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Molecular memory and dynamic cooperativity in monomeric enzymes

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The classical Michaelis-Menten (MM) equation, represents a non-cooperative kinetic response of enzymes with either a single or non-interacting multiple binding site(s). The mean catalytic rate of several monomeric enzymes, however, shows deviations from this classical behavior. This effect, termed as dynamic cooperativity, is believed to be associated with molecular mechanisms, stochastic reaction networks, that include enzymatic conformational fluctuations in product formation pathways. In spite of the latter, however, the present understanding of dynamic cooperativity is confined to mean kinetic measures, obtained from deterministic rate equations, which can not account for fluctuations. Here, we consider a stochastic reaction network for a special class of monomeric enzyme, called mnemonical enzymes, which are known to exhibit both positive and negative (dynamic) cooperativity. We model their kinetics using the chemical master equation (CME) to show how the emergence of dynamic cooperativity, at the molecular level, is inextricably linked to the multiplicity of monomeric enzyme numbers, enzymatic conformational fluctuations and molecular memory. Our results show that dynamic cooperativity is a transient phenomenon, which emerges due to temporal correlations between enzymatic turnovers, and vanishes as these correlations decay and molecular memory fades.

Keywords: Stochastic enzyme kinetics, enzyme cooperativity, mnemonical enzymes, chemical master equation, waiting time distributions.

1. Introduction

The classical Michaelis-Menten (MM) equation predicts a hyperbolic dependence of the mean catalytic rate on substrate concentration[1]. The latter represents a non-cooperative kinetic response of enzymes with either a single or non-interacting multiple binding site(s). Deviations from hyperbolicity, displayed by several enzymes with multiple binding sites, is then a signature of interactions between binding sites. From the perspective of classical kinetics, thus, enzyme cooperativity is inherently linked to the multiplicity of binding sites and interactions between them[2,3]. As a corollary, an enzyme with a single binding site, a monomeric enzyme, or an enzyme with non-interacting multiple binding sites, studied in recent single-molecule experiments[4,5], can not show “cooperativity”.

While the classical description of enzyme cooperativity is confined to substrate binding affinity at equilibrium, there are several monomeric enzymes which show dynamic (or kinetic) cooperativity[2,3]. In particular, several monomeric enzymes show positive cooperativity, in which the variation of product formation rate with substrate concentration is more steeper than is allowed by the MM equation. This results in a speeding up of the MM kinetics. In the opposite case of negative cooperativity, the substrate variation of product formation rate is less steeper than the MM equation, resulting in a slowing down of the MM kinetics. Deviations from the MM equation, and hence non-hyperbolicity in monomeric enzymes, is believed to be associated with molecular mechanisms, stochastic reaction networks, that allow enzymatic conformational fluctuations in product formation pathways[2,3,6–9].

The landmark experiment on the turnover kinetics of a single tetrameric enzyme, β-galactosidase, has directly measured fluctuations in the catalytic rate to reveal that the MM equation is not obeyed at the molecular level[4,5]. β-Galactosidase is an enzyme with four independent and identical binding sites, which is known to follow the MM equation in bulk amounts. At the single-molecule level, however, devia-

tions from the MM equation are believed to be the result of temporal fluctuations due to interconversions between multitude of enzymatic conformational states before forming a product through one of the several possible MM pathways. These temporal fluctuations can now be measured and characterized in terms of new statistical measures – distributions of waiting times between consecutive product formation and their moments[4,5]. The latter suggests that multiplicity of binding sites in a single fluctuating enzyme, even if non-interacting, is necessary to yield deviations from the MM equation. In the same vein, thus, the emergence of dynamic cooperativity in monomeric enzymes can be traced to the multiplicity of monomeric enzyme numbers and enzymatic conformational fluctuations in product formation pathways.

While the mechanistic origin of dynamic cooperativity has long been known, most previous studies have used deterministic approach to understand dynamic cooperativity. These studies have assumed, implicitly, that distinct conformational states of enzymes and enzymes-substrates, at any time *t*, can be written as their respective concentrations. The time evolution of these concentrations follows the classical deterministic mass action kinetics. While it is not clear how deterministic rate equations can account for conformational fluctuations in reaction pathways, deviations of the mean product formation rate from the MM equation, a result of MM-like but not MM mechanisms, is considered to be a signature of dynamic cooperativity[3,6–9]. Strictly speaking, however, the number of enzymes in a given conformational state and the lifetime of each conformational state are fluctuating quantities, the time evolution of which can only be described probabilistically, and not deterministically. This naturally demands a stochastic kinetic description, within which a generic link between intrinsic temporal fluctuations in the reaction pathway and dynamic cooperativity can be established.

