process (MRP) model, also known as the extended kinetics model in the theory of motor proteins [5]. In terms of the MRP, the kinetics of a single enzyme becomes a stochastic sequence of forward and backward cycles as a function of time. We shall denote the number of forward and backward cycles by $\nu_+(t)$ and $\nu_-(t)$, as shown in Fig. 1.

It is obvious that the cycle time distributions give information on the kinetics. In this Rapid Communication we show that the key nonequilibrium thermodynamic quantity, $\Delta \mu$, can be obtained from stochastic data on single-enzyme cycle $\nu(t) = \nu_+(t) - \nu_-(t)$ via two equalities

$$\Delta \mu = k_B T \ln[\langle \nu_+(t) / \nu_-(t) \rangle],$$

$$\langle e^{-\nu(t)k_B T} \rangle = 1 \quad \forall t,$$
mediately yields that of KLS. The symmetry in the generating function implies the FT for $Q$ \cite{12}.

The symmetry implies that $\ln(e^{-Q(0)/k_BT})=0$. This is analogous to the Jarzynski equality \cite{13}, which is surprising since $\langle \overline{Q(t)} \rangle=-k_BT \ln e^{-Q(0)/k_BT}$ is the mean heat dissipated from the NESS, which certainly is not equal to 0; it should always be greater than 0. The Jarzynski equality provides the possibility obtaining a function of state such as the free energy from a nonstationary heat functional $Q(t)$ with finite $t$. This was proposed and experimentally tested for the mechanical work functional on single biological macromolecules such as RNA \cite{14,10}.

The difference between the FTs for $W(t)$ in the limit of infinite $t$ and for $Q(t)$ with any finite $t$ is crucial to real experiments. In heuristic thermodynamic terms, the work functional $W(t)$ \cite{8} is related to the $\Delta \mu_0$ of a reaction and the heat functional $Q(t)$ \cite{11} to $\Delta \mu$. While the former is determined by the transition rate constants, and hence is experimentally accessible in short time, the latter depends on the stationary probability. For cyclic enzymatic turnovers, however, $W=Q$. Hence, the FT associated with enzyme cycle kinetics is particularly simple, and experimentally accessible \cite{3}. Generalizing the Jarzynski equality to open systems, Hatano and Sasa’s equality for the NESS \cite{13} also suggested the possibility of the computing chemical driving force for single-molecule chemical reactions in NESS (see \cite{3,15}).

To show Eqs. (1) and (2), there are two strategies. One is based on traditional Markov models, i.e., master equations, for single-enzyme kinetics. Then both equations can be shown as consequences of the existing FTs \cite{8,11}. An alternative, the more insightful approach is to model the kinetics in terms of a MRP with cycle kinetics. In our model, we shall show a surprising equality between the forward and backward cycle time distributions: $\Theta_+(\tau) = \Theta_-(\tau)$. With this equality, Eq. (1) becomes obvious, and Eq. (2) can be shown in elementary terms, in Eqs. (7)–(11) below.

The equality $\Theta_+(\tau) = \Theta_-(\tau)$ turns out to be a very important relation in enzyme kinetics. This is a key result of this work. It has to do with microscopic reversibility. There is experimental evidence for it, as well as theoretical models proving equal mean time $\langle \Delta T_\tau \rangle = \langle \Delta T_{\tau^-} \rangle$ \cite{16,17}. We shall give a proof for the equal distribution with continuous $\Delta T$ and binary $\Delta \nu$.

\begin{equation}
\omega_\nu(t) = \Pr(\Delta \nu = \pm 1, \Delta T_\ell = t) \quad (\ell \geq 1).
\end{equation}

The equation $\Theta_+(\tau) = \Theta_-(\tau)$ leads to $\omega_\nu(t) = p_\omega \omega(t)$. That is, the random variables $\Delta \nu_\nu$ and $\Delta T_\ell$ are statistically independent.

To show the equality $\Theta_+(\tau) = \Theta_-(\tau)$ for forward and backward cycles, we consider a sequential enzyme reaction as shown in Fig. 3(a) and a corresponding exit problem \cite{23} shown in Fig. 3(b). Starting at the central position $E$, $\omega_\nu(t)$ and $\omega_{\nu^-}(t)$ are the cumulative probabilities of reaching $B+E$ and $A+E$. Since only the first and last steps are irreversible,

\begin{figure}[ht]
\includegraphics[width=\textwidth]{fig2.png}
\caption{(a) Schematics for an enzyme reaction converting substrate $A$ to product $B$. In a NESS, the concentrations for $A$ and $B$, $c_A$ and $c_B$, are controlled through feedback by an experimenter. The cumulative number of $B$ taken out by the time $t$ is denoted by $n(t)$, $-\infty < n(t) < \infty$. (b) The integer-valued $n(t)$ is most naturally modeled by a random walk with forward and backward time distributions $\omega_\nu(t)$ and $\omega_{\nu^-}(t)$ \cite{5}.
\end{figure}

