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Michaelis−Menten Equation and Detailed Balance in Enzymatic Networks

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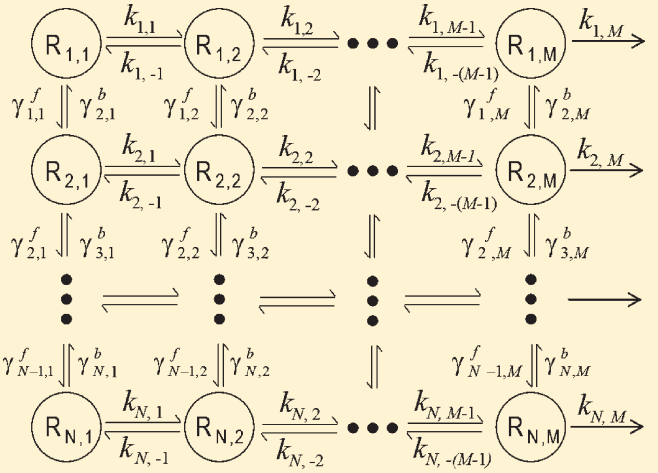
ABSTRACT: Many enzymatic reactions in biochemistry are far more complex than the celebrated Michaelis−Menten scheme, but the observed turnover rate often obeys the hyperbolic dependence on the substrate concentration, a relation established almost a century ago for the simple Michaelis−Menten mechanism. To resolve the longstanding puzzle, we apply the flux balance method to predict the functional form of the substrate dependence in the mean turnover time of complex enzymatic reactions and identify detailed balance (i.e., the lack of unbalanced conformtional current) as a sufficient condition for the Michaelis−Menten equation to describe the substrate concentration dependence of the turnover rate in an enzymatic network. This prediction can be verified in single-molecule event-averaged measurements using the recently proposed signatures of detailed balance violations. The finding helps analyze recent single-molecule studies of enzymatic networks and can be applied to other external variables, such as force-dependence and voltage-dependence.



I. INTRODUCTION

For nearly a century, the classic Michaelis−Menten (MM) mechanism has been the foundation for studying the steady-state kinetic behavior of enzymatic reactions.[1−3] In the basic MM scheme illustrated in Figure 1a, a substrate, S, binds reversibly with a free enzyme to form a substrate−enzyme complex, ES, which reacts irreversibly to yield product. One can show the hyperbolic relationship between the steady-state turnover velocity (i.e., the rate of product formation), v, and substrate concentration, [S]:

$$v = \frac{k_2[\mathrm{S}]}{[\mathrm{S}] + K_\mathrm{M}} \tag{1}$$

where $K_\mathrm{M} = (k_{-1} + k_2)/k_1^0$ is the Michaelis constant and k_2 is the catalytic rate constant. Equation 1 is the celebrated MM equation, one of the most cited results in basic sciences. Enzymatic reactions in biochemistry are often far more complicated than the basic MM mechanism in Figure 1a; yet a surprising number of such reactions are found to follow the functional form of the MM equation,[2,3]

$$v = \frac{[\mathrm{S}]}{a_0[\mathrm{S}] + b_0} \tag{2}$$

where a_0 and b_0 are fitting parameters that may not be interpreted as k_2 and K_M as in eq 1. Why do some enzymatic reactions follow the hyperbolic substrate dependence while many others deviate from the simple functional form? What can we say about an enzymatic reaction if the MM equation fails to describe its turnover rate? Here we address these important questions by relating the hyperbolic form of enzymatic turnover rates to the detailed balance (DB) condition of the underlying kinetics, thus establishing a connection to a fundamental property of nonequilibrium thermodynamics.

This study is also motivated by recent single-molecule studies of enzymatic reactions.[4−8] These studies reveal that proteins fluctuate over a wide range of time scales, but turnover reactions of fluctuating enzymes are often found to obey the MM relation, which was originally derived for enzymes with a unique conformation. This finding has stimulated several theoretical studies to examine various scenarios for which the validity of the MM relation can be established.[4,9,10] Yet, a question remains about how these seemingly different scenarios can be unified on the basis of a generic feature of reaction thermodynamics. Below, we will show that the hyperbolic form is valid as long as there is no circulating current between bindings of different conformations of the fluctuating enzyme, which is a consequence of the detailed balance (DB) condition.