Here, we present a generalized theoretical formalism, based on the chemical master equation (CME)[10,11], to understand dynamic cooperativity at the molecular level. We consider a special class of monomeric enzymes, called mnemonic enzymes or enzymes with memory[9], to show how the emergence of dynamic cooperativity is inextricably linked to the multiplicity of monomeric enzyme numbers, enzymatic conformational fluctuations and molecular memory.

2. Modeling dynamic cooperativity in monomeric enzymes

A class of monomeric enzymes, called mnemonic enzymes or enzymes with memory, are known to exhibit dynamic co-operativity[9]. Monomeric cooperativity is best described by a simple mnemonical model that includes interconversions between two conformational states of a free enzyme, one less stable than the other, shown in Fig. 1. The term mnemonic refers to a memory of the enzyme that "remembers" for a while the conformation stabilized by the product. Transient kinetic studies on wheat germ hexokinase, a mnemonical enzyme, have provided evidence of both positive and negative co-operativity in these class of enzymes[12]. From a theoretical point of view, the latter makes them "model enzyme systems" to investigate the link between conformational fluctuations and nature (positive and negative) of dynamic cooperativity in monomeric enzymes.

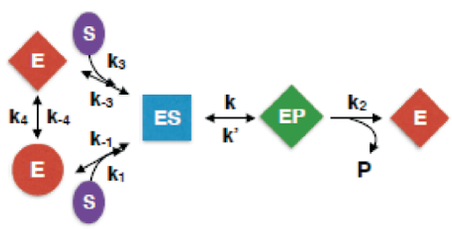

Fig. 1. Mnemonical enzyme model is a stochastic reaction network that includes fluctuations between two conformational states of a free enzyme before forming a product through single pathway. Red circle and rhombus represent two conformational states of a free enzyme, E_1 and E_2. Blue square and green rhombus represent enzyme-substrate *ES* and enzyme-product E_2P conformational states. The term mnemonic refers to a kind of memory of the enzyme which remembers the conformation stabilized by the product (rhombus).

We first outline the classical approach, based on deterministic mass action kinetics, to show how the existing kinetic measure based on the mean product formation rate, the steady-state enzymatic velocity, is used to discern positive and negative cooperativity. This is followed by a stochastic approach, based on the chemical master equation (CME), which provides new statistical measures of fluctuations to quantify dynamic cooperativity at the molecular level.

A. Deterministic kinetics

The mnemonical model includes enzymes in two confor-

mational states of a free enzyme, E_1 and E_2, represented by solid circle and rhombus in Fig. 1, respectively[9]. The substrate binding yields a different conformational state ES, represented by solid square. The product binding, on the other hand, stabilizes one of the two conformational states (represented by rhombus) of a free enzyme, E_2P, which dissociates to form product P and free enzyme E_2.

The rate equations for the mnemonical model are given by

$$d_t[E_1] = -(k_a + k_{-4})[E_1] + k_{-1}[ES] + k_4[E_2]$$
$$d_t[E_2] = -(k_b + k_4)[E_2] + k_{-3}[ES] + k_{-4}[E_1] + k_2[E_2P]$$
$$d_t[ES] = k_a[E_1] + k_b[E_2] + k'[E_2P] - (k_{-1} + k_{-3} + k)[ES]$$
$$d_t[E_2P] = k[E_1S] - (k' + k_2)[E_2P],$$

where $k_a = k_1[S]$, $k_b = k_3[S]$ and $[E]_0 = [E_1] + [E_2] + [ES] + [E_2P]$ is the total enzyme concentration.

The steady-state enzymatic velocity is given by $V = k_2[E_2P]_{ss}$, where $[E_2P]_{ss}$ is the steady-state concentration of enzyme-product complex. The steady-state approximation for E_1, E_2, ES, E_2P reduces the above set of coupled ordinary differential equations into algebraic equtions[9]. The latter can be easily solved to yield the steady-state enzymatic velocity V as

$$\frac{[E]_0}{V} = \frac{\alpha + \beta[S] + \gamma[S]^2}{\delta[S] + \in[S]^2} \qquad (1)$$

where α, β, γ, δ and $\in$ are effective rate coefficients whose explicit functional form is provided in Table 1.

