Anomalous fluctuation scaling laws in stochastic enzyme kinetics: Increase of noise strength with the mean concentration

Ramon Grima
School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JZ, United Kingdom
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It is commonly thought that if a rate constant is perturbed such that the intracellular concentration of a certain species increases, then the fluctuations in the concentration will correspondingly decrease in strength. We here test whether this conventional wisdom generally holds true. We study the dependence of the noise strength (the coefficient of variation) in protein concentrations as a function of the mean protein concentration for a system in which protein is transported in and out of an intracellular compartment and it is catalyzed into a product by a multisubunit enzyme inside the compartment. The mean protein concentration is varied through perturbation of one of the rate constants. For low protein concentrations, the noise strength scales as $[P]^{-1/2}$, where $[P]$ is the mean concentration; this is the conventional fluctuation scaling law. However, we show that over a wide range of physiological concentrations, there are manifest anomalous fluctuation scaling laws proportional to $[P]^0$ and $[P]^{(N-1)/2}$, where N is the number of binding sites of the multisubunit enzyme. These laws are particularly conspicuous when the rate of protein import into the compartment is much larger than its export rate out of the compartment and when the enzyme exhibits positive cooperativity. The results imply that over a certain range of physiological concentrations, noise strength remains the same or increases with the mean protein concentration. This contradicts the popularly held notion that noise strength decreases with increasing concentration and suggests that noise can be important even when the number of molecules is large.

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I. INTRODUCTION

Molecular noise originates from the random timing of the biochemical events of unimolecular dissociation and bimolecular binding [1]. These events lead to fluctuations in the number of molecules by one or two molecules and, hence, an argument has frequently been made that the dynamics of chemical systems with a small mean number of molecules is stochastic. By this reasoning, noise is considered to be of principal importance for gene regulatory networks (since genes are present in few copies per cell) [2–6] and of lesser importance for metabolic networks where the proteins numbers can be of the order of thousands.

This intuitive understanding of noise is encapsulated in the often-quoted, classical rule of thumb, namely that the size of intrinsic noise (the coefficient of variation which is the standard deviation of number fluctuations divided by the mean number of molecules) is proportional to the inverse square root of the mean concentration [7]. We shall refer to this as the classical or conventional fluctuation scaling law. This law implies that for a given cell, if a rate constant is perturbed such that the concentration in a certain species increases, then the fluctuations in the concentration will correspondingly decrease.

In this article we show that deviations from this classical scaling law are common and that in some instances noise can even increase with protein concentration, which in turn implies that noise can be highly relevant to the dynamics of species characterized by a large copy number of molecules. The paper is divided as follows. In Sec. II, we introduce a stochastic model of enzyme-mediated protein catalysis and develop a simple effective approximation of the chemical master equation for this model. In Sec. III we utilize this effective method to study the dependence of the coefficient of variation of protein fluctuations on the mean protein concentration. We uncover several new anomalous scaling laws proportional to $[P]^{-1}$, $[P]^0$, and $[P]^{(N-1)/2}$, where N is the number of binding sites of the multisubunit enzyme and $[P]$ is the protein concentration. These predictions are verified by stochastic simulations. We conclude by a discussion of our results in Sec. IV in the context of recent single-cell studies.

II. THE ENZYME MODEL

We start by considering the following model of protein influx, outflux, and catalysis,

$$\emptyset \underset{k_1}{\overset{k_0}{\rightleftharpoons}} P, \quad P + C_i \underset{k_{-(i+1)}}{\overset{k_{(i+1)}}{\rightleftharpoons}} C_{i+1}, \quad C_{i+1} \xrightarrow{m_i} C_i + X, \quad (1)$$

where $i \in (1,2,\ldots,N)$, and P refers to the protein. The protein is input into a compartment at a rate k_0, leaves the compartment at a rate k_1 and is catalyzed into another species X by a multisubunit enzyme with N binding sites. The label C_i represents enzyme-protein complex species composed of an enzyme molecule and $i-1$ protein molecules (C_1 is the free enzyme species). An illustration of the catalytic process for the case of a two-subunit enzyme is shown in Fig. 1. Note that the total concentration of enzyme molecules is a constant at all times (denoted as E_T), since enzyme species shuffle between various complex states but are not degraded. Note also that the case $N = 1$ corresponds to catalysis via the Michaelis-Menten mechanism while for larger N the catalysis can, for certain parameter values, be cooperative with Hill coefficient N [8]. Examples of multisubunit enzymes abound, e.g., the mammalian TRiC enzyme ($N = 16$ subunits), which plays an essential role in the folding of actin, tubulin, and a large number of cell cycle regulators [9,10] and cytochrome P450 ($N = 2$ subunits), a family of enzymes playing a prominent role in the metabolism of a large number of substrates [11,12]. Hence, the