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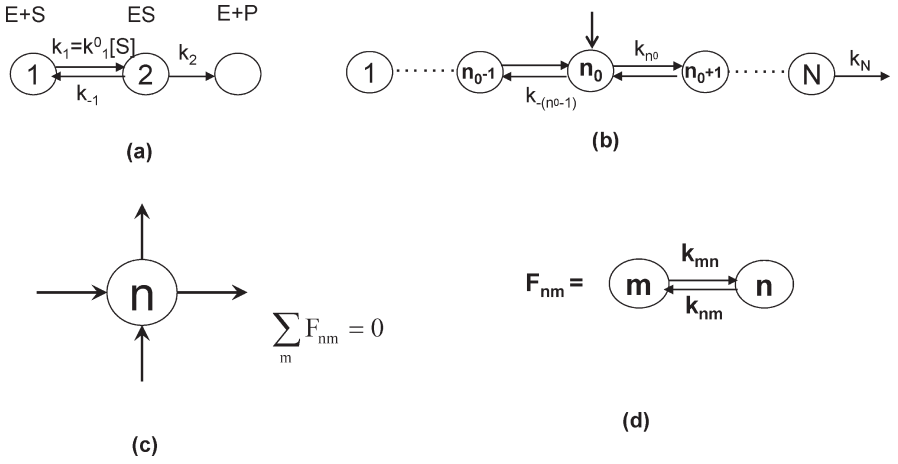

Figure 1. Kinetic models of enzymatic reactions: (a) the generic Michaelis−Menten kinetic scheme, with k_1^0 the binding rate, k_2 the catalytic rate, and k_{-1} the dissociation rate. (b) Linear chain reaction where n_0 is the initial state and two decay rate constants, k_{n_0} and $k_{-(n_0-1)}$, are substrate-dependent. (c) Conservation of integrated flux: $\Sigma_m F_{nm} = 0$ if there is no initial population. (d) Definition of integrated flux F_{nm}.

II. FLUX BALANCE METHOD

The flux balance method allows us to solve for the turnover rate using population fluxes instead of rate constants and establish a direct connection to population current and detailed balance. The standard procedure to solve for the turnover velocity almost always involves matrix inversion, which becomes tedious as the number of states increases and is not necessary if we are only concerned with the functional form of the substrate dependence. As discussed later, there are also other approaches for deriving rate constants, such as pathway analysis and Derrida's method. These two approaches can provide both the average rate and higher order moments but do not explicitly yield substrate dependence, which is the goal of our calculation.

Let us follow the trajectory of a single enzyme in the turnover reaction. Sampling from the initial probability distribution $P_n(0)$, the enzyme propagates through the network and finally reaches the exit states. For a first-order kinetic network, the master equation of the population evolution reads[11]

$$\dot{P}_n = \sum_m k_{nm}P_m - \sum_m k_{mn}P_n \tag{3}$$

where P_n is the conditional probability at state n, m is all the states linked to state n, and k_{mn} is the rate constant from state m to state n. As shown in Figure 1c,d, integration of the evolution equation from $t = 0$ to $t = \infty$ leads to the flux balance condition for state n,

$$-P_n(0) = \sum_m F_{nm} \tag{4}$$

where the terminal distribution vanishes, $P_n(t{=}\infty) = 0$, due to the irreversible depletion step during one turnover reaction. Here, F_{nm} is the integrated flux from state m to state n,

$$F_{nm} = k_{nm}\tau_m - k_{mn}\tau_n = \int_0^\infty k_{nm}P_m - k_{mn}P_n dt \tag{5}$$

and τ_n is the mean residence time at state n,

$$\tau_n = \int_0^\infty P_n(t)\, dt \tag{6}$$

which is the average time that the enzyme spends on the nth state during the turnover process. The sum of the residence times gives the mean turnover time $\langle t\rangle = \Sigma_n\tau_n$. The concept of mean residence time has previously appeared in literature,[11−14] for example, in the paper by Bar-Haim and Klafter.[13] Here, we use the concepts of mean residence time and integrated flux to formulate the flux balance method, which directly relates the mean first passage times (MFPTs) with the integrated flux.