Table 1. Explicit functional form of the effective kinetic coefficients of eq. (1)

Coefficient	Functional form
α	$(k_4 + k_{-4})[(k_{-1} + k_{-3})(k' + k_2) + kk_2]$
β	$(k_1k_4 + k_3k_{-4})(k + k' + k_2) +$ $(k' + k_2)(k_1k_{-3} + k_3k_{-1}) + kk_1k_2$
γ	$k_1k_3(k + k' + k_2)$
δ	$kk_2(k_1k_4 + k_3k_{-4})$
$\in$	$kk_1k_2k_3$

B. Stochastic kinetics

Before turning to the stochastic kinetic description of the mnemonical enzyme model, a few points of distinction between deterministic and stochastic kinetics are worth highlighting. Deterministic mass action kinetics, valid for macroscopic amounts of reactants, describes the change of concentrations with time. This implicitly assumes that all reactants, described collectively in terms of their concentrations, combine and transform continuously in time to form products, such that both $[P]$ and its rate of change $d_t[P]$ can be defined.

In stochastic kinetics, however, molecular noise, originating from fluctuations of both quantum mechanical and thermal origin, imparts stochasticity to each step of the reaction mechanism. As a result, the number of reactants, complexes and products change in discrete integer jumps, occurring randomly in time, such that neither $[P]$ nor its rate of change $d_t[P]$ can be defined. Instead, the kinetics is described by a series of waiting times τ_1, τ_2, ... between two consecutive product turnovers $\tau_p = T_p - T_{p-1}$, with turnover number, p = 1, 2, ..., where T_p is the turnover time for the p-th product formation[13].

Table 2. Rate parameters for three kinds of dynamic cooperativity in the mnemonic model

Cooperativity	k	k'	k_1	k_{-1}	k_2	k_3	k_{-3}	k_4	k_{-4}
Positive	10^3	1	1	10^{-3}	10^6	1000	10^{-6}	10^5	0.1
Negative	10^3	1	10^3	10^{-3}	10^6	1	10^{-6}	10^2	10^2
Zero	10^3	1	2	0.2	10^6	2	0.2	1	1

A schematic of the mnemonical model for a single enzyme forming products, one at a time, in discrete turnover events is depicted in Fig. 2. The presence of molecular noise ensures that both τ_p and T_p are stochastic quantities with probability distributions of waiting times, $w(\tau_p)$ and joint distributions of p-th and q-th waiting times, $w(\tau_p, \tau_q)$, which can be obtained from the chemical master equation (CME) approach, described below. Kinetic information contained in these distributions, for given p, q = 1, 2, ... , N and $[S]$ is, then, extracted in terms of their moments.

The CME accounts for inherent stochasticity of a chemical reaction in terms of the time evolution of the joint probability of the number of molecular species in the reaction mechanism[10,11]. The CME assumes occupancy time for each kinetic state as exponentially distributed, and for a given mechanism can be written as the gain (first line) and loss

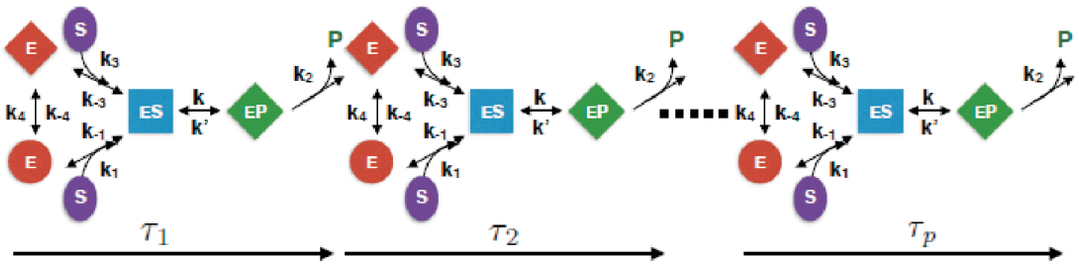

Fig. 2. A schematic of the mnemonical model for a single enzyme forming products, one at a time, in discrete turnover events. Here, $\tau_p = T_p - T_{p-1}$ is the waiting time between two consecutive product turnovers and T_p with p = 1, 2, ... is the p-th turnover time.