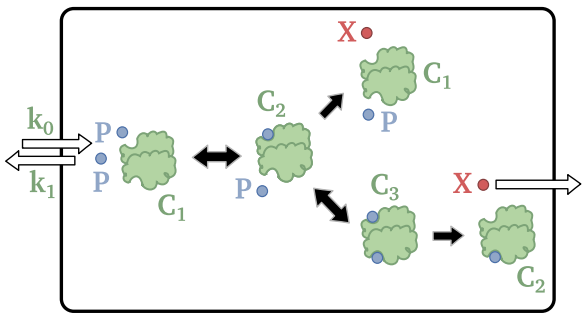

FIG. 1. (Color online) Illustration of substrate catalysis mediated by a two-subunit enzyme. This is the reaction scheme (1) for the case $N = 2$. The rectangle represents the compartment in which the reaction is confined.

proposed reaction scheme (1) can be thought of as a simple generic means of modeling post-translational modification of proteins via enzyme reactions.

The stochastic description of this reaction system, as given by the chemical master equation (CME) (a time-evolution equation for the probability of the system being in its various states [1]) cannot be exactly solved. Applying the linear-noise approximation (LNA) to the CME [7] (excluding species X from this description since it does not interact with any of the other components) leads to a system of linear equations for the $(1/2)(N+1)(N+2)$ second moments of the noise. Given that a large number of equations are obtained even for small N, analytical insight is very difficult with such a method as well.

To circumvent these difficulties we here propose an alternative approach: (i) We postulate an effective CME for just the protein rather than the $N+2$ species of the original reaction scheme (1). (ii) We apply the LNA to this effective CME to obtain an expression for the protein fluctuations. (iii) The scaling laws obtained by this method are then compared with numerical results obtained using the standard LNA and also using the stochastic simulation algorithm applied to reaction scheme (1). As we shall see the good match between the latter and our approach *a posteriori* justifies the use of the effective method. The relevance of our results to biochemical systems are guaranteed by use of physiological rate constants in stochastic simulations (see Ref. [13] for details).

A. Effective approximation of the CME

We start our analysis by ignoring noise and solving the deterministic rate equations of the concentrations of species involved in reaction scheme (1). For the moment we shall set the protein export rate k_1 to zero (later we will relax this assumption). This is the case of unidirectional active transport whereby the protein molecules are moved across the compartment membrane in only one direction, i.e., an energy-dependent process leading to a transport asymmetry such that molecules accumulate on one side of a compartment membrane. Such mechanisms are common inside cells; see, for example, Ref. [17] for an introduction to such transport systems.

We define the convenient set of constants $g_i = \prod_{j=1}^{i}(k_{-(j+1)} + m_j)/k_{j+1}$ for $i \in (1,\ldots,N)$. The $N+1$ rate equations for the complex concentrations $[C_i]$ can be solved in steady state and after substitution in the rate equation for the protein concentration $[P]$ we obtain

$$\partial_t[P] = 0 = k_0 - [P]\frac{E_T\sum_{i=1}^{N} m_i g_i^{-1}[P]^{i-1}}{1+\sum_{i=1}^{N} g_i^{-1}[P]^i}. \quad (2)$$