The solution to eq 4 is supplemented by the normalization condition,

$$\sum_N F_N = 1 \tag{7}$$

which is imposed by the fact that population depletes at the exit states N. Here, $F_N = k_N\tau_N$ is the integrated reactive flux associated with the reaction step exiting from the network, i.e., probability of population depletion from the Nth exit state, and k_N is the reactive rate constant from the exit state to form the product. Under the steady-state condition, the enzyme is recycled constantly so that the population will reach a steady state, and the integrated reactive flux is set equal to the initial population distribution,

$$F_N = P(0) \tag{8}$$

which was used as the initial condition for calculating the distribution function of single molecule events.[15−17] Applying the balance condition and boundary condition to the network, we can obtain the integrated flux for each kinetic step, F_{nm}; then the residence time, τ_n; and finally the first passage time, $\langle t\rangle = \Sigma_n\tau_n$. We demonstrate the flux balance method with a simple example of chain reactions and then present an analysis of the Michaelis−Menten equation for fluctuating enzymes with implications for detailed balance.

III. EXAMPLE I: CHAIN REACTIONS AND MICHAELIS−MENTEN EXPRESSION

Our first example is a linear chain reaction in Figure 1b, which has been solved many times in the literature[11−14] and is a benchmark to illustrate the conceptual simplicity of the flux balance method. As will be shown later, the turnover rate of an enzymatic network with microscopic balance is the sum of the contributions from individual chain reactions that compose the network; therefore, the solution for the chain reaction is useful and instructive for further analysis.

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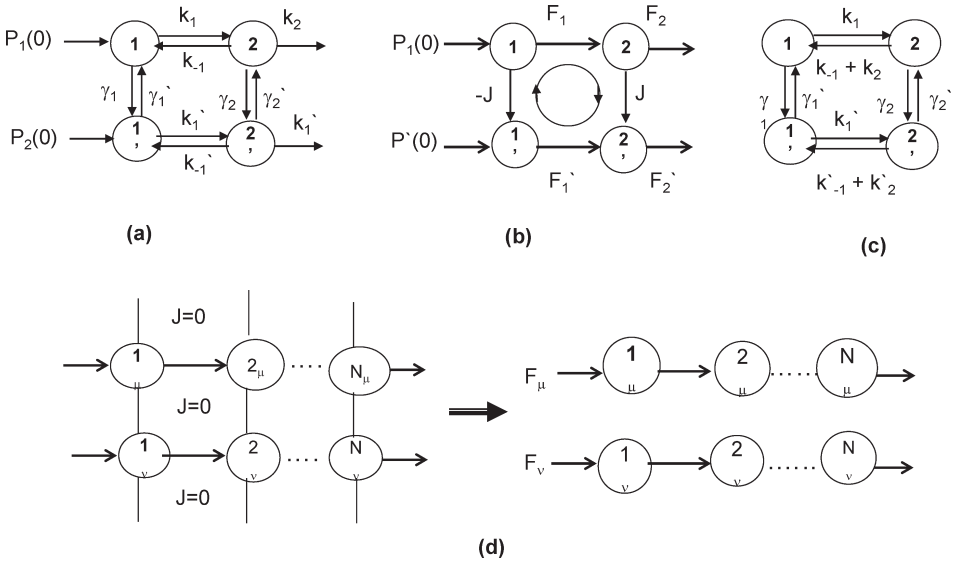

Figure 2. Kinetic models of enzymatic reactions with conformational fluctuations: (a) a generic scheme of an fluctuating enzyme with two conformational channels; (b) the flux−current plot of the generic scheme in (a); (c) an equivalent representation of the generic scheme in (a); (d) an enzymatic reaction network on the left-hand-side, which under the DB condition reduces to a network of effective uncoupled chain reactions on the right-hand-side.