(second line) in probability due to the forward and backward reactions:

$$\partial_t P(\boldsymbol{n},t) = \sum_\sigma t_\sigma^{\rightarrow}(\boldsymbol{n}-\boldsymbol{r}_\sigma)P(\boldsymbol{n}-\boldsymbol{r}_\sigma,t) - t_\sigma^{\leftarrow}(\boldsymbol{n})P(\boldsymbol{n},t)$$

$$+ \sum_\sigma t_\sigma^{\leftarrow}(\boldsymbol{n}+\boldsymbol{r}_\sigma)P(\boldsymbol{n}+\boldsymbol{r}_\sigma,t) - t_\sigma^{\rightarrow}(\boldsymbol{n})P(\boldsymbol{n},t) \qquad (2)$$

For the mnemonical model, $P(\mathbf{n},t)$ is the joint probability distribution of the state vector $\mathbf{n} = (n_{E_1}, n_{E_2}, n_{ES}, n_{E_2P}, n_p)$ representing the number of enzymes, complexes and products in various conformational states at time t, subject to the constraint $n_{E_1} + n_{E_2} + n_{ES} + n_{E_2P} = N$, where N is the total number of enzymes. Here, $t_\sigma^{\rightarrow}(\mathbf{n})$ and $t_\sigma^{\leftarrow}(\mathbf{n})$ are the rates of the σ-th forward and backward reaction steps, which takes the state $\mathbf{n}$ to the state $\mathbf{n} + \mathbf{r}_\sigma$ and $\mathbf{n} - \mathbf{r}_\sigma$, respectively. For the mnemonical enzyme model, the number of reaction steps and the corresponding $\mathbf{n}$, $\mathbf{r}_\sigma$, $\mathbf{t}_\sigma^{\rightarrow}(\mathbf{n})$ and $\mathbf{t}_\sigma^{\leftarrow}(\mathbf{n})$ are summarized in Table 3.

The waiting time distribution for the p-th product formation is related to the solution of the above CME through

$$w(\tau_p; N) = -\Sigma^* \partial_t P(\mathbf{n}^*, p-1, t)|_{t=\tau_p} \qquad (3)$$

where the summation is over the number of reactants and

Table 3. Reaction steps and the corresponding $\mathbf{r}_\sigma$, $\mathbf{t}_\sigma^{\rightarrow}(\mathbf{n})$, $\mathbf{t}_\sigma^{\leftarrow}(\mathbf{n})$ for the mnemonical enzyme model

Step	$\mathbf{r}_\sigma$	$\mathbf{t}_\sigma^{\rightarrow}(\mathbf{n})$	$\mathbf{t}_\sigma^{\leftarrow}(\mathbf{n})$
$E_2 \rightleftharpoons E_1$	(1, –1, 0, 0, 0)	$k_4 n_{E_1}$	$k_{-4} n_{E_2}$
$E_1 \rightleftharpoons ES$	(–1, 0, 1, 0, 0)	$k_a n_{E_1}$	$k_{-1} n_{ES}$
$E_2 \rightleftharpoons ES$	(0, –1, 1, 0, 0)	$k_b n_{E_2}$	$k_{-3} n_{ES}$
$ES \rightleftharpoons E_2P$	(0, 0, –1, 1, 0)	$k n_{ES}$	$k' n_{E_2P}$
$E_2P \rightarrow P + E_2$	(0, 1, 0, –1, 1)	$k_2 n_{E_2P}$	0

intermediates in the mnemonic model, described by the state vector $\mathbf{n}^* = (n_{E_1}, n_{E_2}, n_{ES}, n_{E_2P})$ and $n_p = p - 1$ with p = 1, 2, ..., as the turnover numbers[14].

A single mnemonical enzyme, at any time t, can occupy only one of the four possible states, specified by the state vector $\mathbf{n}^*$. This makes the catalytic turnovers a renewal process, in which the waiting times between consecutive turnovers are independently and identically distributed. From this, it follows that the identity $w(\tau_1) = w(\tau_p)$ holds for all p = 2, 3, ..., and specification of turnover index is not necessary. The constraint of mutual exclusivity for N = 1 implies that $P_{E_1} = P(1, 0, 0, 0, 0)$, $P_{E_2} = P(0, 1, 0, 0, 0)$, $P_{ES} = P(0, 0, 1, 0, 0)$, $P_{EP_2} = P(0, 0, 0, 1, 0)$ for the first product formation. For a single enzyme, thus, the CME decouples to a set of ordinary differential equations (ODEs),

$$d_t P_{E_1} = -(k_a + k_{-4})P_{E_1} + k_{-1}P_{ES} + k_4 P_{E_2}$$
$$d_t P_{E_2} = -(k_b + k_4)P_{E_2} + k_{-3}P_{ES} + k_{-4}P_{E_1}$$
$$d_t P_{ES} = k_a P_{E_1} + k_b P_{E_2} + k' P_{E_2P} - (k_{-1} + k_{-3} + k)P_{ES}$$
$$d_t P_{E_2P} = k P_{ES} - (k' + k_2)P_{E_2P}$$

The constraint of mutual exclusivity of states and the renewal nature of turnover statistics for N = 1, when used in eq. (3), yields the following expression for the waiting time distribution:

$$w(\tau; N = 1) = -\partial_t (P_{E_1} + P_{E_2} + P_{ES} + P_{E_2P})|_{t=\tau} \qquad (4)$$

which using the above set of ODEs simplifies to

$$w(\tau; N = 1) = k_2 P_{E_2P}(\tau) \qquad (5)$$

The Laplace transform of the above ODEs yields a set of coupled algebraic equations which can be easily solved to obtain an exact expression for the waiting time distribution, $W(s) = k_2 P_{E_2P}(s)$ for N = 1[17].