\begin{figure}[ht]
\includegraphics[width=\textwidth]{fig3.png}
\caption{(a) A schematic for an enzyme reaction converting $A$ to $B$. The transition time distribution of a single enzyme converting $A$ to $B$, $\omega_\nu(t)$, and converting $B$ to $A$, $\omega_{\nu^-}(t)$, is intimately related to the exit problem shown in (b) in which $u_1$ and $w_n$ are pseudo-first-order rate constants that depend on the concentrations of $A$ and $B$, respectively: $u_1 = u_1 c_A$, $w_n = w_n c_B$. The scheme in (b) has been used to compute steady-state one-way flux in Hill’s theory on biochemical cycle kinetics \cite{25,6}.
\end{figure}
where \( w_+(t) \) and \( w_-(t) \) both have \( 2n+1 \) exponential terms with the same eigenvalues, one of which is 0. Thus both can be written as \( a_0 + a_1 e^{-\lambda t} + a_2 e^{-2 \lambda t} + \cdots + a_{2n} e^{-2n \lambda t} \). With some straightforward algebra, it can be shown that for all \( 0 \leq m \leq 2n \) [24]

\[
\frac{1}{w_1 w_2 \cdots w_n} \frac{d^m w_+(0)}{dt^m} = \frac{1}{u_1 u_2 \cdots u_n} \frac{d^m w_-(0)}{dt^m}.
\]

Since the functions \( w_+(t) \) and \( w_-(t) \) are completely determined by these initial conditions, which satisfy the linear algebraic system, we have

\[
w_+(t) = \prod_{i=1}^{n} \left( \frac{w_i}{u_i} \right) = e^{-\lambda \mu k_B T},
\]

independent of \( t \). That is, \( \Theta_+(t) = \Theta_+(t) \).

The meaning of the equality now becomes clear: We recall that \( u_1 \) and \( w_1 \) are pseudo-first-order rate constants: \( u_1 = u^0_1 c_A \) and \( w_1 = w^0_1 c_B \). In a chemical equilibrium,

\[
e_B = \frac{u^0_1 w_2 \cdots w_n - 1}{w_1 w_2 \cdots w_n - 1} e^\mu k_B T,
\]

that is, \( w_+(t) = w_-(t) \). Therefore, in a chemical equilibrium not only does the average \( w_+(\infty) = w_-(\infty) \), i.e., the forward flux equals the backward flux, but the detailed kinetics for the transition time distributions has to be equivalent: There is absolutely no statistical difference between the forward and backward reactions. In a NESS when Eq. (6) does not hold true, \( w_+(t) \neq w_-(t) \). But the difference is only in the total probability \( p_+ = w_+(\infty) \) and \( p_- = w_-(\infty) \), the distribution functions \( \Theta_+(t) = \Theta_-(t) \) still hold true. This equality is essential to the KLS symmetry below. It is known that microscopic reversibility has to be satisfied even when a mesoscopic system is in a nonequilibrium steady state [8].

For the number \( k \) of successive renewal events (forward plus backward turnovers) within time \( [0, t] \), let us denote \( (v_k, T_k) = \sum_{i=1}^{k} (\Delta v_i, \Delta T_i) \). The moment-generating function for \( \nu(t) \) is

\[
g_\lambda(t) = \langle e^{-\lambda \nu(t)} \rangle = \sum_{n=0}^{\infty} e^{-\lambda n} \sum_{k=0}^{\infty} \text{Pr}(v_k = n, T_k \leq t, T_{k+1} > t)
\]

\[
\leq \sum_{k=0}^{\infty} \left( \sum_{n-k}^{k} e^{-\lambda n} \text{Pr}(v_k = n) \right) \\
\times \text{Pr}(T_k \leq t, T_{k+1} > t)
\]

\[
= \sum_{k=0}^{\infty} (p_+ e^{-\lambda} + p_- e^{\lambda})^k \text{Pr}(T_k \leq t, T_{k+1} > t).
\]

Equation (8) is obtained because of the independence between \( v_k \) and \( T_k \). Then from Eq. (9) we have the KLS symmetry

\[
\lambda^* = \ln(p_+/p_-).
\]

If \( \ln(p_+/p_-) = \Delta \mu / k_b T \) holds true. We recognize that \( \nu(t) \Delta \mu / k_b T \) is the external chemical work done to the system in a NESS. Hence Eq. (11) is analogous to the Jarzynski equality for a cycle.

If we let \( t \rightarrow \infty \) in Eq. (5), we have \( \ln(p_+/p_-) = \lambda^* = \Delta \mu / k_b T \), which is needed in deriving Eq. (11). This generalizes the well-known result for single-step chemical reactions [25, 6] to any complex enzyme reaction cycle.