To simplify the notation, we denote $k_n = k_{n+1,n}$, $k_{-n} = k_{n,n+1}$, and $F_n = F_{n+1,n} = k_n\tau_n - k_{-n}\tau_{n+1}$. Then, the flux balance condition in eq 4 reduces to

$$F_n - F_{n-1} = \delta_{nn_0} \tag{9}$$

where n_0 is the entrance state at which the initial population is prepared. For a network with a single exit state, the integrated flux at the exit state is normalized, giving $F_N = 1$ for the linear chain with N the number of states on the chain. From the flux balance condition, we have for $F_n = F_N = 1$ for $n \geq n_0$, giving

$$\tau_n = \frac{k_{-n}}{k_n}\tau_{n+1} + \frac{1}{k_n} \tag{10}$$

and for $n < n_0$, $F_n = 0$, giving

$$\tau_n = \frac{k_{-n}}{k_n}\tau_{n+1} \tag{11}$$

Iterating eqs 10 and 11, we obtain τ_n and the mean first passage time $\langle t\rangle = \Sigma_n\tau_n$, which recovers the results obtained earlier.[11−14] Though the current derivation is simpler, the analysis in ref 14 is more general as it is not limited to rate processes but applicable to any waiting time processes. Note that τ represents the mean residence time in this paper but is used to represent the average lifetime (i.e., the inverse rate constant in rate processes) in ref 14. For the purpose of our analysis, substrate concentration dependence can be obtained from the flux balance equations without writing out the explicit and often lengthy velocity expression:

- The MM mechanism is simply a chain reaction with $N = 2$, $n_0 = 1$, and $k_1 = k_1^0[S]$. Application of eq 10 results in the celebrated Michaelis−Menten (MM) rate equation for enzymatic turnover reactions,

$$\langle t\rangle_M = \frac{1}{k_2} + \frac{k_{-1} + k_2}{k_1k_2} = \frac{1}{k_2} + \frac{K_M}{[S]k_2} \tag{12}$$

which is related to eq 1 via $v = 1/\langle t\rangle_M$.

- For multiple intermediate conformational states along the linear chain in Figure 1b, the mean turnover time takes the MM functional form as

$$\langle t\rangle = \langle t_2\rangle + \frac{k_{-1}\tau_2 + 1}{[S]k_1^0} = \frac{1}{k_2} + \frac{K_M}{[S]k_2} \tag{13}$$

with $k_2 = 1/\langle t_2\rangle$ and $K_M = (k_{-1}\tau_2 + 1)/(k_1^0\langle t_2\rangle)$. Since subsequent steps after binding are decoupled from substrate binding events, both $\langle t_2\rangle$, the first passage time starting from the second state along the chain, and τ_2, the mean residence time at state 2, are independent of the substrate concentration [S].

- If the substrate binds at an intermediate conformational state $n = n_0$ along the chain and the [S]-dependent rate constants are $k_{n_0} = k_{n_0}^0[S]$ and $k_{-(n_0-1)} = k_{-(n_0-1)}^0[S]$, the mean turnover time becomes

$$\langle t\rangle = \langle t_{n_0+1}\rangle + \frac{k_{-n_0}\tau_{n_0+1} + 1}{[S]k_{n_0}^0} + \sum_{n=1}^{n_0-1}\tau_n \tag{14}$$

where the contribution from the states to the left of the binding site, τ_n for $n < n_0$, is [S]-independent. Since both the first and last terms are independent of [S], the MM equation in the form of eq 12 remains valid.

In general, the mean turnover time $\langle t\rangle$ derived from eqs 10 and 11 maintains the MM form of eq 12 regardless of the number of binding sites and their locations along the chain. This conclusion is not surprising as our recent analysis of generic kinetic schemes[14] has demonstrated that enzymatic reactions along a chain can be reduced to the simple generic form in Figure 1a when waiting time distribution functions are used instead of rate constants. Further, the analysis below will rationalize the validity of the MM expression on the basis of the detailed balance condition, thus relating the topology of reaction networks to the functional form of the turnover rate.