The fluctuation analysis proceeds as follows. We start by noting that Eq. (2) suggests an effective reaction (in steady-state conditions) of the type

$$\emptyset \xrightarrow{k_0} P, \quad P \xrightarrow{k'} \emptyset, \quad (3)$$

where k' is an effective rate constant equal to $k'(n) = [E_T\sum_{i=1}^{N} m_i g_i^{-1}(n/\Omega)^{i-1}]/[1+\sum_{i=1}^{N} g_i^{-1}(n/\Omega)^i]$, n is the discrete number of molecules of protein P, and Ω is the compartment volume. The CME for the above effective reaction scheme is

$$\partial_t\Pi(n,t) = k_0\Omega(\Pi(n-1,t) - \Pi(n,t)) + (n+1)k'(n+1)\Pi(n+1) - nk'(n)\Pi(n), \quad (4)$$

where $\Pi(n,t)$ is the probability that there are n proteins at time t. We proceed by applying the LNA to the above effective CME [7]. We shall be exclusively working in the steady-state regime. This leads to an equation for the rate of change of the mean protein concentration which is precisely given by Eq. (2) and to a linear Langevin equation for the fluctuations η about this mean concentration,

$$\partial_t\eta_p = J\eta_p + \Omega^{-1/2}B\Gamma(t), \quad (5)$$

where $\Gamma(t)$ is Gaussian white noise with zero mean and a time correlation function $\langle\Gamma(t)\Gamma(t')\rangle = \delta(t-t')$, $J = -d/d[P]([P]k'(\Omega[P]))$, and $B = \sqrt{k_0 + [P]k'(\Omega[P])}$. We shall consider the case where the concentration is varied through the protein influx rate k_0 and, hence, we can write k_0 in terms of $[P]$ using Eq. (2), which leads to $B = \sqrt{2[P]k'(\Omega[P])}$.

Justifiably, one may question the validity of this effective CME approach (and the corresponding LNA) given that the effective CME Eq. (4) was written down on purely heuristic grounds. Nevertheless, it has been shown that such effective approaches can be rigorously derived from the CME under quasiequilibrium conditions [18–21], which for enzyme systems is enforced whenever the turnover numbers, m_i, are much smaller than the rates at which complex decays back into protein and enzyme, $k_{-(i+1)}$. In the absence of such conditions, the effective CME will in some cases give the correct qualitative description and in other cases not [20]; the main plus of this approach is its simplicity. It is from the latter perspective that we here utilize the effective CME approach: We are not concerned with its rigorous derivation but rather we want to use this CME to derive simple scaling relationships between the size of the noise and the concentrations which will be later checked via stochastic simulations.

In what follows we shall use the effective CME approach to study fluctuations in the protein concentration. Fluctuation scaling laws in the enzyme and product concentrations are not available for the following reasons. The effective method we use throws away any information about the enzyme fluctuations, as can be seen from the fact that only the protein

species features in the effective reaction scheme (3). As for the product X, in our model it accumulates with time and thus is not amenable to the steady-state treatment presented in this article. Of course in reality this product would be feeding into some downstream reactions which would then ensue into a steady state for the product; however, explicit details of this connectivity would be needed before one can explicitly analyze its fluctuations.

III. SCALING ANALYSIS OF THE COEFFICIENT OF VARIATION

It can be shown by taking moments of Eq. (5) that the variance of the noise in steady-state conditions is given by $\sigma^2 = B^2/2|J|\Omega$. Hence, it follows that the coefficient of variation is given by

$$C_V = \frac{\sigma}{[P]} = (\Omega[P]s)^{-1/2}, \tag{6}$$

where $s = ([P]/x)(dx/d[P])$ is the susceptibility [22] which in this context is defined as the relative change in the effective protein removal flux $x = [P]k'(\Omega[P])$ following a change in the protein concentration $[P]$. Next we consider the limit of small and large concentrations of s from which we can deduce the scaling of C_V in the same limits.

In the limit of small protein concentrations, we have

$$s = 1 + O([P]). \tag{7}$$

In contrast there are two distinct large $[P]$ limits depending on the values of the turnover numbers m_i. If they are all equal to each other, then we have

$$s = Ng_N[P]^{-N} + O([P]^{-(N+1)}), \tag{8}$$

whereas if m_i all differ from each other, then we have

$$s = \frac{g_N(m_N - m_{N-1})}{g_{N-1}m_N}[P]^{-1} + O([P]^{-2}), \tag{9}$$

with $m_0 = 0$ and $g_0 = 1$.