For multiple enzyme numbers, N > 1, the catalytic turn-

overs form a non-renewal process, in which waiting times between consecutive turnovers are neither independent nor identically distributed[14]. As a result, an exact analytical solution of the CME is difficult to obtain. However, the method of superposition of renewal processes[18] can be used to obtain the first waiting time distribution for any $N > 1$, provided the waiting time distribution for $N = 1$, eq. (5), is known from the solution of the ODEs

$$w(\tau_1; N) = N\, w(\tau_1; N = 1)\left(\int_{\tau_1}^{\infty} w(\tau'_1; N = 1)\, d\tau'_1 \right)^{N-1} \quad (6)$$

The analytical expression for $w(\tau, N = 1)$, and thus $w(\tau_1, N)$, is unwieldy. Therefore, we avoid presenting their functional form, and directly compute their first two moments.

The first moment of $w(\tau, N = 1)$ yields the mean waiting time for a single enzyme,

$$\langle \tau \rangle = \frac{\alpha + \beta[S] + \gamma[S]^2}{\delta[S] + \in[S]^2} \quad (7)$$

which exactly recovers the inverse steady-state enzymatic velocity, $[E]_0/V$. The variance of the distribution, $\tau_\tau^2 = \langle \tau^2 \rangle - \langle \tau \rangle^2$, provides a statistical measure of intrinsic temporal fluctuations, called the randomness parameter, $r = \dfrac{\sigma_\tau^2}{\langle \tau \rangle^2}$.

Another statistical measure of intrinsic temporal fluctuations that can be derived from $w(\tau_p, \tau_q)$ is the correlation, $C_q = \langle \delta\tau_p \delta\tau_{p+q} \rangle$, between a waiting time, τ_p, and another, τ_{p+q}, q turnovers apart, where $\delta\tau_p = \tau_p - \langle \tau_p \rangle$, $p = 1, 2, \ldots$. A non-zero value of C_q indicates that enzymatic turnovers are temporally correlated, implying that the waiting time duration of first turnover influences the waiting time duration of subsequent turnovers. This "molecular memory" effect implies that sequences of waiting times shorter or longer than the mean are more probable than sequences with uniformly distributed waiting times[13,14].

To obtain $w(\tau_p, \tau_q)$, we carry out exact stochastic simulations of the CME[15,16]. For this we generate typically 10^6 stochastic trajectories of the mnemonic enzyme model for rate parameters given in Table 2.

3. Results

The moments of $w(\tau_p)$ and $w(\tau_p, \tau_q)$ provide kinetic measures of means and fluctuations, which we describe below.

A. Kinetic measures of means

Classical limit: Dynamic cooperativity, in the classical sense, is described as deviation of the steady-state enzymatic velocity $V/[E]_0$ from the MM equation. This, best represented in terms of the double reciprocal plot of $[E]_0/V$ versus $1/[S]$, the Lineweaver-Burk plot, is shown in the left panel of Fig. 3. In the latter, the linear dependence of $[E]_0/V$ on $1/[S]$ is a signature of zero-cooperativity, arising from the hyperbolicity of the MM equation. A speeding up or slowing down of the kinetics with respect to the MM equation, shown by red and blue curves respectively, thus, indicates positive or negative (dynamic) cooperativity.