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IV. EXAMPLE II: FOUR-STATE MODEL AND DETAILED BALANCE

Now we analyze a simple model of fluctuating enzymes with two conformational channels, a model extensively investigated in the context of single-molecule enzymology. Although the turnover velocity for this model has been derived before,[4,12,18,19] the final expression is often too complicated to determine the general dependence on the substrate concentration. As depicted in Figure 2a, the turnover reaction proceeds from two conformational states with two different sets of rate constants, $\{k_1, k_{-1}, k_2\}$ and $\{k'_1, k'_{-1}, k'_2\}$, respectively, and the two conformational channels interconvert with rate constants γ_1 and γ'_1 between the two free-enzyme states and γ_2 and γ'_2 between the two substrate-enzyme states. The kinetic scheme depicted in Figure 2a includes a closed loop that allows for a nonequilibrium circulating population current J. Thus, we immediately identify the two integrated fluxes associated with conformational fluctuations as J and $-J$, which yield the balance conditions for the enzyme−substrate complex $F_1 = F_2 + J$ and $F'_1 = F'_2 - J$. Here we assume the stationary condition where the fluctuating enzyme after releasing the product will combine with another substrate molecule and start another turnover cycle. We note that the stationary conditions $F_2 = P_1(0)$ and $F'_2 = P'_1(0)$ have been introduced earlier as the initial condition for event-averaged single-molecule measurements of enzymatic turnover reactions.[15]

Given the integrated fluxes, it is straightforward to obtain the following expressions for residence times: $\tau_2 = F_2/k_2$, $\tau'_2 = F'_2/k'_2$, $\tau_1 = F_2K_M/k_2[S] + J/k_1$, and $\tau'_1 = F'_2K'_M/k'_2[S] - J/k'_1$. Summing over the mean residence times, we arrive at the formal expression for the mean turnover time

$$\langle t\rangle = F_2\langle t\rangle_M + F'_2\langle t'\rangle_M + J\left(\frac{1}{k_1} - \frac{1}{k'_1}\right) \tag{15}$$

where $\langle t\rangle_M$ is the MM expression for the mean turnover time. The current J is determined from explicit expressions for the integrated flux of conformational fluctuations, i.e., $\gamma_2\tau_2 - \gamma'_2\tau'_2 = J$ and $\gamma_1\tau_1 - \gamma'_1\tau'_1 = -J$, giving

$$J = \frac{\gamma_2}{k_2}F_2 - \frac{\gamma'_2}{k'_2}F'_2 = \frac{1}{D}\frac{r_2r'_1K'_M - r'_2r_1K_M}{k_2k'_2[S]} \tag{16}$$

with the determinant

$$D = \left(\frac{\gamma_2}{k_2} + \frac{\gamma'_2}{k'_2}\right)\left(1 + \frac{\gamma_1}{k_1} + \frac{\gamma'_1}{k'_1}\right) + \left(\frac{\gamma_1}{k_2}\frac{K_M}{[S]} + \frac{\gamma'_1}{k'_2}\frac{K'_M}{[S]}\right) \tag{17}$$

The ratio of the two conformational channels is determined from

$$\left[\frac{\gamma_2}{k_2}\left(1 + \frac{\gamma_1}{k_1} + \frac{\gamma'_1}{k'_1}\right) + \frac{k_1}{k_2}\frac{K_M}{[S]}\right]F_2 = \left[\frac{\gamma'_2}{k'_2}\left(1 + \frac{\gamma_1}{k_1} + \frac{\gamma'_1}{k'_1}\right) + \frac{k'_1}{k'_2}\frac{K'_M}{[S]}\right]F'_2 \tag{18}$$

which, in combination with normalization $F_2 + F'_2 = 1$, yields the values of F_2 and F'_2. These equations provide the explicit formula for the overall turnover rate as well as the integrated fluxes associated with all the steps in the kinetic scheme. Yet, to establish the validity of the MM expression, we need to examine the substrate dependence, which involves the mean turnover time and current equations in eqs 15 and 16, respectively, but does not require the explicit solution in terms of the rate constants. The explicit solution for the model system has been reported before using the polynomial dependence on the substrate concentration,[18,19] the pathway solution,[12,14] the direct matrix inversion,[4] and Derrida's method.[20,21] Our derivation is simple and relates directly to the unbalanced current J in the cyclic kinetics.