We can now deduce the following scaling laws for the coefficient of variation, C_V with protein concentrations. Substituting Eq. (7) in Eq. (6) we find that for small protein concentrations we have the standard scaling law:

$$C_V = \Omega^{-1/2}[P]^{-1/2}. \tag{10}$$

Substituting Eq. (8) and Eq. (9) in Eq. (6), we find the surprising result that for large protein concentrations we have two possible anomalous scaling laws,

$$C_V \propto \Omega^{-1/2}[P]^{(N-1)/2}, \quad m_i = m_{i-1}\ \forall i \tag{11}$$

$$C_V \propto \Omega^{-1/2}[P]^{0}. \quad m_i \neq m_{i-1}\ \forall i \tag{12}$$

Hence, our effective theory predicts a transition in the exponent of the fluctuation scaling power law as the protein concentrations change from small to large. These theoretical predictions are confirmed by stochastic simulations using the stochastic simulation algorithm and by numerically solving the LNA of the full scheme (1); the results are shown in Fig. 2 and Fig. 3. Note that for values of m_i which are close together in Fig. 3, the power law shows characteristics of

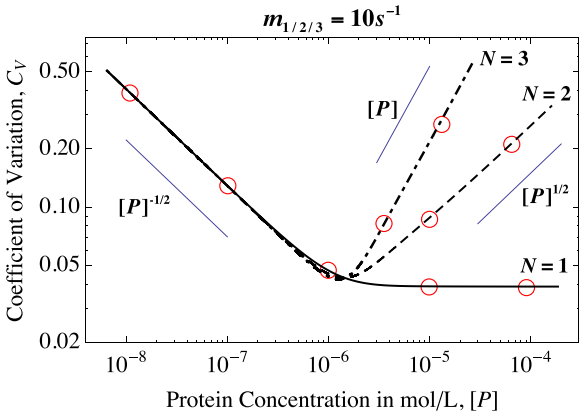

FIG. 2. (Color online) Plot of the coefficient of variation of fluctuations in protein concentration C_V versus the mean protein concentration $[P]$ for the catalytic reaction scheme (1) with $N = 1,2,3$ with equal turnover numbers m_i and in the absence of protein outflux. The lines show the predictions of the LNA (solid for $N = 1$, dashed for $N = 2$, and dot dashed for $N = 3$) while the circular points are obtained from stochastic simulations of the CME using the stochastic simulation algorithm (the moments are calculated by time averaging over long trajectories; error bars are not visible on this plot). Note that both the LNA and the stochastic simulations of the CME are for the full reaction scheme (1). The parameters are $k_1 = 0$, $m_1 = m_2 = m_3 = 10$ s^{-1}, $k_2 = k_3 = k_4 = 1 \times 10^7$ M^{-1} s^{-1} and $k_{-2} = k_{-3} = k_{-4} = 1$ s^{-1} where the units of time and concentration respectively are seconds (s) and mol/L or molar (M). The total enzyme concentration is fixed to 1.7×10^{-6} M and the compartment volume to 1 fl (corresponding to roughly 1000 enzyme molecules in the compartment). The protein concentration is varied by changing the protein production rate k_0 over the range 0–1.7×10^{-5} M s^{-1}. These results confirm the anomalous scaling law Eq. (11).

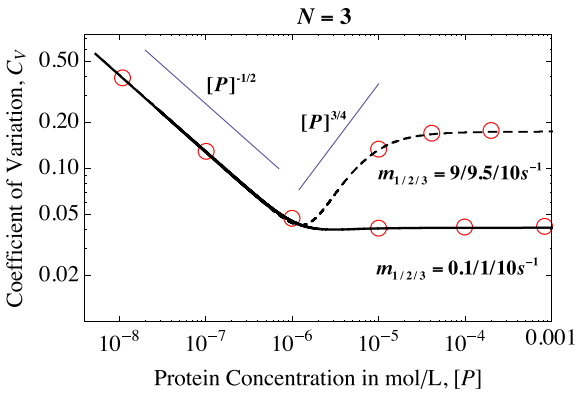

FIG. 3. (Color online) Plot of the coefficient of variation of fluctuations in protein concentration C_V versus the mean protein concentration $[P]$ for the catalytic reaction scheme (1) with $N = 3$ and unequal turnover numbers in the absence of protein outflux. Note that both the LNA (lines) and the stochastic simulations of the CME (points) are for the full reaction scheme (1). The turnover numbers are shown in the figure while the rest of the parameters are as in Fig. 2. The results confirm the anomalous scaling law Eq. (12). Note that for unequal but closely separated turnover numbers, the scaling law shares characteristics of the scaling laws for unequal and equal turnover numbers: it roughly follows Eq. (11) for intermediate concentrations and Eq. (12) for large concentrations.