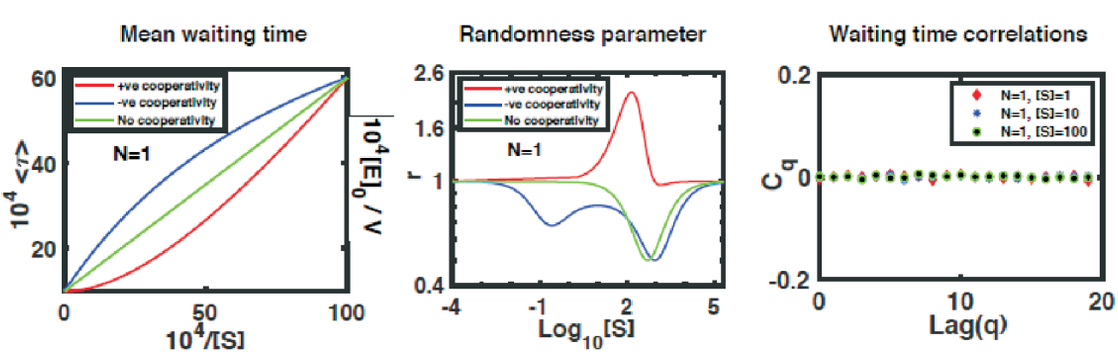

Fig. 3. Single monomeric enzyme: variation of the kinetic measures of mean – the mean waiting time $\langle \tau \rangle$ for a single enzyme and the inverse steady-state velocity $[E]_0/V$ for macroscopic amounts of enzymes – as a function of substrate concentration is shown in the left panel. Variation of the randomness parameter r with $[S]$, and waiting time correlations C_q with turnover lag $q = 1, 2, \ldots$ is shown in the middle and right panels.

Single enzyme: At the single enzyme level, the substrate variation of the mean waiting time $\langle\tau\rangle$ has the same functional form as $[E]_0/V$. The variation of $\langle\tau\rangle$ versus 1/[*S*] and $[E]_0/V$ versus 1/[*S*], shown as a combined plot in the left panel of Fig. 3 for common rate parameters listed in Table 2, is thus identical. This shows that a single monomeric enzyme shows dynamic cooperativity in the same way as macroscopic amounts of enzymes. However, since "cooperativity" is a collective phenomenon, requiring multiplicity of enzyme numbers or binding sites, indicates that the kinetic measures of means – the mean waiting time at the single enzyme level or steady-state enzymatic velocity for macroscopic amounts of enzymes – are not sufficient to quantify dynamic cooperativity.

B. Kinetic measures of fluctuations

The randomness parameter, $r = \dfrac{\sigma_\tau^2}{\langle\tau\rangle^2}$, and the correlation, $C_q = \langle\delta\tau_1\delta\tau_q\rangle$, between the waiting times of first and *q*-th turnovers, with q = 1, 2, 3, ... lags, provide new kinetic measures of intrinsic temporal fluctuations that have no classical analog. We compute these measures for single and multiple enzyme(s) for rate parameter conditions, Table 2, that yield (i) zero, (ii) negative and (iii) positive (dynamic) cooperativity for the mean waiting time, the left panel of Fig. 3.

Single monomeric enzyme: The magnitude of *r* is linked to mechanism topologies[19]. For mechanisms with linear topologies, in which the terminal step is irreversible, the randomness parameter is always less than one, $r < 1$. For mechanisms with branched topologies, the rate parameter conditions that favor dynamic disorder always yield $r > 1$. The absence of dynamic disorder in mechanisms with branched topologies, implies $r < 1$.

The mnemonical enzyme model is a stochastic reaction network in which fluctuations between two conformational states of a free enzyme connects two linear MM pathways. This yields a molecular mechanism with branched topology. The magnitude of *r* for rate parameter conditions in Table 2 is depicted in the middle panel of Fig. 3. (i) For zero cooperativity, the rate parameters for two linear MM pathways and the rate of interconversions between two conformational states are symmetrical. This yields an effective linear MM pathway with $r < 1$. This is the condition for zero cooperativity (green line) in a network with branched topology[18]. (ii) For negative cooperativity, the substrate binding step for one of the two possible MM pathways is rate limiting at lower [*S*], yielding a minimum for $r < 1$. With the increase in [*S*], *r* increases gradually while remaining less than unity, until [*S*] attains a value at which the rate parameters of the mnemonical model become symmetrical. This corresponds to condition (i) of zero-cooperativity, which results in a second minimum for $r < 1$ in higher [*S*] range. Thus, negative cooperativity (blue line) in the mnemonical enzyme network is signaled by $r < 1$. (iii) For positive cooperativity, the competition between the rates of conformational fluctuations and substrate binding yields $r > 1$ as long as both the substrate binding steps are rate limiting. This is the condition for dynamic disorder, $r > 1$, in the mnemonical enzyme network with positive cooperativity (red line). In all three cases, as soon as product formation becomes the rate limiting step, $r = 1$ is attained.

The right panel of Fig. 3 shows that waiting time correlations, C_q, irrespective of the type of cooperativity, are identically zero for all [*S*]. This follows from the renewal nature of the turnover statistics for a single enzyme. The correlation between first and *q*-th waiting times, q = 1, 2, ... turnovers apart, thus, provides a new statistical measure of dynamic cooperativity. Since $C_q = 0$ for $N = 1$, the waiting time correlations capture the essential idea that a single enzyme can not show dynamic cooperativity.