An implication of the turnover rate expression is an intuitive interpretation of the validity of the hyperbolic form in the presence of conformational fluctuations, a key issue in single-molecule enzymology. To address the issue, we examine eq 15 in the limit of zero current, $J = 0$, when the last term in eq 15 vanishes. The probabilities F_2 and F'_2 are determined by $\gamma_2F_2/k_2 = \gamma'_2F'_2/k'_2$, where γ_2 and γ'_2 are the interconversion rate constants at the enzyme−substrate complex. Then, F_2 and F'_2 are independent of [S], and the turnover rate is an inhomogeneous sum of the MM rate associated with each conformation channel, i.e, $\langle t\rangle = F_2\langle t\rangle_M + F'_2\langle t'\rangle_M$. Consequently, a sufficient condition for the validity of MM equation is detailed balance, defined explicitly as

$$\frac{\gamma_1}{\gamma_2}K_M = \frac{\gamma'_1}{\gamma'_2}K'_M \tag{19}$$

which is obtained by setting $J = 0$ in eq 16. In fact, the enzymatic turnover reaction can be described as a reversible reaction shown in Figure 2c such that eq 19 is equivalent to the detailed balance (DB) condition

$$k_1\gamma_2(k'_{-1} + k'_2)\gamma'_1 = k'_1\gamma'_2(k_{-1} + k_2)\gamma_1 \tag{20}$$

where the left side is the product of clockwise rate constants, and the right side is the product of counterclockwise rate constants. The simple four-state model in Figure 2c and the corresponding detailed balance condition has been discussed in theoretical analysis of on−off blinking traces.[22]

The DB condition in eq 19 or eq 18 suggests several possible scenarios: (i) $\gamma_1 = \gamma'_1 = 0$; (ii) $\gamma_2 = \gamma'_2 = 0$; (iii) $\gamma_1/\gamma'_1 = \gamma_2/\gamma'_2$ and $K_m = K'_M$. The first two scenarios correspond to kinetic schemes without any closed loops, i.e., linear kinetics, whereas the third scenario corresponds to a kinetic scheme where the population distribution remains constant between conformational channels at all chemical states involved in the reaction. The latter case has been studied extensively in the analysis of event-averaged measurements of single-molecule turnover experiments. When the microscopic DB condition (strong condition) is not satisfied, we can identify approximate conditions (weak conditions) when the hyperbolic form remains a reasonable description. Close examination of eqs 15−18 suggests the following scenarios: (iv) $\gamma_2/k_2 \to 0$ and $\gamma_1/k_1 \to 0$; (v) $\gamma_1/k_1 \to \infty$ and $\gamma'_1/k'_1 \to \infty$; (vi) $\gamma_2/k_2 \to \infty$, $\gamma'_2/k''_2 \to \infty$, and $k_1 = k'_1$. Both scenarios (iv) and (v) correspond to the weak condition of $D \propto 1/[S]$ so that J is independent of [S]. The last scenario (vi) corresponds to the case of vanishing last term in eq 15 and [S]-independence in eq 18. These special scenarios have been previously proposed by the Xie group and by Gopich and Szabo,[4,9,10] and are now interpreted on the basis of flux conservation in eq 4 and the detailed balance condition in eq 15. Our solution suggests a general condition for the validity of the MM expression, which not only unifies the kinetic schemes

considered previously but also applies to arbitrarily complex enzymatic networks.

V. GENERALITY

We now generalize the conclusion derived for the four-state model in Figure 2a to an enzymatic network consisting of many conformations of linear chain reactions, as illustrated in Figure 2d: The MM relation in the form of eq 1 remains valid as long as there is no unbalanced current in the kinetic steps associated with substrate binding. As a result, the DB condition is obeyed in every conformational kinetic loop so the enzymatic turnover reaction (i.e., the chain reaction) is decoupled from conformational fluctuations, and the mean turnover rate is simply the weighted sum of the contributions of chain reactions associated with conformational states (i.e., the right-hand-side of Figure 2d). Explicitly, under the DB condition, the mean turnover time in the enzymatic network can be written in a general form as