both anomalous scaling laws predicted above: at intermediate concentrations the law is roughly $[P]^{3/4}$, which is close to the $[P]$ law predicted by Eq. (11) for $N = 3$ while at large concentrations we have the $[P]^0$ law predicted by Eq. (12). The size of the region of intermediate concentrations over which noise increases with concentration is found to increase with decreasing differences between the values of m_i and tends to infinity in the limit of zero differences. This necessarily follows from the fact that Eq. (12) must "switch" to Eq. (11) as the differences between the turnover numbers goes to zero.

It is also found that the anomalous phenomenon of fluctuation size increasing with concentration, which is observed at intermediate concentrations for unequal turnover numbers, is enhanced by positive cooperativity [see Fig. 4(a)] and is attenuated by negative cooperativity [see Fig. 4(b)], where positive cooperativity implies a situation in which substrate binding to an enzyme facilitates the next binding event and negative cooperativity implies the opposite. Positive cooperative phenomena are very common in biochemistry [15] and, hence, the anomalous effect we have identified here could be relevant to a wide range of biochemical systems.

We stress that though the size of the fluctuations may increase or remain the same with increasing protein concentration, the macroscopic limit is still well defined; this is since the latter is given by the limit of infinite volume Ω at constant concentration [1,7] and the scaling laws in all cases are proportional to $\Omega^{-1/2}$.

An intuitive explanation for the transition in the fluctuation scaling law is as follows. From Eqs. (2) and (3) one deduces that the effective rate parameter k' is a constant for small protein concentrations and scales as $k' \sim 1/[P]$ for large protein concentrations $[P]$. The latter implies that the effective protein removal rate due to catalysis decreases with increasing concentration, when the concentrations are sufficiently large. Hence, in this regime, large fluctuations in protein concentrations can be considerably long lived and correspondingly their magnitude can be quite large compared to the case where the removal rate is independent of $[P]$, namely the case of small concentrations. In agreement with this picture, one notes that large steady-state protein concentrations correspond to the region of parameter space close to a bifurcation; this is characterized by a small real and negative eigenvalue of the Jacobian of the effective deterministic equation which in turn implies very weak dampening of the fluctuations and, hence, to large fluctuations in the protein concentrations.

A. The case of bidirectional protein movement into the compartment

Thus far we have assumed unidirectional transport of protein into the compartment. We now treat the case in which protein can move in and out of the compartment via diffusion or active processes, i.e., we allow k_1 to take a nonzero value. The reduced rate equation model for scheme (1) now reads as follows:

$$\partial_t[P] = k_0 - [P](k_1 + k'(\Omega[P])). \tag{13}$$

Recall that the nonlinear catalytic rate $k'(\Omega[P])$ is proportional to a constant for low concentrations and of order $[P]^{-1}$ for high concentrations [see just after Eq. (3) for the definition of k'].