At the single molecule level, thus, deviations of the mean waiting time from the MM equation, is merely a reflection of distinct product formation pathways, as captured by *r*, requiring different mean waiting time for product formation.

Multiple monomeric enzymes: For $N > 1$, the non-renewal nature of turnover statistics yields $C_q \neq 0$, implying that enzymatic turnovers are correlated in time. A finite value of C_q indicates that the time duration of first turnover influences the time duration of subsequent turnovers. This yield a "molecular memory" effect in which positive correlations between waiting times suggest that a long (or short) first waiting time (compared to its mean value) is more likely to be followed by a long (or short) second waiting time. Similarly, negative correlations between waiting times imply that a long (or short) first waiting time is more likely to be followed by a short (or

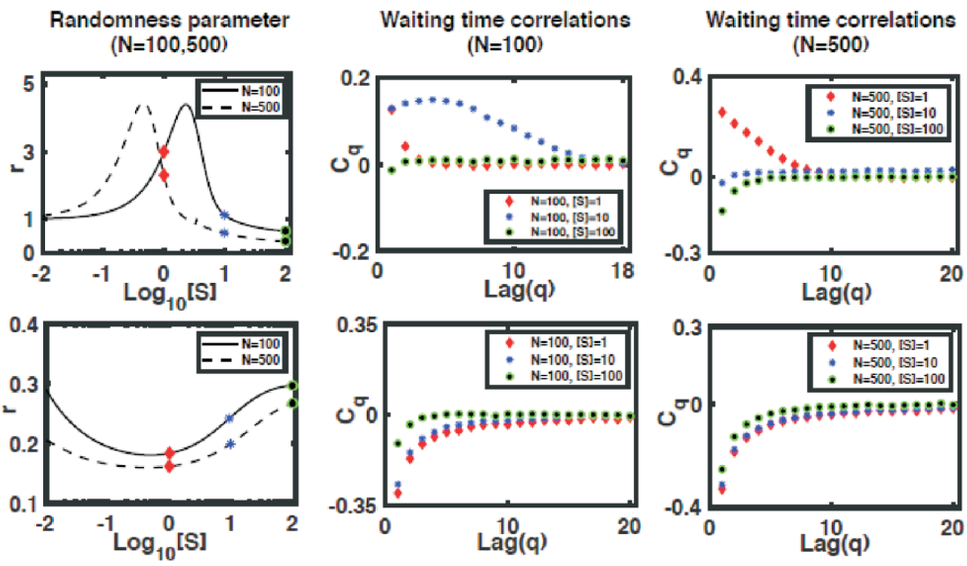

Fig. 4. Left panels show variation of the randomness parameter r versus [S] for N = 100, 500. Red, blue and green symbols represent the substrate concentrations for which waiting time correlations C_q for N = 100 (middle panels) and N = 500 (right panels) have been evaluated. Rate parameter values for top and bottom panels are given in the first and second column of Table 2. For the mnemonic enzyme model, the simultaneous observation of $r > 1$ and $C_q > 0$ is a signature of positive cooperativity. Similarly, $r < 1$ and $C_q < 0$ implies negative cooperativity.

long) second waiting time. The critical turnover number q^* beyond which temporal correlations decay and molecular memory fades demarcates a transient regime $q \ll q^*$ from a steady state regime $q \gg q^*$ [20]. In the transient regime $C_q \neq 0$ and molecular memory persists. In the steady-state regime, in contrast, $C_q = 0$ with no molecular memory.

$C_q \neq 0$ ensures that the multiplicity of enzyme numbers is necessary to yield a form of "cooperativity" in which product turnovers are not independent, as in classical deterministic kinetics, but are correlated in time. To understand the link between molecular memory and dynamic cooperativity, Fig. 4 presents a combined plot of r versus [S] (left panel) and C_q versus q (right panels). Interestingly, the rate parameters for positive cooperativity Table 2, for which the presence of dynamic disorder yields $r > 1$, also yield $C_q > 0$ in same substrate range. This implies that positive cooperativity in mnemonical enzyme model is related to the simultaneous observation of $r > 1$ (dynamic disorder) and $C_q > 0$ (positive memory). Similarly, the bottom panel of Fig. 4 shows that negative cooperativity in mnemonical enzyme model is related to the simultaneous observation of $r < 1$ (absence of dynamic disorder) and $C_q < 0$ (negative memory).