$$\langle t\rangle = \sum_{\mu} F_{\mu}\langle t\rangle_{\mu} = \frac{1}{\bar{k}_2} + \frac{K_M}{[S]\bar{k}_2} \tag{21}$$

where index μ denotes the conformational channel. In eq 21, $\langle t\rangle_{\mu}$ is the mean turnover time associated with each channel as given in eq 13, and F_{μ} is the corresponding exit probability (i.e., the integrate reactive flux), which is independent of the substrate concentration in the absence of current. Therefore, in eq 21, we introduce the effective catalytic rate

$$\bar{k}_2 = 1/\sum_{\mu} F_{\mu}\langle t_2\rangle_{\mu} \tag{22}$$

and the effective Michaelis constant

$$K_M = \sum_{\mu} F_{\mu}K_{\mu,M}\langle t_2\rangle_{\mu}/\sum_{\mu} F_{\mu}\langle t_2\rangle_{\mu} \tag{23}$$

Similar relations have been previously suggested for specific models of enzymatic turnovers[4,9] and earlier for on−off reactions,[22] which can now be obtained generally from eq 21.

An interesting application of eq 21 is enzyme specificity, where the single enzyme in Figure 2e binds with different substrates $[S_{\mu}]$ in different conformational channels. By setting a zero interconversion rate for the substrate−enzyme complex state, we have the flux balance relation $F_{\mu}\langle t_{\mu}\rangle = F_{\nu}\langle t_{\mu}\rangle$ and thus the general result for enzyme specificity

$$\frac{P_{\mu}(0)}{P_{\nu}(0)} = \frac{F_{\mu}}{F_{\nu}} = \frac{[S_{\mu}]k_{\mu,2}/K_{\mu,M}}{[S_{\nu}]k_{\nu,2}/K_{\nu,M}} \tag{24}$$

which applies to a steady-state enzymatic reaction with an arbitrary number of substrates, each with an arbitrary number of enzyme conformations.

When the DB condition is violated, the MM functional form will break down, and the turnover rate will depend on the current. Though explicit rate expressions are difficult to obtain, we observe a functional form for the mean turnover time as

$$\langle t\rangle = a_0 + \frac{b_0}{[S]} + \frac{b_1}{c_1 + [S]} + \frac{b_2}{c_2 + [S]} + \cdots \tag{25}$$

where a, b, c are undetermined coefficients. For systems with zero current, only the two leading terms with a_0 and b_0 in eq 25 survive, and we recover the standard MM equation in eq 21. For the four-state model in Figure 2d, we can confirm the non-MM correction term with b_1 and c_1 using the solution in eqs 15−18 and relate this correction term to the presence of the nonequilibrium current J in the cyclic loop. For a general network, since unbalanced currents in different kinetic loops are independent, we can add a non-MM correction term for each nonequilibrium current to the mean turnover time, thus justifying eq 25. In fact, we can rigorously prove the one-to-one correspondence between the unbalanced currents and the independent hyperbolic terms in eq 25 and use this result to demonstrate a broad range of cooperative behaviors in nonequilibrium enzymes.[23] Further, we note that our analysis of the substrate concentration dependence can be extended to other external variables, including voltage in ion channels, workload in molecule motors, and force applied to proteins.[24]

VI. CONCLUDING REMARK

As a final remark, we point out that the nonequilibrium current investigated in this work cannot be measured in ensemble experiments. Because conformational fluctuations are not directly probed experimentally, the macroscopic balance is always maintained between the free enzyme and substrate−enzyme complex.[15] However, in a recent study,[25] we proposed three signatures of DB violations in single-molecule event-averaged measurements: (i) peaks in the waiting time distribution; (ii) asymmetry in two-event histograms; (iii) disappearance in the diagonal dominance of joint-event histograms. As a result, if the single-molecule histogram displays one of these signatures, the enzymatic reaction violates detailed balance, and we may expect deviations from the MM equation. On the other hand, if the enzymatic reaction is in equilibrium, the MM equation will hold and no signatures of DB violations can be observed. Therefore, the relationship between detailed balance and the validity of MM equation for the ensemble turnover rate can be verified by means of single-molecule event-averaged measurements.

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