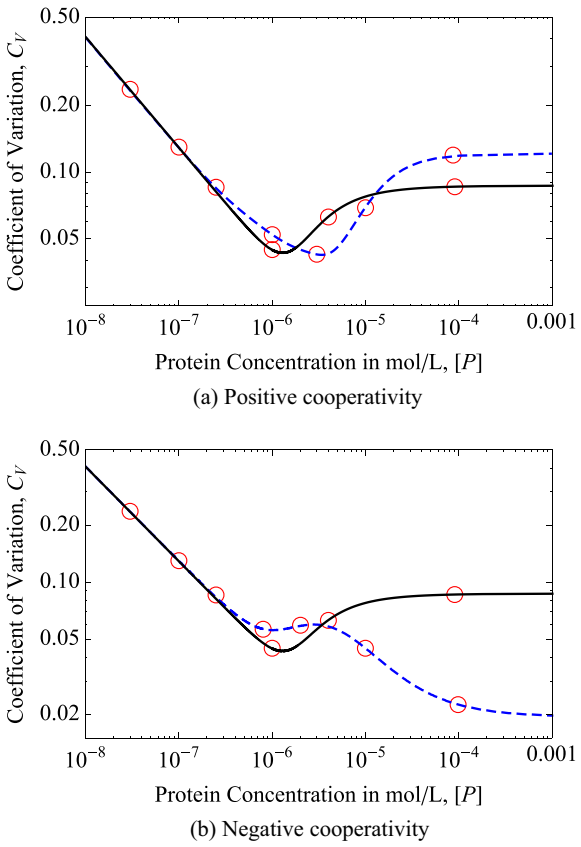

FIG. 4. (Color online) Influence of cooperativity on the anomalous scaling laws at intermediate concentrations for the case of unequal turnover numbers. Panels (a) and (b) show plots of the coefficient of variation of fluctuations in protein concentration C_V versus the mean protein concentration $[P]$ for the catalytic reaction scheme (1) with parameters $k_2 = 0.05 \times 10^7$ s^{-1} M^{-1}, $k_3 = 1 \times 10^7$ s^{-1} M^{-1}, $k_4 = 1.95 \times 10^7$ s^{-1} M^{-1} (blue dashed line in panel a), $k_2 = k_3 = k_4 = 1 \times 10^7$ s^{-1} M^{-1} (solid black line in panels a and b) and $k_2 = 1.95 \times 10^7$ s^{-1} M^{-1}, $k_3 = 1 \times 10^7$ s^{-1} M^{-1}, $k_4 = 0.05 \times 10^7$ s^{-1} M^{-1} (blue dashed line in panel b). These respectively represent the cases of positive cooperativity, no cooperativity, and negative cooperativity. The rest of the parameters are $N = 3$, $m_1 = 6$ s^{-1}, $m_2 = 8$ s^{-1}, $m_3 = 10$ s^{-1}, $k_{-2} = k_{-3} = k_{-4} = 1$ s^{-1}. The total enzyme concentration and the compartment volume are as in Fig. 2. Note that both the LNA (lines) and the stochastic simulations of the CME (points) are for the full reaction scheme (1). A comparison of the black and blue lines in both panels shows the anomalous phenomenon of increasing C_V with increasing protein abundance (apparent at intermediate concentrations) is amplified in the presence of positive cooperativity and diminished in the presence of negative cooperativity.

Hence, for small and large concentrations, the second term in Eq. (13) is directly proportional to the protein concentration, i.e., the protein removal kinetics is first order. Thus the kinetics can only be nonlinear for intermediate concentrations; this behavior stems from the dominance of the $k'(\Omega[P])$ term over the rate k_1 in Eq. (13). On this basis, one expects the anomalous fluctuation scaling laws that we found earlier, Eqs. (11) and (12), to be valid in some intermediate concentration range and the conventional scaling law Eq. (10) to manifest for small and large concentrations.