4. Conclusion

In this work, we use the CME description to present a stochastic reformulation of a mnemonical enzyme network, which is known to exhibit dynamic cooperativity, both positive and negative, albeit in a deterministic sense. The inclusion of stochasticity in the mnemonical enzyme model allows us to introduce two statistical measures of intrinsic temporal fluctuations – the randomness parameter r and waiting time correlations between enzymatic turnovers C_q – to quantify dynamic cooperativity at the molecular level. While the magnitude of r signals a branched or linear product formation pathway, with or without dynamic disorder, the sign of C_q indicates the nature of molecular memory in the transient regime.

Our analysis shows that a single mnemonical enzyme exhibits dynamic cooperativity in the same way as a macroscopic amounts of enzymes. However, since "cooperativity" necessarily requires multiplicity of enzyme numbers or binding sites, implies that kinetic measures – the mean waiting time for a single monomeric enzyme level or steady-state enzymatic velocity for macroscopic amounts of monomeric enzymes – are not sufficient to quantify dynamic cooperativity.

This motivates us to identify C_q, a statistical measure of correlations between enzymatic turnovers, as a new kinetic measure to quantify the nature and extent of dynamic cooperativity. $C_q = 0$ for $N = 1$ and $C_q \neq 0$ for $N > 1$ ensures that the multiplicity of enzyme numbers is necessary to yield a form of "cooperativity" in which product turnovers are temporally correlated in the transient regime. For the mnemonical enzyme model, the relation between r and C_q suggests that $C_q > 0$ is a signature of positive cooperativity in which a long (or short) first waiting time is more likely to be followed by a long (or short) second waiting time. Similarly, $C_q < 0$ is a signature of negative cooperativity in which a long (or short) first waiting time is more likely to be followed by a short (or long) second waiting time.

Dynamic cooperativity, at the molecular level, thus, emerges as a transient phenomenon, the duration of which is determined by the time required for temporal correlations between enzymatic turnovers to vanish and molecular memory to fade.

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References

1. L. Michaelis and M. L. Menten, *Biochem. Z*., 1913, **49**, 333.
2. A. Cornisn-Bowden, "Fundamentals of Enzyme Kinetics", 3rd ed., Portland Press, London, 2004.
3. A. Cornish-Bowden and M. L. Cardenasm, *J. Theor. Biol*., 1987, **124**, 1.
4. B. P. English, W. Min, A. M. van Oijen, K. T. Lee, G. Luo, H. Sun, B. J. Cherayil, S. C. Kou and X. S. Xie, *Nat. Chem. Biol*., 2006, **2**, 87.
5. W. Min, B. P. English, G. Luo, B. J. Cherayil, S. C. Kou and X. S. Xie, *Acc. Chem. Res*., 2005, **38**, 923.
6. J. R. Sweeny and J. R. Fisher, *Biochemistry*, 1968, **7**, 561.
7. C. Frieden, *J. Biol. Chem*., 1970, **245**, 5788.
8. G. R. Ainslie, J. P. Shill and K. E. Neet, *J. Biol. Chem*., 1972, **247**, 7088.
9. J. Ricard, J.-C. Meunier and J. Buc, *Eur. J. Biochem*., 1974, **49**, 195.
10. N. G. Van Kampen, "Stochastic Processes in Physics and Chemistry", 3rd ed., Elsevier, 2007.
11. C. W. Gardiner, "Handbook of Stochastic Methods for Physics, Chemistry and Natural Sciences", 2nd ed., Springer, 2004.
12. J. Ricard, J. Buc and J.-C. Meunier, *Eur. J. Biochem.*, 1977, **80**, 581.
13. A. Dua, *Resonance*, 2019, **24**, 297.
14. S. Saha, S. Ghose, R. Adhikari and A. Dua, *Phys. Rev. Lett.*, 2011, **107**, 218301.
15. D. T. Gillespie, *J. Phys. Chem*., 1977, **81**, 2340.
16. D. T. Gillespie, *Annu. Rev. Phys. Chem*., 2007, **58**, 35.
17. A. Kumar, H. Maity and A. Dua, *J. Phys. Chem. B*, 2015, **119**, 8490.
18. A. Kumar, S. Chatterjee, M. Nandi and A. Dua, *J. Chem. Phys*., 2016, **145**, 085103.
19. J. R. Moffit and C. Bustamante, *FEBS J*., 2014, **281**, 498.
20. A. Kumar, R. Adhikari and A. Dua, *Phys. Rev. Lett*., 2017, **119**, 099802.