We are now also in a position to show Eq. (1). The mean number of net turnovers can be computed from the \( g_\lambda(t) \) given in Eq. (9):

\[
\langle \nu(t) \rangle = \langle \nu_+(t) \rangle - \langle \nu_-(t) \rangle = - \left[ \frac{dg_\lambda(t)}{d\lambda} \right]_{\lambda=0}
\]

\[
= (p_+ - p_-) \sum_{k=0}^{\infty} k \text{Pr}(T_k \leq t, T_{k+1} > t)
\]

\[
= (p_+ - p_-) \\
\times \text{(mean no. of cycles in time } t)\).
\]

Therefore, \( \langle \nu_+(t) \rangle = \frac{n_T}{p_+} \). Furthermore, in the limit of a large \( t \),

\[
\langle \nu(t) \rangle = \langle \nu_+(t) \rangle / \langle \nu_-(t) \rangle \text{, where } \langle \nu_+(t) \rangle = \int_0^t \nu(t) \text{d}t \text{ is the mean time for one cycle, forward or backward. When } p_+ = p_-, \text{ the steady-state flux } J = \lim_{t \rightarrow \infty} \langle \nu(t) \rangle / t = 0 \text{ as expected. When } p_+ > p_-, J > 0.
\]

Studying enzyme-catalyzed biochemical reactions in situ requires methods for measuring \( \Delta \mu \), the NESS chemical driving force. Currently none exists. We propose obtaining \( \Delta \mu \) from stochastic cycle data of a single-enzyme molecule, \( \nu(t) \), via (i) an equality similar to that of Jarzynski and Hatano-Sasa, \( \langle e^{-\nu(t) \Delta \mu / k_B T} \rangle = 1 \); or simply (ii) \( k_b T \ln[\langle \nu_+(t) \rangle / \langle \nu_-(t) \rangle] \). We developed a MRP model for enzyme cycles with arbitrary complex mechanism, and found an equality between the forward and backward cycle time distributions based on microscopic reversibility. This equality is a generalization of what is known as the Haldane relation for reversible enzyme kinetics and recent results in [17]. The model enables us to establish a FT and above equalities (i) and (ii) for any \( t \). Noting that \( (1/\nu(t)) = J \), one thus obtains both the flux \( J \) and the driving force \( \Delta \mu \) for a reaction in a NESS from the fluctuating \( \nu(t) \). The statistical accuracies associated with these measurements were discussed in [3].

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ski’s equality was first established for time-dependent conser-
vation of the FT. For recent reviews, see H. Qian, J. Phys.: Condens. Matter 17, S3783 (2005); H. Qian and D. A. Beard, Biophys. Chem. 114, 213 (2005).


[12] Symmetry \( c_{\lambda} = c_{-\lambda} \) means \( \Sigma_{\lambda} e^{-\lambda a} \Pr(Q/k_bT = a) = \Sigma_{\lambda} e^{-\lambda a} \Pr(Q/k_bT = -a) \) \( \forall \lambda \). Hence \( \Pr(Q/k_bT = a) = \Pr(Q/k_bT = -a) = e^a \). The symmetry is a mathematical version of the FT.

[13] C. Jarzynski, Phys. Rev. Lett. 78, 2690 (1997). While Jarzynski’s equality was first established for time-dependent conser-
vative systems, it was later shown that it is also valid for the NESS of open systems; see T. Hatano and S. I. Sasa, ibid. 86, 3463 (2001).


journ time for each transition. It becomes a Markov process if the distributions of the sojourn times are all exponential and independent of the next state; it becomes a Markov chain if the sojourn times are all equal to 1, and it becomes a renewal process if there is only one state.


[22] The Haldane relation in reversible enzyme kinetics [19] states that the steady-state velocities for forward and backward en-
yzme reactions, \( V_f \) and \( V_r \), have the same saturation dependence on the substrate and product concentrations: \( V_f = \alpha_f [S] / (1 + [S]/K_f + [P]/K_p) \), \( V_r = \alpha_r [P] / (1 + [S]/K_s + [P]/K_p) \), where \( \alpha_f/\alpha_r = K_{eq} \), the equilibrium constant between \( S \) and \( P \). Also see [17].


[24] The initial condition leads to \( \Sigma_{\lambda} e^{-\lambda a} = \Sigma_{\lambda} e^{-\lambda a} = \cdots = \Sigma_{\lambda} e^{-\lambda a} = 1 \) for \( a \). But \( d^n w(0)/da^n = \Sigma_{\lambda} e^{-\lambda a} = \cdots = w_0 \). Furthermore, \( d^n w(0)/da^n = \Sigma_{\lambda} e^{-\lambda a} = \cdots = w_0 \). If \( a \) \( a \) \( a \), then \( d^n w(0)/da^n = \Sigma_{\lambda} e^{-\lambda a} = 0 \). Hence, \( d^n w(0)/da^n = \Sigma_{\lambda} e^{-\lambda a} = 0 \). If \( a \) \( a \) \( a \), then \( d^n w(0)/da^n = \Sigma_{\lambda} e^{-\lambda a} = 0 \). Hence, \( d^n w(0)/da^n = \Sigma_{\lambda} e^{-\lambda a} = 0 \).