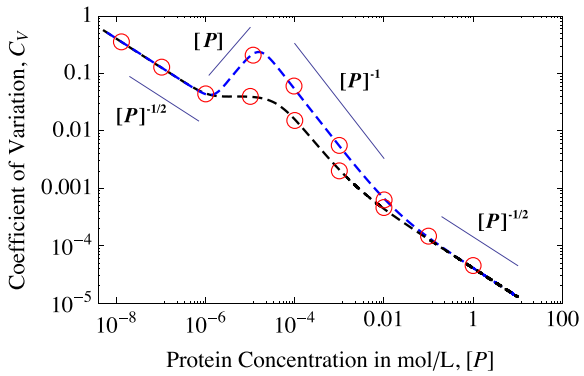

FIG. 5. (Color online) Influence of bidirectional protein transport on anomalous scaling laws for intermediate and large protein concentrations. We show a plot of the coefficient of variation of fluctuations in protein concentration C_V versus the mean protein concentration $[P]$ for the catalytic reaction scheme (1) with nonzero k_0 and k_1. Note that both the LNA (lines) and the stochastic simulations of the CME (points) are for the full reaction scheme (1). Two cases are shown: (i) the blue dashed line is for $N = 3$, $m_{1/2/3} = 10\ \mathrm{s}^{-1}$, $k_1 = 0.001\ \mathrm{s}^{-1}$; (ii) the black dashed line is for $N = 3$, $m_{1/2/3} = 0.1/1/10\ \mathrm{s}^{-1}$ and $k_1 = 0.01\ \mathrm{s}^{-1}$; the rest of the parameters are as in Fig. 2. Protein concentration is varied by changing k_0 over the range 0–$10^{-3}\ \mathrm{M\ s}^{-1}$. The results confirm that in the presence of bidirectional transport, (i) the anomalous scaling laws at intermediate concentrations ($C_V \propto [P]^0$ and $\propto [P]$ for unequal and equal turnover numbers respectively) are the same as those predicted for large concentrations for unidirectional transport and (ii) the conventional scaling law ($C_V \propto [P]^{-1/2}$) is obtained for large concentrations.

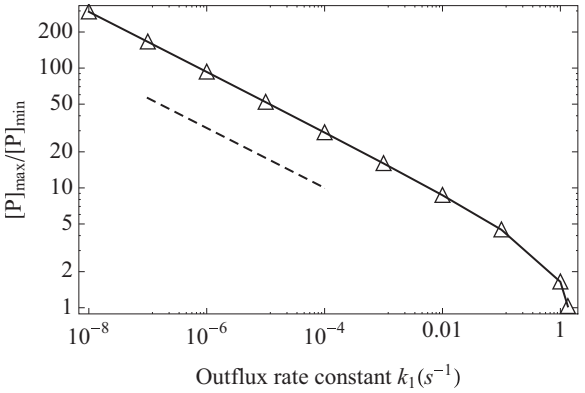

FIG. 6. Dependence of the range of anomalous behavior on the outflux rate constant k_1. By anomalous behavior here we specifically mean the increase of C_V with protein concentration over a range of intermediate concentrations, as, for example, seen in Fig. 5. $[P]_{\mathrm{min}}$ and $[P]_{\mathrm{max}}$ are, respectively, the protein concentrations at which the coefficient of variation reaches a minimum and a maximum which correspond to the two concentrations between which the C_V increases with protein concentration (see Fig. 5). Hence, the ratio $[P]_{\mathrm{max}}/[P]_{\mathrm{min}}$ is a measure of the size of the region of protein concentrations over which anomalous behavior is observed. The parameters are $N = 3$, $m_{1/2/3} = 10\ \mathrm{s}^{-1}$ and the rest are as in Fig. 2. The points are obtained using the LNA of the effective CME, Eq. (6), and the lines connecting them are simply a guide to the eye. The dependence is found to be $[P]_{\mathrm{max}}/[P]_{\mathrm{min}} \propto k_1^{-1/4}$ for small k_1 (dashed line), which confirms that the anomalous behavior becomes manifest over larger ranges of the concentration as the outflux decreases, i.e., as the transport switches from bidirectional to unidirectional. For $k_1 \gtrsim 4/3\ \mathrm{s}^{-1}$ the anomalous behavior disappears.

This hypothesis is confirmed by LNA and stochastic simulations of the full scheme (1), which are shown in Fig. 5. For the case $N = 3$, and equal turnover numbers, the exponent of the scaling law changes from $-1/2$ to 1 (over one order of magnitude) to -1 (over three orders of magnitude) and, finally to $-1/2$ as protein concentration increases. The relevance of anomalous behavior to real systems stems from the fact that simulations show that it is observed over the micromolar to millimolar regime (Fig. 5), which is a subset of the typical physiological range of concentrations (nanomolar to millimolar [16]).

The exponent of 1 stems from the nonlinear catalytic mechanism; indeed, this is the same as predicted in the absence of protein export by Eq. (11) (see also Fig. 2). The exponent of $-1/2$ for large concentrations is clearly due to the first-order protein export. The exponent of -1 originates from contributions to the fluctuations due to both the nonlinear catalysis and the first-order export process. In Fig. 5 we also show the effect of adding protein export to the case $N = 3$ with unequal turnover numbers; we see that the scaling law for intermediate protein concentrations is 0 in accordance with what is predicted for large concentrations in the absence of protein outflux by Eq. (12) (see also Fig. 3).

In Fig. 6 we explore how the size of the regime in which fluctuations increase with concentration varies with k_1; it is shown that the size of this regime increases with decreasing k_1 and is zero for k_1 above a certain threshold value. Hence, the aforementioned type of anomalous behavior is conspicuous whenever the rate of protein import into the compartment considerably exceeds the rate of protein export out of the compartment

IV. DISCUSSION

Summarizing, we have shown the existence of anomalous scaling laws in a model of enzyme-mediated catalysis. These laws emerge due to the nonlinearity in the law of mass action characterizing the bimolecular binding of protein and enzyme. The anomalous laws have been found to manifest over at least a decade of physiological protein concentrations and, hence, they are probably of relevance to real biological systems. We have also shown that the degree of observability increases with the asymmetry of the transport process carrying protein across the compartment membrane and with the extent of positive cooperativity.

Our study has made use of the LNA to derive the anomalous scaling laws. In recent years it has been shown that corrections to the LNA predictions of mean concentrations, of the covariance of fluctuations, and of higher moments of the probability distribution can differ considerably from those of the CME whenever there is at least one bimolecular reaction in the chemical reaction system [23–27]. The excellent agreement between the LNA prediction for the C_V and stochastic simulations in the figures is in some sense due to a cancellation of two errors since the LNA underestimates both the protein concentration and the variance of fluctuations

in enzyme systems [23,25]. It is, however, plausible that in different systems than the one considered here, the anomalous scaling laws are modified when the next order correction to the LNA is taken into account.

Here we have presented an analysis of a specific model of enzyme catalysis. However, it is to be noted that the results derived are more general than this model. In particular, the nonlinear catalytic rate [second term in Eq. (2)] is of the same form as the Adair equation [28] and that obtained from the Koshland-Nemethy-Filmer sequential model [29], which are commonly used mathematical descriptions of allosteric regulation [15]. It is also important to note that the effects elucidated stem from the nonlinear dependence of k' on the protein concentration in reaction scheme (3). Hence, one would expect that independent of the precise reaction mechanism at work, any chemical system which can be roughly approximated as (3) with a nonlinear k' will display anomalous fluctuation scaling laws in some range of protein concentrations. Such concentration-dependent degradation rates are common in nature [30,31] and, hence, it stands to reason that the effects elucidated here for a specific model of catalysis maybe found in other biochemical systems. Indeed it can be shown that similar anomalous scaling laws also manifest in simple gene regulatory networks; this will reported elsewhere.

We finish by noting that a transition from a $[P]^{-1/2}$ to a $[P]^0$ scaling law has been observed in single-cell studies [32,33]. The authors attributed the origin of the $[P]^0$ scaling law to heterogeneity in the rate constants of a linear genetic network in a population of cells. Our results, on the other hand, can be interpreted to hold for heterogeneity in the rate constants of a nonlinear enzymatic reaction in a population of cells. The heterogeneity that we consider is specifically in the rate constant k_0 since each point in the C_V versus $[P]$ plots corresponds to a particular value of k_0. While for linear systems only a transition from $[P]^{-1/2}$ to a $[P]^0$ scaling law is observed, in our nonlinear system a diversity of transitions have been elucidated, including the aforementioned transition, a transition from $[P]^{-1/2}$ to $[P]^{(N-1)/2}$ scaling law (where N is a positive integer), a transition from $[P]^{(N-1)/2}$ to $[P]^0$ or $[P]^{-1}$ scaling law, and a transition from $[P]^{-1}$ to $[P]^{-1/2}$ scaling law. One may ask why such transitions have not been observed thus far. The data in Refs. [32,33] are not for one protein but rather collated from that of all detectable protein species and, hence, are only reflective of the dominant transition in the scaling law. In contrast, our theory is for fluctuations in a single protein species involved in a particular catalytic reaction and, hence, it is plausible that various transitions in the fluctuation scaling law of a single protein species are masked by the averaging over all protein species and reactions, which is inherent in the published data.

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